Synthesis of Heterocyclic Compounds Using ketoacids-based Multicomponent Reactions and Evaluation of Their Anti-prion Activities

Submitted in partial fulfilment of the requirements for the degree

Doctor of Philosophy

by

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Declaration

I declare all presented that work in this thesis is my own research work that has been carried out in the Chemistry department at the University of Sheffield between October 2014 and August 2018 under the supervision of Professor Beining Chen. I also declare that this thesis is submitted to the University of Sheffield for obtaining of Ph.D. degree and has not been submitted elsewhere to any University or any other educational institution for obtaining any degree.

Ahmed M.J. Hsskia

August 2018

Abstract

The work outlined in this thesis consists of **ten chapters**:

Chapter one contains a general introduction of heterocyclic compounds and their use in drug discovery. It also contains an overview of ketoacids chemistry, isocyanides and multicomponent reactions as efficient ways for synthesising diverse and various heterocyclic compounds. In addition, explains subject aims and objectives. **Chapter two** describes a method for the synthesis of keto acids, isocyanides and convertible isocyanide from cheap stating materials and the synthesis of cyclic amides (lactams) by multicomponent reactions (3C-4C-Ugi reaction). The methodology which depends on the activation of keto acids (Carboxylic group) using PPTS is an excellent route to accomplish Meyer's lactamaztion in good yields. The optimisation and diastereoselectivity of all reactions are also discussed. **Chapter three** describes the synthesis of Ugi-tetrazole derivatives and information about using flow chemistry in this work. The results were investigated of similar reactions achieved using either batch or flow machines. **Chapter four** describes efforts towards using a microwave technique for synthesis a group of pyrazol-oxopyrrolidine derivatives with satisfactory yields. **Chapter five** includes an efficient methodology utilized to synthesize isoqunoline and isoindoline libraries from α-ketoacids and β-ketoacids in order to showcase the benefit of α- and β-ketoacids for synthesis of diverse heterocyclic compounds. **Chapter six** outlines exploiting post-transformations of multicomponent products for synthesis of diverse and various heterocycles with good yields. A central goal of further expansion is to provide collections of stereo chemically and structurally diverse compounds in order to evaluate their biological systems. The reaction of convertible isocyanide for synthesising pyroglutamic acid analogues is described. A developed method includes using bifunctional keto acids or amines *via* multicomponent reactions and their post-transformations for synthesis of tetrazole-lactams, diones, pyrrole-oxopyrrolidine and a set of medicinally-valuable pyrrolo-pyridine derivatives under mild conditions are also described. **Chapter seven** describes the main target of all synthesised compounds evaluated as anti-prion diseases. These compounds are designed to bind a putative binding pocket of PrP^{C} and stabilise the normal isoform. This should prevent its conversion into the infectious isoform (PrP^{S}) in brain. **Chapter eight**, **nine** and **ten** contain conclusion and future work, data of all experimental procedures and references respectively.

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Abbreviations

A549	Cell line lung carcinoma
AD	Alzheimer's disease
Ac	Acetyl
Aβ	Amyloid beta
Ar	Aryl
BBB	Blood brain barrier
Bn	Benzyl
Boc	*tert*-Butyoxycarbonyl
br.	Broad
BSA	Bovine serum albumin
BSE	Bovine Spongiform Encephalopathy
Cbz	Carboxybenzyl
CDI	1,1'-Carbonyldiimidazole
CDK2	Cyclin dependent kinase 2
Cdc7	Cell division cycle 7-related protein kinase
CJD	Creutzfeldt-Jackob Disease
Con.	Concentrated
CSA	Camphor sulfonic acid
Cy	Cyclohexyl
d	doublet
DBU	1, 8-Diazabicycloundec-7-ene
d.d	doublet of doublets
ddd	doublet of doublet of doublets
DIPEA	Diisopropylethylamine
DMAP	Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide

DNA	Deoxyribonucleic acid
Dp	receptor Prostagl and in D_2 receptor
de.	diastereomeric excess
E	Electrophile
EC_{50}	Half maximal effective concentration
EI	Electron ionization
ELOVL3	Elovel fatty acid elongase 3
ES	Electrospray
Et	Ethyl
EtOAc	Ethyl acetate
FBS	Foetal Bovine Serum
FTIR	Fourier-transform infra-red
GABAA	Gamma-aminobutyric acid
GHz	Gigahertz
GPCR	G protein coupled receptor
HBSS	Hanks balanced salt solution
Hela	Henrietta lacks human cell
HIV	Human immunodeficiency virus
HMDS	Hexamethyldisilazane
HMPA	Hexamethylphosphoramide
HOMO	Highest occupied molecular orbital
HPLC	High performance liquid chromatograph
HRMS	High-resolution mass spectrometry
HTAC	Hexa decyltrimethyl ammonium chloride
Hz	Hertz
iPr	Isopropyl
J	Coupling constant
KHMDS	Potassium bis(trimethylsilyl)amide
LDA	Lithium diisopropylamide

LiHMDS	Lithium hexamethyldisilazide
LUMO	Low unoccupied molecular orbital
MCR	Multicomponent reaction
Me	Methyl
MeCN	Acetonitrile
MK2	Mitogen-activated protein kinase
mol	Moles
MS4A	Molecular sieves 4A
MsCl	Methanesulfonyl chloride
MTT	Thiazolyl blue tetrazolium bromide
MW	Microwave
M/Z	Mass to charge ratio
NBCS	New-born Calf Serum
*n*Bu	*normal*-Butyl
nBuLi	t-butyllithium
NCS	*N*-chlorosuccinimide
NMR	Nuclear magnetic resonance
Nu	Nucleophile
P388	P388 leukaemia cell
Ph	Phenyl
PivCl	Pivaloyl chloride
PK	Proteinase K
PLK1	Polo-like kinase 1
PMB	*para*-Methoxybenzyl
PMSF	Phenylmethylsulfonyl fluoride
ppm	Parts per million
PPTS	Pyridinium *p*-toluenesulfonate
PrP^{C}	Normal isoform protein isoform
PrP^{Sc}	Abnormal form protein

PTC	Phase transfer catalyst
PTSA	*para*-toluene sulfonic acid
QSAR	Quantitative structure-activity relationship
R_f	Retention factor
r.t.	Room temperature
SMB	Scrapie mouse brain
t	triplet
TBAb	Tetra-n-butyl ammonium bromide
TBAF	Tetra-n-butyl ammonium fluoride
TBD	1,5,7-Triazabicyclo[4.4.0]dec-5-ene
TBS	*t*-Butyldimethylsilyl
TEBA	Benzyl-triethyl ammonium chloride
TEBAC	Triethylbenzylammonium chloride
TFA	Trifluoroacetic acid
TFE	Triflouroethanol
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TMA	Tissue microarrays
TMEDA	Tetramethylethylenediamine
TMSCl	Trimethylsilyl chloride
TMSOTf	Trimethylsilyltrifluoromethane sulfonate
$TMSCHN_2$	Trimethylsilyldiazomethane
TNF inhibitor	Tumour necrosis factor
tRNA	Transfer RNA
TSEs	Transmissible spongiform encephalopathies
UV	Ultraviolet
VHTS	Virtual high throughput screen
XtalFluor-E	Diethylaminodifluorosulfniumtetrafluoroborate

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Part I. Introduction

Chapter 1. Introduction

1.1. General introduction

Heterocycles are one of most popular structural features in drugs. Pharmaceutical companies have invested a great deal to develop efficient synthetic approaches to prepare these compounds with sufficient diversity and structural complexity.[1] Ideally, a typical synthetic approach should produce the desired product in as few steps as possible and in a satisfactory yield by utilizing environmental friendly reagents.[2] However, common synthetic methods are usually carried out in multi-steps and in linear fashion between reactants with lower overall yields, high costs and large quantity of waste being generated. To successfully deal with these limitations, efforts have been made to develop reaction routes which are able to produce multiple bonds in one reaction step.

Combinatorial chemistry has attracted much interest and been the focus of lot of synthetic efforts. It has also been employed as an active tool to synthesize heterocyclic compounds with required properties in cost-effective manner.[3] However, it provides limited diversity, the development of more efficient and new synthetic approaches is still a major challenge to most chemists.[4]

One alternative to improve the efficiency and diversity of heterocyclic synthesis is to use multicomponent reactions (MCRs). MCRs are reactions that combine at least three starting materials in a one-pot reaction to produce a new product, and all or most of the atoms are converted to the new product. In comparison with traditional synthetic approaches which involve multistep linear organic syntheses[5], MCRs are more beneficial due to their tremendous atom efficiency.[6] MCRs have been widely used as efficient ways to generate chemically diverse heterocyclic compounds to cover a large chemical space.[7]

Keto acids play a vital role in Ugi reactions. They can react with diverse reagents to furnish new heterocyclic chemo types. Keto acids that were used as essential component to form heterocycles with high diversity and complexity will discussed in more details in section **1.2**.

On the other hand, isocyanides have been employed for many years as a key component in MCRs to construct different libraries and scaffolds, [8] especially as building blocks for the synthesis of heterocyclic synthesis. Due to its intriguing electronic structure, isocyanides can act as either an electrophile or a nucleophile, [9] which will discussed in more details in section **1.3**.

Flow chemistry and microwave synthesis are modern techniques that have found many applications in organic synthesis. They have advantages of speeding up the reactions, increasing the reaction yield, improving selectivity, saving reagents and solvents etc.[10]

However, their applications in MCRs have been somewhat limited. In this project the production of Ugi-tetrazole derivatives by using the flow reactor (compared the same reactions that performed under batch conditions) was performed, with the aim to improve products yields, to shorten reactions time and to achieve better selectivity. Also in this project, microwave methodology was used in the synthesis of number of pyrazole-oxopyrrolidine derivatives as fast as possible with high yield, shorter reaction time and solvent free conditions.

As post-condensation transformations can give high yields of functionalized heterocycles,[11] a wide range of tethered keto-acids was successfully exploited in intramolecular Ugi reactions which could be followed by either one or two consecutive expansions to afford a range of unique heterocyclic scaffolds with satisfactory yields. The combination of MCRs with post-MCR transformation has been usefully viewed as an excellent synthetic approach for the production of highly functionalized compounds such as pyroglutamic acid analogues lactam-tetrazole derivatives, diones derivatives, pyrrole-oxopyrrolidine derivatives and pyrrolo-pyridine derivatives, through C–N bond coupling by using either bases or acids.

Prion disease is also known as transmissible spongiform encephalopathies (TSEs). They are incurable and fatal neurodegenerative diseases influencing both humans and animals. There is no effective cure for the TSEs currently available and this is of special concern in European countries.[12] TSEs can be potentially represented as inherited, sporadic and iatrogenic. In this project a library of heterocyclic compounds synthesised was screened in scrapie cell line (SMB) hoping to find novel anti-prion lead compounds.

1.2. Keto acids

Keto acids or ketoacids, also called oxoacids, can be defined as organic compounds containing a ketone and carboxylic acid group, which are not only useful chemical reagents, but also biologically active themselves. [13] They can be classified according to the positions of the keto group in the molecule. α-Keto acids, or 2-oxoacids, for example: pyruvic acid **1** has the keto group adjacent to the carboxylic acid group. The β-keto acids are key components in the citric acid cycle, [14] 3-Oxoacids, such as acetoacetic acid **2**, 3-oxo-3-phenylpropanoic acid **3** and oxaloacetic acid **4** contain a ketone group at the second carbon from a carboxylic acid group. The ketoacid **2** and **3** have been used in the synthesis of active molecules such as anti-HIV drug and DPC **083**.[15] Oxaloacetic acid **4** is also found in the Krebs cycle. 4-Oxoacids or γ-keto acids contain a ketone group that is located at the third carbon from the carboxylic acid. 4-Oxopentanoic acid **5** has been derived from deamination of glutamic acid; 2-oxopentanedioic acid **6** participates in cell signalling *via* functioning as a cofactor for different iron-containing redox enzymes.

Although proteins and stored fats are used as primary source for the production of energy, some keto acids are produced metabolically in ammouliani cells when carbohydrate and sugars levels are low. The ketogenic amino acids can be employed to produce α-keto acids. These keto acids are essentially used as energy source for liver cells and in the synthetic approach of fatty acids.[16]

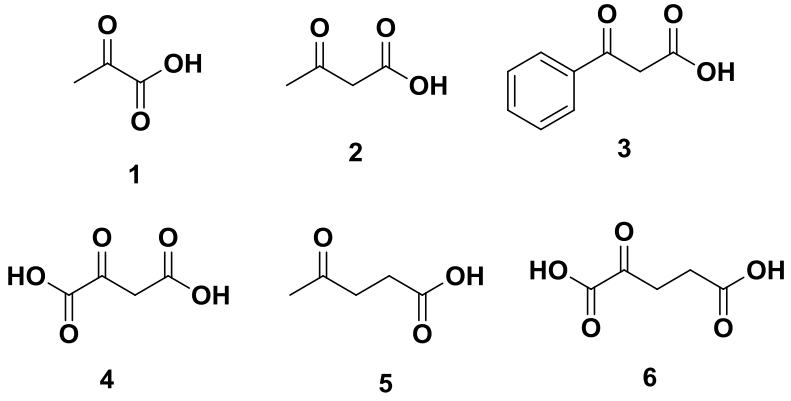


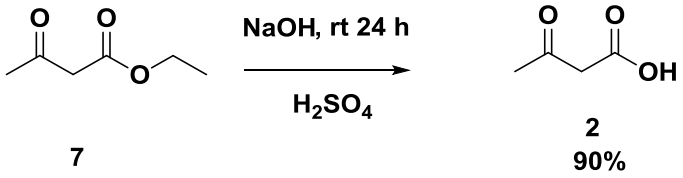
Figure 1: Some keto acids.

Most keto acids used in organic synthesis as reagents are produced using chemical synthesis.

1.2.1. Synthesis of keto acids

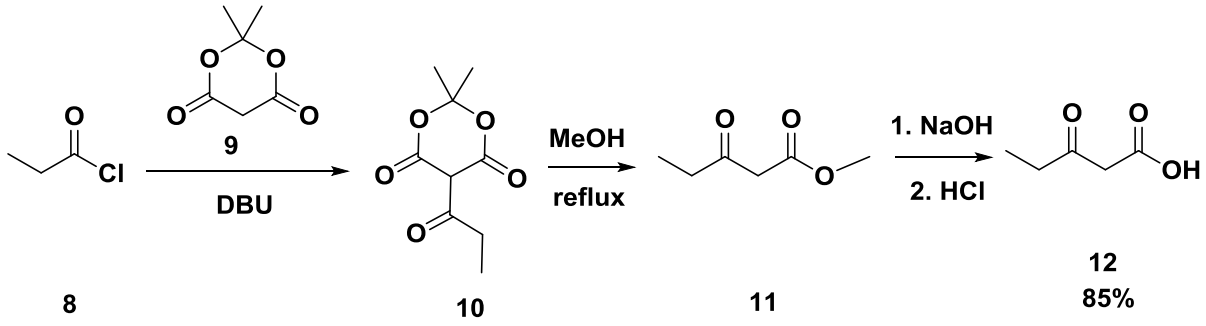
In 1952, Kreuger isolated 3-oxobutanoic acid **2** as crystalline compound by hydrolysis of ethyl-3-oxobutanoate **7** in aqueous sodium hydroxide (Scheme **1**). Acidifying with sulphuric-

acid afforded keto acid **2**.[17]



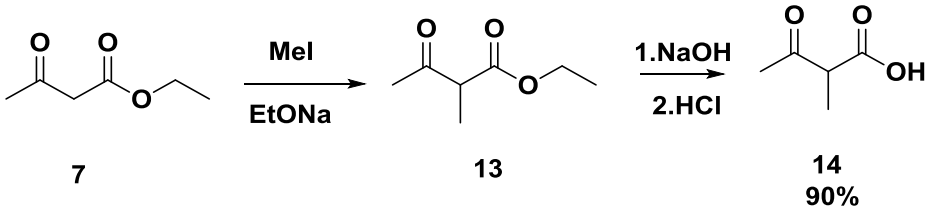
Scheme 1: Kreuger's approach to synthesise 3-oxobutanoic acid **2**.

3-Oxopentanoic acid **12** was obtained in a 3-step synthesis which propanoyl chloride **8** and 2, 2-dimethyl-1,3- dioxane-4,6-dione **9** react to form 2,2-dimethyl-5-propionyl-1,3-dioxane-4, 6-dione **10**.[18] The compound **10** was then successfully methanolysed in reflux with methanol to obtain methyl-3-oxopentanoate **11** in good yield. The ester **11** was then stirred overnight with aqueous solution of sodium hydroxide followed by acidification by HCl to give free keto acid **12** as white solid after recrystallization (Scheme **2**).



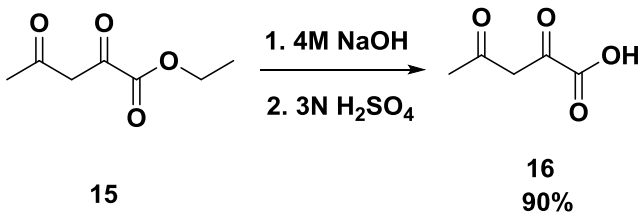
Scheme 2: A synthetic approach to synthesise 3-oxopentanoic acid **12**.

2-Methyl-3-oxobutanoic acid **14** was produced from ethyl-3-oxobutanoate **7**. It was alkylated with methyl iodide in the presence of sodium ethoxide to form ethyl-2-methyl-3-oxobutanoate **13**. This ester **13** was then exposed to sodium hydroxide in water to provide the desired oxoacid **14** (Scheme **3**).[19]



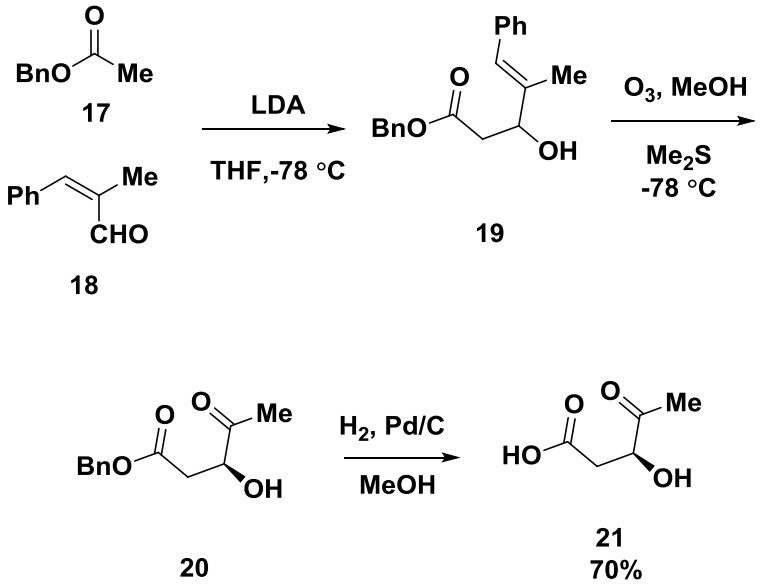
Scheme 3: A synthetic approach to produce 2-methyl-3-oxobutanoic acid **14**.

The free acid of 2,4-dioxopentanoic acid **16** was obtained by employing one equivalent of ethyl acetopyruvate **15** in distilled water and equal equivalent of 4M sodium hydroxide followed by after acidifying with 3N sulfuric acid (Scheme **4**).[20]



Scheme 4: A synthetic approach of 2,4-dioxopentanoic acid **16**.

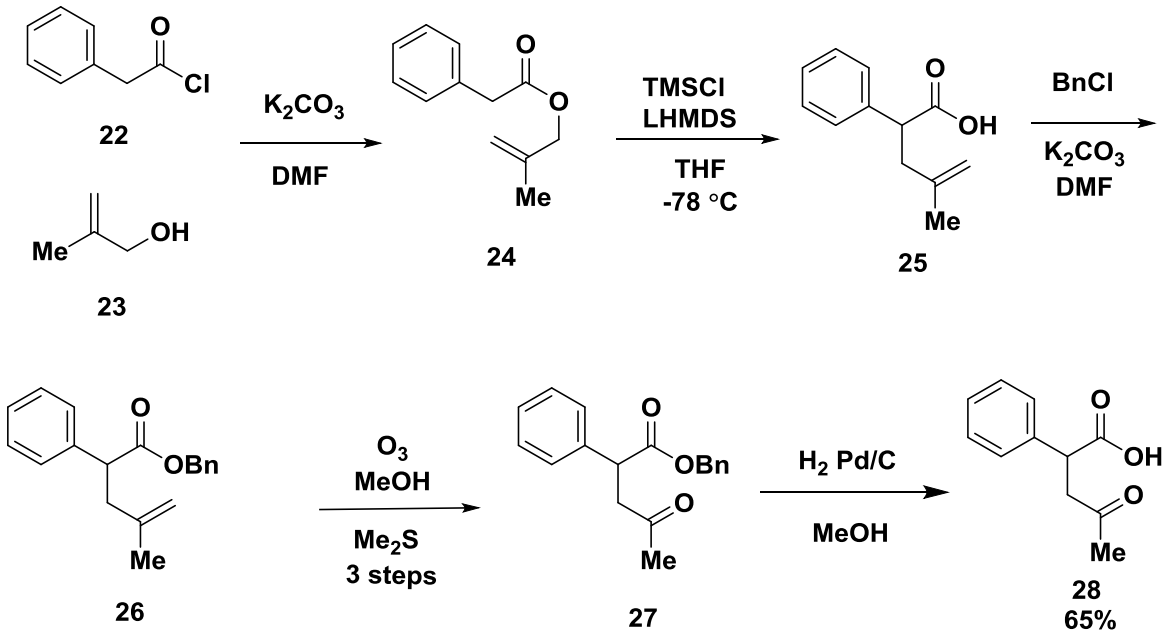
The synthesis of 3-hydroxy-4-oxopentanoic acid **21** can be started from an aldol reaction of lithium enolate of benzyl acetate **17** with (*E*)-2-methyl-3-phenylacrylaldehyde **18**, which yielded aldol product **19** in good yield. The aldol product **19** was conveniently exposed with ozone to provide *β*-hydroxy-*γ*-ketoester **20** in satisfactory yield. Finally, 3-hydroxy-4-oxoketoacid **21** was successfully obtained in quantitative yield by hydrogenation of ester **20** under neutral conditions to generate the keto acid **21** (Scheme **5**).[21]



Scheme 5: A synthetic approach of 3-hydroxy-4-oxopentanoic acid **21**.

Some studies have been carried out to investigate reactions consisting of Ireland-Claisen rearrangement of an allyl ester, which was then followed by oxidative olefin cleavage through ozonolysis to prepare the various *γ*-ketoacids. For example, 2-phenylacetyl chloride **22** can couple with 2-methyl-2-propenol **23** to furnish ester **24**. Ireland-Claisen rearrangement

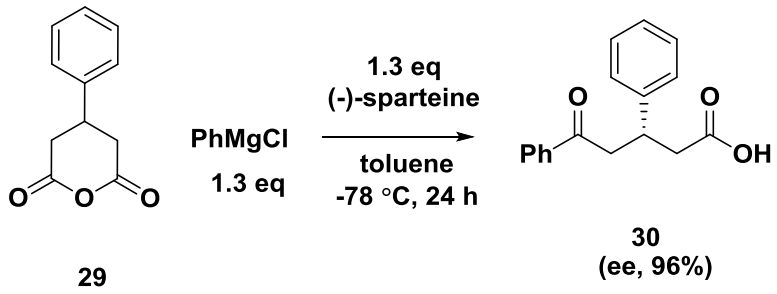
was initiated by adding of trimethylsilylchloride and then lithium bis (trimethylsilyl) amide at very low temperature to afford **25**. The resulting carboxylic acid was successfully protected to afford benzyl ester **26**. The corresponding ketone **27** was formed by cleaving exo-olefin of **26** with ozonolysis. The benzyl ester of **27** was then hydrolysed with palladium-charcoal to yield the corresponding γ-ketoacid **28** (Scheme **6**).[22]



Scheme 6: A synthetic approach to synthesise γ-ketoacid **28**.

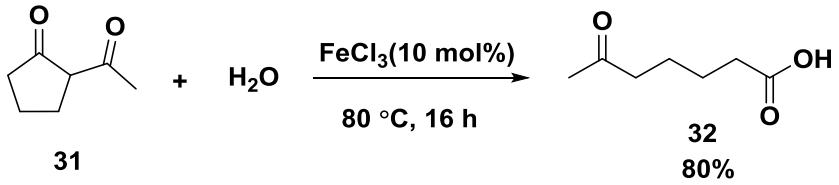
It was also mentioned that an array of cyclic anhydrides was effectively de-symmetrized by sparteine-bound Grignard reagents to prepare keto acids in good enantiomeric excess. Major research have been focused on the use of (-)-sparteine to provide an asymmetric reaction of organolithium reagents. The result demonstrated that (-)-sparteine yields good enantiocontrol for a reaction of a Grignard reagent.[23] For example, the initial step includes opening a ring of 3-phenylglutaric anhydride **29** by using phenylmagnesium chloride. 3-Phenylglutaric anhydride was desymmetrized by phenylmagnesium chloride and the outcome showed that using a slight excess of Grignard reagent/(-)-sparteine can increase stereo selectivity by 88%. It was also noted that the changing in the electronic nature of the Grignard agent has moderate influence on enantioselection, while an increase in the steric demand has substantial impact. Another example, benzyl-substituted with anhydrides were successfully reacted to furnish keto acids with plausible enantioselectivity. The ring-opening of heteroatom-substituted anhydrides also proceeded with excellent stereo selection. It was demonstrated that efficient desymmetrization not only occurs in monocyclic, but also in bicyclic

anhydrides. Hence, the 3-phenylglutaric anhydride **29** was treated with phenyl magnesium chloride/(-)-sparteine to furnish the chiral keto acid **30** (Scheme **7**) in good enantiomeric excess (ee, 96%).[24]



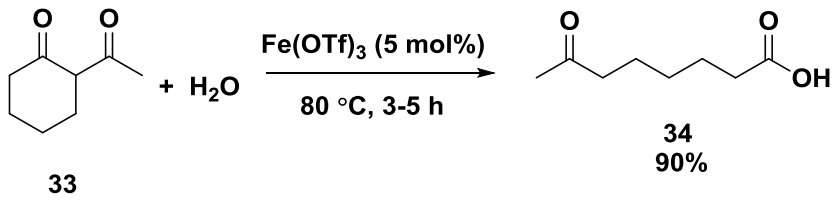
Scheme 7: A synthetic approach for the chiral keto acid **30**.

Iron is beneficial and the most abundant metals on the earth. An iron-based catalyst is commonly used in organic synthesis due to its low cost and a good reactivity. [25] Chemists have inspired to synthesise esters using ferric chloride as a catalyst. This reaction reveals that carbon–carbon bonds can be easily cleaved by a metal-induced six-membered cyclic transition state in good yields. The scope of the catalytic reaction was investigated using 1, 3-diketones as a model reaction. The catalytic activity of anhydrous ferric chloride differs significantly from that of ferric chloride hexa hydrate, while iron(III) trifluoromethane sulfonate can afford approximately the same yields as that of ferric chloride. The optimisation was carried out in the presence of ferric chloride as the catalyst; the corresponding esters were formed in high yields. Therefore, some cyclic 1,3-diketones for instance 2-acetylcyclopentanone **31** was treated with ferric chloride in water to furnish the corresponding keto acid **32** in good yields (Scheme **8**). The etherification reaction was exclusively located on the carbonyl group in the cyclohexanone ring to form the corresponding ring-opened oxo esters in an excellent yield. The reaction proceeded *via* metal-induced construction of a 6-membered cyclic transition state and Csp2–Csp3 bond cleavage.[26]



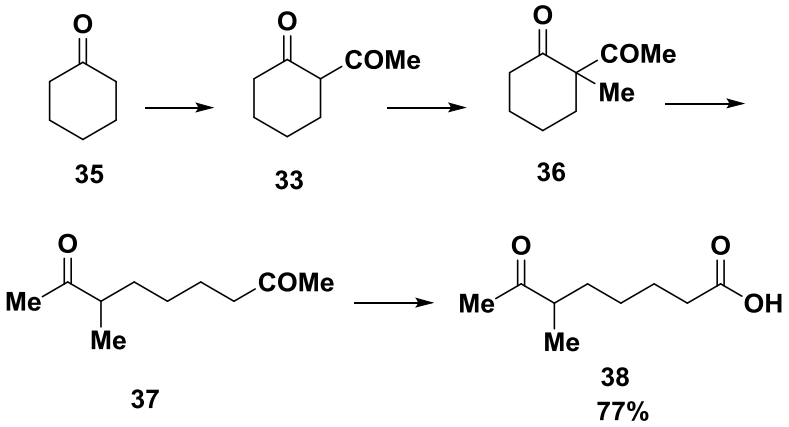
Scheme 8: A synthetic approach of keto acid **32**.

Iron(III) trifluoromethane sulfonate cleavage of the carbon–carbon bond occurs *via* a retro-Claisen condensation reaction.[27] This reaction is beneficial for synthesising various kinds of esters and ketones under mild conditions.[28] The keto acid **34** was obtained in quantitative yield from 2-acetylcyclohexanone **33** when iron (III) trifluoromethane sulfonate (5 mol%) was exploited as the catalyst (Scheme **9**). This may be due to the fact that irontriflate is more stable in protic media such as water and alcohol. It was also observed that the reaction of cyclic 1,3-diketones just with water catalysed by iron (III) triflate yielded the corresponding oxo acids in an excellent yield because it is a stronger Lewis acid than ferric chloride.[29]



Scheme 9: A synthetic approach of keto acid **34**.

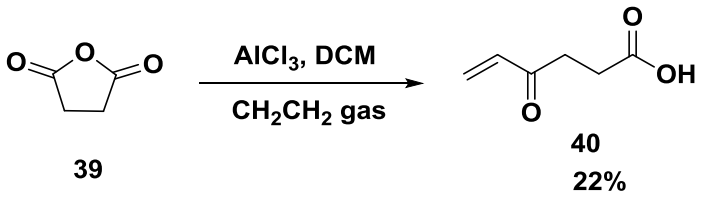
For the production of 6-methyl-7-oxononanoic acid **38**, cyclohexanone **35** was used as a starting material. Pritzkow has proposed to produce methyl-6-methyl-7-oxooctanoate **37** starting from cyclohexanone **35**, [30] where cyclohexanone **35** was converted to 2-acetylcycloalkanone **33** which was then methaylated and underwent Claisen retro condensation to furnish keto acid **38** after acidification of ester **37** with HCl.[31]



Scheme 10: A synthetic approach of 7-methyl-8-oxononanoic acid **38**.

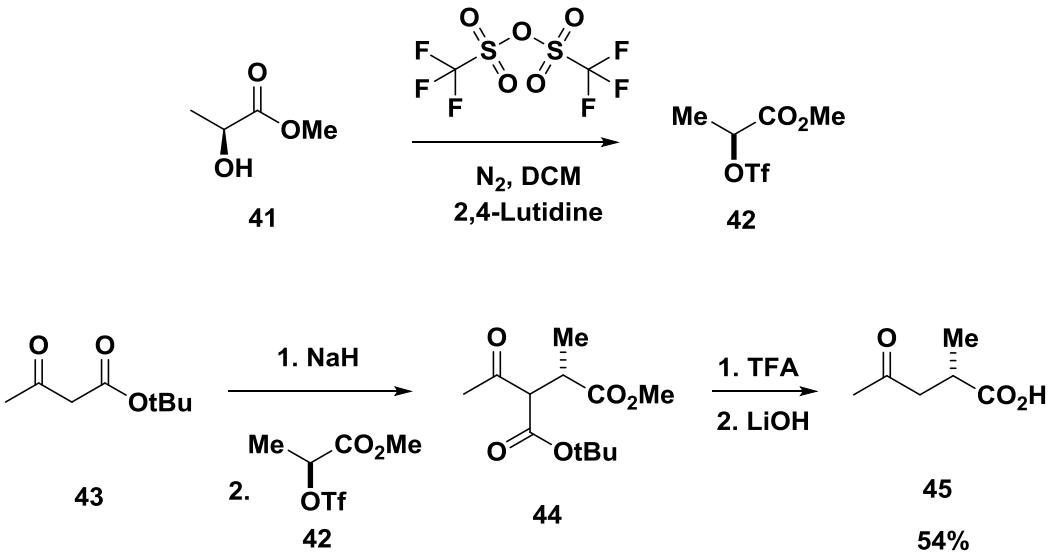
Friedel-Crafts reaction can be exploited as a convenient procedure to obtain enones from acylation of ethylene with anhydrides. A suspension of anhydrous aluminium chloride and succinic anhydride **39** in dry 1,2-dichloroethane was vigorously stirred. Ethylene was cautiously bubbled through the resultant solution for at least 5 hours. The resulting solution

was then poured into 5% hydrochloric acid and extracted with ethyl acetate to afford 4-oxo-5-hexenoic acid **40** as a white solid 22% yield (Scheme **11**).[32]



Scheme **11**: A synthetic approach of 4-oxo-5-hexenoic acid **40**.

A chiral keto acid **45** was prepared from (*S*)-methyl-2-hydroxypropanoate **41** and *tert*-butyl-*β*-keto ester **43**. Firstly, one equivalent of (*S*)-methyl-2-hydroxypropanoate **41** reacts with one equivalent triflic anhydride at 0 °C in dry dichloromethane under a nitrogen atmosphere to give a solution of 2-triflyloxy ester **42**. The ester **42** reacted with a solution of two equivalents of *tert*-butyl-*β*-keto ester **43** and two equivalents of sodium hydride in dry tetrahydrofuran at 0 °C over a period of 10 minutes. The resulting solution was further stirred at room temperature for 24 hours to yield (3*S*)-1-*tert*-butyl-4-methyl-2-acetyl-3-methylsuccinate **44**. The intermediate **44** was then treated with TFA (24 h) and saponified with LiOH to produce (*S*)-2-methyl-4-oxopentanoic acid **45** in 54% yield as a colourless oil (Scheme **12**).[33]



Scheme 12: A synthetic approach of (*S*)-2-methyl-4-oxopentanoic acid **45**.

1.3. Isocyanides

Isocyanides, also called isonitriles, have been considered to be structurally similar to that of carbon monoxide.[34] An isocyanide contains a N-C bond, which displays a sharp single infrared peak at the region approximately 2130 cm^{-1}while is similar to the C-O bond of carbon monoxide, exhibiting its peak at 2143 cm^{-1}.[35] Isocyanides are represented as the only class of stable organic compounds which also have a formal divalent carbon.[36] The isocyanide group has different properties than other functional groups. The two resonance forms can be clearly noted for isocyanide. Firstly, the triple bond between the nitrogen atom and the carbon atom with a positive charge on the nitrogen atom, (Figure **2**, **A**) and a negative charge on the carbon atom. Secondly, a carbon-nitrogen double bond is featured with a non-bonding pair of electrons on the terminal carbon (Figure **2**, **B**).[37] These two forms co-exist and are similar to CO.

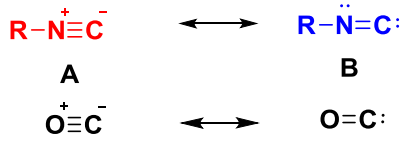
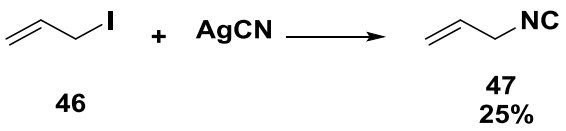


Figure 2: Resonance structure of isocyanide and carbon monoxide.

Due to the reactivity of isocyanides, they are increasingly employed as key building blocks in organic synthesis. However, they are volatile, with a distinct pungent odour.[38]

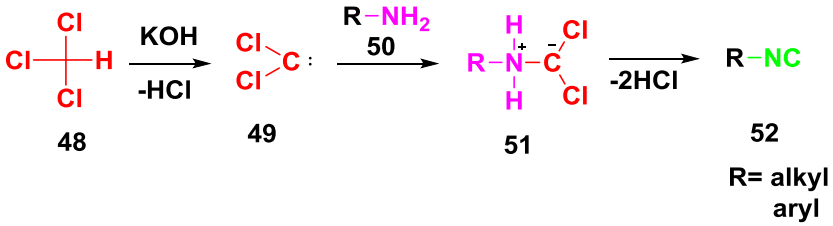
1.3.1. Synthesis of isocyanides

In 1859, Lieke synthesized isocyanide **47**. He obtained it unexpectedly from allyl iodide **46** and silver cyanide, noting the awful smell of the products (Scheme **13**).[39]



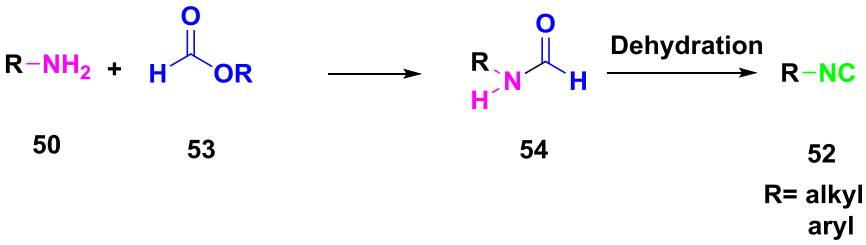
Scheme 13: Lieke's synthetic approach of isocyanide.

A new procedure to synthesise isocyanides **52** was found by Hoffmann in 1867.[40] The condensation of a primary amine **50** with a dichlorocarbene **49**, which was generated *via* heating of chloroform **48** with potassium hydroxide as depicted (Scheme **14**).



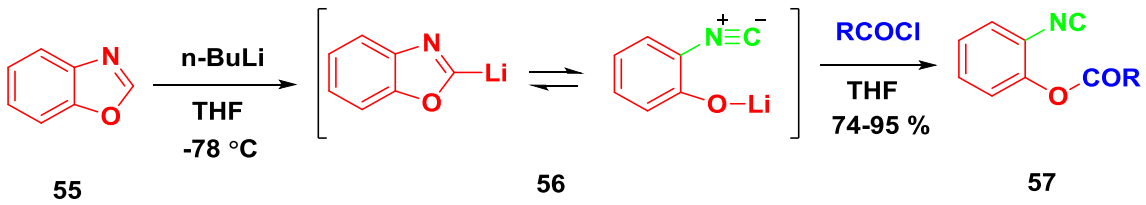
Scheme 14: Isocyanides was made by Hoffmann.

In 1958, the isocyanides **52** have become easily accessible by dehydration of formylamides **54**.[41] Ivar Ugi made significant efforts to develop an optimized approach for formation of isocyanide **52** by using the dehydration of *N*-mono substituted formamides, which can be generated from primary amines and methyl or ethyl formate or formic acid [42], as shown in Scheme **15**.



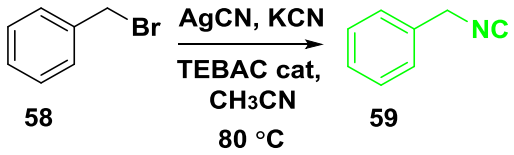
Scheme 15: Ugi's approach to synthesize of isocyanides by dehydration of formamides.

The other synthetic strategy to produce isocyanide **57** uses organolithium compounds reacting with benzoxazole **55**. In this reaction, the H-2 of benzoxazole **55** is firstly deprotonated by the base to form 2-isocyanophenolate **56**. This intermediate then traps an electrophile, for instance an acyl chloride to produce the corresponding isocyanide **57** [43] as illustrated in Scheme **16**.



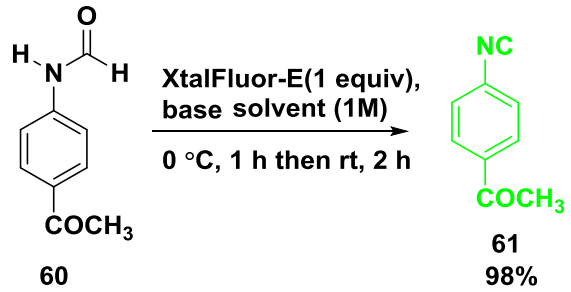
Scheme 16: Synthetic route of isocyanides from benzoxazole **57**.

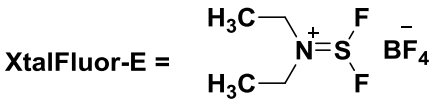
Some isocyanides such as benzyl isocyanide **59** have revolting odours. A reaction between a benzyl bromide **58** and silver and potassium cyanide in acetonitrile at 80 °C is widely used to prepare isocyanide **59** (Scheme**17**).[44]



Scheme 17: Isocyanide from alkyl halides depends on silver and potassium cyanide.

Isocyanides are well known to be unstable. They are normally freshly synthesised prior to their use in the reaction. A straightforward new approach is utilized to prepare isocyanides **61** by also using the dehydration of formamide **60** in up to 98% yield [45] (Scheme **18**).





Scheme 18: The scope of the dehydration of formamide with XtalFluor-E.

1.3.2. Electronic structure and reactivity of isocyanides

Isocyanides exist as resonance forms between divalent carbon forms and zwitterions **A** and **B**, the carbon atom of the isocyano group displays carbene-like reactivity, which is represented in the resonance, as shown (Figure **2**, **B**). Structurally, the linear form of isocyanides is well noted by the dipolar resonance structure (Figure **2**, **A**).The results showed that 50% of it existed as carbene, 30% zwitteriomic form and the viewing 20% as complex [46] (Figure **3**).

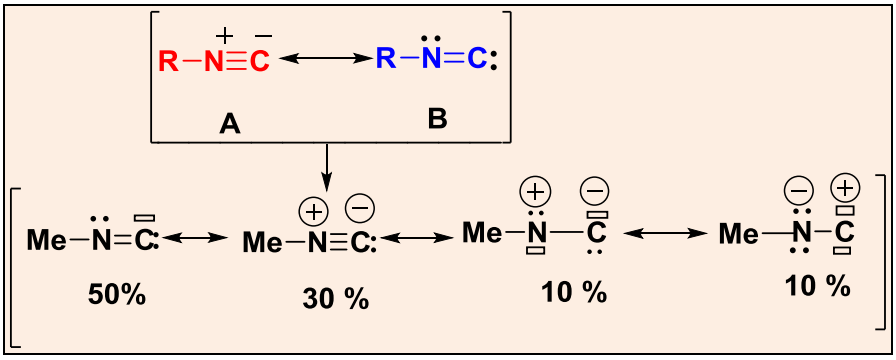


Figure 3: Valence bond theory with regard to resonance structures of isocyanides.

Isocyanides are stable under basic condition and they are often experimentally made under basic conditions, but they are very sensitive to acids. The corresponding formamides are produced by isocyanides with aqueous acidic solutions. Although, the acidic hydrolysis is apparently a suitable way to eliminate the horrible odour of isocyanides, most isocyanides can polymerize in the presence of acids.[47]

Isocyanides are able to behave as radical by forming imidoyl radical fragments (Figure **4**), these species can be further cleaved into a nitrile and an alkyl radical or may react with an unsaturated compound to form heterocyclic compounds.[48]

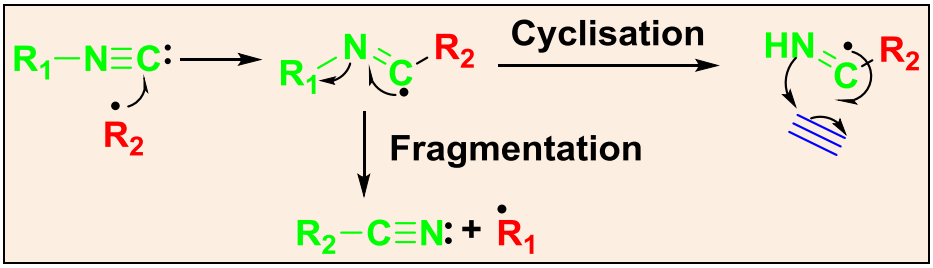


Figure 4: Addition of a radical on an isocyanide.

As isocyanides exist in zwitterionic form, this means that isocyanides are able to react with both electrophiles and nucleophiles. The carbon atom of isocyanides can be attacked by nucleophiles because it has a large coefficient in the LUMO (π^*) orbital when a nucleophile is attacked on the isocyanide. The divalent carbon behaves as a nucleophilic which is also conversely attacked by an electrophile where it interacts with the HOMO (σ) orbital on isocyanide at the same terminal carbon atom. Therefore, both electrophiles and nucleophiles will attack on the same terminal carbon.

A nucleophile interacts on the carbon atom (nitrile) because it has the highest coefficient in LUMO while an electrophile interacts simultaneously with the nitrogen atom which has the highest coefficient in HOMO (Figure **5**).

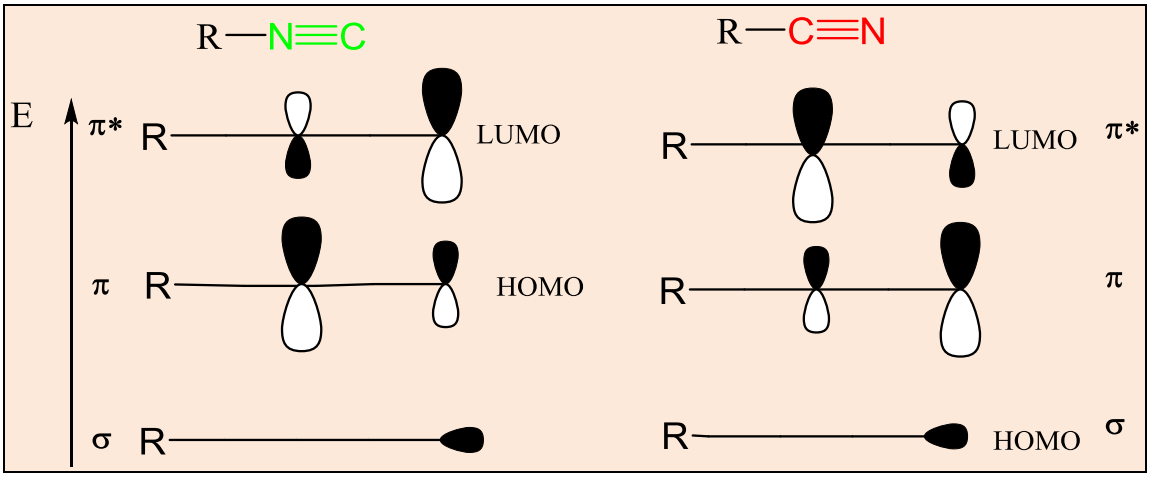


Figure 5: Frontier orbitals of isocyanides and cyanides[46].

There has been an issue with regard to the unusual electronic structure of isocyanides and they could be described in Figure **6** as they appear as a standard Lewis structure. Conventionally, isocyanides are presented in two resonance forms, the linear zwitterionic form **A** and the carbene form **B**. Although X-ray diffraction techniques confirmed its the linear structure and the nucleophilic, this zwitterionic structure fails to display the carbene-feature of the isocyanide carbon atom.[46]

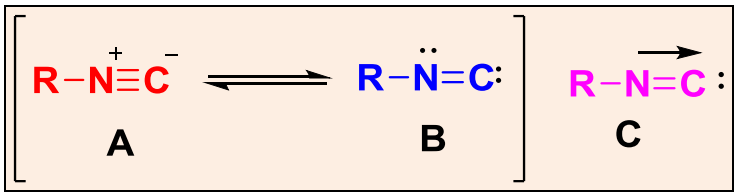


Figure 6: The dative bond of isocyanide and linear zwitterionic resonance form.

Recently, a high-level computational study has confirmed that representation **C** is more accurate in illustrating the electronic structure of isocyanides. This has never extensively been accepted by chemists because the dative bond may be misconstrued. Most synthetic chemists still prefer to use the zwitterionic form **A** instead because the reaction between isocyanides and a nucleophile takes place the isocyanides carbon and a formal negative charge on **A** is easier to be accepted.[49]

1.4. Multicomponent chemistry

Three or four even six different components can be brought together in a one-pot reaction forming a single product which may be entitled under the term ‘multicomponent reactions’. Multicomponent reactions (MCRs) are represented as perfected tools to generate libraries of small-molecule compounds. Many reviews on multicomponent reactions have been published

with specific focuses on their applications in the drug discovery process or total synthesis of natural products or developing strategies for constructions of new structural frameworks.[50] MCRs possess many benefits over stepwise linear synthesis (Figure **7**) including atom economy, reduced waste, saving time, energy and facile purification methods.[9]

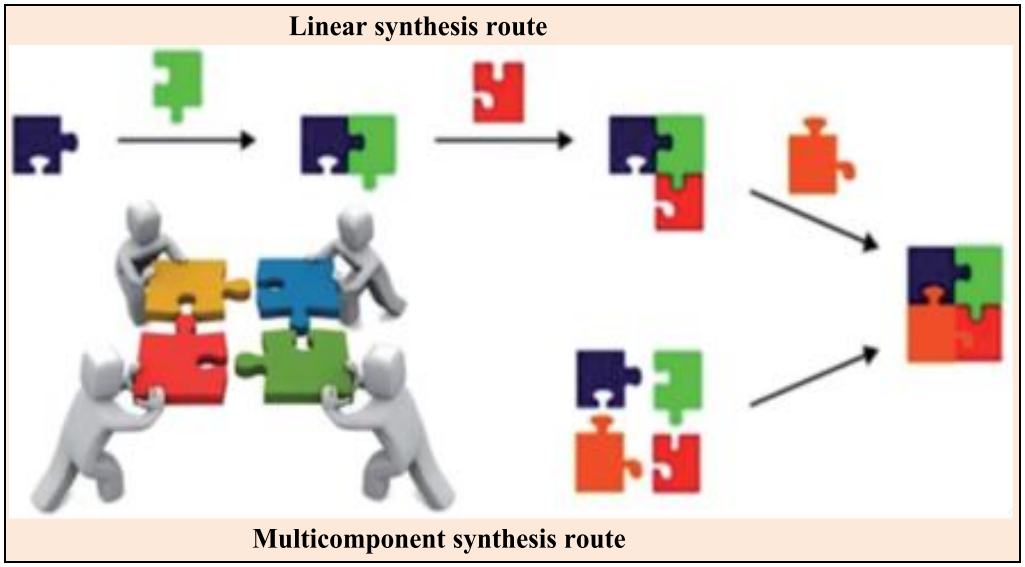


Figure 7: Comparison between multicomponent reaction approach and linear synthesis.

MCRs have emerged as a powerful tool for the synthesis of both simple and complex organic compounds. In the MCRs, elaborated molecular scaffolds can be easily accessed, the combination of structural diversity and eco-compatible methodologies may be achieved in a single-pot. Most atoms found in the reagents should be structurally assembled in the product structure.[51]

MCR mechanisms can be illustrated as a cascade of successive bimolecular reactions, although sometimes intermolecular steps are required to allow at least three components involved in the reaction, which are then progressed to final products.[52]

Multicomponent reactions include sequential transformations between three or more components, which are simultaneously converted into a product that contains some or all fragments from each reactant [53] (Figure **8**). The mechanisms of these reactions are undoubtedly complex which require a good understanding of how individual molecules react in a specific sequence to create desired products.[54] A linear way to assemble four components into a reaction will require three consecutive steps, and certainly the yield with each step is important and some purification is likely required during work-up.[55]

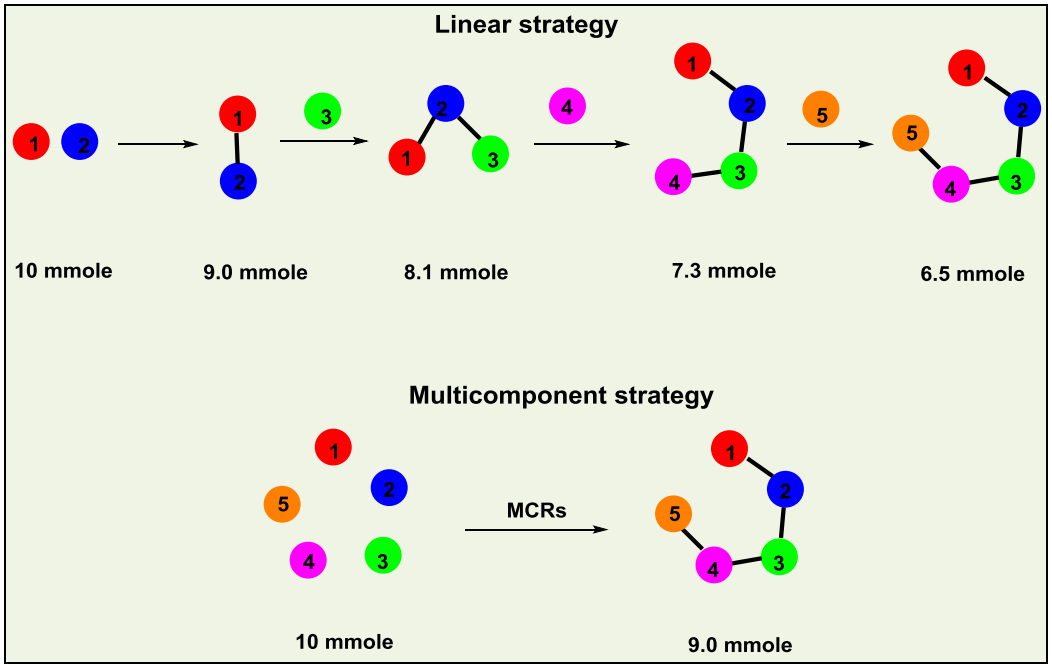


Figure 8: Strategic benefits of multicomponent synthesis.

The advantages of MCRs can be illustrated as followed.[56]

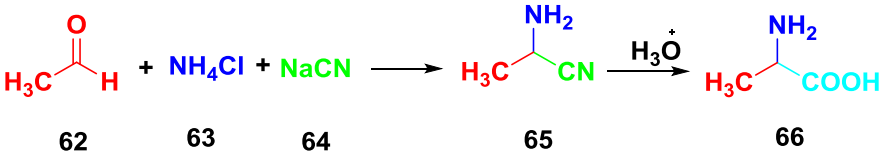
1- MCRs can afford high yield of products, with a minimum effort.
2- MCRs provides a powerful tool for the pharmaceutical industry to construct compound libraries and to synthesise complex structures by simple single-pot methods.[57]
3- A structural product is easily diversified by variations of each component.
4- The starting materials which are used in MCRs can be either commercially available or easily prepared.
5- The number of products which are obtained by MCRs depends on the number of reactants incorporating successfully in the reaction.[58]

While a MCR has many advantages, the success of a MCR relies on several factors:

1- The number of bonds that are generated in one sequence.
2- The complexity in structure.
3- The MCRs' suitability of specific reactions.

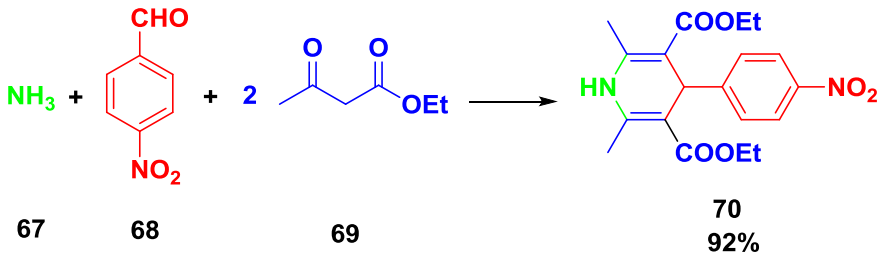
1.4.1. History of multicomponent reactions

Multicomponent reactions were firstly introduced over 150 years by Strecker.[59] The aldehyde **62** was condensed with ammonium chloride **63** in presence of sodium cyanide **64** to produce an α-amino nitrile **65**; the α-amino acid **66** was formed after hydrolysis of **65** (Scheme **19**).



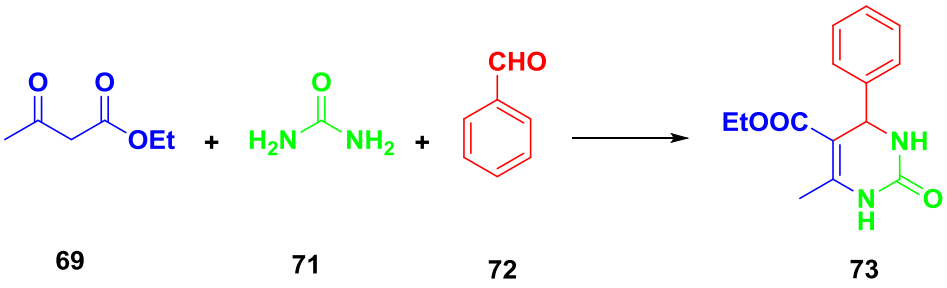
Scheme 19: Strecker's synthetic approach of the *α*-amino acid alanine **66**.

Many important heterocyclic compounds were synthesised by MCRs. For example, dihydropyridine **70** was synthesized by Hantzsch in 1882[60] using ammonia **67**, 4-nitro benzaldehyde **68** and ethyl acetoacetate **69** (Scheme **20**).



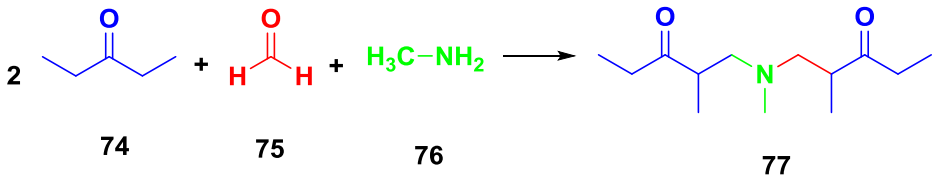
Scheme 20: Hantzsch synthetic approach for dihydropyridine **70**.[60]

In 1891, Biginelli[61] identified that 3,4-dihydropyrimidin-2(1H)-ones **73** can be synthesised from ethyl acetoacetate **69**, urea **71** and an benzaldehyde **72** (Scheme **21**).



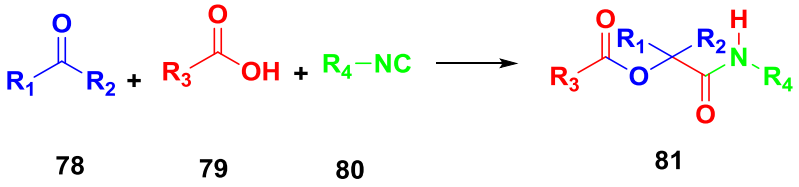
Scheme 21: Biginelli's synthetic route for 3,4-dihydropyrimidin-2(1*H*)-one **73**.[61]

An amino alkylation reaction of an enol **74** with formaldehyde **75** and methylamine **76** to give a tertiary amine **77** was successfully achieved by Mannich in 1912 [62](Scheme **22**).

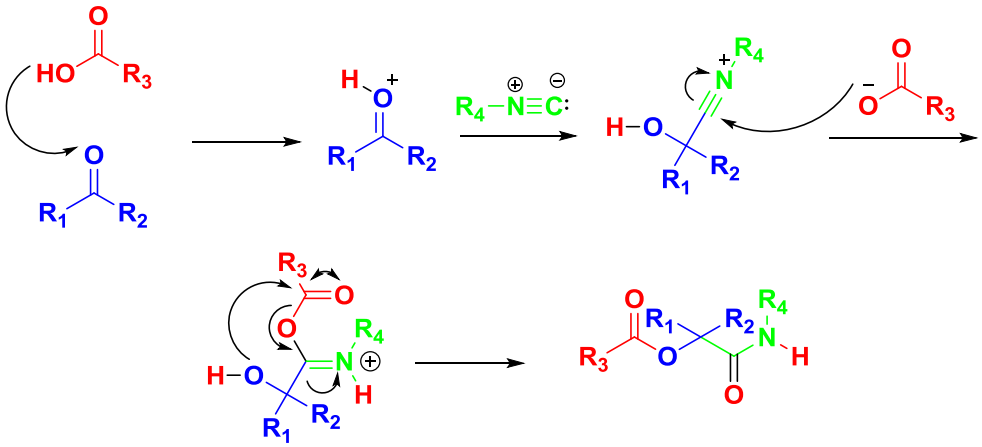


Scheme 22: Mannich reaction.[62]

The isocyanide-based MCRs and their applications first appeared in the literature in 1921, when the production of *α*-acyloxycarbonamides **81** was accomplished by Passerini using carboxylic acids and carbonyl compounds. Mario Passerini discovered the first isocyanide based multi-component reaction.[63] This reaction included three components, a carbonyl compound like a ketone **78**, a carboxylic acid **79** and an isocyanide **80**, Passerini described these components react together to form *α*-hydroxycarboxamides (*α*-acyloxycarboxamide) **81** (Scheme **23**). The reaction exhibits a facile process to form a product which contains every portion of the three components.



Scheme 23: Passerini's route for synthesis of α-acyloxycarboxamides.

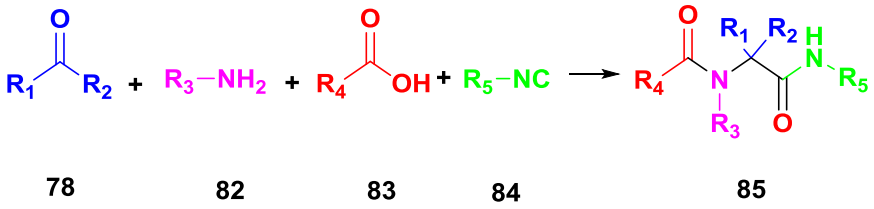


Scheme 24: A plausible mechanism of the Passerini reaction.

Although the Passerini reaction was carried out in non-polar solvents at mild conditions, products were provided in excellent yields. It was proposed that the Passerini reaction cannot

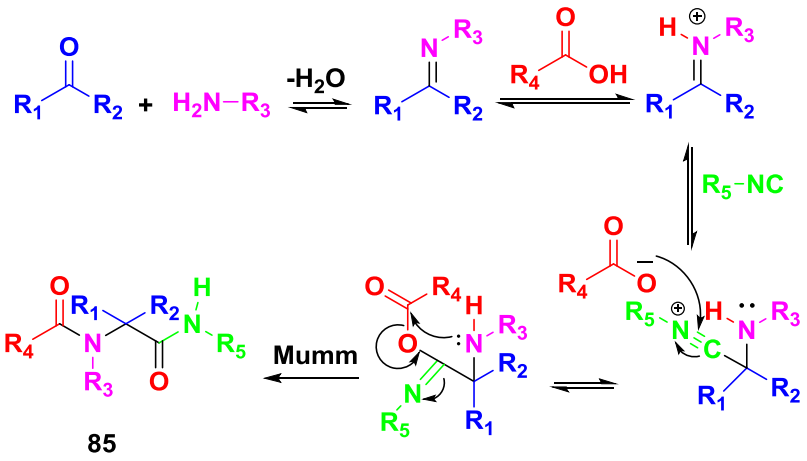
follow an ionic pathway and the hydrogen bonding was also thought to offer a crucial role in the formation of a cyclic intermediate during isocyanide insertion (Scheme **24**).

In 1960's, the scope of the Passerini reactions was extended by Ivar Ugi [64],[65], who firstly used an isocyanide to generate a bis-amide **85** as shown in Scheme **25**.



Scheme 25: Ugi reaction.

The ketone **78** and the amine **82** react to give an imine with one equivalent water being eliminated. The imine was protonated by carboxylic acid **83** to generate as iminium ion which is able to act as a nucleophile to react with the isocyanide **84** to form a nitrilium ion. The nucleophilic trapping process of this intermediate *via* a carboxylic acid undergoes the acyl imidoyl fragments. A Mumm rearrangement occurs in the final step through transformation of the acyl group from the oxygen atom to the nitrogen atom to yield an amine as shown in Scheme **26**.



Scheme 26: The plausible mechanism of Ugi reaction.

It is known that in the Ugi reaction the acide component plays a vital role. It is able to protonate imine and the carboxylate can trap the nitrilium intermediate (Scheme **26**). Finally, the Mumm rearrangement leads to the Ugi adduct (Figure **9**). The general proposition

elucidates a transition state which bears the imine or iminium, the iminium may be trapped by the isocyanide, then is followed by addition of the acid.[66]

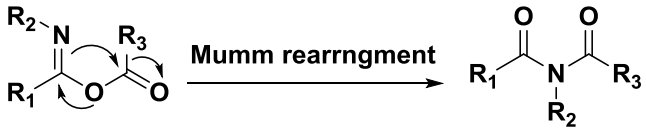


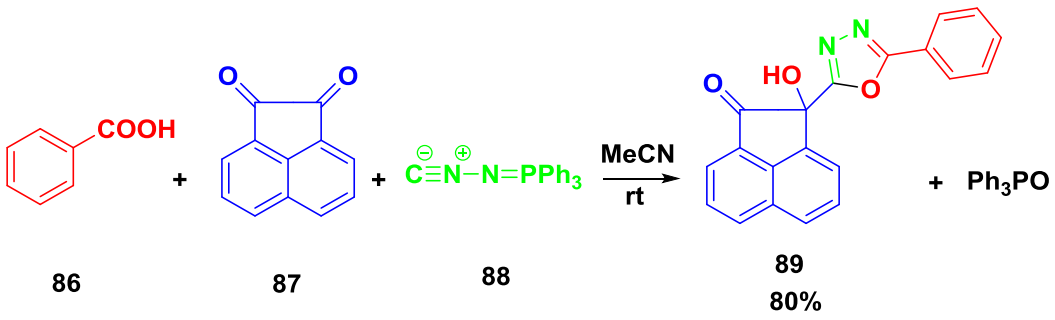
Figure 9: Mumm rearrangement.

In addition, the acyl moiety was shifted by the acidic structure and Ugi developed this strategy by using hydrazoic acid.[67] Since then, U-4CRs have received considerable attention because the products were biologically active amino acids, their applications have attracted a lot of interested in the synthetic field of natural and unnatural amino acids. The MCRs have been used to synthesise a large number of different scaffolds. In addition, most of these compounds are produced from commercially available starting materials, this means that compounds are largely accessible through a single type of reaction.[68] Although some limitations that restrict the full utilization of MCRs, they are still today under exploited in both target-oriented and diversity-oriented syntheses.[69]

In more recent years, Domling has discovered that thiocarboxylic acids could be used in the Ugi reaction to form thioamides[70],[71]. Because IMCR is the main focus of this Ph.D. project, it will be discussed in more details in the following section.

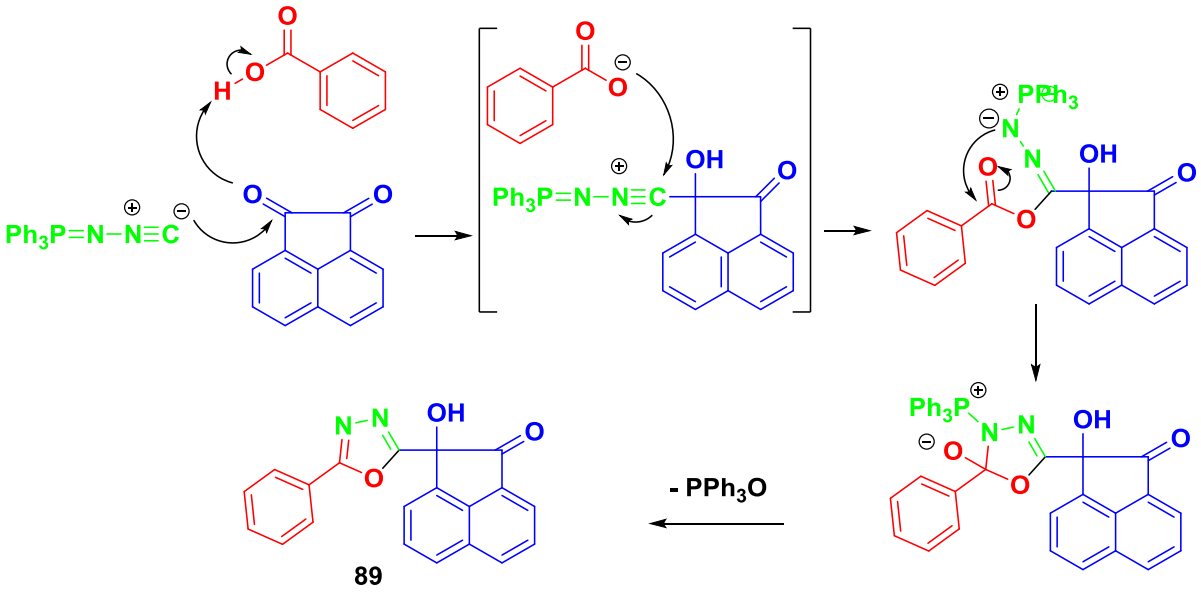
1.4.2. Isocyanides-based multicomponent reactions (IMCRs)

Isocyanides in MCRs control the formation of diverse bond types, leading to magnificent levels of chemo-, regio-, and stereoselectivity. Isocyanide-based multicomponent reactions (IMCRs) can be achieved using easily available starting materials with a wide range of functional groups. Ultrasound irradiation is a significant and innocuous tool for reagent activation in organic synthesis.[72] Isocyanides based-MCRs initiated by ultrasound irradiation have been identified as a clear and beneficial strategy in organic synthesis compared with traditional methods. Ultrasound irradiation in isocyanides-based multicomponent reactions has been used to develop a novel method for the formation of 1,3,4-oxadiazole derivative **89** *via* the condensation reaction between benzoic acid **86**, acenaphthoquinone **87** and (*N*-isocyanimino) triphenylphosphorane **88** under ultrasound irradiation (Scheme **27**).[73]



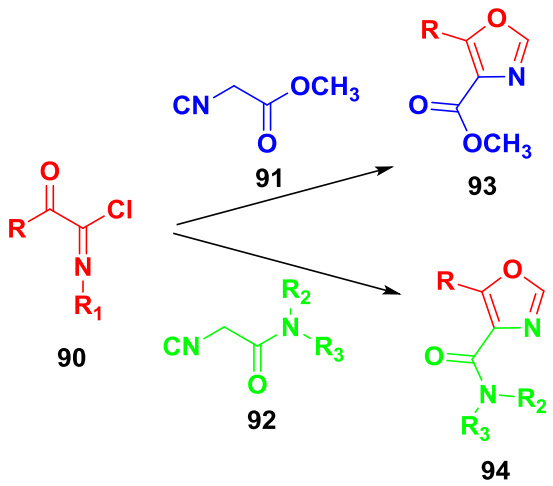
Scheme 27: Synthetic approach of 1,3,4-oxadiazole derivative **89** depends on ultrasound irradiation.

The protonated carbonyl group is attacked by the isocyanide, followed by nucleophile attack on the carbonyl group by the acetate anion; intermolecular cyclization and subsequent aromatization by loss of triphenylphosphene oxide result in the desired compound **89** (Scheme **28**).



Scheme 28: Mechanism was proposed for the synthesis of 1,3,4-oxadiazole derivative **89**.[73]

In the exploitation of nucleophilic characteristics on the carbon of isocyanoesters **91** or isocyanoacetamides **92** when reacting with **90** under basic conditions such as DBU (Scheme **29**), it was found that only the path **A** led to the formation of 4-acyl-1,3-oxazole derivatives (Figure **10**), where the nucleophile attacks a carbonyl group of *α*-ketoimidoyl chlorides **90**. The nucleophile can also pursue path **B**, then followed by the hydrolysis of *α*-ketoimidoyl chlorides to *α*-ketoamides in the presence of acetic acid or formic acid.



Scheme 29: Preparative approach of 4-acyl-1,3-oxazole derivatives.

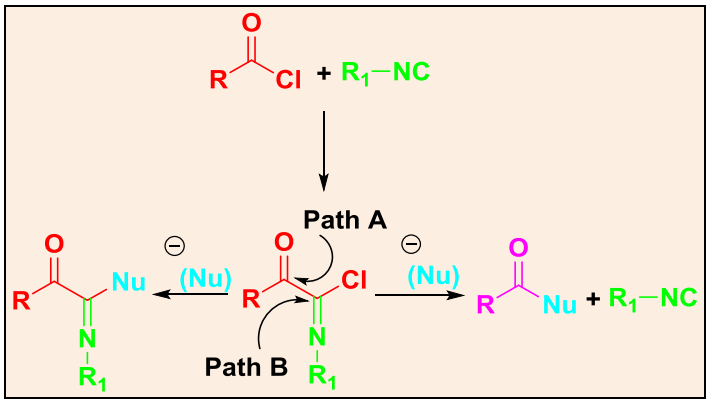
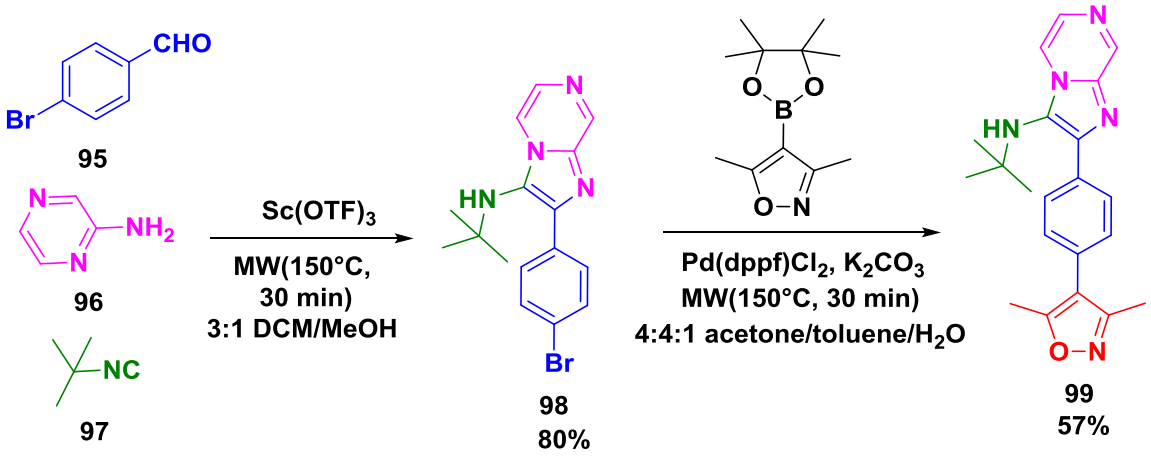


Figure 10: Synthetic ways for the nucleophilic attack to *α*-ketoimidoyl chlorides.[74]

Bromodomain inhibitor (BET) has emerged as a promising therapeutic strategy in cancer. The BET family comprises BRD2, BRD3 and BRD4 which function as transcriptional coactivator proteins and relay signals from master regulatory transcription factors, such as NFkB in inflammation and MYC in cancer.[75] Using isocyanides-based multicomponent reactions novel epigenetic inhibitors such as bromodomain inhibiting compounds were developed. The use of isocyanide as a key component in the synthesis of isoxazole **99** derivative was evaluated. The isoxazole **99** was prepared in a two-step reaction; the first step involves a one-pot reaction of 4-bromobenzaldehyde **95**, 2-aminopyrazine **96** and *t*-butylisocyanide **97** under microwave at 150 °C for 30 minutes to give **98** in 80% yield. Compound **98** was subsequently coupled with 3,5-dimethylisoxazole-4-boronic acid pinacol ester (Suzuki coupling) to give 3,5-dimethylisoxazole derivative **99** in 57% yield (Scheme **30**).[76]



Scheme 30: Synthetic approach of dimethylisoxazole derivative **99**.

The 3,5-dimethylisoxazole derivative **99** was shown to bind BRD4 (cellular potency in BRD4 lines) and shows potency against TAF1. The cocrystalization of the compound **99** with BRD4 was performed and the crystal structure was solved at 1.56 Å resolutions (Figure **11**).

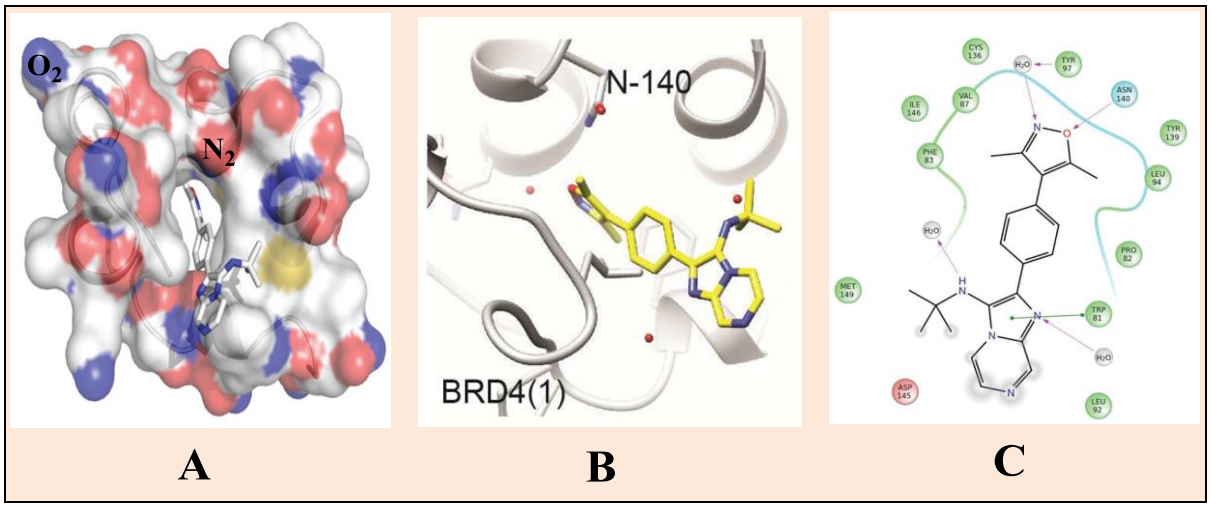
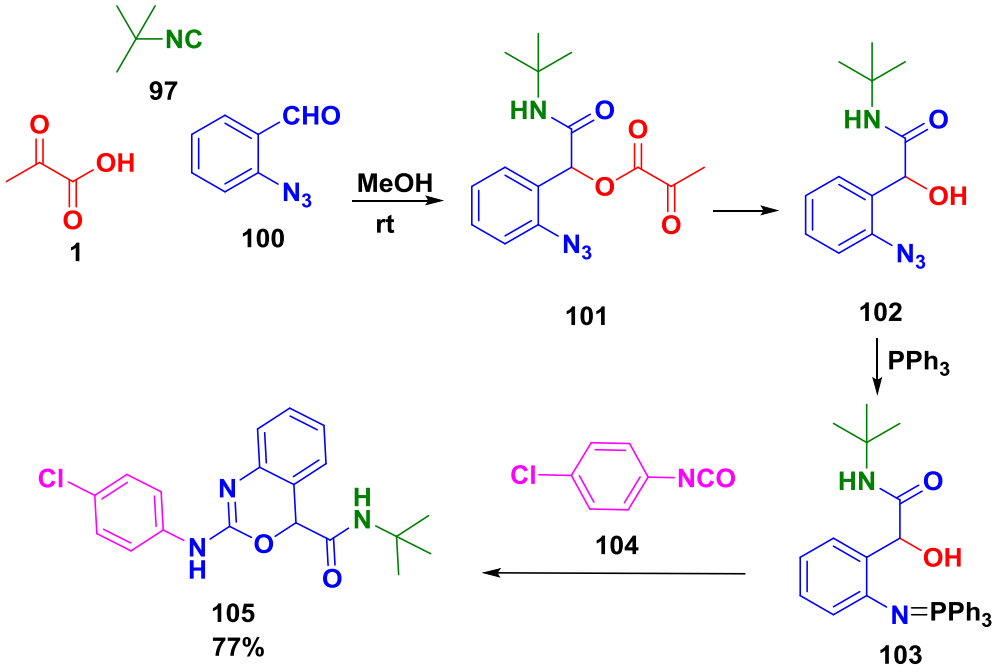


Figure 11: (**A**) Compound **99** is a potent and selective inhibitor of BRD4, (**B**) The Crystal structure of **99** bound to BRD4 and (**C**) The interaction diagram of **99** from the cocrystal structure with BRD4.[76]

1.4.3. Keto acids-based multicomponent reactions

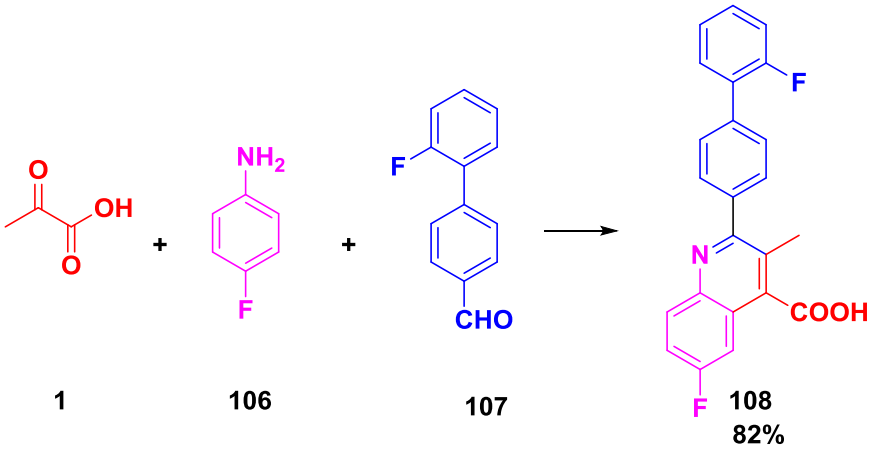
Recently, chemists have been interested in the synthesis of amide derivatives due to their biological activities. The pyruvic acid **1** was employed in the Passerini reaction as one of the three components to react with 2-azidobenzaldehyde **100** and *t*-butylisocyanide **97** to afford an initial Passerini product **101** in moderate yield. The product **101** has further undergone hydrolysis during work-up to obtain azide **102**. The azide **102** was then treated with triphenyl

phosphine to afford iminophosphorane **103** in excellent yield *via* the Staudinger reaction. To a solution of iminophosphorane **103** in dry toluene was added isocyanate **104** under reflux to form 2-amino-4-aminocarbonyl substituted 4H-3,1-benzoxazines **105** as crystalline solids in satisfactory yields (Scheme **31**). It was noted that the transformation of **103** into **105** includes aza-Wittig reaction between iminophosphorane **103** and isocyanate **104** to furnish a carbodiimide, which was easily undergone by ring closure to form **105**.[77]



Scheme 31: A synthetic approach of benzoxazines **105**.

Doebner *et al* employed *α*-keto acid **1**, *para*-fluoroaniline **106** and 2'-fluoro- [1,1-biphenyl]-4-carbaldehyde **107** in multicomponent reactions to synthesise DHODH inhibitor **108** (Scheme **32**). DHODH inhibitor **108** has been tested in multiple clinical trials for cancer treatment and immune suppression.[78] A cocrystal structure of DHODH inhibitor has been published and revealed that the inhibitor occupies a long hydrophobic channel and induces a significant charge-charge interaction with the Arg**136** (Figure **12**). DHODH inhibitor was synthesised by Doebner-**3**CR. Doebner‘s product is situated in a deep and hydrophobic protein binding site (PDB ID 1UUO). The salt bridges are formed between a carboxylic acid and the guanidine unite of Arg**136**. Notable is the interaction between the two fluorine atoms located on the outskirt of biphenyl ring and the isoquinoline which binds to targets suites in a hydrophobic environment.[79]



Scheme 32: A synthetic approach of DHODH inhibitor **108**.

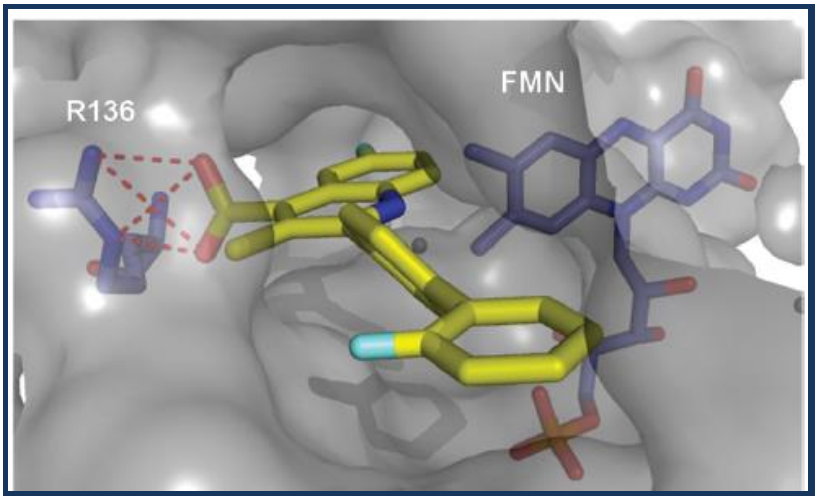
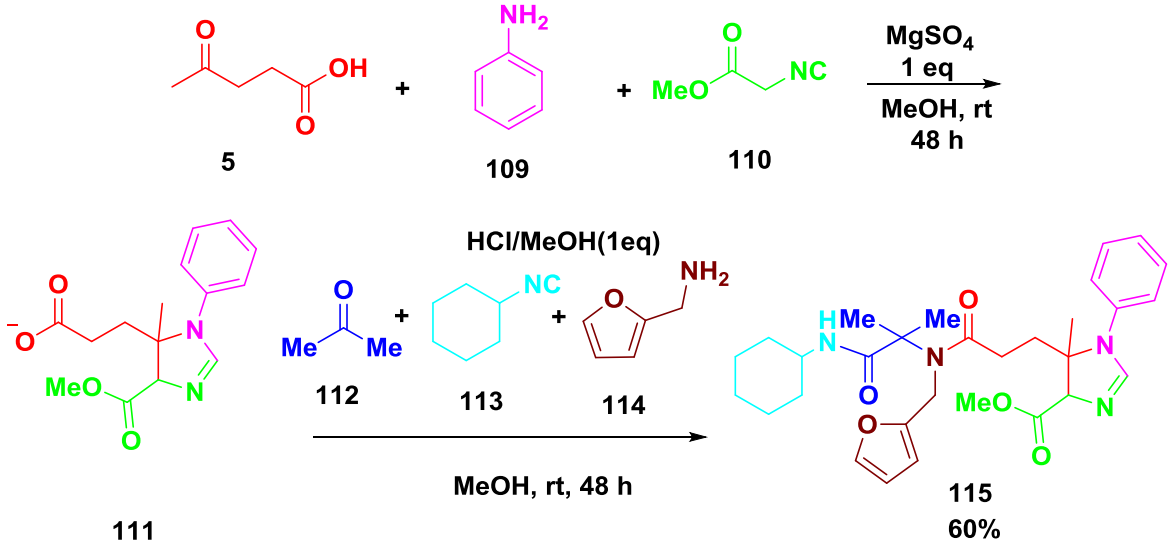


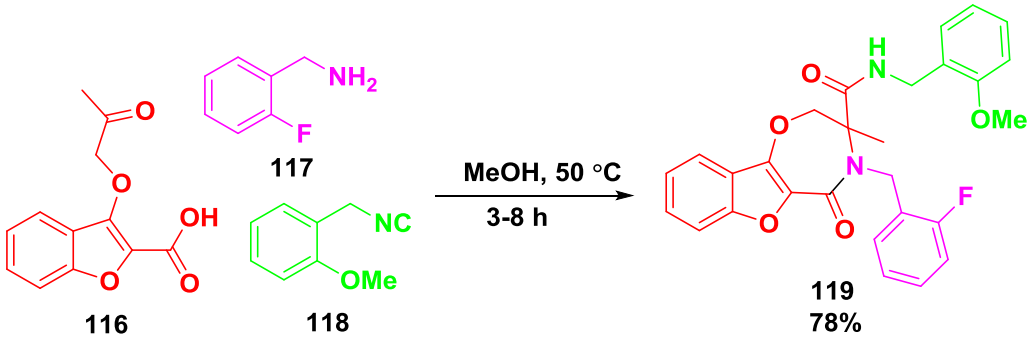
Figure 12: A cocrystal structure of DHODH inhibitor.[80]

The synthetic approach for complex molecules with unpresented properties is still determined by rather inefficient step-by-step reaction outcomes. The orthogonal functional groups may be assembled into one molecule to allow multiple reactions taking place on one molecule to form a more complex structure. This protocol avoids the use of protective groups and increases the efficiency of reactions. The keto acid **5** can be converted to 2-imidazoline scaffold *via* a carbonyl component. 4-Oxopenatnoic acid **5**, aniline **109** and isocyanide **110** were efficiently reacted in one pot in the presence one equivalent of base to afford an intermediate carboxylate **111**. This intermediate **111** reacted with different components **112**, **113**, and **114** in another one pot to result complex product **115** in moderate yield (Scheme **33**).[81]



Scheme 33: A synthetic route of complex product **115**.

Elena *et al* have developed four-component Ugi reaction to synthesize of novel aryl- and heteroaryl-fused derivatives such as 3-methyl-5-oxo-1, 4-oxazepine-3-carboxamides **119** where a variety of starting materials are used. It was observed that a reaction of keto acid **116**, (2-fluorophenyl) methanamine **117** and isocyanide **118** in methanol at 50 °C within 3–8 hours gave 3-methyl-5-oxo-1,4-oxazepine-3-carboxamides **119** as novel heteroaryl-fused derivatives (Scheme **34**). Presumably, the reaction followed the same initial step as the classical Ugi condensation to produce an intermediate imine, which is then attacked by the isocyanides to form a nitrilium intermediate. The nitrilium intermediate is then undergone with intramolecular cyclization to yield oxazepine **119**.[82]

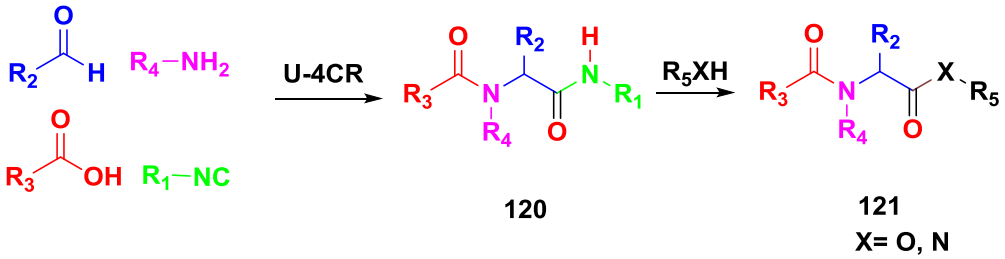


Scheme 34: A synthetic route of 3-methyl-5-oxo-1, 4-oxazepine-3-carboxamides **119**.

1.5. Convertible isocyanide

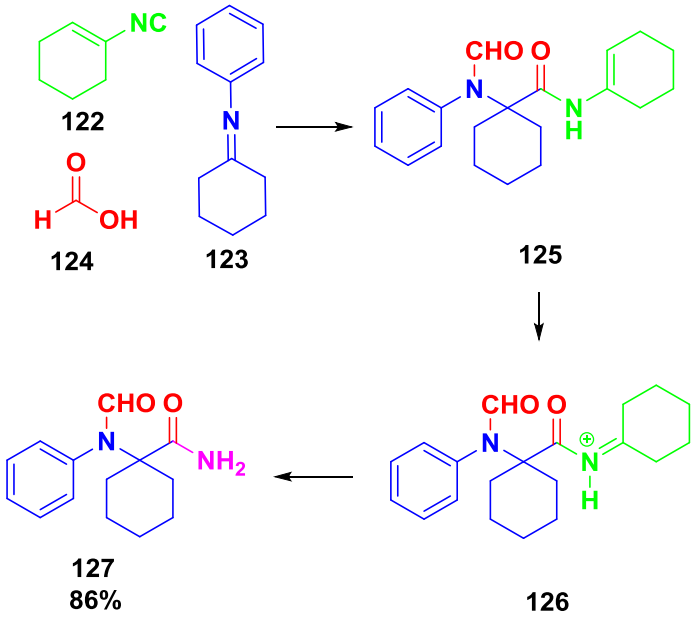
Efficient conditions to provide the hydrolysis of amides to carboxylic acids can be achieved by heating with strong base or acid.[83] Several convertible isocyanides have been specifically

investigated to allow the selective cleavage of the *C*-terminal amide bond under milder conditions. The *C*-terminal amide bond in **120** formed from isocyanides, the **120** can be converted into different functionalities **121**; have the term "Convertible Isocyanide" as shown in (Scheme **35**).



Scheme 35: Identification of convertible isocyanide.

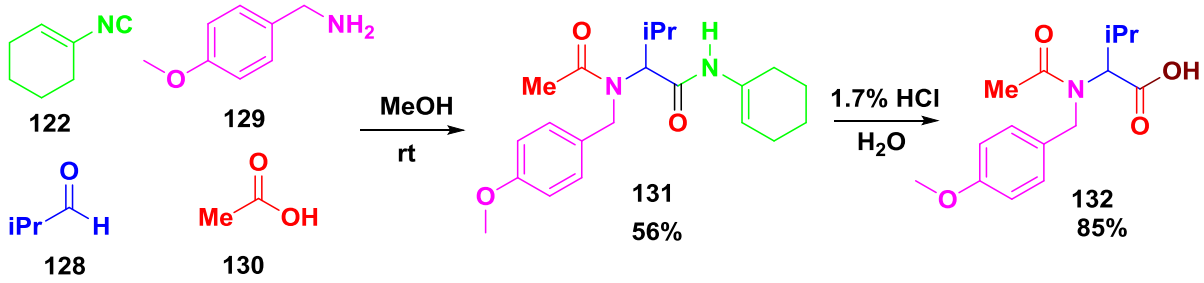
In 1963, Ugi introduced 1-cyclohexen-1-yl isocyanide **122** as an efficient starting material of unknown hydrogen isocyanide.[84] The reaction between isocyanide **122**, cyclohexanone *N*-phenyl imine **123** and formic acid **124** yielded *N*-cyclohexen-1-yl amide **125** (Scheme **36**). The secondary amide was transformed to primary *α*-acylamino amide **127** in 86% yield *via* acidic activation of the *N*-acyl enamide **126** followed by a hydrolysis.



Scheme 36: 1-Cyclohexen-1-yl isocyanide **122** in Ugi reaction.

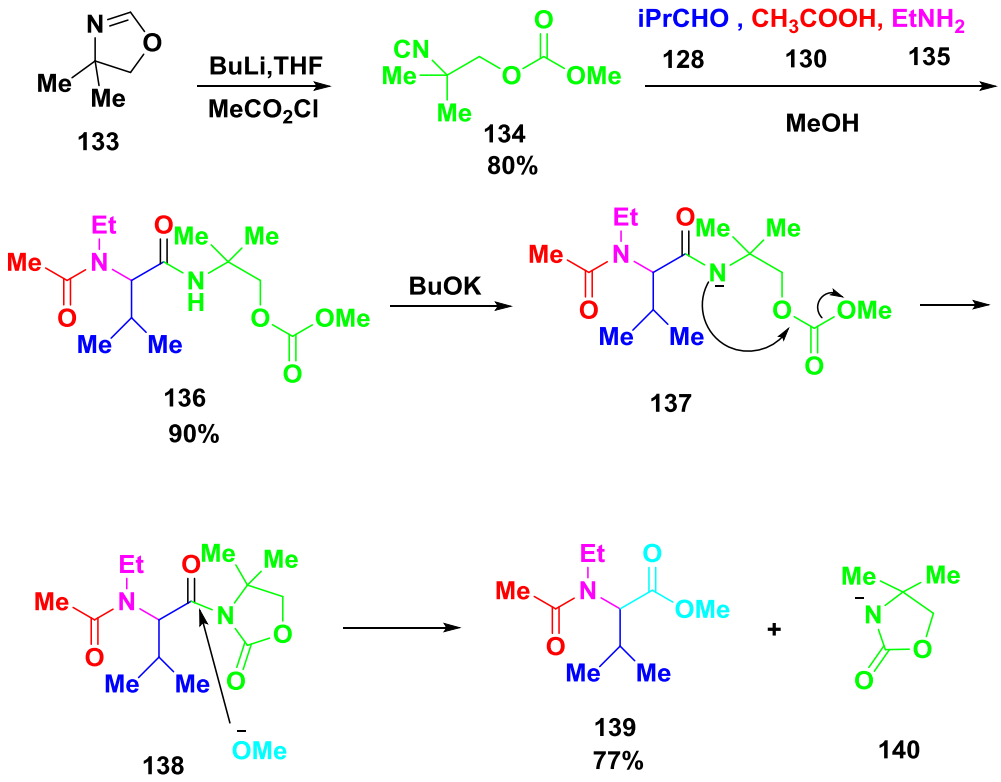
In 1995, Armstrong introduced isocyanide **122** for the synthesis of a new adduct **132**, which was formed from reaction of **122** with isobutyraldehyde **128**, *p*-methoxybenzylamine **139**, and acetic acid **130** to afford diamide **131**. The diamide **131** can then be converted in the

corresponding carboxylic acid **132** using HCl (1.7%) in THF at room temperature (Scheme **37**).[85]



Scheme 37: 1-Cyclohexen-1-yl isocyanide **122** has been applied by Armstrong.

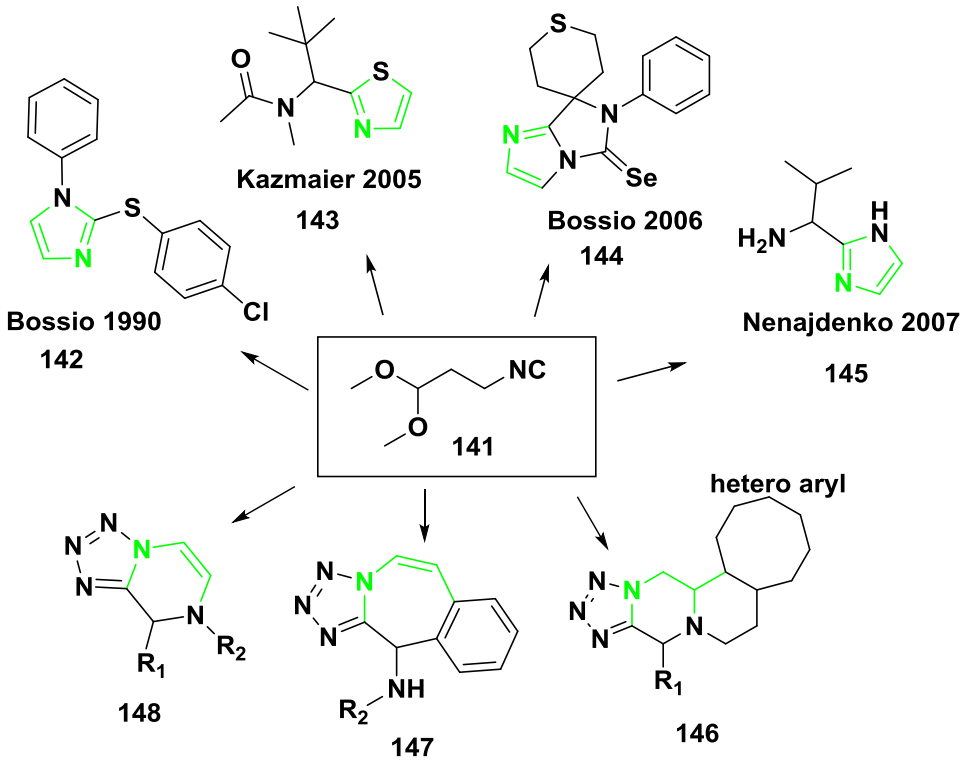
A convertible isocyanide methyl 2-isocyano-2-methylpropylcarbonates **134** was developed by Ugi *via* one step reaction where 4,4-dimethyl-2-oxazoline **133** (Scheme **38**) was deprotonated by nBuLi in THF at -78 °C, followed by reaction with methyl chloroformate. The Ugi reaction of isocyanide **134** with isobutyraldehyde **128**, acetic acid **130** and ethyl amine **135** furnishes the expected Ugi product **136**, which would be converted into *N*-acylα-aminoester **137**. The reaction also involves cyclization of the amide anion into the carbonate of **137** to give *N*-acyloxazolidinone **138**, which was then followed by the addition of methoxide to form **139** and *via* an elimination process afforded oxazolidinone **140**.[86]



Scheme 38: Ugi's convertible isocyanide **134**.

The use of universal building blocks in the synthetic field has many advantages, particularly in the parallel synthesis of larger libraries. For instance, unprotected α-amino acids have recently used in different multicomponent reactions as stereoselective species to yield a novel cyclic diverse compounds.

Hardtke described isocyanide diethyl acetal **141**, which was exploited to prepare for the building block in heterocyclic compounds (Scheme **39**).[87] These heterocyclic compounds (**142-148**) can be synthesised from isocyanide diethyl acetal **141**.[88]

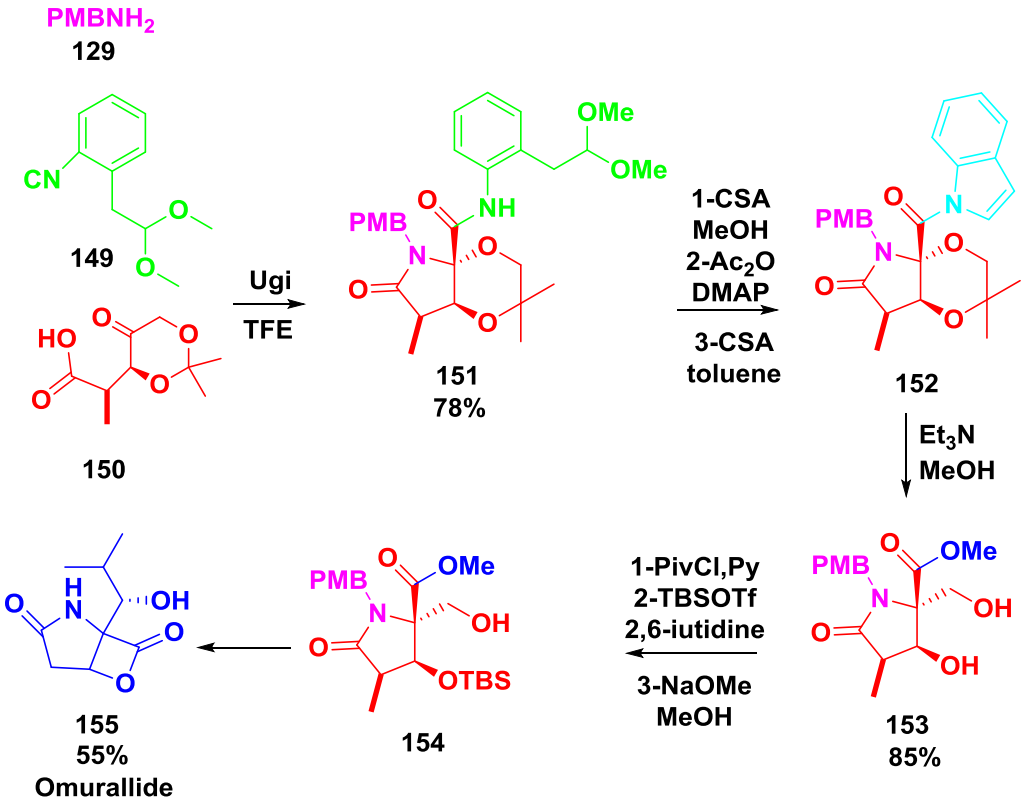


Scheme 39: Heterocyclic scaffolds in previous work using isocyanide **141**.

1.6. Ketoacids and convertible isocyanides in the synthesis of biologically active molecules

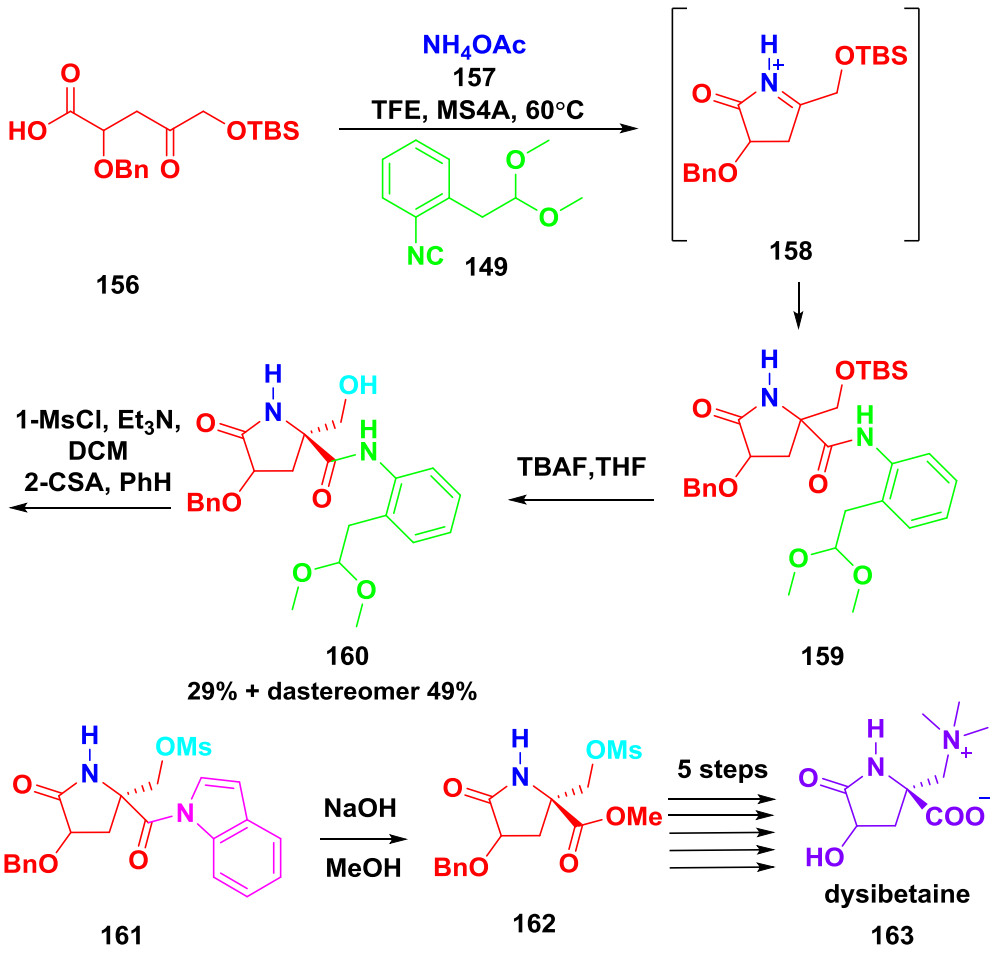
Multicomponent reactions have been considered as a facile and efficient approach to synthesise structurally diverse large libraries and complex molecules for biological screenings.[89]1-Isocyano-2-(2,2-dimethoxyethyl) benzene **149** as a convertible isocyanide has been employed by the Kobayashi group to build the main framework of the proteasome inhibitor omuralide **155** (Scheme **40**).[90] The keto acid **150** has been chosen to control the stereoselective Ugi 4C-3C reaction, providing **151** in 78% yield. 1,3-Dioxan-5-one **150** is a stereoselective ring system, which allows a small nucleophile such as isocyanide **149** to

approach from the axial direction to form omuralide **155**.[91] The Ugi product **151** was treated with catalytic CSA in methanol to yield the corresponding diol without formation of *N*-acylindole. The protection of diol formed a diacetate, which was not affected by the acid catalysed indole formation. The desired *N*-acylindole **152** can be obtained, which was converted directly to the methyl esterdiol **153** in good yield. Sequentially, the formal total synthesis of omuralide **154** was followed by pivaloate formation of alcohol of **154**, TBS protection of the secondary alcohol and NaOMe-mediated removal of the pivaloate.



Scheme 40: Synthetic total route of omuralide features a stereoselective of Ugi 4C-3C reaction.

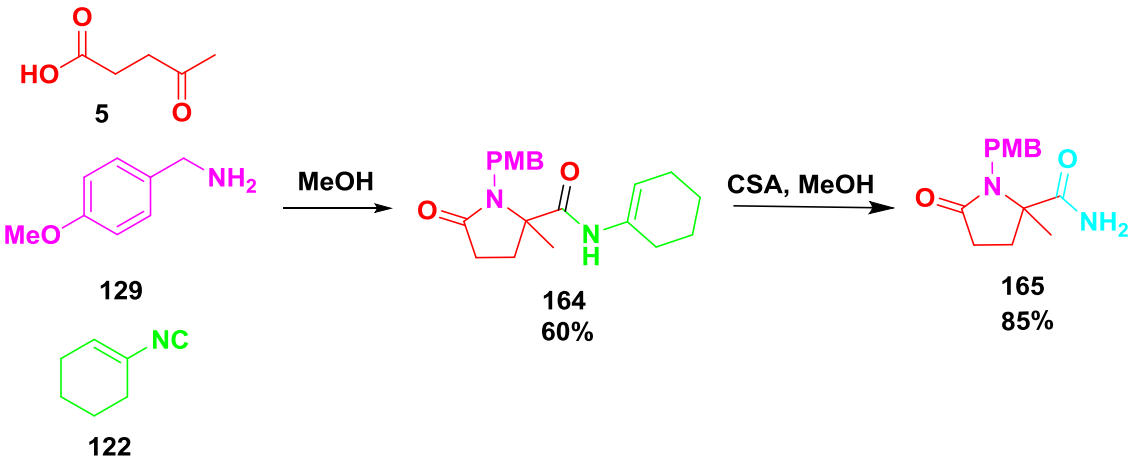
In 2008, the total synthesis of (-)-dysibetaine as a neuroexcitotoxin was published by Kobayashi.[92] The (-)-dysibetaine **163** is known as a member of a group of natural products which was isolated by Sakai *et al* in 1990 from the micronesian sponge.[93] The cyclic amide **159** was formed by an Ugi 4-centered 3-component reaction (Scheme **41**). The TBS group of **159** was efficiently removed by using TBAF and the diastereomers **160** were then separated at this stage by silica column chromatography. The isocyanide **149** was selected as it can be converted to the acylindole **161** which was feasibly hydrolysed to the methyl ester **162**. The racemic dysibetaine **163** could then be obtained by using several functional group interchanges.



Scheme 41: The total synthetic route of (±)-dysibetaine **163**.

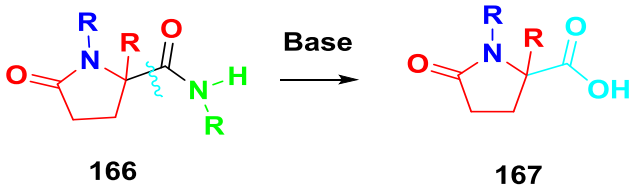
The selective cleavage of one amide in an Ugi product can be achieved through using "convertible isocyanides". As the isocyanide component in the Ugi 4C reaction has become the *C*-terminus for *α*-acylamino amide product therefore, a convertible isocyanide allows the *C*-terminal amide to be selectively cleaved.[94],[95]

A known convertible isocyanide **122** was applied to synthesise a 2-alkylpyroglutamic acid derivatives by Ugi reaction using *γ*-keto acids **5** and amine **129** (Scheme **42**). It is noted that the convertible isocyanide **122** could form an intermediate amide **164**, which can then be easily hydrolysed to yield pyroglutamic amide analogue **165**. This derivative **165** was generated by the U-4C-3C through *C*-terminal amide **164** which was selectively converted to the 2-alkylpyroglutamic amide **165**.[96]



Scheme 42: Isocyanide **122** has been used to synthesise pyroglutamic amide **165**.

It was reported that convertible isocyanides such as **122** failed to hydrolyse the *C*-terminal amide of pyroglutamic acid amides **165**. Therefore, an alternative convertible isocyanide is required to allow selective activation under mild conditions and hydrolysis of the *C*-terminal amide of acid amides **166** to form pyroglutamic acid **167** (Scheme **43**).



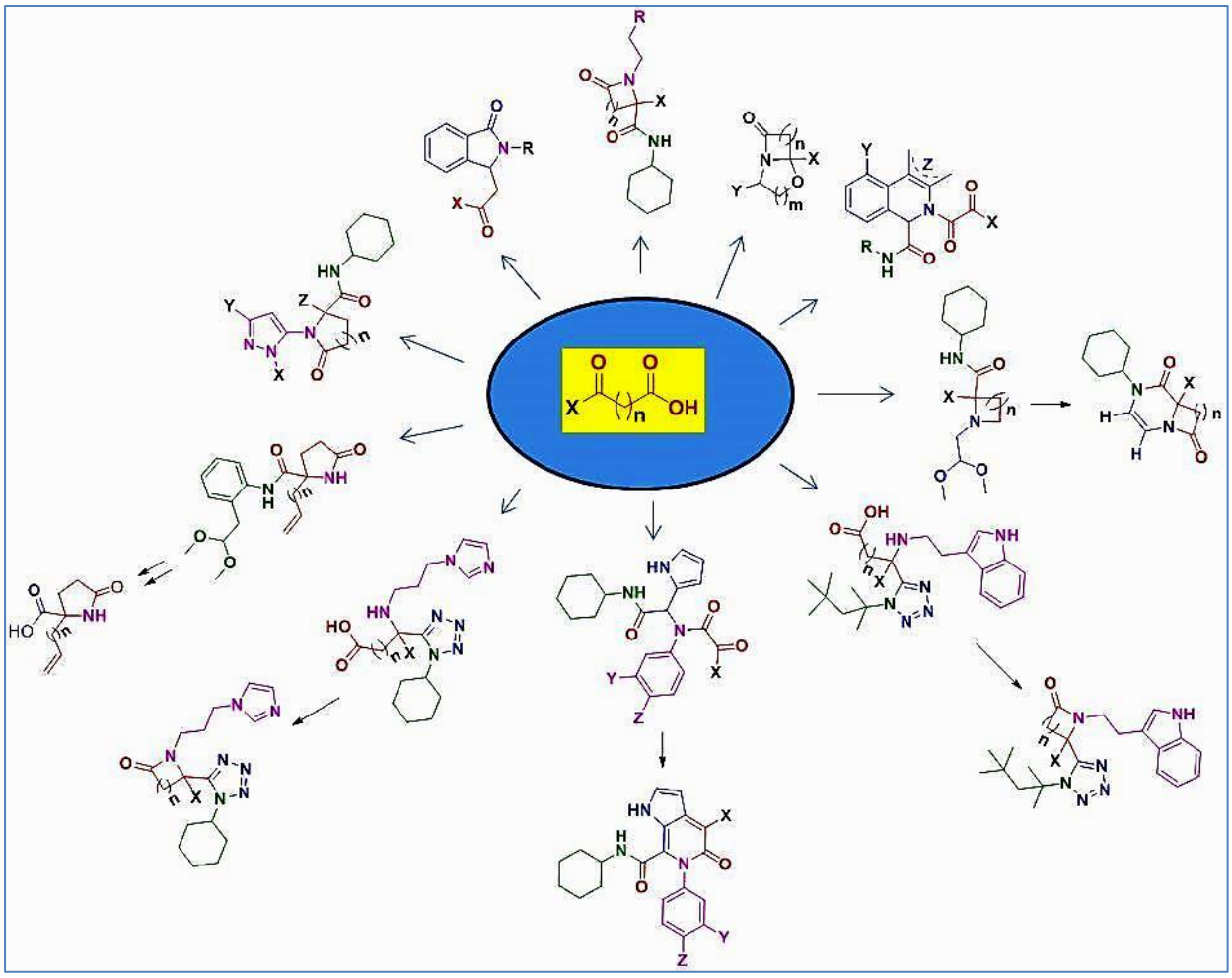
Scheme 43: Hydrolysis process of *C*-terminal amide.

1.7. Aims and objectives

The aim of my PhD project was to synthesise a range of heterocyclic compounds using MCRs (Scheme **44**) and screen their biological activities against prion diseases. To achieve this goal, the following objectives needed to be met:

1- Synthesis of keto acids from cheaper commercially available starting materials.
2- Synthesis of benzyl isocyanide **59**, cyclohexylisocyanide **113** and convertible isocyanide **149** using cheaper commercially available compounds.
3- Utilize keto acids and isocyanides to synthesize cyclic amides (*β*-, *γ*-, and *δ*-lactam amides) using multicomponent Ugi reactions.

4- Develop continuous flow processes to improve productivity of some Ugi-tetrazole derivatives with high yields and reduced reaction times.
5- Develop microwave techniques (MW) as conventional technology to synthesise pyrazole-oxopyrrolidine derivatives and improve productivity.
6- Synthesis of isoquinoline and isoindoline derivatives from α- and β-ketoacids.
7- Use convertible isocyanide **149** and keto acids to synthesise pyroglutamic acid analogues using multicomponent Ugi reactions.
8- Develop post-Ugi transformation reactions towards the synthesis of derivatives, diones derivatives, pyrrole-oxopyrrolidine derivatives and pyrrolo-pyridine derivatives.
9- Screen all synthesised compounds for anti-prion activity.



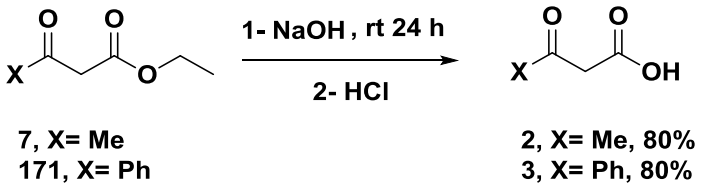
Scheme 44: Schematic diagram of project objectives.

Part II. Results and Discussions

Chapter 2. Synthesis of cyclic amides (Lactams)

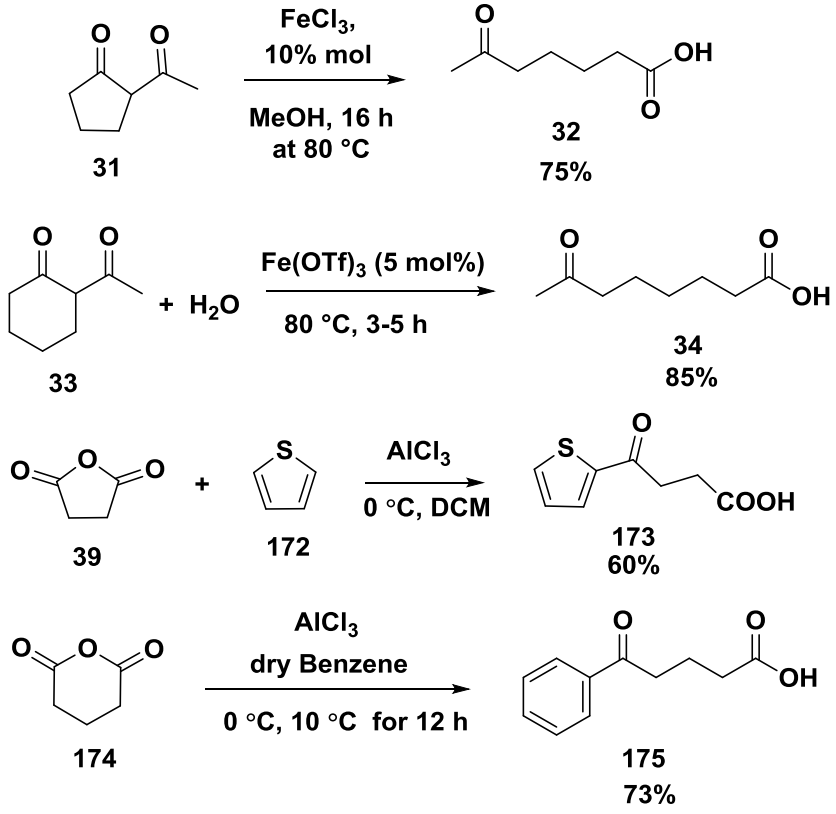
2.1. Synthesis of keto acids

All keto acids used in this thesis are either commercially available such as, 4-oxopentanoic acid **5**, 5-oxohexanoic acid **168**, 2-oxo-2-phenylacetic acid **169** and 2-acetylbenzoic acid **170** or synthesised by myself. Keto acids were made by alkali hydrolysis from esters such as 3-oxobutanoic acid **2**, 3-oxo-3-phenylpropanoic acid **3** (Scheme **45**).



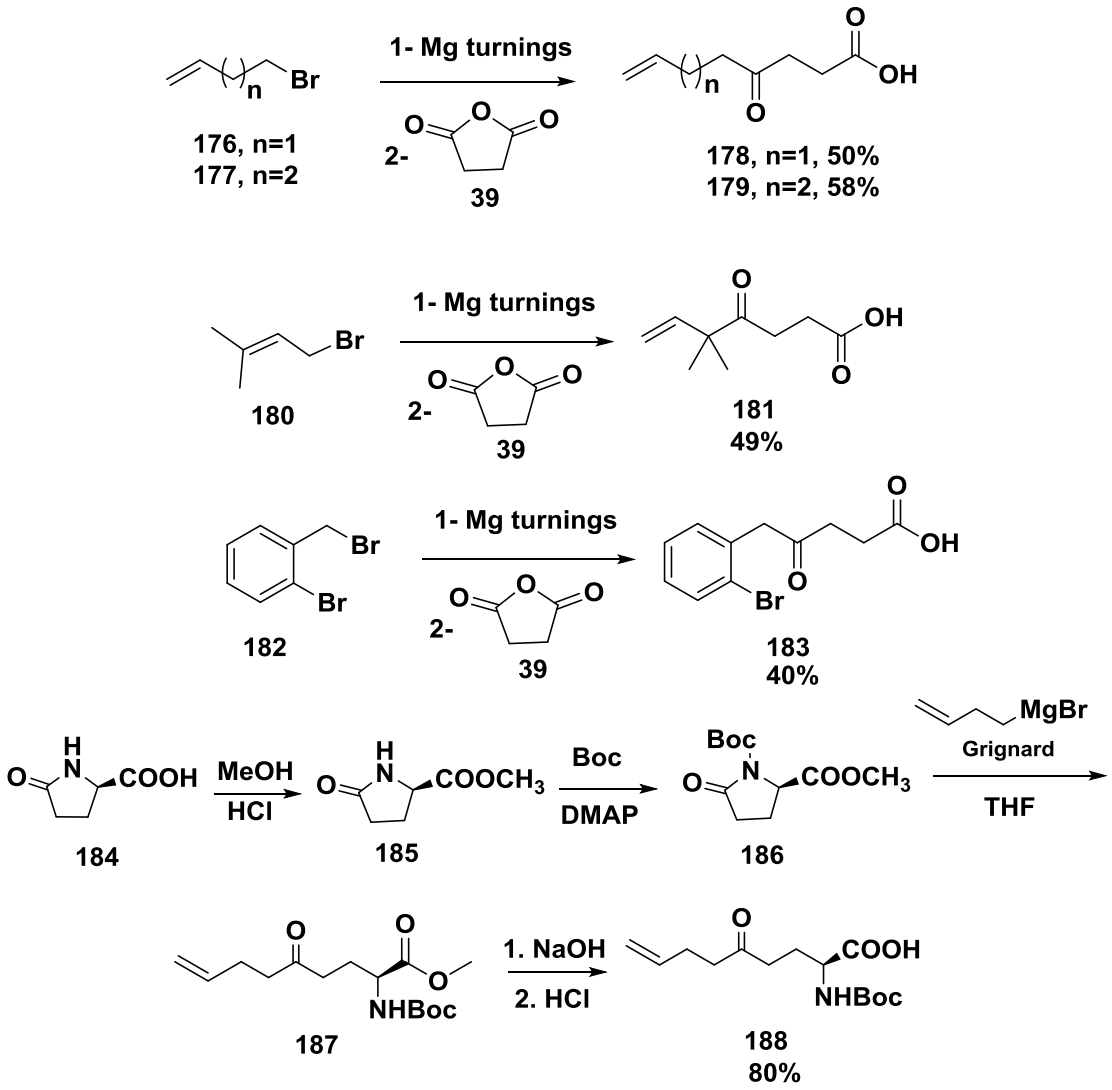
Scheme 45: Synthesis of keto acids **2** and **3**.

Ketoacids have also been synthesised from an array of cyclic anhydrides. They were effectively synthesised by catalytic reagents such as $FeCl_3$, $Fe(OTf)_3$ or $AlCl_3$ to prepare 6-oxoheptanoic acid **32**, 7-oxooctanoic acid **34**, 4-oxo-4-(thiophen-2-yl) butanoic acid **173** and 4-benzoylbutyricacid **175** in good yields (Scheme **46**).



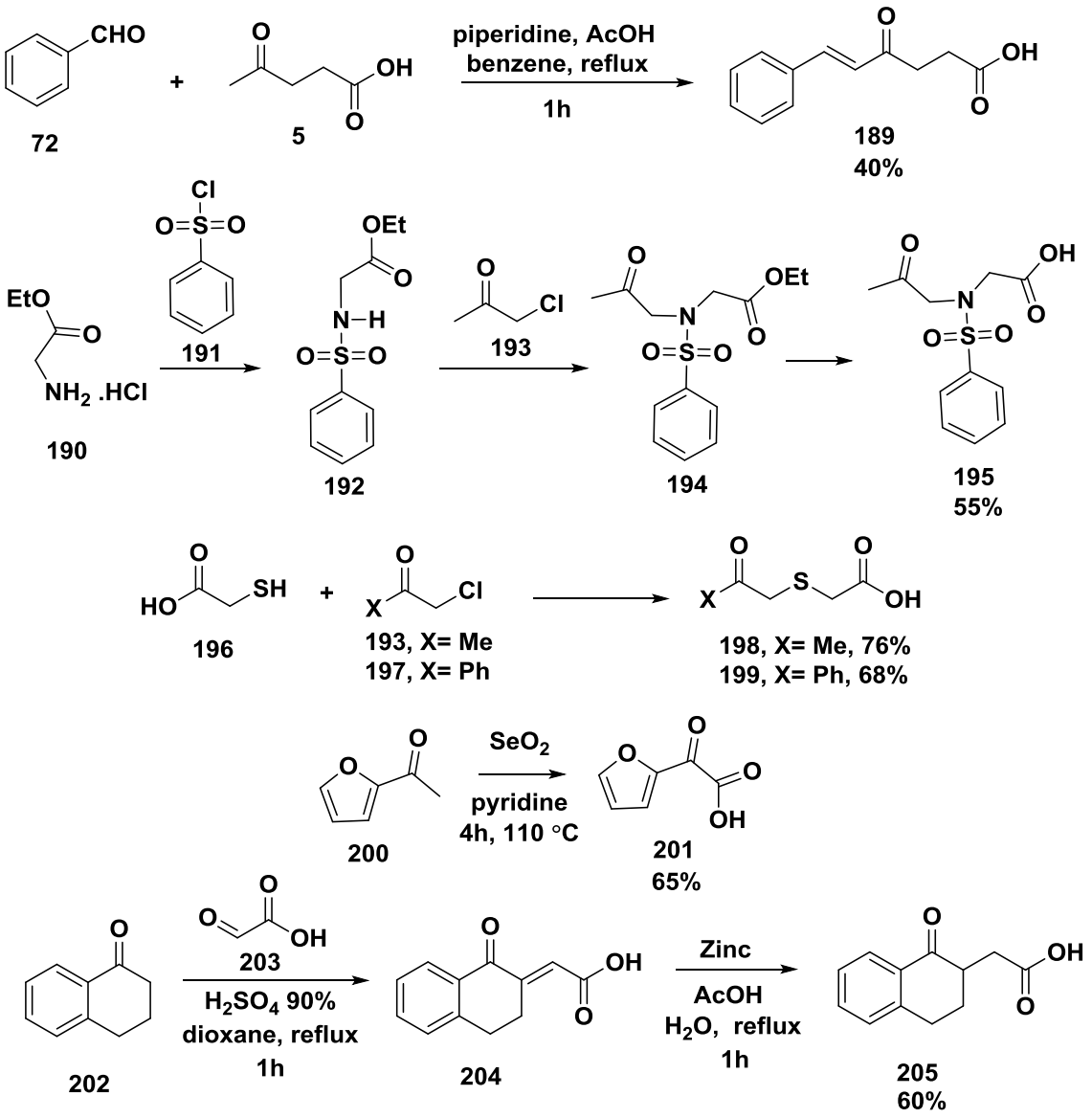
Scheme 46: Synthesis of keto acids **32**, **34**, **173** and **175**.

The ring-opening of succinic anhydride by using Grignard reagents was used to synthesise keto acids such as 4-oxooct-7-enoic acid **178**, 4-oxonon-8-enoic acid **179**, 5,5-dimethyl-4-oxohept-6-enoic acid **181**, 5-(2-bromophenyl)-4-oxopentanoic acid **183** and 2-((*tert*-butoxycarbonyl) amino)-5-oxonon-8-enoic acid **188** which was synthesised in five steps (Scheme **47**).



Scheme 47: Synthesis of some keto acids by ring-opening with Grignard reagent.

(*E*)-4-Oxo-6-phenylhex-5-enoic acid **189**, 2-(*N*-(2-oxopropyl) phenylsulfonamido) acetic acid **195**, 2-((2-oxopropyl)thio) acetic acid **198**, 2-((2-oxo-2-phenylethyl)thio) acetic acid **199**, 2-(furan-2-yl)-2-oxoacetic acid **201** and 2-(1-oxo-1, 2,3,4-tetrahydronaphthalen-2-yl)acetic acid **205** were prepared by different methods according to literature (Scheme **48**).

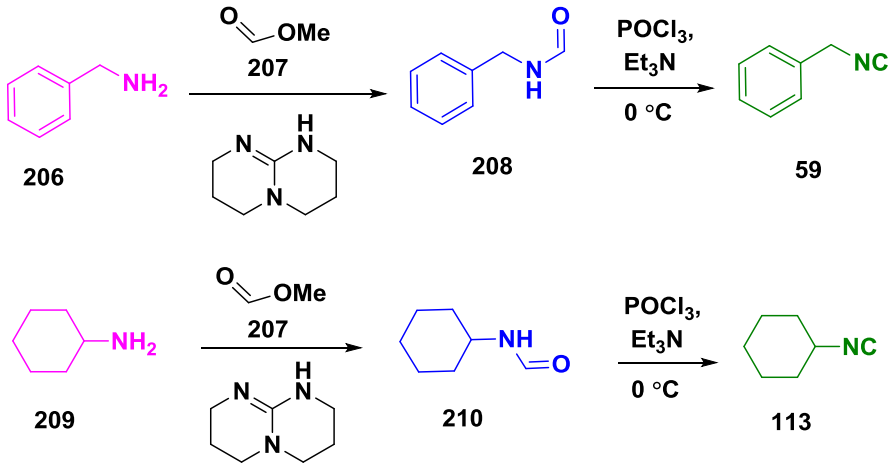


Scheme 48: Synthesis of some keto acids with different methods.

2.2. Synthesis of isocyanides

Approaches for preparing isocyanides have been developed by using common reagents for formylation of a primary amine to formamide followed by dehydration to result in corresponding isocyanides. The most convenient approach of dehydration is with phosphorus oxychloride ($POCl_3$). Isocyanides are well known due to the reactivity of the isocyanide functional group, but most isocyanides are not commercially available. Even if they are available, they are normally very expensive. For example, 5 g of benzyl isocyanide **59** is priced at £300, while starting material, 2500 g of benzyl amine from the same supplier is priced at £225. Therefore, benzyl isocyanide **59** was freshly synthesised by using cheaper commercially available benzyl amine as a starting material. The same method was followed

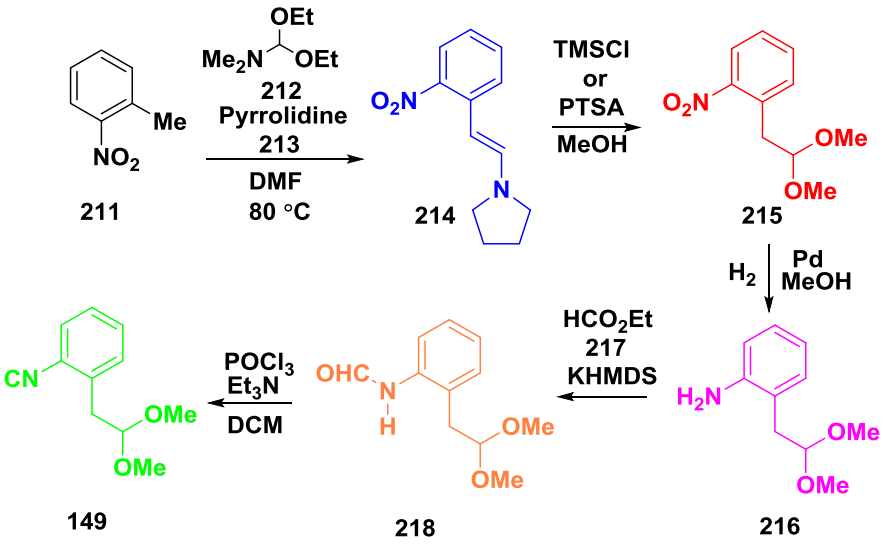
to synthesise cyclohexylisocyanide **113** by using cheaper commercially available cyclohexylamine as a starting material (Scheme **49**).



Scheme 49: Synthesis of isocyanides **59** and **113** from a primary amine.

2.3. Synthesis of convertible isocyanide 149

A convertible isocyanide can provide a handle for further reactions to take place. A convertible isocyanide **149** can be obtained in five steps (Scheme **50**). Enamine **214** was synthesised from *o*-nitrotoluene **211**[90] which can then be successfully converted to the methyl acetal **215** using chlorotrimethylsilane. The hydrogenation process of **215** with palladium as catalyst provided aniline **216**, [97] which was then formylated with methyl or ethyl formate **217** using KHMDS to produce formamide **218**. The formamide **218** was dehydrated to give 1-isocyano-2-(2,2-dimethoxyethyl)benzene **149** by using $POCl_3$ and Et_3N.[98]



Scheme 50: Preparative route of convertible isocyanide **149**.

2.4. Cyclic amides (Lactams)

Natural products are rich sources for lead discovery of many pharmaceutical drugs. Most natural products have been successfully separated from organisms inhabiting terrestrial environments. The cyclic amide motif is a backbone of a significant part of natural products.[99] Key structural penicillin families **219** are well known as *β*-lactams which contain a core cyclic amide feature and are still used today as antibiotics to treat different types of bacterial infections. Lactacystin **220** was discovered to entice neuritogenes in neuroblastoma cell lines activity. Salinosporamide **221** is a proteasome inhibitor which are being developed as a therapeutic agent for malaria[100] and multiple myeloma[101] (Figure **13**).

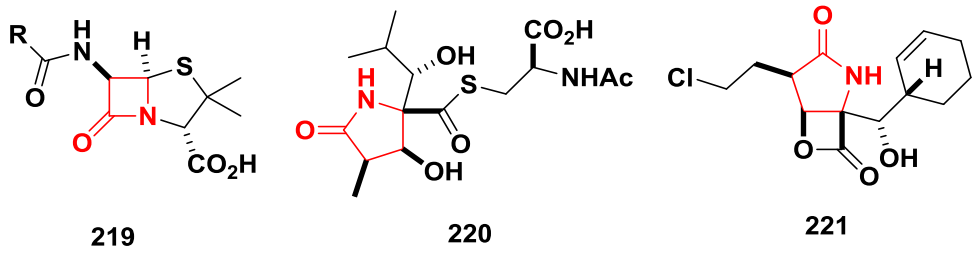


Figure 13: Examples of important cyclic amides.

Bryozoans have been proved to be a useful source for biologically active compounds. The majority of compounds isolated to date from bryozoans are alkaloids. A bromopyrrole alkaloid, aspidostomide **222** (Figure **14**) was first isolated from patagonian bryozoans (A. spidostomagiganteum).[102] Aspidostomide **222** is derived from either dibromotyrosine or bromo tryptophan biochemically.

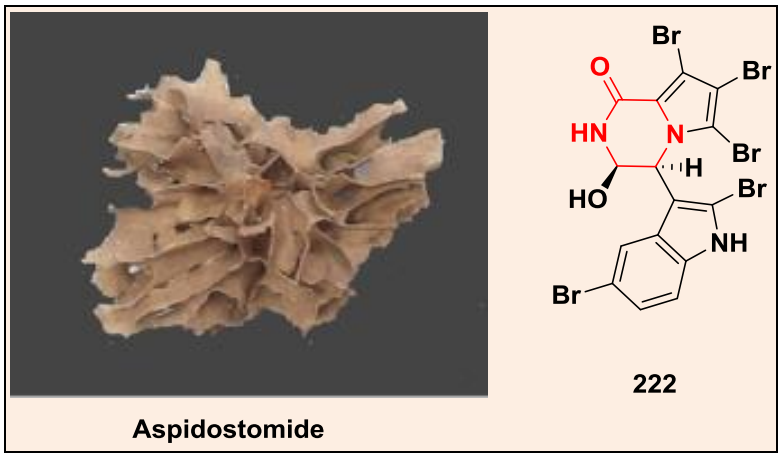


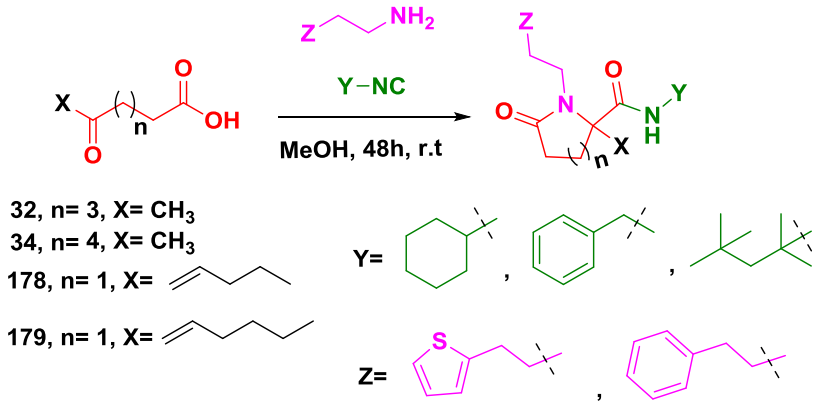
Figure 14: Aspidostomide **222** isolated from bryozoans as cyclic amide.[102]

Therefore, the synthesis of new bioactive cyclic amides has prompted chemists to employ multicomponent reactions such as Ugi reaction which reported the synthesis of amides from carbonyl components, amines and isocyanides which tether together to give access to cyclic amides in satisfactory yields.[103] In this project, the synthesis of cyclic amides was explored using keto acids as key component reactions.

2.4.1. Synthesis of cyclic amides (Lactams)

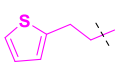
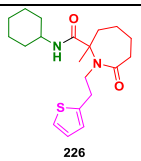
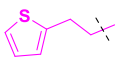
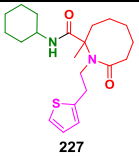
Several characteristics of the Ugi reaction have been noted in cyclic amide synthesis: first of all the reaction does not proceed in the absence of protic solvent such as methanol, secondly the yield of reaction decrease as ring size increases.

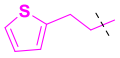
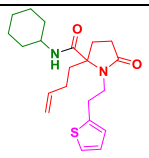
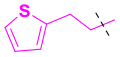
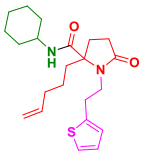
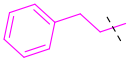
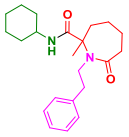
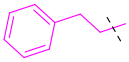
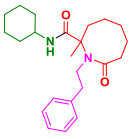
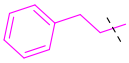
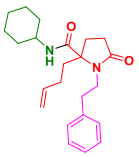
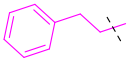
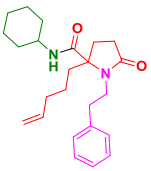
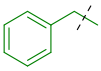
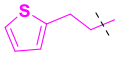
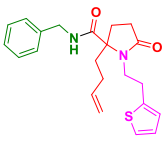
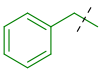
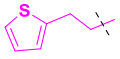
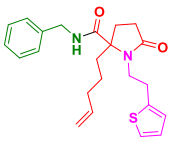
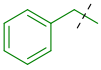
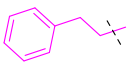
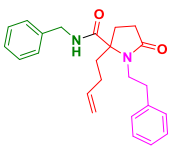
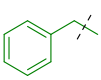
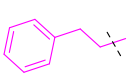
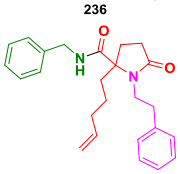
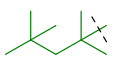
A library of cyclic amides was synthesised using six different ketoacids: 6-oxoheptanoic acid **32**, 7-oxooctanoic acid **34**, 4-oxo-4-(thiophen-2-yl) butanoic acid **173**, 4-benzoylbutyric acid **175**, 4-oxooct-7-enoic acid **178** and 4-oxonon-8-enoic acid **179**, three isocyanides (benzyl isocyanide **59**, cyclohexylisocyanide **113** and 2-isocyano-2,4,4-trimethylpentane **223**), and two amines; (2-(thiophen-2-yl) ethanamine **224**, 2-phenylethanamine **225** as shown in Scheme **51**.

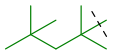
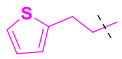
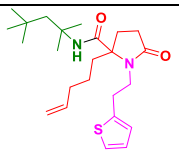
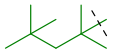
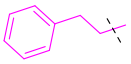
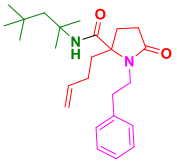
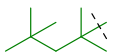
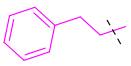
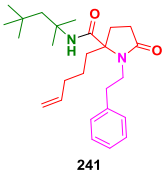


Scheme 51: Synthesis of cyclic amides.

Table 2.1: Synthesis of cyclic amides

Entry	n	X	Y	Z	Product	yield[a]
1	3	CH_3			 226	40%
2	4	CH_3			 227	-

3	1				 228	45%
4	1				 229	55%
5	3	CH_3			 230	35%
6	4	CH_3			 231	-
7	1				 232	40%
8	1				 233	35%
9	1				 234	62%
10	1				 235	55%
11	1				 236	45%
12	1				 237	50%
13	1				238	46%

14	1				 239	40%
15	1				 240	50%
16	1				 241	47%

[a] yield of isolated product

Cyclic amide compounds with yields of 35-62% were illustrated in Table **2. 1**. Attempts were made to synthesise 7-membered cyclic amides **226** and **230** (7-oxoazepane, entry **1** and **5**) by using 6-oxo-heptanoic acid **32**, cyclohexylisocyanide **113** and 2-(thiophen-2-yl) ethanamine **224**, 2-phenylethanamine **225** under high dilution with yields ranging between 35 and 40%. While many trials were carried out to synthesise 8-membered cyclic amides (azocane-2-carboxamide **227** and **231**, entry **2** and **6**) from 7-oxooctanoic acid **34**, cyclohexylisocyanide **113** and 2-(thiophen-2-yl) ethanamine **224**, 2-phenylethanamine **225** under the same reaction conditions, this yielded a complex mixture of products. These mixtures were examined but none of them afforded the expected azocane-2-carboxamide. The reaction intermediate to form the nine-membered is considered to be even more disfavoured from both enthalpic and entropic standpoints.[104] It was investigated that transannular interactions contradict the energy evolved during formation of new bonds, and 8-membered rings are entropically disfavoured as the cyclic compounds have fewer degrees of conformational freedom when compared to the acyclic precursors (Figure **15**).

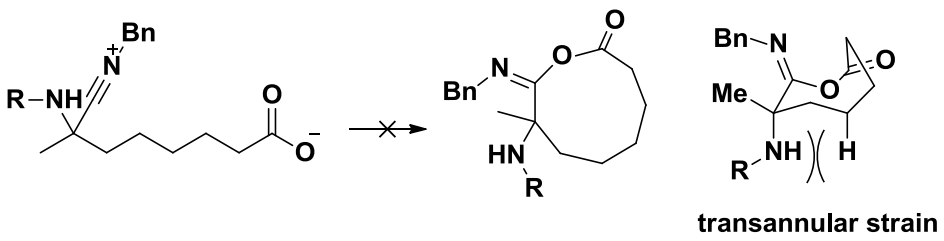
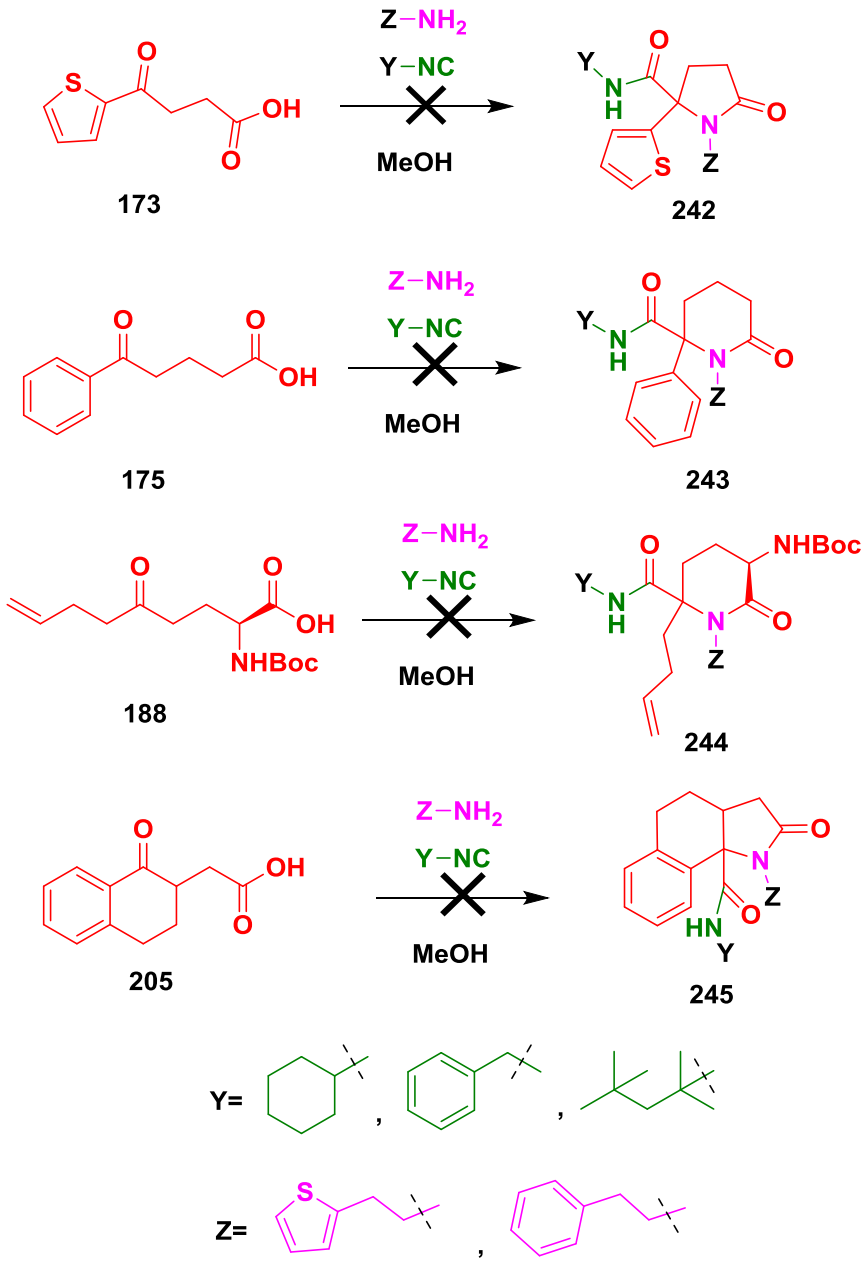


Figure 15: The postulated reasons to interpret the failed synthesis of azocane-2-carboxamide.

Pyrrolidine cyclic amide compounds **228-233** (except **230** and **231**) were successfully synthesised in yields of 35-62 % using keto acids containing double bonds, (2-(thiophen-2-yl) ethanamine **224**, 2-phenylethanamine **225** and cyclohexylisocyanide **113**.

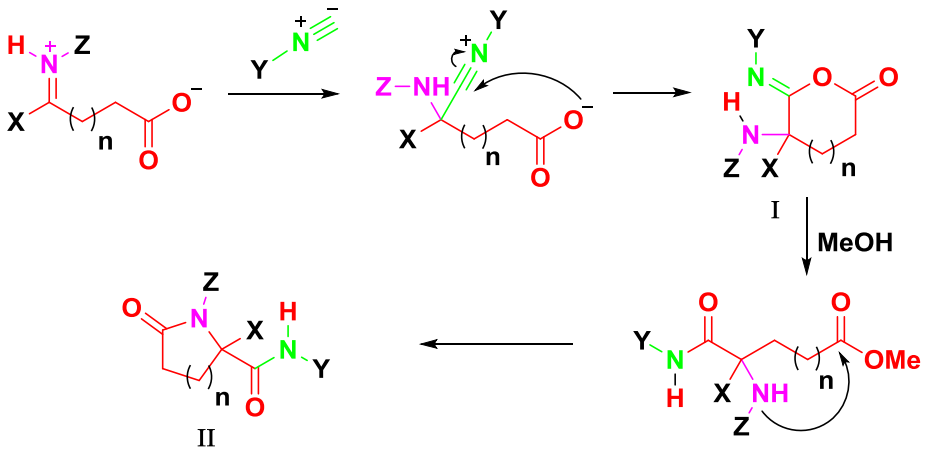
The reactions using 2-isocyano-2,4,4-trimethylpentane **223** afforded cyclic amides in moderate yields 40-50 % in all cases and the reactions using benzyl isocyanide **59** and keto acids containing double bonds gave moderate yields (45-62%). The yields of the reaction seem to be related to the stability of the isocyanide starting material. Therefore, the most stable isocyanides such as cyclohexyl isocyanide **113** produce moderate yields

Synthesis of cyclic amides **242**, **243**, **244** and **245** were repeatedly attempted from 4-oxo-4-(thiophen-2-yl)butanoic acid **173**, 4-benzoylbutyricacid **175**, 2-((*tert*-butoxycarbonyl) amino)-5-oxonon-8-enoic acid **188** and 2-(1-oxo-1,2,3,4-tetrahydronaphthalen-2-yl)acetic acid **205** as shown in Scheme **52**, unfortunately the trials failed. It may be interpreted that 4-oxo-4-(thiophen-2-yl) butanoic acid **173** does not dissolved in solvents such as methanol. While a complex mixture was yielded under the reaction conditions when use 4-benzoylbutyric acid **175**, 2-((*tert*-butoxycarbonyl) amino)-5-oxonon-8-enoic acid **188** and 2-(1-oxo-1,2,3,4-tetrahydronaphthalen-2-yl) acetic acid **205** as starting material. These reactions were carefully monitored but none gave the cyclic amide possibly due to a steric hindrance from phenyl group of keto acid **175**, Boc group of keto acid **188** and bulk cyclic of keto acid **205** which prevents the cyclisation in the proposed Ugi reaction.



Scheme 52: The failed trails for cyclic amides from keto acids.

These observations have led to a plausible mechanism which involves the condensation of an amine with the carbonyl yields an imine which is then protonated by the carboxylic acid. An addition of isocyanide follows a cyclisation of the carboxylate moiety to provide a cyclic intermediate **I**. An attack of methanol forms an amino ester which then undergoes cyclisation to afford the product **II** (Scheme **53**).[103] The number of carbon atoms between the carbonyl group and carboxylic group in keto acids dictates the size of ring which can be formed.



Scheme 53: The potential mechanism for Ugi products.

2.5. **Bi-and tri-cyclic amides (Lactams)**

Nonracemic (bi- and tri-cyclic lactams) pyrrolidinones and substituted pyrrolidines are of great relevance in drug development. They are the core to antibiotic compounds, for example, bicyclomycin **246** which is extensively utilized for treatment of diarrhoea.[105] 7,5-Fused membered amides **247** are present in a number of compounds with important biological applications for example some seven-membered lactams have been investigated as potential peptide turn mimetics. [106] Tricyclic compound **248** was employed as anti-inflammatory [107] and tricyclic compound **249** which has been recently evaluated as HIV-1 reverse transciptase inhibitor.[108] Interestingly, tetralone template **250** is found to be valuable drugs for serotonine receptor ligands[109] (Figure **16**). Bicyclomycin **246** has been synthesised from isoleucine and leucine in multistep reactions and biosynthetically isolated from *Streptomyces aizumenses*.[110] Racemic product **247** was synthesised from the trialkylaluminum under Beckman rearrangement of cyclohexanone oxime sulfonates and the alkylation reaction of dipole stabilized carbanions. Tricyclic compound **248** was synthesised in moderate yield from *N*-(ω-bromoalkyl) phthalimide treated with lithium phenylacetylide. Tricyclic compound **249** was prepared with a high enantioselectivity of 1,2-addition of thiol to ketimine. The ketimine has been derived from 3-hydroxy-3-arylisoindolinones catalyzed by a chiral phosphoric acid to furnish tricyclic compound **249** (Isoindolinone-based N, S-ketals).[111] While tricyclic compound **250** has been synthesised under enantioselective controll using a multistep synthesis.

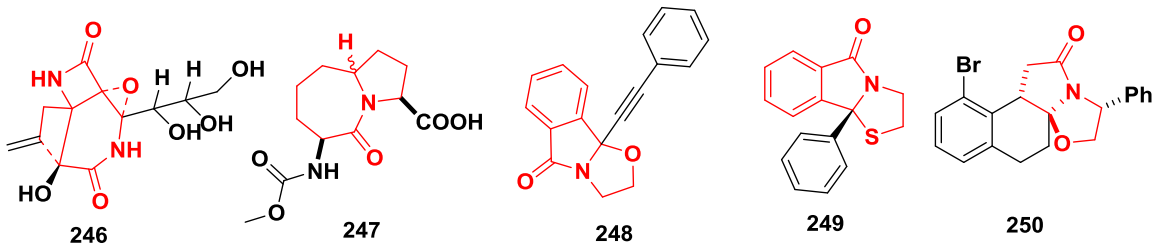


Figure 16: Bi- and tri-cyclic substructure amides have been used to clinical evaluation.

2.5.1. Synthesis of bi-and tri-cyclic amide (Lactams)

Meyers' lactamization is a bielectrophile-binucleophile reaction (BiE-BiNu reactions) that is used as access to design complex bicyclic amides with quaternary centres.[112] These lactams are considered as important key for the synthesis of natural products, particularly alkaloids such as: Amaryllidaceae **251** [113] and Erythrina **252** [114] (Figure **17**).

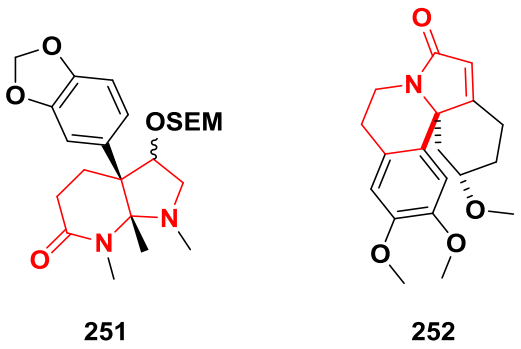
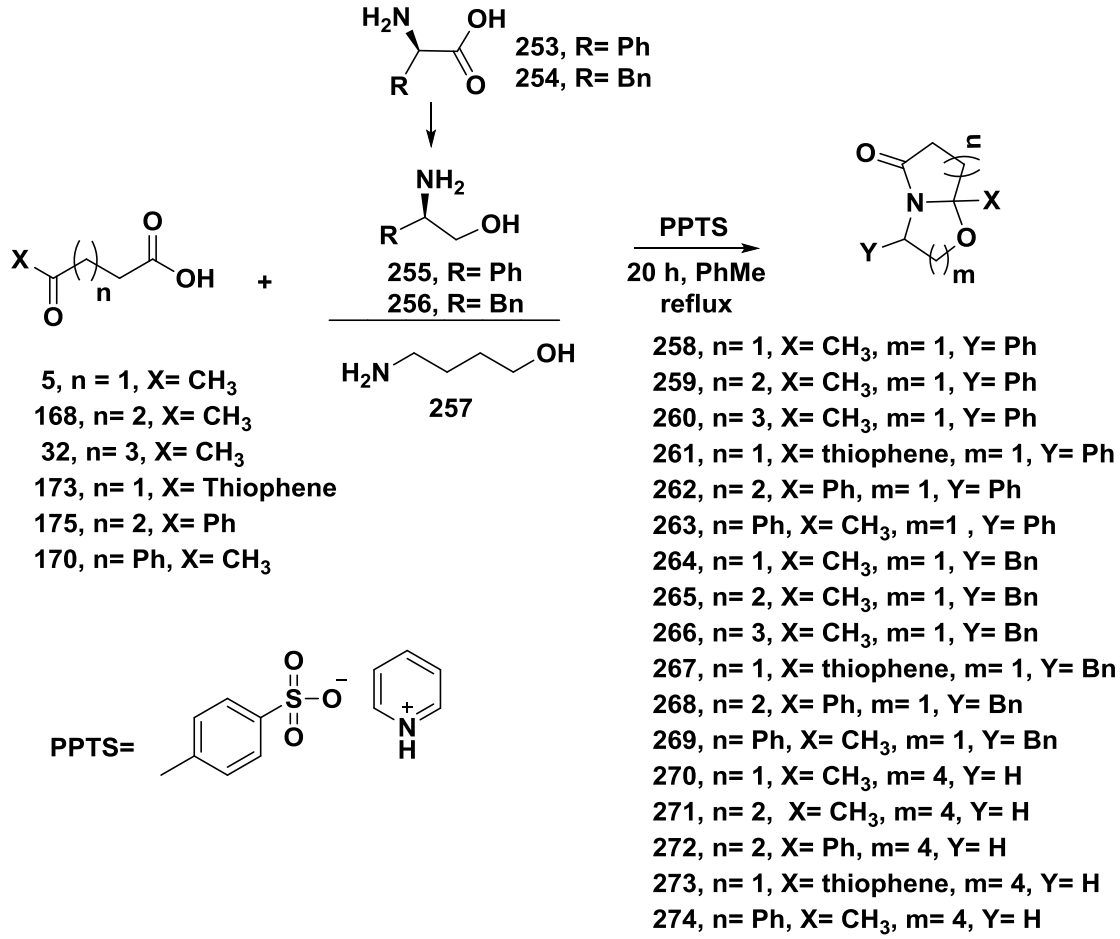
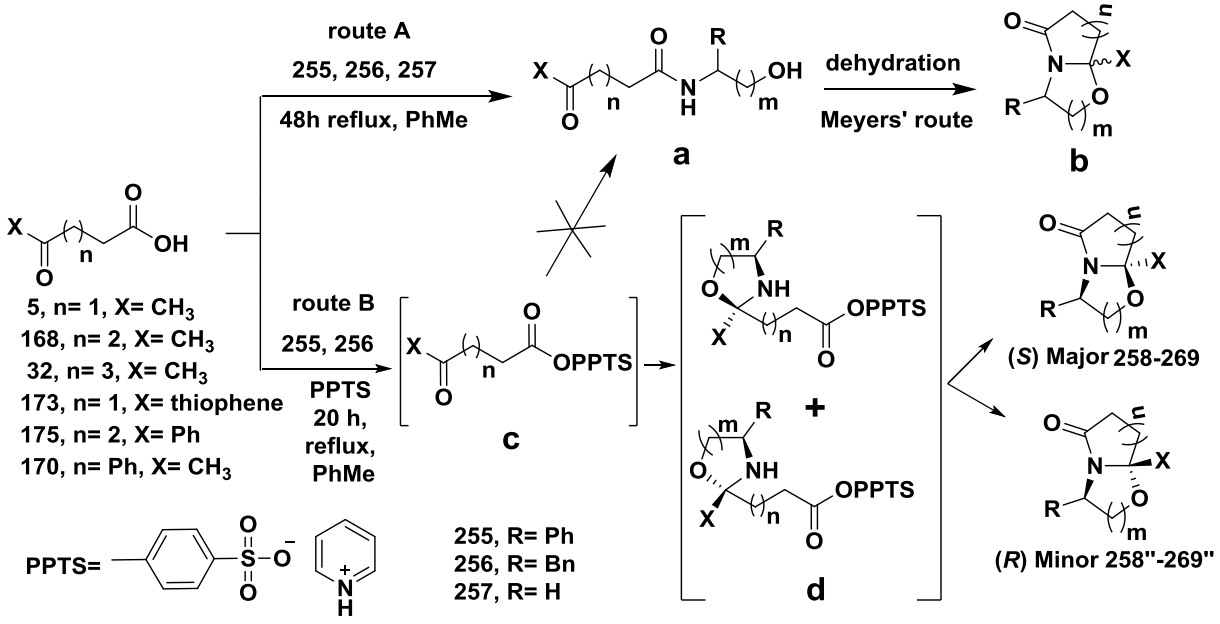


Figure 17: Shows alkaloid production from bicyclic amides.

Meyers' methodology has proven to be useful in creating chiral quaternary centres of bicyclic lactams with the stereoselective constructions. The Meyers' reaction consists of cyclodehydration of chiral or non-chiral amino alcohols and keto acids by refluxing in toluene for 48 h with azeotropic removal of water (Dean Stark). Apart from the conditions of these reactions that are often incompatible with high functionalization of constructions, the serious limitations of this methodology include a mixture of two or more stereoisomers being formed, long time and moderate yields etc. These limitations should be taken into account when searching for other and more desirable reactions. As an efficient route to stereochemical control in Meyers' approach; a route developed in this project involves an activation of the keto acids by means of pyridinium *p*-toluenesulfonate (PPTS) as a catalyst yielding higher diastereoselectivity of bicyclic in good yield after 20 h (Scheme **54**).



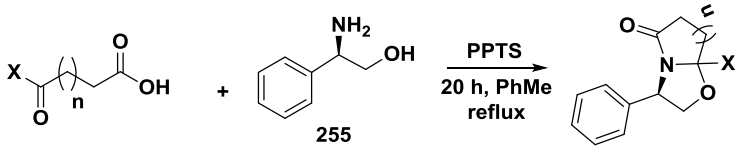
Scheme 54: Synthesis of bi- and tri-cyclic amides derivatives.

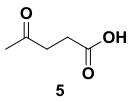
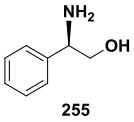
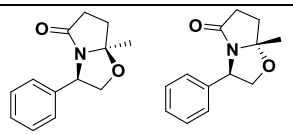
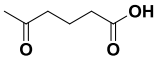
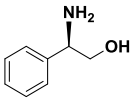
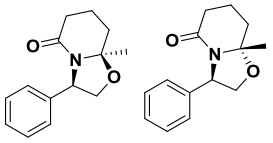
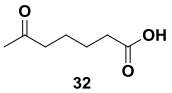
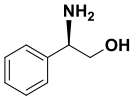
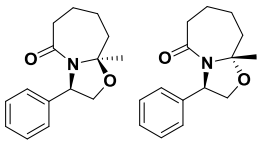
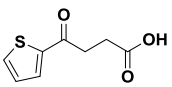
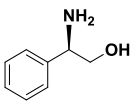
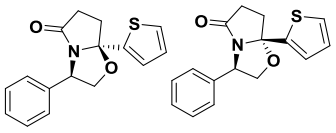
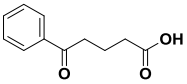
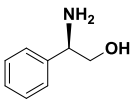
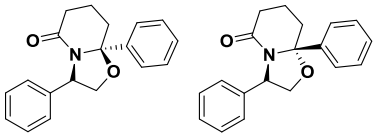


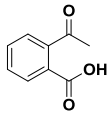
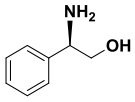
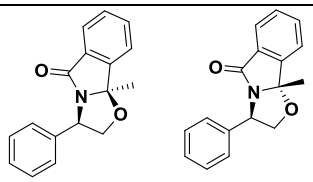
Scheme 55: The plausible mechanism of catalytic methodology **PPTS**.

The proposed mechanism (Scheme **55**) shows comparison between the previous cyclization (Meyer) conditions route **A** and route **B**. The route **B** has demonstrated to be efficient and superior for yielding the desired bicyclic amides with excellent stereoselectivity. This is due to the ketoacids which reacted with PPTS in toluene at reflux to afford the corresponding activated ester **c**. In this case, no trace of the expected intermediate *β*-ketoamide-alcohol **a** in the crude reaction mixture, but the bicyclic amides **258-269** were further chromatographed and found as a single diastereoisomer in high yield. The activated ester **c** reacts with aminoalcohlols **255** and **256** to afford the oxazolidine intermediate **d** faster rather than the expected *β*-ketoamide-alcohol **a**. Two types of aminoalcohols (aromatic and aliphatic) were subjected to lactamization in toluene under optimized conditions. It was found that the use of PPTS reagent gave comparable results to those obtained under Meyers' conditions with good yields and highly stereoselective manner to axially chiral bi- and tri-cyclic amides (Table **2.2** and table **2.3**).

Table 2.2: Formation of various bi- and tri-cyclic amides from aromatic amino alcohol **255**.

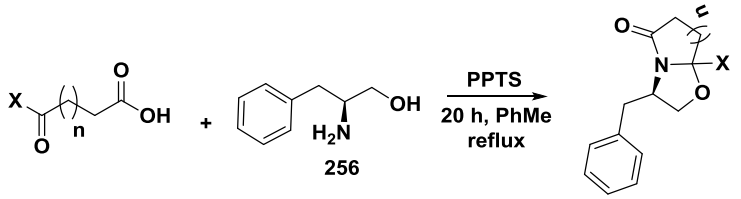


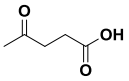
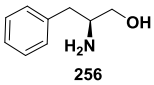
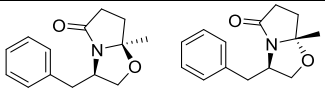
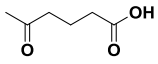
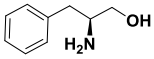
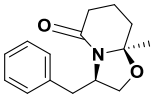
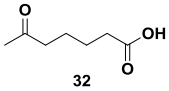
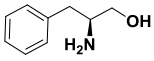
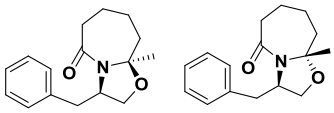
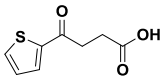
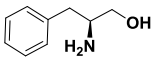
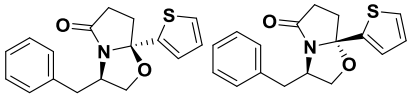
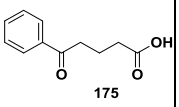
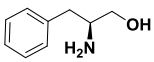
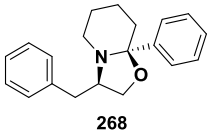
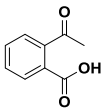
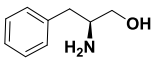
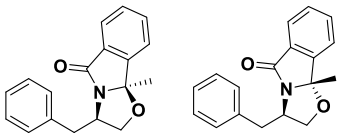
Entry	Ketoacids	Amino alcohol	Bicyclic lactams	de (%)[a]	Yield (%)
1	 **5**	 **255**	 **258 / 258"**	96	86
2	 **168**	 **255**	 **259 / 259"**	92	78
3	 **32**	 **255**	 **260 / 260"**	88	70
4	 **173**	 **255**	 **261 / 261"**	78	60
5	 **175**	 **255**	 **262 / 262"**	98	72

6	 170	 255	 263 / 263"	96	92

de (%)[a] were measured by HPLC

Table 2.3: Formation of various bi- and tri-cyclic amides from aromatic amino alcohol **256**.



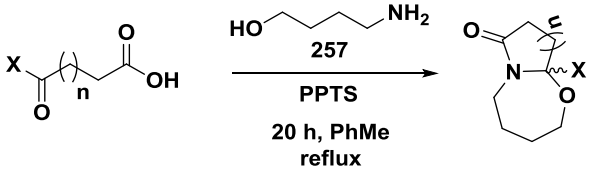
Entry	Ketoacids	Amino alcohol	Bicyclic lactams	de (%)[a]	Yield (%)
1	 5	 256	 264 / 264"	76	90
2	 168	 256	 265	100	80
3	 32	 256	 266 / 266"	92	65
4	 173	 256	 267 / 267"	82	61
5	 175	 256	 268	100	91
6	 170	 256	 269 / 269"	94	92

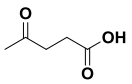
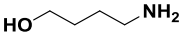
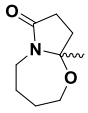
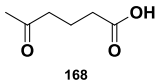
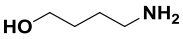
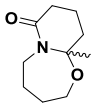
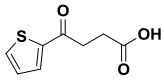
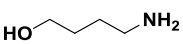
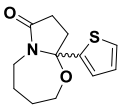
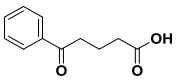
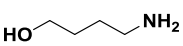
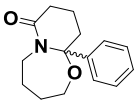
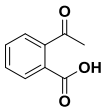
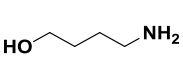
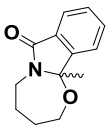
de (%)[a] were measured by HPLC

Table **2.2** and table **2.3** show that amino alcohol **255** and **256** were reacted with different ketoacids in the presence of one equivalent of PPTS in toluene under reflux for 20 h yielding corresponding bicyclic amides. The bicyclic amides derived from amino alcohols **255** and **256** were obtained with good yields (65-92%) as mixtures of diastereoisomers (Table **2.2**, and table **2.3** (except entries **2** and **5**)). The diastereoisomeric excess (de) is around (76-98%) and depends on functional group **X**. It was revealed that no equilibration occurs between

diastereoisomers which were isolated by chromatographic purification. Interestingly, NMR and HPLC studies confirmed that only one diastereoisomer excess (de. superior to 100%) of bicyclic amides (Table **2**.**3**, entries **2** and **5**) were obtained. The bicyclic amides (Table **2**.**2**, entry **4** and table **2**.**3**, entry **4**) were collected in medium yields due to low solubility of keto acid **173** in toluene. It was noted that 5,7-bicyclic amides (Table **2**.**2**, entry **3**) which derived from amino alcohol **255** the protons of benzylic group in the major amide diastereomers **260** appeared downfield (7.30 (0.04 ppm) from that for the minor lactam **260"** (7.19 (0.02 ppm). This distinctive behaviour was also observed with regard to tricyclic amides (Table **2**. **2**, entry **6** and table **2**. **3**, entry **6**) which were derived from amino alcohol **255**, **256** and keto acid **170** (*syn*-alkyl, 5.38 ppm, *anti*-alkyl, 4.96 ppm). It was stereochemically confirmed to be 4:1 favouring the *syn*-alkyl.[115] Therefore, the majority of constructions of **263** and **269** must have the (*S*)-configuration.

Table 2.**4**: Formation of various bi- and tri-cyclic amides from aliphatic amino alcohol **257**.



Entry	Ketoacids	Amino alcohol	Bicyclic lactams	Yield (%)	ee(%)
1	 5	 257	 270	65	0
2	 168	 257	 271	60	0
3	 173	 257	 272	40	0
4	 175	 257	 273	45	0
5	 170	 257	 274	70	0

ee (%) were confirmed by HPLC

On the other hand, the bicyclic amides **270**-**274** (Table **2**. **4**, entries **1**-**5**) were expected to be enantiomers by catalytic methodology. The enantiomers could not be produced presumably due to intermolecular reactions and the major products were racemic (Figure **18**, **A**). Therefore, the lowest conversions were noted for 4-amino butanol **257** due to the construction of an amide as a side product as shown in (Figure **18**, **B**).

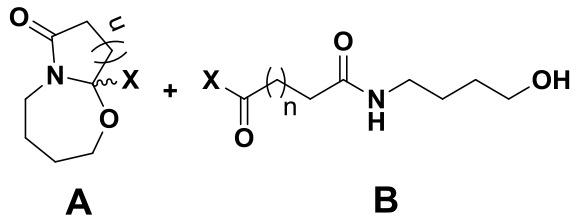


Figure 18: The expected non-cyclic amide byproduct and bicyclic lactams.

The poorer yield and racemic products were observed for bicyclic amides (Table **2**. **4**, entries **1**-**5**) due to 7-membered rings are entropically disfavoured as the bicyclic compounds [112] which have fewer degrees of conformational freedom leading to the presence of non-cyclic amide byproduct. X-ray analysis of bicyclic lactams **262**, **273** and **274** confirmed the correct structures as illustrated in **Figure 19**.

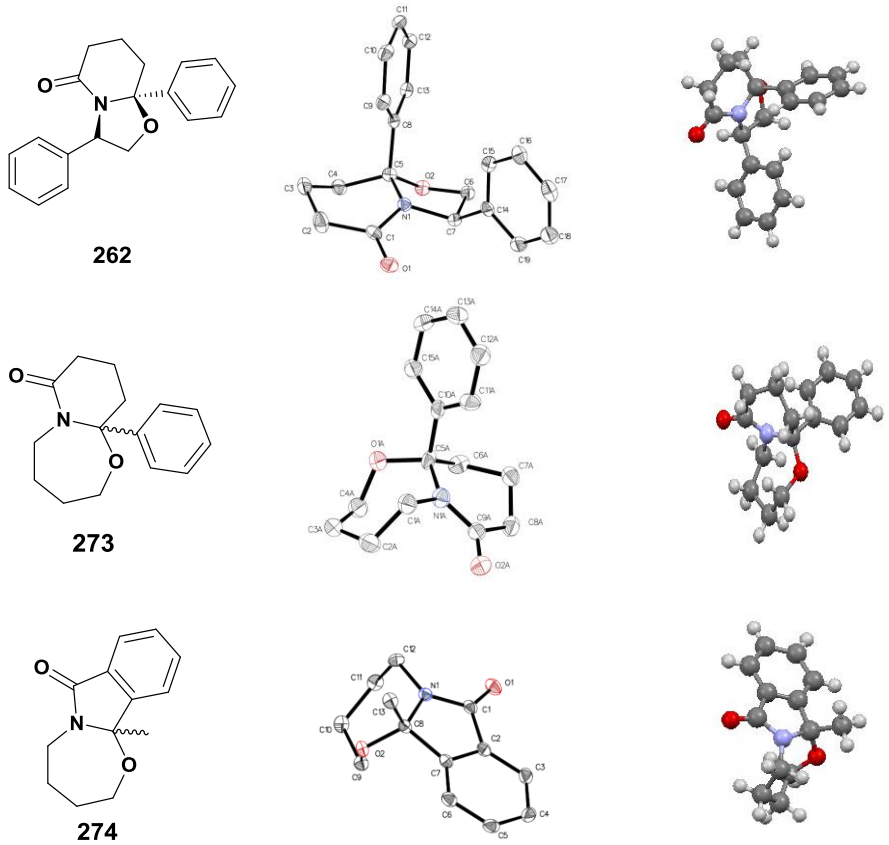


Figure 19: X-ray of crystal structure of bi- and tricyclic amides.

Chapter 3. Synthesis of Ugi-tetrazole derivatives and using flow chemistry

3.1. Flow chemistry

Flow chemistry has become an attractive methodology in organic synthesis with an increase publications in the past two decades since 1998.[116] It was reported that about 50% organic reactions could be conducted using continuous flow chemistry. Reactions can be performed using a network of interconnecting channels where solutions of starting materials and the reagents can meet and react to form products. Continuous flow reactions have several advantages including efficient mixing, precise control of physiochemical parameters such as residence time, temperature, pressure and heat transfer etc. Therefore the accumulation of un-reacted reagents, reactive intermediates or side products which can occur in batch reactions can be minimised. Selectivity and yield of reactions can also be improved.[117]

The scales of flow chemistry are classified by the diameter of the reaction channels; microfluidic (10 μm to 500 μm) or mesofluidic (500 μm to 5 mm). The range in diameter of a microfluidic system is sufficient to allow compounds synthesised at a rate of over 10 milligram/hour while a mesofluidic system is suitable for 100 gram/hour scale synthesis. Although a mesofluidic system has less efficient mixing and less efficient heat transfer, this machine can offer improvement of flow capacity and fewer tendencies to blockages. A microfluidic system contains smaller size, it offers higher surface-to-volume ratios and more efficient mixing have higher reaction yield and product purity. However this system suffers from low flow capacity and a higher prone to blockages.[118]

For my thesis, a mesofluidic system was used for improving yields of some Ugi-tetrazole derivatives, therefore my work will focus on this type of system.

3.1.1. Advantages of flow chemistry

Flow chemistry has several important advantages: the flow reactor contains tubing with a range of diameters between 500 μm and 5 mm, therefore the mixing of starting materials becomes very fast. A predictable mixing would be described as combination two or more reagents to result one uniform material. The mixing in a closed channel occurs *via* two mechanisms: laminar and turbulent flow. Laminar flow represents that fluids flow in parallel manner with no disruption between the layers therefore the mixing occurs through diffusion. Turbulent flow represents the fluids flow in a chaotic manner, causing vortexes of swirling fluids with large surface area where mixing occurs through diffusion (Figur**e 20**).

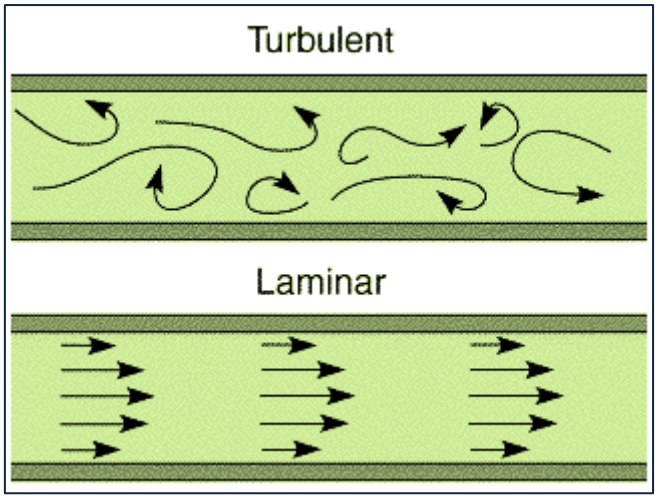
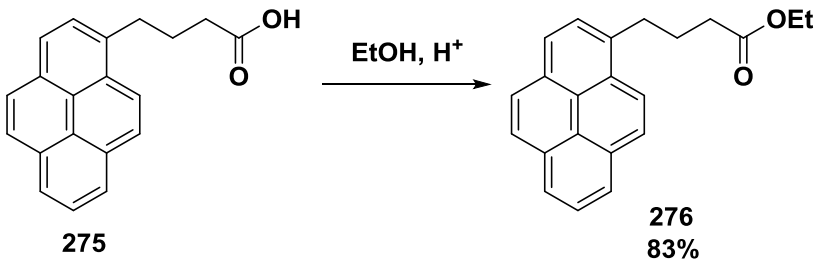


Figure 20: Laminar and turbulent manners for fluids flow.[119]

The flow machine has a thermal control system when the contents of the reaction are heated *via* conduction or convection. Verboom *et al*. reported the yield of esterification reaction of 9-pyrenebutyric acid **275** with ethanol to afford pyrenebutyric acid ethyl ester **276** [120] (Scheme **56**).



Scheme 56: Esterification reaction of 9-pyrenebutyric acid **275** catalysed by acid.[120]

A residence time for producing 83% of ester **276** was 40 minutes under flow machine at 50 ºC, while only 15% of the same ester was produced under batch reaction at the same time.

The ratio of flow rate to capacity of the channels tubes represents the residence time of the solution passing in a flow reactor according to the following equation:

$$\boldsymbol{Residence\ time\ (min) = Channel\ capacity\ (mL) / Flow\ rate\ (mL/min)}$$

Increasing the flow rate and the length of the channels tubes affords the scalability of flow rate yields. Therefore, a 10 mL reactor and a 20 mL reactor operating at a flow rate of 1 mL/min and 2 mL/min respectively will give the same residence time, the same residence

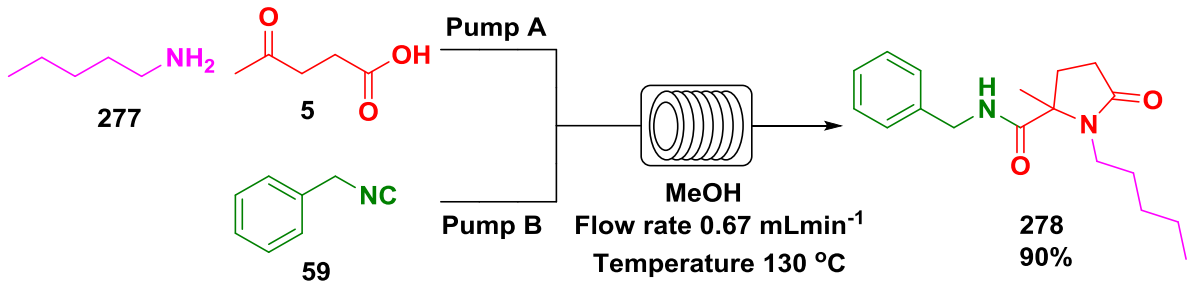
time can be achieved by varying accordingly time. The flow reactor used in this project is a Vapourtec E-series flow reactor as shown in Figure **21**.

Figure 21: Vapourtec E-series flow system is equipped with three pumps.

The tube reactor is made from fluoropolymer PTFE for a typical 10 mL reactor. Therefore the flow reactors has a tremendous surface-area-to-volume ratio in PTFE tubing which enables a rapid heat transfer and precise control of reaction temperature compared with conventional batch reactors.[121] These tubes are coded by blue and red, which are also chemically inert under most synthetic conditions. It is particularly suitable for the reaction that is rapid and highly exo- or endothermic because of effective mixing and heat exchange. [122] Furthermore, the flow rate of reaction can be adjusted to accurately control the residence time. The optimized conditions for a given reaction can be rapidly determined by changing parameters such as flow rate, temperature and reactor size, followed by monitoring the reaction outcome by TLC, NMR and Mass spectrometry.[123]

3.1.2. **Multicomponent reactions in flow chemistry**

Flow chemistry has been used by the Chen group as efficient methodology for the synthesis of compounds using isocyanide-based multicomponent reaction.[124] A cyclic amide **278** was synthesised by a previous member of the group in yield 48% using batch method. The yield of this product was increased to 90% using flow chemistry (Scheme **57**).



Scheme 57: Synthesis of cyclic amide **278** by flow chemistry.

The Vapourtec R-series machine was configured with a 10 mL reactor and a 100 psi back-pressure regulator. A pump **A** was charged with 1.3 M 4-oxopentanoic acid **5** and 1.3 M pentylamine **277** in methanol. A pump **B** was charged with 1.1 M benzyl isocyanide **59** in methanol and the reaction was then performed at different reactor temperatures with different flow rates. The solutions of reagents combined at a T-piece, passed through the 10 mL flow reactor and heated to 130 ^{o}C with flow rate of 0.67 mL/min (a residence time 15 minutes) led to a 90% yield of product **278**.

3.2. Tetrazoles derivatives

The tetrazoles are important synthetic substructure for a wide range in a medicinal and organic chemistry. Some medicinally active compounds contain tetrazole substructure have been synthesized in multicomponent reactions. Tetrazole **279** was reported as an antagonist (GPCR) of melanin concentrating hormone **1** (MCH1) which is an important drug target for obesity diseases.[125] *β*-Carboline-tetrazole **280** has been tested as an active anti-leishmanial compound.[126] Tetrazolyl oxime **281** has been screened as effective activity as antifungal.[127] Compound **282** has been prepared by Ugi reaction as a selective and potent agonist for a new remedy of endometriosis and recently has been used as a reagent for P450 inhibition in an *in vivo* pharmacological tests.[128] Protease inhibitors **283**, **284** and **285** have been synthesised based *via* Ugi reaction comprise hydroxymethyl-tetrazoles core[129] (Figure **22**).

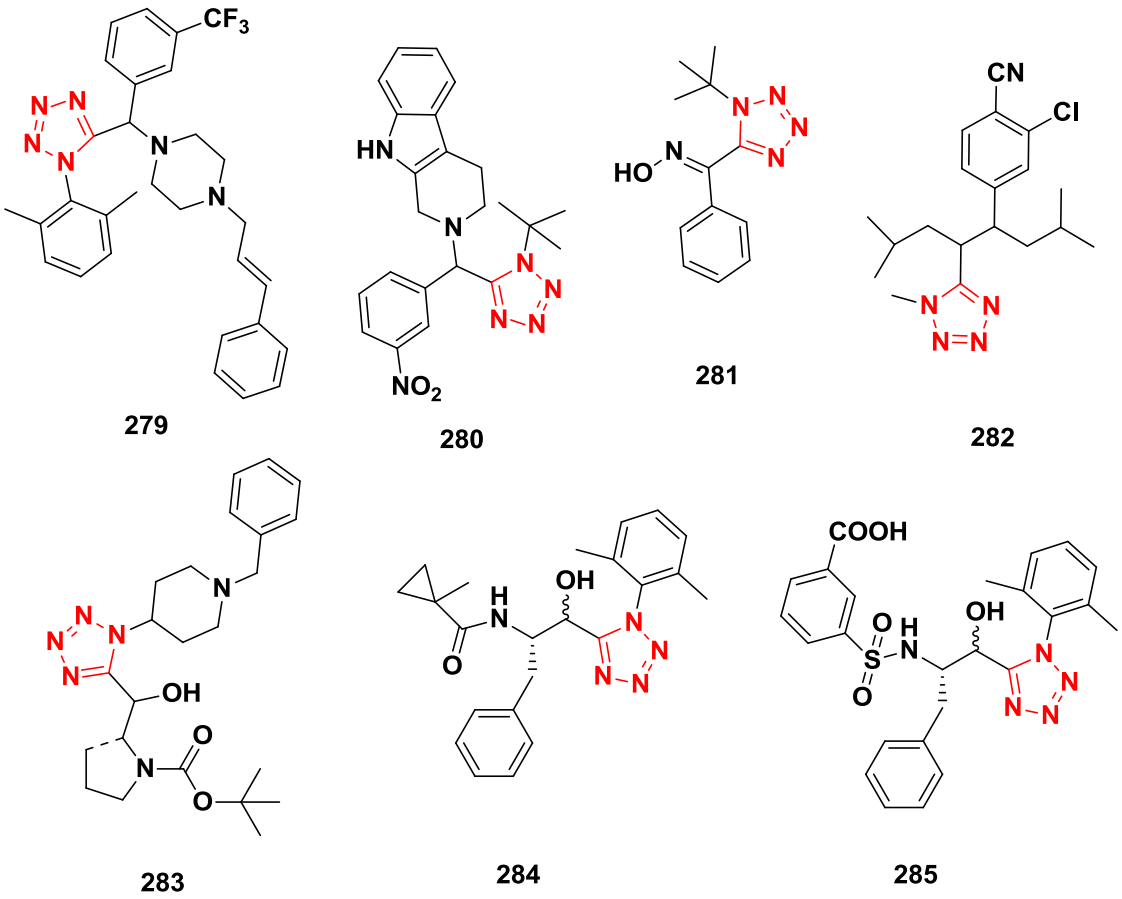


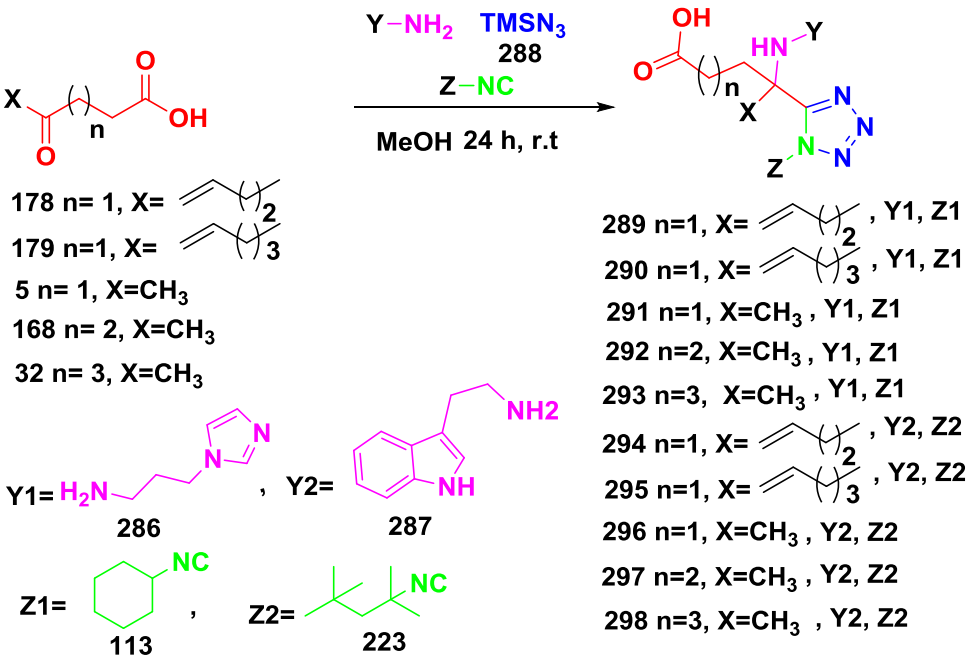
Figure 22: Some pharmacological compounds include tetrazoles substructure.

3.2.1. Synthesis of tetrazoles derivatives

Two approaches have commonly been used in the synthesis tetrazoles. Firstly, an addition of an azide source to a nitrile and secondly an azide source is added to an amide *via* the reaction with its imidoyl derivative.[130] However, these approaches have several limitations including multistep synthesis of starting materials and large amount of chemical waste. In this aspect, the use of trimethylsilyl azide ($TMSN_3$) in the Ugi multicomponent reaction is a valuable because it is an efficient method to overcome these limitations.

Continuous flow chemistry has found applications in organic synthesis with distinctive benefits of being an efficient, green, selective and improved productivity in comparison with traditional batch approaches.[131] It has been successfully utilized to synthesise a wide range of heterocyclic compounds.[132] However, this methodology has never been reported for the synthesis of 1,5-disubstituted tetrazole compounds. In this project, we firstly want to address the issue of the synthesis of Ugi-tetrazoles in batch. The methodology of the synthesis of 1,5-disubstituted tetrazoles moiety using on the Ugi-azide multicomponent reaction is outlined in

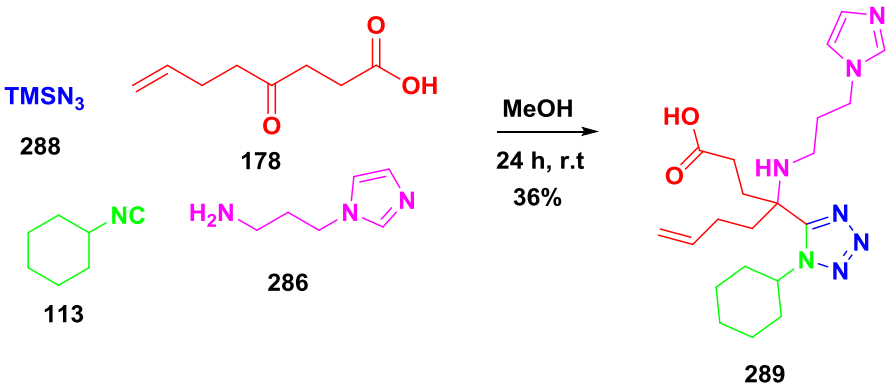
Scheme **58**. This reaction includes four components, namely keto acids as bifunctional reagents, primary amines, isocyanides and trimethylsiliyl azide. Then, measures for improving productivity of the Ugi-tetrazoles reactions will be explored and optimized using continuous flow chemistry. This study also revealed that the scope of Ugi-tetrazoles products can be expanded to five, six and seven membered-rings, furnishing unprecedented γ-, δ- and ε-lactam tetrazoles (Chapter **6**).



Scheme 58: Synthetic approach of Ugi-tetrazole derivatives.

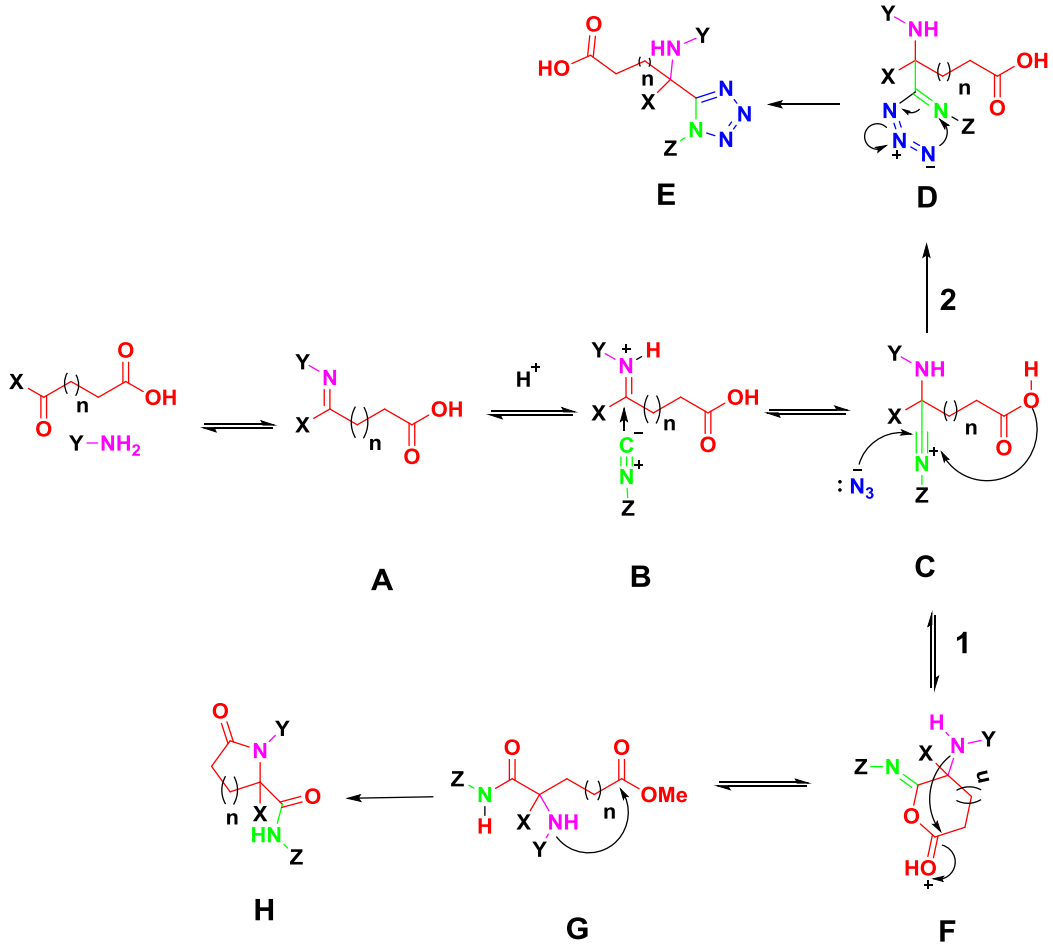
3.2.1.1. Batch approach

The tetrazoles derivatives are synthesised from the combination of the keto acids component amines, isocyanides and trimethylsilyl azide in Ugi reaction to afford the Ugi-tetrazole products. Interestingly, our efforts were firstly focused on the synthesis of Ugi-tetrazole **289** as a model reaction. The reactants were stirred in methanol for 24 h at room temperature, the increasing the reaction temperature over room temperature for long time is not preferable due to the decomposition of isocyanide component at high temperature. Ugi-tetrazole **289** was successfully isolated in 36% yield after purification by column chromatography (Scheme **59**).



Scheme 59: Synthesis of Ugi-tetrazole **289** in a bath reaction.

The mechanism of reaction is outlined in scheme **60**. Imine **B** was afforded *via* condensation of a keto-acid and a primary amine. The intermediate nitrilium ion **C** was formed by addition isocyanides to the protonation of imine. The ultimate intramolecular Ugi reactions *via* path **1** can afford cyclic amide **H** after Mumm rearrangement. It was proposed that small and highly nucleophilic azide ion can intercept **C** (path **2**) which prefers to intramolecular ring closure with the free carboxylic acid to furnish **E**.



Scheme 60: A postulated mechanism of Ugi-tetrazole condensation.

The competition occurs between path **1** and path **2** in one-pot reaction in batch as illustrated in Scheme **60** can potentially produce a mixture of products. Subsequently, the yield of desired product was poor. Therefore, due to this low yield, we turned our attention to finding an efficient and method that can be utilized to improve productivity of Ugi-tetrazoles.

3.2.1.2. Optimisation of conditions using flow reactor

In model reaction, Ugi-tetrazole **289** was synthesised in a batch reaction of 24 hours from 4-oxooct-7-enoic acid **178**, amine **286**, cyclohexylisocyanide **113** and $TMSN_3$ **288**. The product was isolated and purified as yellow oil in 36% yield (Scheme **59**). Known concentrations of **289** were analysed by HPLC (Waters Xbridge a C18 5 μm column (4.6 mm × 250 mm); 5−95% MeCN/ aqueous TFA (0.1%) over 20 mins, hold 10 mins; UV detection at 215 nm) and a calibration curve was constructed (Figure **23**).

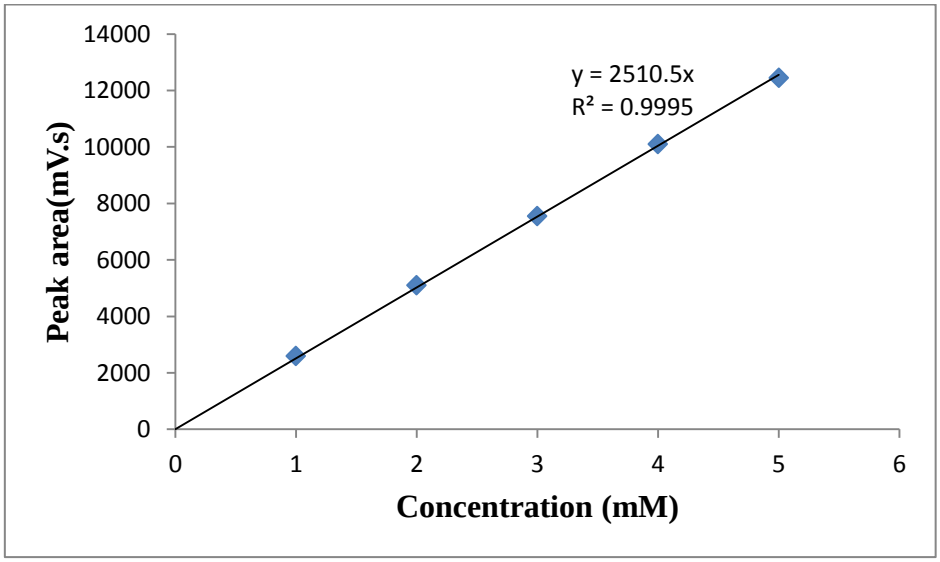


Figure 23: The curve of calibration (concentration vs area) for **289** analysed by HPLC.

Ugi-tetrazole **289** was then repeatedly synthesised using a continuous flow reactor. The flow reactor was configured according to Figure **24**. A back-pressure regulator was fitted to allow that the reaction temperature exceed to the boiling point of solvents used can be achieved. A pump **A** was charged with 1M 4-oxooct-7-enoic acid **178**, 1M 1-(3-aminopropyl) imidazole **286** and 1M cyclohexylisocyanide **113** in methanol, and input to pump **B** was charged with 1 M $TMSN_3$ **288** in methanol. The reaction was then optimised by ranging temperatures and flow rates (Table **3.1**). The mixture of reaction was then collected and purified by column chromatography. The isolated yield is recorded in Table **3.1**.

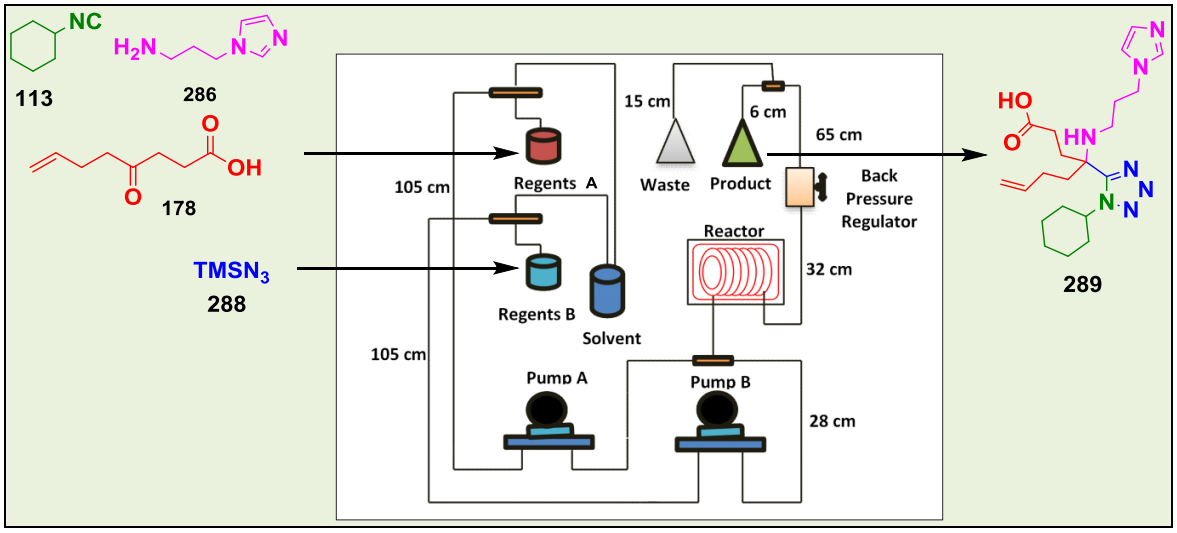
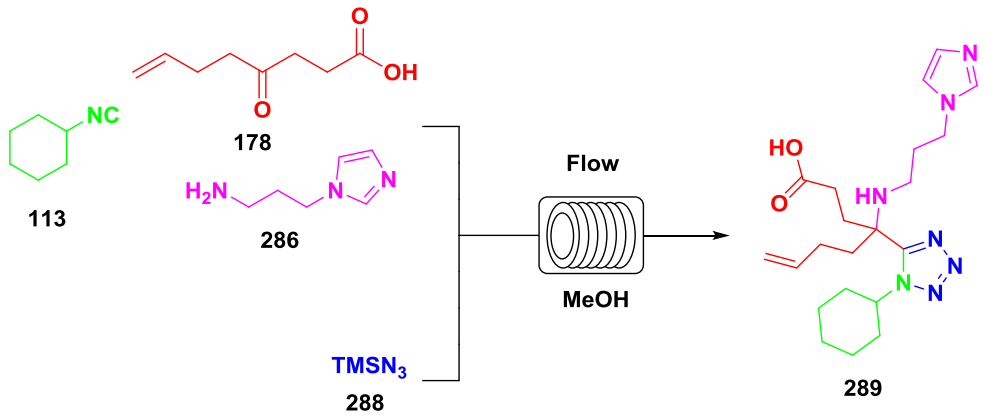


Figure 24: Diagram shows continuous flow equipment for synthesis of Ugi-tetrazole **289**.

Table 3.1. The optimized parameters of the synthesis of Ugi-tetrazole **289**.



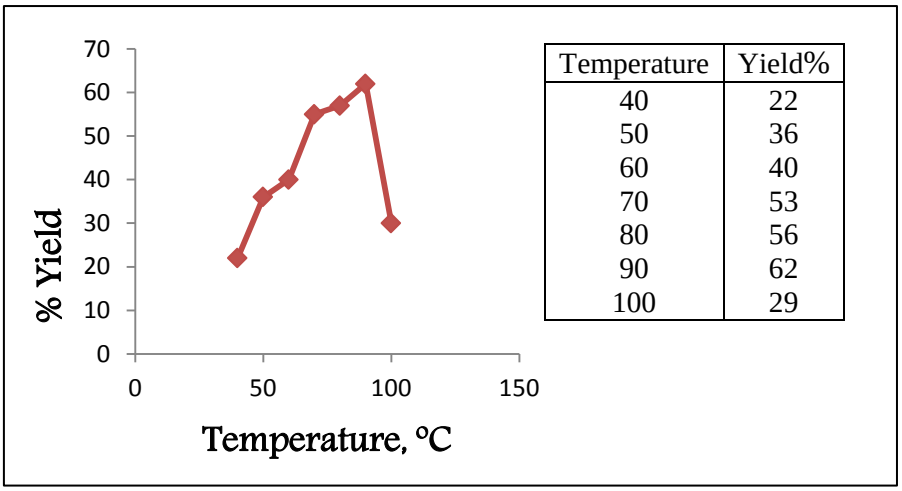
Entry	Residence time [min]	Reactor temperature	yield [%][a]
1	5	40	-
2	10	50	-
3	10	60	15
4	10	70	22
5	10	80	32
6	10	90	38
7	15	90	41
8	20	90	62
9	25	90	48
10	25	100	27

[a] Calculated by column chromatography

The table **3.1** shows that an increase in temperature increases the conversion of **289** until 100 ºC. The methanol (b.p 64 ºC) solvent boils at temperature above 100 ºC under the reaction conditions causing irregular flow rates due to the formation of solvent bubbles. A back pressure regulator of 300 psi was therefore applied to allow overheating methanol without boiling up to 100 ºC. However, the reactor started to encounter when it was heated above 100

°C. Therefore, temperatures above 100 °C not applied. Increasing the residence time at 90 °C led to a conversion of 62 % at 20 minutes residence time (Table **3.1**, entry **8**). Further increases in residence time (Table **3.1**, entry **9**) with maintaining temperature at 90 °C afforded lower conversion of (48%).

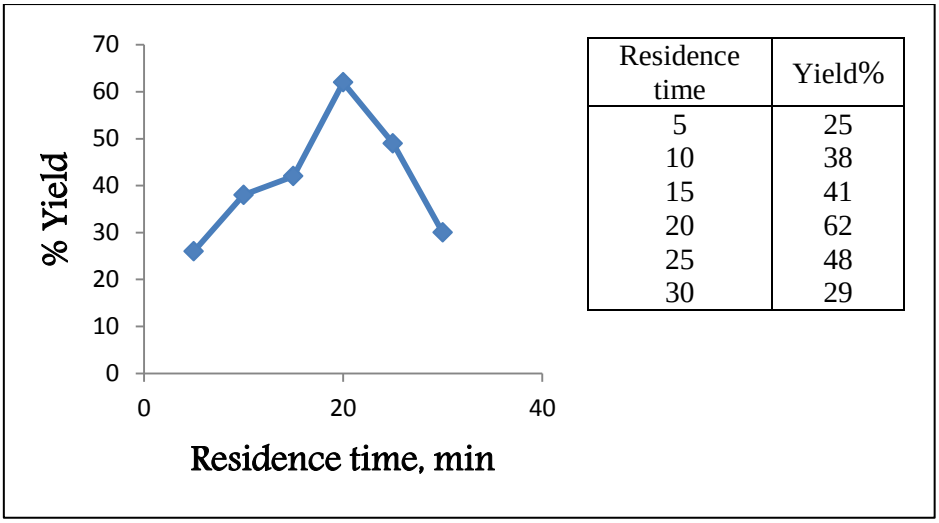
The synthesis of Ugi-tetrazole **289** can be performed under flow chemistry varies 15% to 62% yield was obtained. It can be seen that both temperature and the residence time affect the reaction yield. The effect of temperature upon reaction yield can be illustrated in Figure **25**. When the reaction temperature was increased at 10 °C interval between 40-100 °C, the yield increased from 22 up to 62% within 20 minutes (residence time). At a reaction temperature of 90 °C, the reaction produced highest yield. When reaction temperature was increased to 100 °C, the yield dropped. Obviously, It was noted that the effect of reaction temperature is less evident from 60 °C to 70 °C than that of 70 °C to 90 °C. This is probably due to the fact that isocyanides were decomposed after 90 °C or the flow system became unstable which could affect the reaction.



Temperature	Yield%
40	22
50	36
60	40
70	53
80	56
90	62
100	29

Figure 25: The outcome for model reaction (Ugi-tetrazole **289**) under flow conditions by varying temperature (residence time was mantained 20 minutes).

The residence time of model reaction was further optimised by carrying reaction at 90°C. As it can be seen from Figure **26**, the reaction was generally completed within 20 minutes at 90°C. Increasing the residence time longer than 20 minutes causes a decrease in reaction yield.

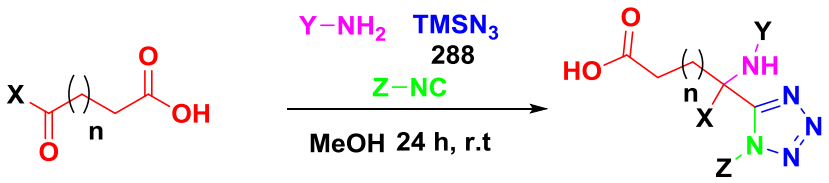


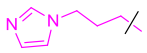
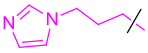
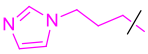
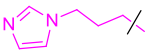
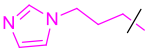
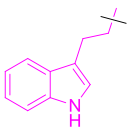
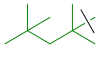
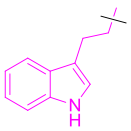
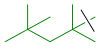
Residence time	Yield%
5	25
10	38
15	41
20	62
25	48
30	29

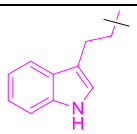
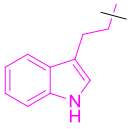
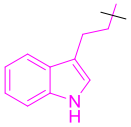
Figure 26. The outcome for model reaction (Ugi-tetrazole **289**) under flow conditions by varying residence time (temperature was mantained at 90 ºC).

The optimized conditions (residence time 20 minutes and temperature at 90 ºC) were used to synthesise of Ugi-tetrazoles compounds **289-298** by continuous flow. The products and yields from batch and flow reactions are illustrated in Table **3.2**.

Table 3.2: Comparison between batch reaction and flow chemistry to improve productivity of Ugi-tetrazoles.



Product	n	X	Y	Z	Flow[%][a]	Batch[%][b]
289	2				62	36
290	2				58	30
291	2	CH_3			77	42
292	3	CH_3			66	36
293	4	CH_3			45	20
294	2				67	25
295	2				53	22

296	2	CH_3			70	39
297	3	CH_3			60	44
298	4	CH_3			42	28

a, b- Calculated by column, Residence time 20 min, Temperature reaction 90 ºC

The table **3.2** shows a library of Ugi-tetrazoles which were synthesised from different ketoacids, primary amines, isocyanides and $TMSN_3$ using batch and flow reactor. The Ugi-tetrazoles (**289**-**293**) from ketoacids, cyclohexylisocyanide **113** and imidazole amine **286** were obtained in high yield than Ugi-tetrazoles (**294**-**298**) which derived from same ketoacids, 2-isocyano-2,4,4-trimethylpentane **223** and indole amine **287** in both methodologies. Moreover, it was noted that the yields of Ugi-tetrazoles (**289**-**298**) affected by the nature of X, Y and Z. In addition to much shortened reaction time (20 minutes using flow, 24 h in batch), it can be seen from table **3.2** that the reaction conversion using continuous flow method is much higher than the batch reaction.

Chapter 4. Synthesis of pyrazole-oxopyrrolidine derivatives using microwave techniques

4.1. Microwave technique

Microwave (MW) irradiation is giving an increasing popularity in organic synthesis. Microwave irradiation utilises radiation between infrared and radio waves to activate a reaction and its working wavelengths is in a range of 1mm-1m which corresponds to frequencies range between 0.3 and 300 GHz.[133] Although MW irradiation has been firstly used in the mid-1980s, a wider application and reliable MW devices in organic synthesis only started from 2003.[134] The annual number of publications documenting the MW technique in heterocyclic increases sharply since 2003 as shown in Figure **27**.

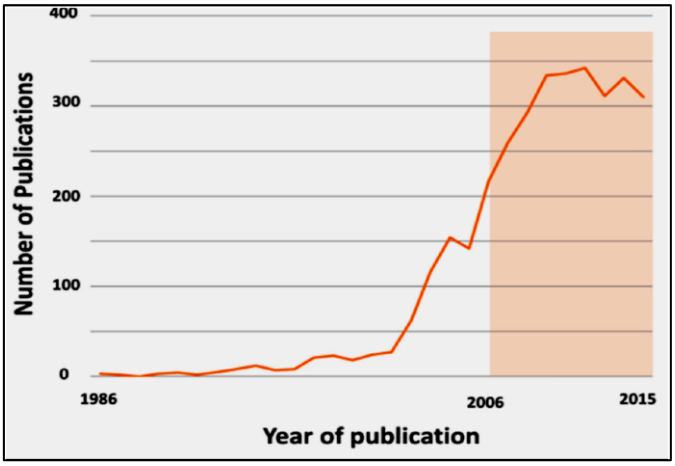


Figure 27: Number of publications of MW irradiation in the synthesis of heterocycles.[135]

4.2. Principles of microwave technique

Generally, the irradiation of microwave (MW) operates by two main principles: the dipolar polarisation and conduction. A dipole moment is generated by heat under MW after polarisation to provide irradiation. The dipole moment is sensitive toward external electric fields and to provide it's align under rotation of MW irradiation (Figure **28**).

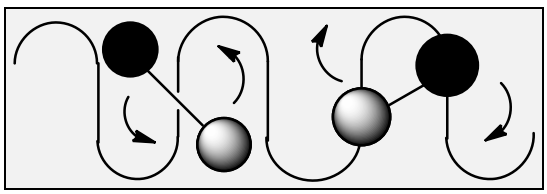


Figure 28: Alignment of dipolar moment in MW irradiation.[136]

The reaction is mostly run in solvent or solvent free array, the alignment of dipolar substances is usually prohibited by the presence of other substances. The dipolar components

are able to align themselves in solvent based on the frequency of irradiation and viscosity of solvents. On the one hand, if the frequency of irradiation is lower; the substances can rotate and acquire some energy from rotation. However, the overall effective heat is too small. If the frequency of irradiation is higher; the components will not have time to response to the oscillating field. Therefore, the dipolar substances can align in the MW irradiation field, which generate different phase between orientation of the MW irradiation field and the dipole. Hence, heating is produced by the collision and fraction of the dipolar compounds.[133]

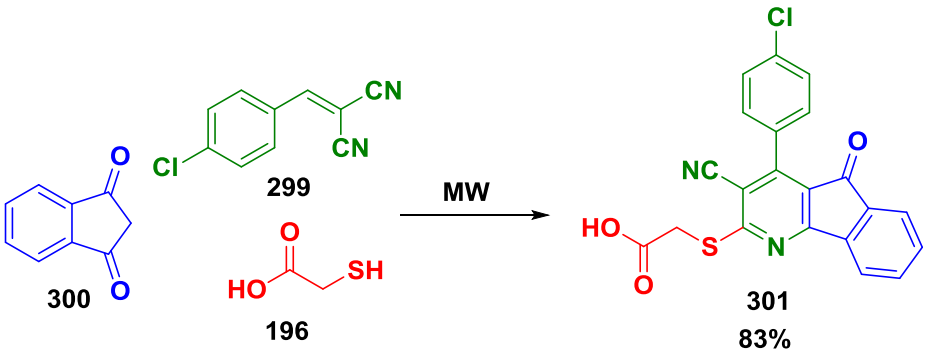
4.3. Advantages of microwave irradiation

MW irradiation not only permits a good transmission of the energy to the reactants but also gives a homogeneous heating to reaction mixtures. In addition, the MW irradiation generates an electric field which causes rotations and oscillations of dipolar and ionic molecules to result in additional local heating.[137] Some reactions can be run in solvent-free conditions, for example heterogeneous reactions are environmentally friendly and give improved selectively, shortened reaction times, raised reaction rates, cleaner products and reduced side-products.[138],[139] MW irradiation in the synthesis of diverse heterocyclic compounds has been extensively reported in the last years as outlined in Figure **27**.

4.4. Application of microwave technique in multicomponent reactions

MW irradiation has been extensively implicated in the scope of combinatorial chemistry and multicomponent reactions.[140] The importance of using MW irradiation in multicomponent reactions including that the reactions can be carried out under solvent free conditions which reduce environmental impact and result in greener chemistry. The absorption of energy by solvents is avoided under solvent free conditions which leads to an effective collision between starting materials, therefore increases the product yields. Several multicomponent reactions were reported using solvent free MW irradiation.

Shujiang Tu *et al* introduced a novel three-component reaction in the synthesis of indeno-pyridine derivative **301** using microwave irradiation (MW).[141] Although some methodologies have been used for the synthesis of indeno-pyridine derivative **301** including oxidative thermal rearrangement and extrusion of organophosphorus compound, these methods are not preferable due to harsh reaction conditions and toxic catalyst. Microwave irradiation (MW) was used as an efficient procedure for the synthesis of indeno-pyridine **301** from mercaptoacetic acid **196**, arylidenemalononitrile **299** and 1,3-indanedione **300** (Scheme **61**).



Scheme **61**: The microwave multicomponent synthesis of indeno-pyridine derivative **301**.

4.5. Pyrazole derivatives

The pyrazole moiety has shown antitumor, [142] anti-fungal [143] and antimicrobial drugs.[144] Pyrazolo-pyridine derivatives have demonstrated to be interesting compounds of heterocycles due to various biological properties involving anti-tubercular, antibacterial and antioxidant activities.[145] A series of pyrazolo-pyridine derivatives **302** as kinase (CDK) inhibitors were synthesized and showed to be potent and selective for CDK inhibitory activities as well as anti-proliferative inhibitors *in vitro* of human tumour cells (cellular proliferation). The key component for the synthesis **302** utilized benzimidazole ring which was subsequently undergone six-step including cyclization and arylation reaction *via* Suzuki coupling. Pyrazole-indole **303** substructure has been synthesised from a nucleophilic fluorination reaction using DAST as fluorinating reagent. Microbiological screening for **303** compounds was screened to evaluate antifungal activity and showed better activity against A niger and C albicans (Greseofulvin as reference). The compounds **303** required eight-steps to synthesise starting from spiro[[1,3]dioxolane-2,3'-indolin]-2'-one.[144] Pyridazine-pyrazole derivatives **304** and **305** were formed in multistep using a pyridazine-hydrazine component. These derivatives were screened at concentration of 0.1 g/mL using DMSO (Solvent) and showed as potent antimicrobial activity (Figure **29**).[143]

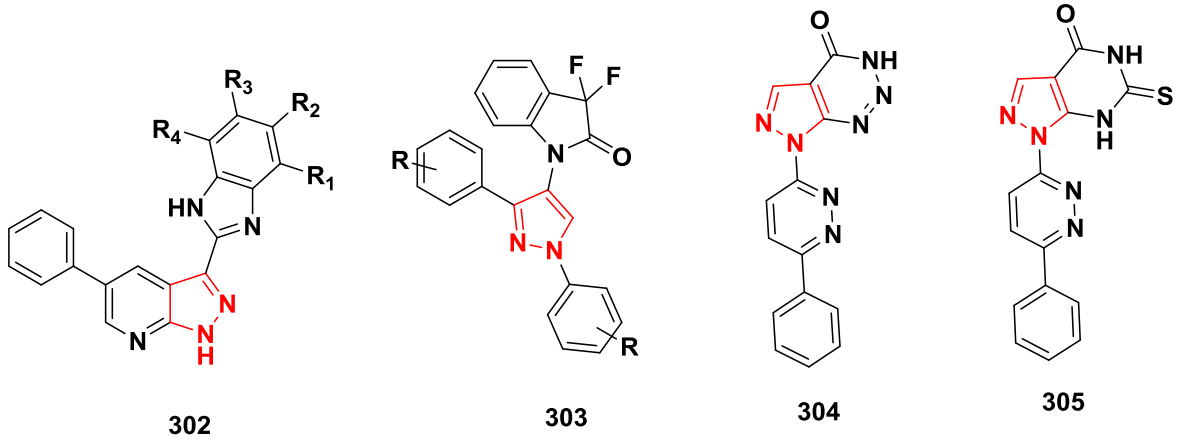
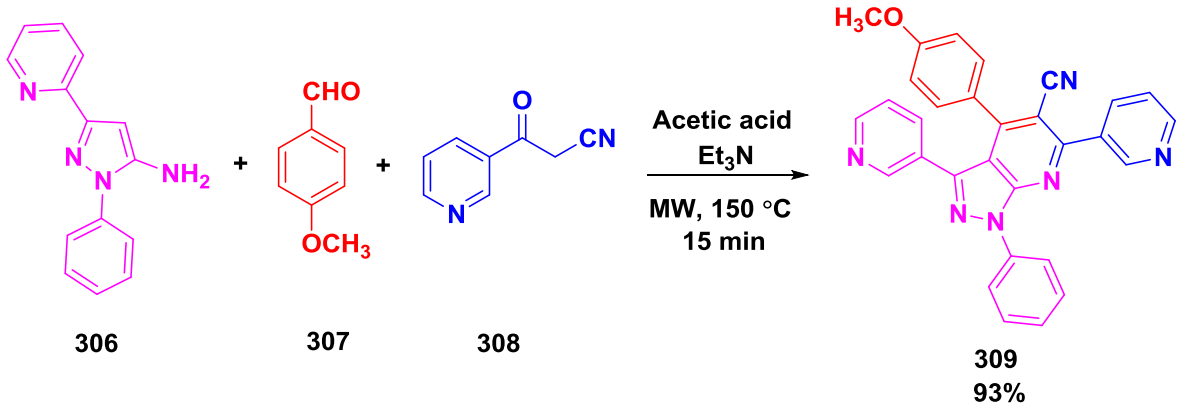


Figure 29: Some pyrazole derivatives possess medicinal properties.

El-borai *et al* reported that pyrazolo-pyridine-carbonitrile derivative **309** could be synthesised *via* multicomponent reactions under MW irradiation (Scheme **62**). This compound **309** has been shown to have anti-tumour activity. [146]

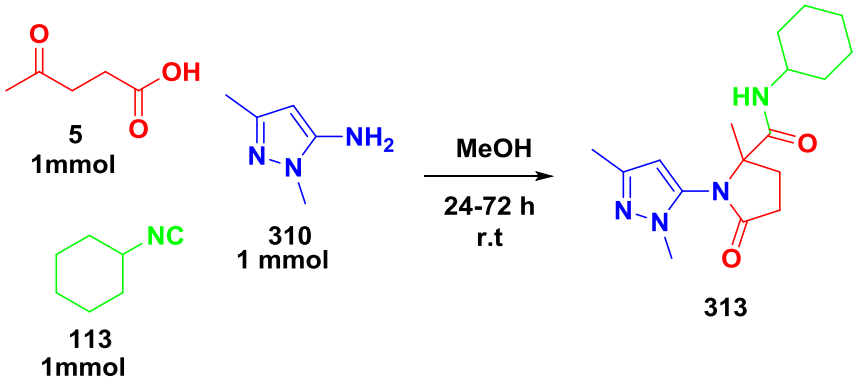


Scheme 62: The microwave multicomponent for the synthesis of **309**.

4.5.1. Synthesis of pyrazole-oxopyrrolidine derivatives

4.5.1.1. Batch method

The pyrazole-oxopyrrolidine derivatives were synthesised from the combination of equal equivalent of the keto acids component, pyrazole amines and cyclohexylisocyanide in Ugi reaction to afford the pyrazol derivatives products. Our efforts were firstly concentrated on the synthesis of pyrazole-oxopyrrolidine derivative **313** as a model reaction. The reactants were stirred in methanol for 24-72 h at room temperature, the increasing the reaction temperature over room temperature for long time is not preferable due to the decomposition of isocyanide component at high temperature. The pyrazole-oxopyrrolidine derivative **313** was isolated in 32% yield after purification by column chromatography (Scheme **63**).



Scheme 63: Batch method for synthesis of pyrazole-oxopyrrolidine derivative **313**.

4.5.1.2. Microwave method

With optimal conditions in hand (Table **4.1**) for the synthesis of pyrazole-oxopyrrolidine **313** (Scheme **64**). MW irradiation was used in multicomponent reactions to synthesise a number of pyrazole-oxopyrrolidine derivatives (Table **4.2**) in reasonable to good yields in short time and solvent free conditions. The same reaction would require 72 h and gave low yield using batch method.

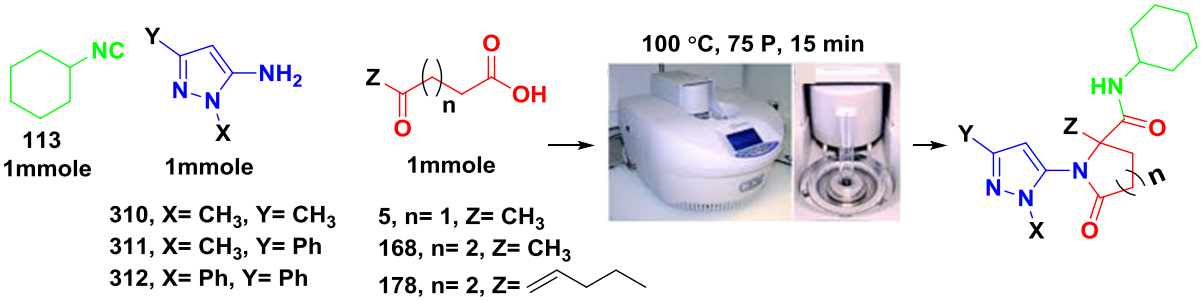
Scheme 64: Synthesis of pyrazole-oxopyrrolidine **313** as model reaction.

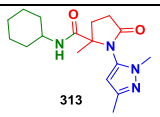
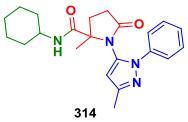
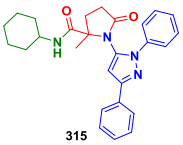
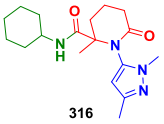
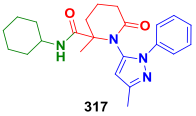
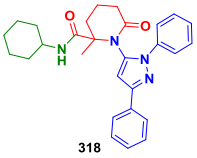
Table 4.1: The optimal conditions for the synthesis of pyrazole-oxopyrrolidine **313**.

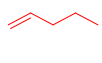
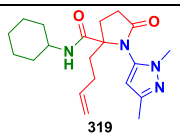
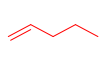
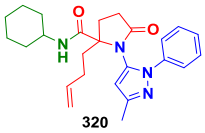
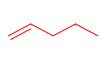
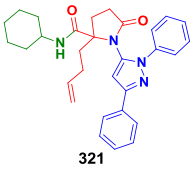
Entry	Method	Solvent	Temperature	Time	yield %
1	Batch	Methanol	rt	24 h	0
2	Batch	Methanol	rt	48 h	0
3	Batch	Methanol	rt	72 h	20
4	Batch	Methanol	60ºC	2 h	-
5	Microwave	-	100, 75 P	5 min	-
6	Microwave	-	100, 75 P	10 min	34
7	Microwave	-	100, 75 P	15 min	72
8	Microwave	-	100, 75 P	20 min	40

The table **4.1** shows that pyrazole-oxopyrrolidine **313** as a model reaction was synthesised under MW irradiation with different conditions. The optimized conditions were investigated for using MW irradiation. It was noted that the 100 ºC, 75 P, at 15 minutes (Table **4.1**, entry **7**) is the best conditions to acquire higher yield of pyrazole-oxopyrrolidine **313**. Therefore the same reaction conditions were used to synthesise the other pyrazole-oxopyrrolidine derivatives **313-321**.

Table 4.2: Synthesis of pyrazole-oxopyrrolidine derivatives **313-321**.



Entry	n	X	Y	Z	Product	yield[a]
1	1	CH_3	CH_3	CH_3	 313	72%
2	1	CH_3		CH_3	 314	65%
3	1			CH_3	 315	55%
4	2	CH_3	CH_3	CH_3	 316	67%
5	2	CH_3		CH_3	 317	50%
6	2			CH_3	 318	60%

7	1	CH_3	CH_3		 319	65%
8	1	CH_3			 320	55%
9	1				 321	45%

[a] yield of isolated product

From the table **4.2**, it can be seen that pyrazole-oxopyrrolidine derivatives **313-321** were synthesised from different keto acids and different amines under MW irradiation. It was observed that the yield of conversion affects with the size ring of oxopyrrolidine and type of amine. Moderated to good yield was noted for five-member ring of pyrazole-oxopyrrolidine derivatives **313-315** (Table **4.2**, entries **1-3**), while moderated yield was shown for six-member ring pyrazole-oxopyrrolidine derivatives **316-318** (Table **4.2**, entries **4-6**). The yield of the conversion was also affected by the substituted amino pyrazole, the amino pyrazole contains dimethyl groups give the higher yield than the amino pyrazole contains diphenyl group due to steric hindrance of phenyl groups which decrease the yield of products.

Chapter 5. Synthesis of isoquinoline and isoindoline derivatives from α- and β-ketoacids

5.1. Isoquinolines derivatives

Isoquinolines are privileged structures that are found in some alkaloids and unnatural bioactive compounds. These compounds contain isoquinoline as cores with fascinating biological activities.[147] Some isoquinoline derivatives work as delivery systems which transport drugs across impermeable the blood brain barrier.[148] For example, 1,2-dihydroisoquinoline-*N*-acetic acid ester **322** is reported as a new carrier for brain-specific delivery **II** model drugs (Figure **30**).[149]

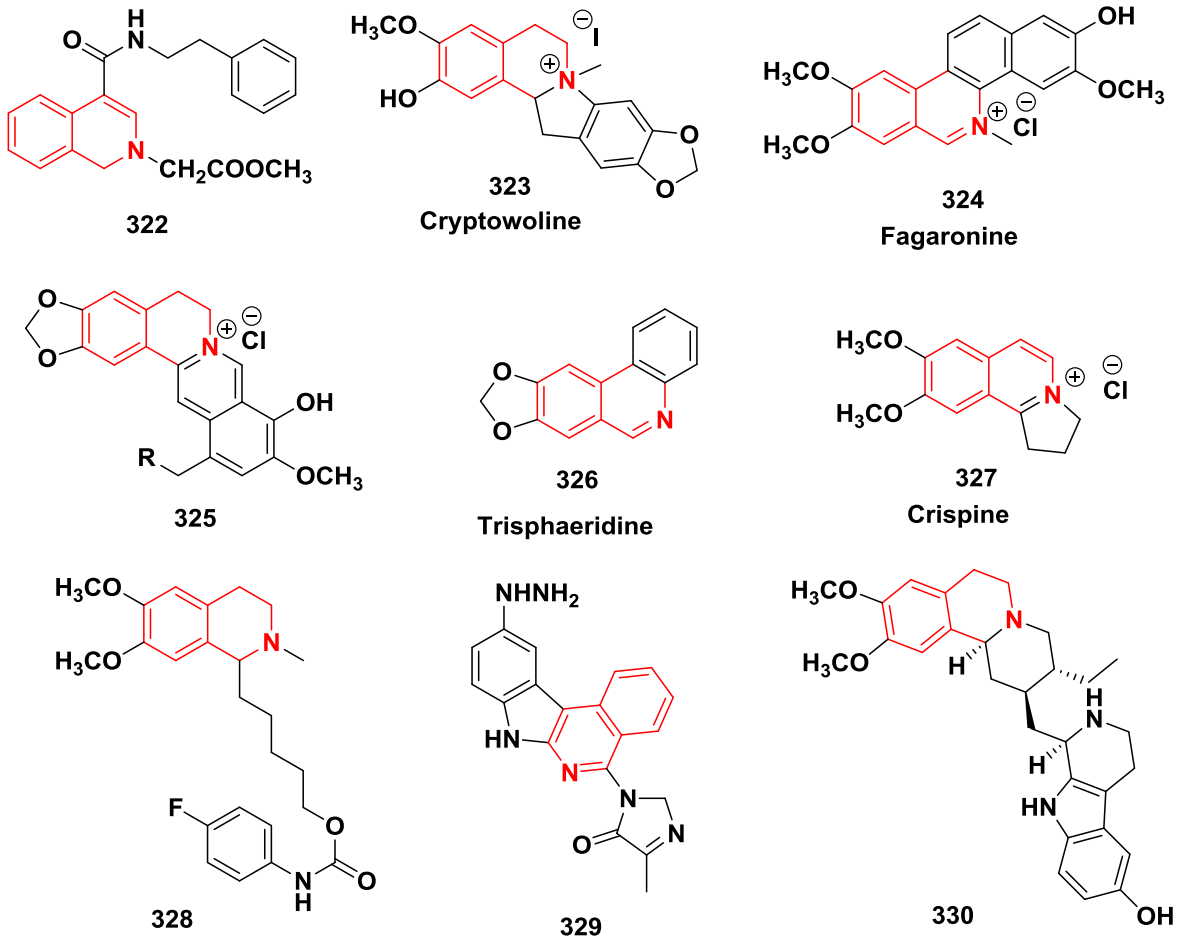


Figure 30: Some isoquinoline derivatives possessing medicinal properties.

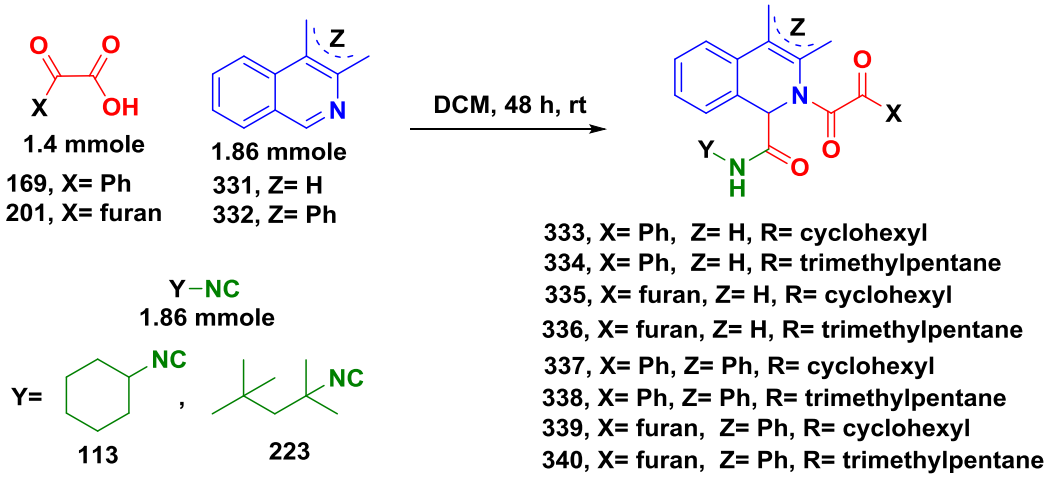
Cryptowoline **323** was screened and displayed a variety of biological activities as anti-leukaemia and anti-tumour agents.[150] Fagronine **324** has solicited pharmaceutical interest as an anticancer drug (P388 cancer cell).[151] Dioxolo-isoquinoline **325** has been demonstrated to have good anticancer efficacies (against HeLa cell line).[152] Trisphaeridine **326** is an isoquinoline alkaloid and possess a wide range of pharmacological potency such as antiviral, antitumor and inhibition of DNA topoisomerase.[153] Crispine **327** was isolated as a natural product to exhibit antidepressant activities.[154]Isoquinoline-carbamate **328** was evaluated as

bactericidal activity.[155] Isoquinoline-fused indole **329** has been reported as a pharmacophore for anti-inflammatory compounds.[156] Alangium alkaloid (tubulosine) **330** was categorized from tetrahydro isoquinoline alkaloids that are formed in nature from dopamine. Tubulosine **330** has been recently isolated from dried fruits of Alangium lamarck and Pogonopus species and used as a bioactive reagent for a variety biological activities such as HIV reverse transcriptase inhibition and inhibition of protein biosynthesis (Figure **30**). [157] The majority of above compounds were synthesised using different methods in four to nine-steps. These methods involve utilizing expensive reagents, catalysts and harsh reaction conditions.

There is no literature precedent for combination of isoquinoline and ketoacids in the presence of isocyanides.

5.1.1. Synthesis of isoquinoline derivatives from *α*-ketoacids

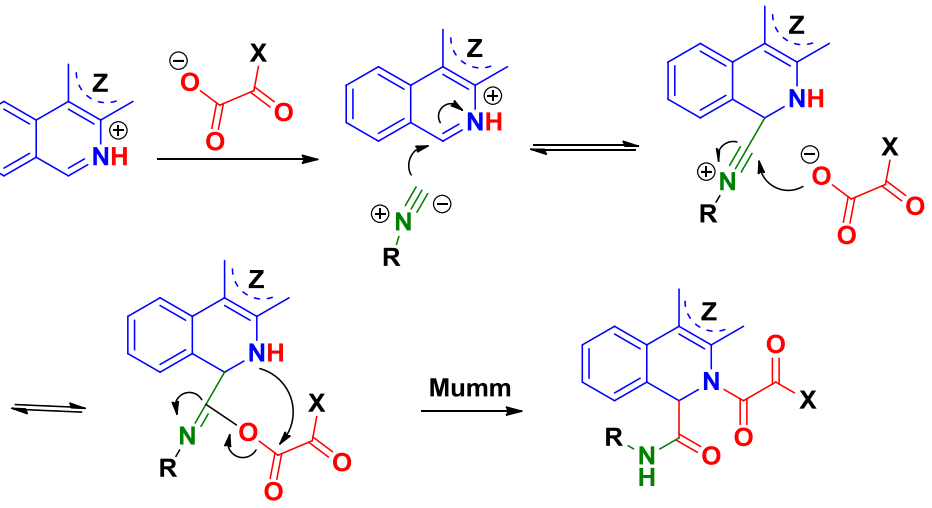
There are two approaches that can be used to synthesise isoquinoline derivatives: One is to incorporate modification prior to isoquinoline ring formation and the other is post transformations of isoquinoline derivatives. In this work, it is proposed that reaction of isoquinolines (an aromatic azine) with *α*-ketoacids would form an *N*-acylazinium which is susceptible to attack by isocyanide moiety to form isoquinoline derivatives in Ugi-like fashion. Further cyclisation of these derivatives were attempted, but failed to carry out post-ring formation modification. Probably, due to the isoquinoline ring-nitrogen acting as a nucleophile, it can therefore be used as a replacement for amine component in Ugi-like reaction (Scheme **65**). Therefore, it was hoped that combination of keto acids with isoquinolines could lead to a library of novel compounds with interesting biological activities.



Scheme 65: Synthesis of isoquinoline derivatives.

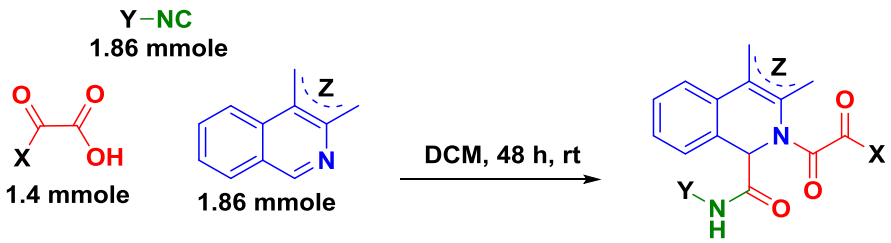
In the first experiment, the reaction of isoquinoline with α-ketoacids and isocyanide in CH_2Cl_2 at room temperature afforded a mixture of the oxoamide and the desired amide in modest yields (Table **5.1**). Although quinoline possesses a convenient reactivity, the multicomponent reaction appears to be restricted to azines which then undergo addition at the α-position. Thus, quinoline was unreactive under these conditions. The same conditions were applied by using phenanthridine instead of isoquinoline and afforded nicely the desired amide. Acridine (linear analogue of phenanthridine) according the same reason above does not afford desired amide under the same conditions.

A mechanism for the synthesis of isoquinoline derivatives is proposed (Scheme **66**). Initially, an isoqunoline was protonated by ketoacid to generate iminum ion that is attacked by isocyanide to form nitrilium ion. This intermediate undergoes acyl imidoyl and a Mumm rearrangement to afford the desired diamide.

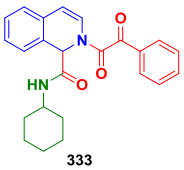
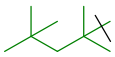
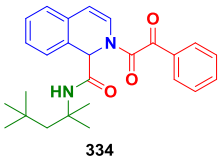
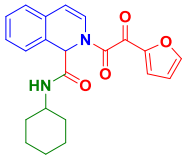
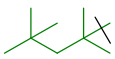
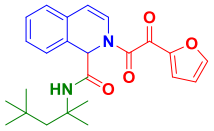
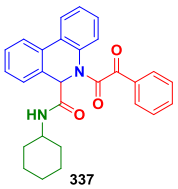
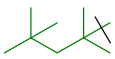
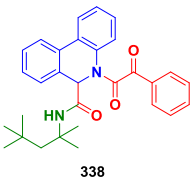
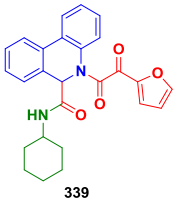
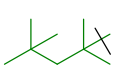
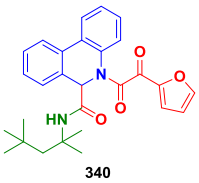


Scheme 66: A plausible mechanism for synthesis of isoquinoline derivatives.

Table 5.1: Synthesis of isoquinoline derivatives.



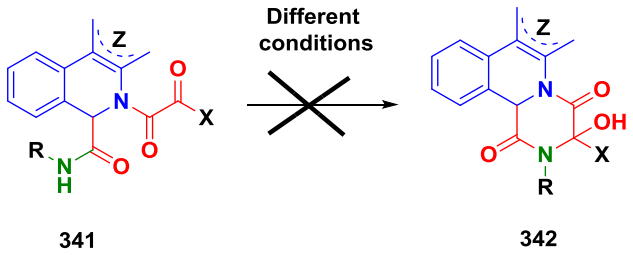
Entry	X	Y	Z	Isoquinoline derivatives	yield[a]

1			H	 **333**	65%
2			H	 **334**	50%
3			H	 **335**	35%
4			H	 **336**	36%
5				 **337**	60%
6				 **338**	45%
7				 **339**	44%
8				 **340**	40%

[a] yield of isolated product

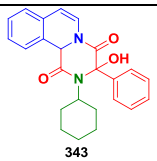
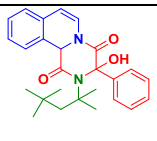
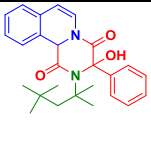
Table **5.1** shows isoquinoline derivatives **333-340** (Table **5.1**, entries **1-8**) that were synthesised from Ugi-like reactions using different keto acids, isoquinoline, phenanthridine and different isocyanides at room temperature for 48 h. It was noted that the yield of conversion affects with nature of isoquinoline and keto acids. Moderate yields were observed for isoquinoline derivatives **333-336** (Table **5.1**, entries **1-4**), while low yields were obtained for phenanthridine derivatives **337-340** (Table **5.1**, entries **5-8**). This could be due to a steric hindrance, due to the bulky nature of phenanthridine.

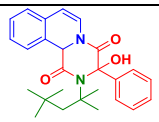
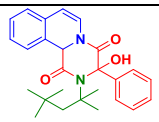
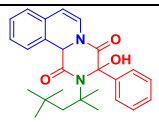
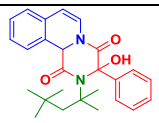
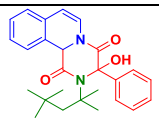
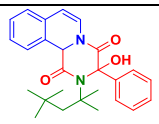
Different conditions have been tried for developing of isoquinoline derivatives **341** using Pictet-Spengler-like cyclization. The cyclization of isoquinoline derivatives was thought to produce tricyclic structures **342** with high diversity and interesting targets with pharmacological activities. The standard reaction conditions for the Pictet-Spengler cyclization were typically followed for screening the suitable conditions using different percentage of TFAA or use of trimethylsilyl triflate (TMSOTf) as Lewis acid for the reaction (Table **5.2**). Unfortunately, all attempts to synthesize the desired compounds failed (Scheme **67**).



Scheme 67: Some attempts for cyclization reactions of isoquinoline derivatives.

Table **5.2**: Trails for the Pictet-Spengler-like cyclization of isoquinoline derivatives.

Entry	Solvent	Additive	Temperature ºC /Time (h)	Product	Yield%
1	TFA:DCM (1:2)	TFAA	r.t / 3 h	 343	-
2	TFA:DCM (1:2)	TFAA	r.t / 3 h	 344	-
3	TFA	TFAA	r.t / 5 h	 344	-

4	TFA	TFAA	r.t / 18 h	 344	-
5	TFA	TFAA	r.t / 24 h	 344	-
6	TFA	TFAA	50 ºC / 4 h	 344	-
7	DCM	TMSOTf	r.t / 3 h	 344	-
8	DCM	TMSOTf	r.t / 10 h	 344	-
9	DCM	TMSOTf	r.t / 24 h	 344	-

5.2. **Isoindolinone derivatives**

The isoindolinone moiety is an important class of building scaffold which is the core unit of an increasing number of natural products [158] and biologically active of synthetic compounds.[159],[160] Isoindolinone derivatives have been extensively used due to their potent therapeutic nature[161]. Isoindolinone derivatives possess anxiolytic activities, for instance, zopiclone **345**, [162] as muscle relaxants, pazinaclone **346** [163] and pagoclone **347** [164] as hypnotics and sedatives. Recently, isoindolinone (5-hydroxypagoclone) **348** has shown inhibition activity of cytotoxic against HeLa, Hep2 and A549 cancer cell lines.[165] In addition, spiro cyclic derivatives containing isoindolinone moiety are represented as important privileged cores in various pharmacophore which exhibit a broad range of therapeutic activities.[166] Compound **349** is found to exhibit as aldose inhibitor,[108] compound **350** gives biological activity toward human ELOVL6 and ELOVL3 inhibitors [167] (Figure **31**). It was reported according to above literatures, the compounds (**345**-**350**) have

been synthesised using different methodologies which include exploiting many reagents, expensive catalyst and harsh reaction conditions.

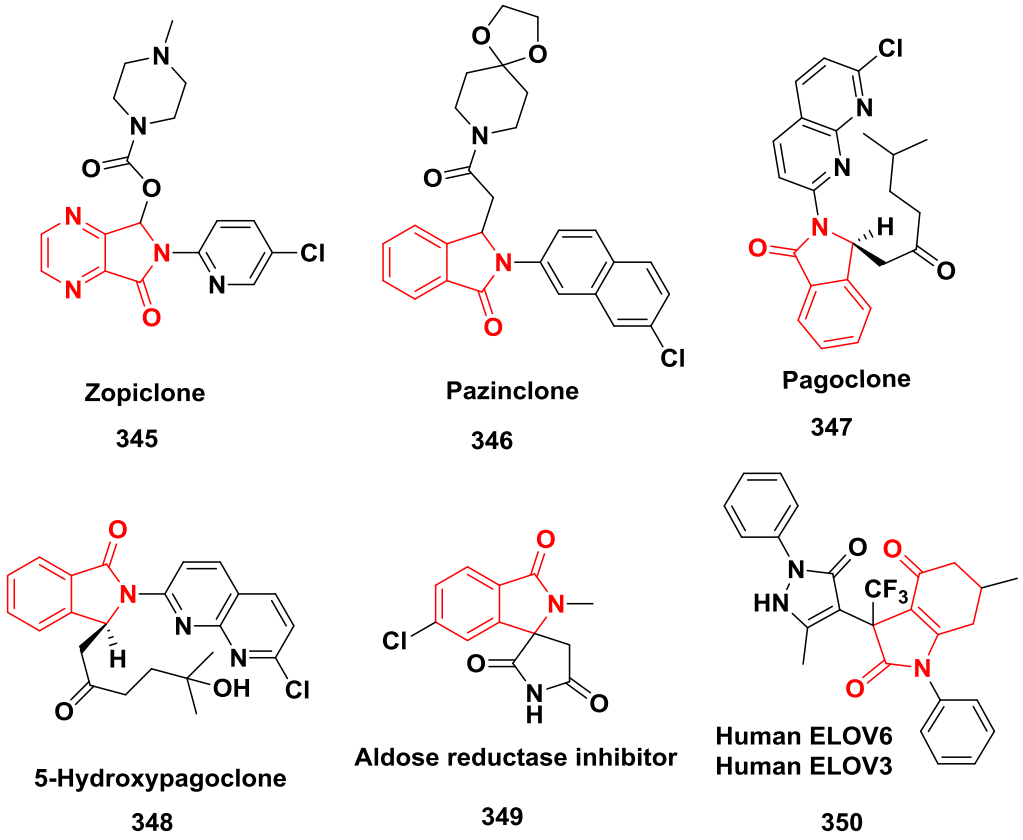


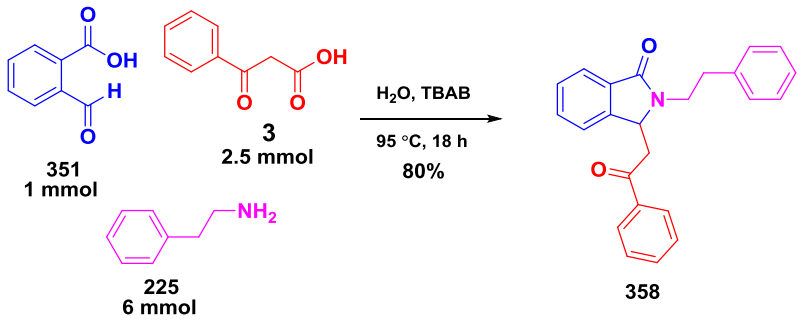
Figure 31: Some isoindolinone derivatives as therapeutic agents.

5.2.1. Synthesis of isoindolinone derivatives from *β*-ketoacids

The synthesis of a small set of novel heterocycle-fused isoindolinone structure for drug discovery was achieved by using a one-pot reaction. This set of compounds may lead to a series of possible pharmacologically exciting heterocycle-fused isoindolinone compounds. Multi-component reactions provide an important service in green reaction media, particularly in water. Multicomponent reactions are utilized to synthesise small drug-like molecules with diverse structure and high atom economy.[168] *β*-Keto acids have been efficiently used to provide ketone enolate *via* the decarboxylation process and this ketone can react with many electrophilic partners and is widely used in variety of decarboxylative transformations.[169],[170] The advantageous importance of the present transformation includes: use of quaternary ammonium salt which is found to significantly activate the alkylation reaction of *β*-keto acids with 2-carboxybenzaldehyde **351** under mild conditions such as using water as nontoxic solvent and only CO_2 generated as non-hazardous waste.

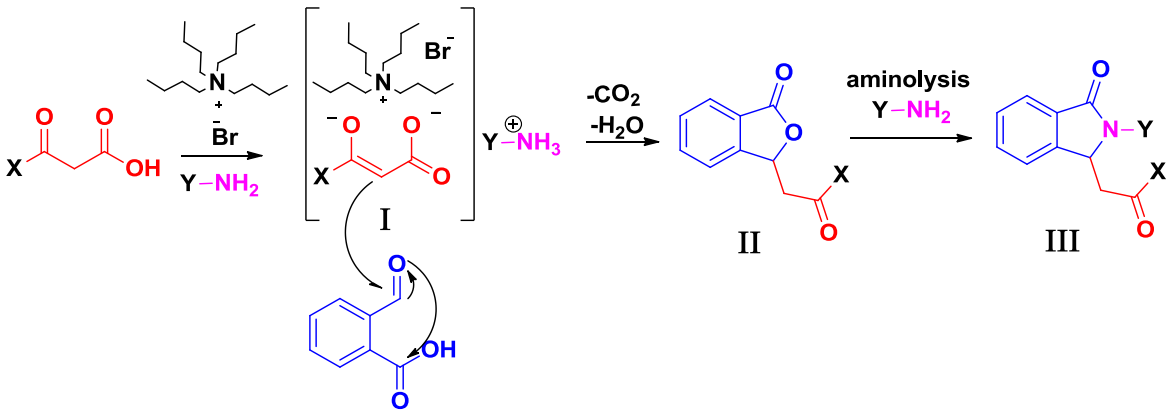
The model reaction was selected from substrate 2-carboxybenzaldehyde **351** (1.0 mmol), 2-oxo-2-phenylacetic acid **3** (2.5 mmol) and 2-phenylethanamine **225** (6 mmol) for 18 h at 90 ºC. The desired product was collected in low yield without any additives (Table **5.3**, entry **1**). An efficient reaction was further improved by using various quaternary ammonium salts as PTC catalyst. Experimental data were revealed that different quaternary ammonium salts (PTC) such as hexadecyltrimethyl ammonium chloride (HTAC), benzyltriethyl ammonium chloride (BTEAC) and tetrabutyl ammonium bromide (TBAB) gave different degrees of activities and afforded the desired products with 55–80% yields (Table **5.3**, entries **2-4**). Interestingly, it is notable that when TBAB (20 mol%) was utilized as the phase-transfer catalyst, the desired product can be yielded to 80% (Table **5.3**, entry **4**).

Table 5.3: Optimized conditions for synthesis of isoindolinone derivative **358**.



Entry	Catalyst	Solvent	Temperature	Time	yield %
1	none	methanol	reflux	18 h	0
2	HTAC	water	95ºC	18 h	55
3	BTEAC	water	95ºC	18 h	68
4	TBAB	water	95ºC	18 h	80
5	TBAB	water	95ºC	24 h	50
6	TBAB	water	reflux	18 h	72

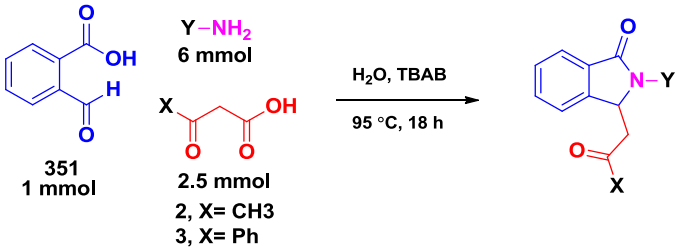
It is thought that the mechanism of this one pot reaction includes an initial deprotonation of 2-oxo-2-phenylacetic acid **3** by the amine in the presence of tetrabutyl ammonium bromide salt (TBAB) to furnish the dianion intermediate **I** due to an electrostatic interaction which forms between the lone pair of two oxygen atoms and the ammonium cation.[171] The condensation between 2-carboxybenzaldehyde **351** and intermediate **I** gives the intermediate **II** which subsequently undergoes decarboxylative addition and further ring-closing reaction. Finally, the extra addition of primary amine to the intermediate **II** leads to the formation of desired products **III** (Scheme **68**).

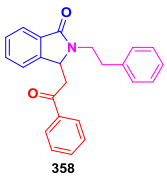
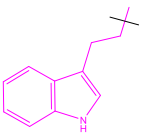
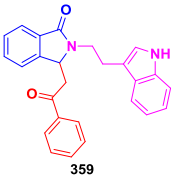


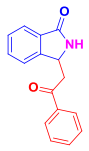
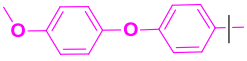
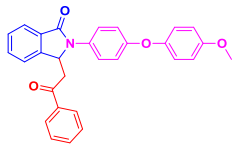
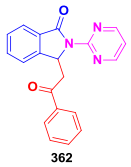
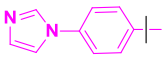
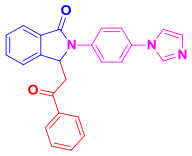
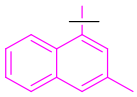
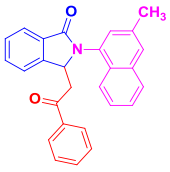
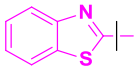
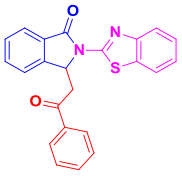
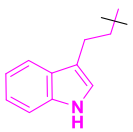
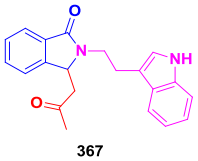
Scheme **68**: A proposed mechanism for synthesis of isoindolinone derivatives.

The further extension of the present methodology was focused on various primary amines including, tryptamine **285**, ammonium hydroxide **352** (32 %), 4-(4-methoxyphenoxy) aniline **353**, 2-aminopyrimidine **354**, 4-(1H-imidazol-1-yl) aniline **355**, 4-aminoquinaldine **356** and 2-aminobenzothiazole **357** under optimized conditions. It was observed that the different yields of corresponding isoindolinone derivatives varied with different amines (Table **5.4**).

Table 5.4: Shows synthesis of isoindolinone derivatives from *β*-keto acids.



Entry	X	Y	Isoindolinone derivatives	Yield[a]
1			 358	80%
2			 359	68%

3		H	 360	75%
4			 361	72%
5			 362	65%
6			 363	77%
7			 364	67%
8			 365	45%
9	CH_3	H	 366	60%
10	CH_3		 367	55%

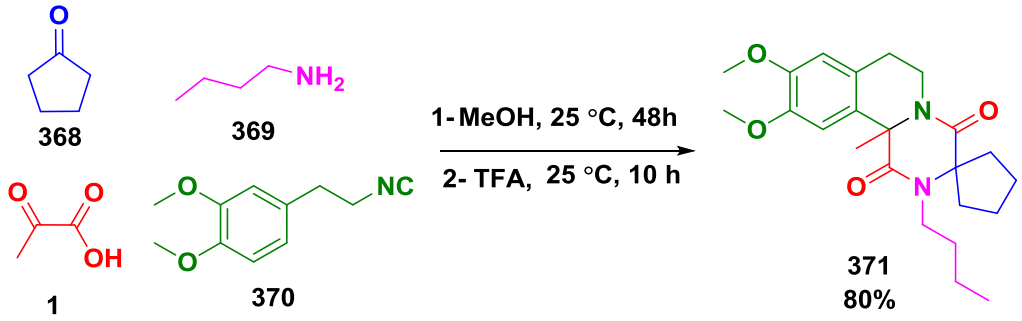
[a] yield of isolated product

From the table **5.4**, it can be deduced that the isoindolinone derivatives **358-367** (Table **5.4**, entries **1-10**) were synthesised from keto acid **3** in ranging between 45%-80% yields. Isoindolinone derivatives **362** and **363** (Table **5.4**, entries **9** and **10**) were synthesised from keto acid **2** in moderate yields. It was observed that isolated yields were dependant on the type of amine used. A variety of primary amines including aryl-alkylamines, ammonium hydroxide and arylamines worked under optimal conditions. It was noted from table that the substituents on the amines have slight effect on the yields of desired products (Table **5.4**). Firstly, in the presence of aryl-alkylamines and ammonium hydroxide, the reaction yield was increased considerably, furnishing the corresponding isoindolinones 80% and 75% yields, respectively (Table **5.4**, entries **1** and **3**). In addition, various aromatic amines which have less nucleophilic were subjected to this process. It can be seen that aromatic amines with a *para*-electron-donating substituent on such as methoxy group (Table **5.4**, entry **4**) the aromatic ring proceed to afford isoindolinone **361** in good yield.

Chapter 6. Post-transformation reactions of multicomponent products

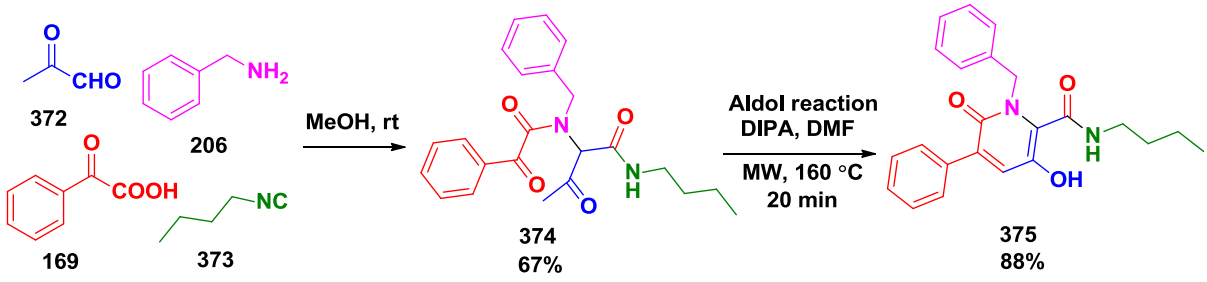
6.1. Post-transformation reactions of multicomponent products

The multicomponent product can be further manipulated to generate molecular diversity and privileged scaffolds. For example, the post-transformation of the Ugi products by Pictet-Spengler reaction (Uig-PS) has been reported and become an efficient method for the access to a family of structurally diverse heterocycles.[172],[173] El Kaim has reported that a Pictet–Spengler cyclisation reaction was efficiently used to synthesise pyrazines derivative **371** (Scheme **69**) [89] as antibacterial,[174] antitumor [175] and chitinase inhibitors.[176]



Scheme 69: Synthesize pyrazines derivative **371**.

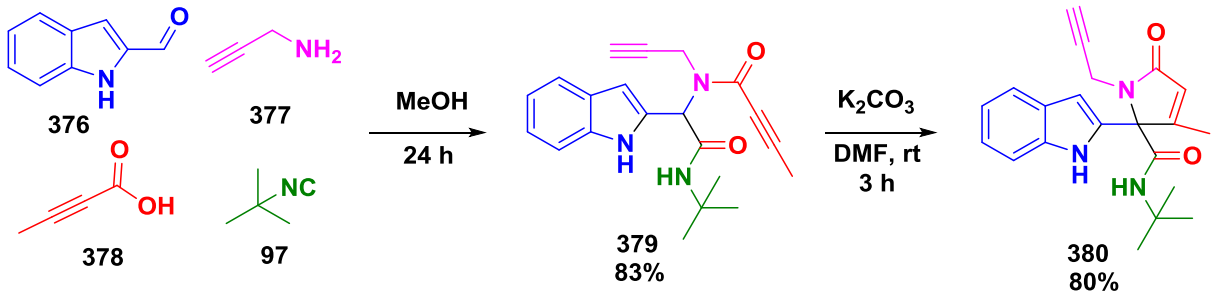
Zhigang *et al*. have reported the possibility to merge conversion of Ugi products into novel heterocycles using the aldol condensation (Scheme **70**). The multicomponent reaction product **374** was smoothly formed in methanol using equal moles of pyruvic aldehyde **372**, benzyl amine **205**, phenylglyoxylic acid **169** and n-butylisocyanide **373**. The compound **374** as a cyclic intermediate which bears an activated methyl and ketonic carbonyl group therefore reacted further (aldol-induced cyclization) under microwave irradiation to afford the desired Ugi/aldol product **375**.[177]



Scheme 70: Synthesis of Ugi/aldol product **375**.

Chao Liu *et al.* exploited indole-2-carbaldehyde **376**, prop-2-yn-1-amine **377**, propiolic acid **378** and isocyanide **97** to synthesise indole-fused backbone **380** *via* two consecutive steps.

First step includes the preparation of Ugi product **379** which can be then converted to final desired product **380** under K_2CO_3-mediated cyclization (Scheme **71**).[178]



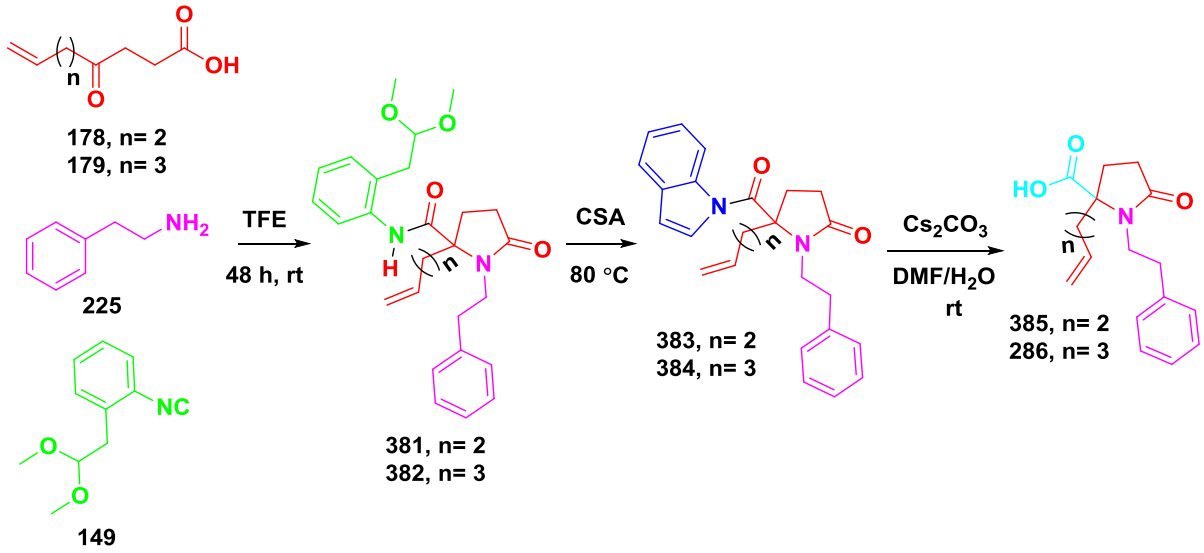
Scheme 71: Synthesis of indole-fused backbone **380**.

6.1.1. Synthesis of pyroglutamic acid analogues

The Ugi 4-center 3-component reactions including a keto acid and convertible isocyanide **149** can result in novel cyclic Ugi products. The Ugi 4-center 3-component reaction has two reactive centres which are present in convertible isocyanide **149** to allow the formation of heterocycles compounds. The advantages of the Ugi 4-center 3-component reactions are mild reaction conditions and the convergent nature of the reaction. These conditions provide a unified strategy towards derivatives through employing functionalized keto acids.[179] This approach might be utilized to synthesise various pyroglutamic acid derivatives through *N*-acylindole intermediates obtained by the Ugi reaction. The pyroglutamic acid moiety is featured in some medicinally natural products: dysibetaine **163**[180] and lactacystin **220**.[181] Pyroglutamic acid derivatives can be functionalized depend on the diastereoselectivity of keto acids. The Ugi 4-center 3-component from keto acid would be an efficient approach to afford pyroglutamic acid analogues.

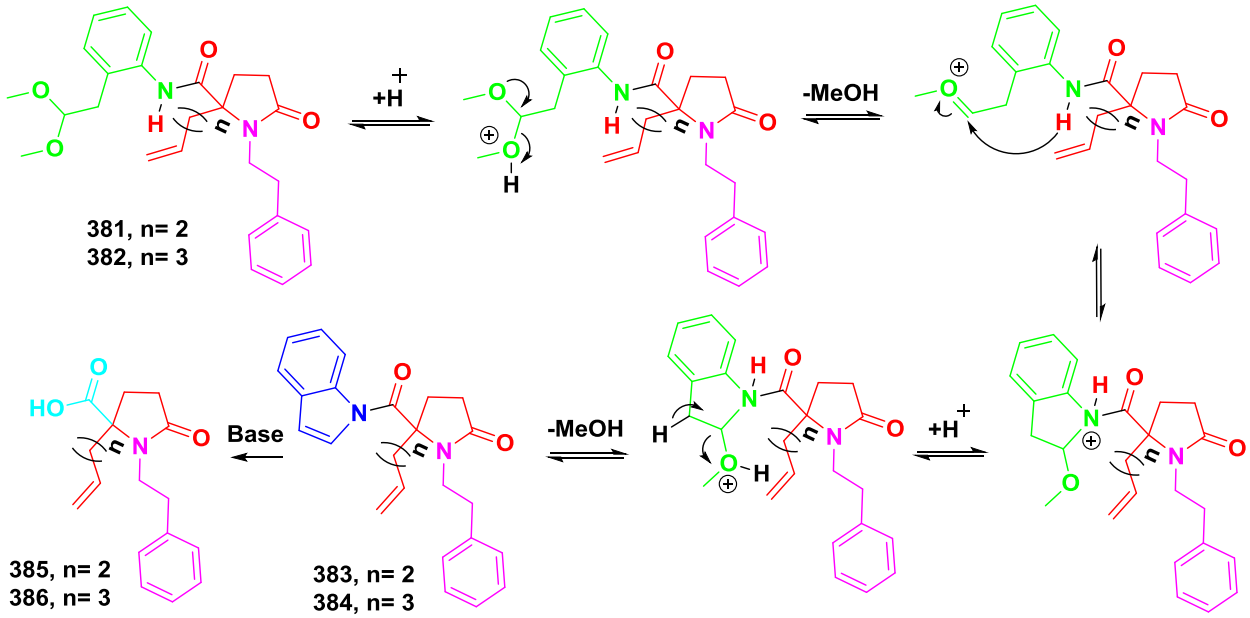
1-Isocyano-2-(2,2-dimethoxyethyl)benzene **149**[182] can participate in the Ugi reaction with keto acids **178** and **179** to afford anilides **381** and **382** that can be successfully converted to the corresponding *N*-acylindoles **383** and **384** which can then be converted to **385** and **386** (Scheme **72**). The synthetic approach of pyroglutamic acid analogues **385** and **386** can be summarised briefly as follows, the first step starts from reaction between γ-keto acids **178** and **179**, 2-phenylethanamine **225** and isocyanide **149** in TFE for **48** h at r.t to produce an initial anilides (Ugi products) **381** and **382** which are easily converted to pyroglutamic acid derivatives **385** and **386** by hydrolysis using Cs_2CO_3 in DMF/H_2O to give *N*-acylindoles **383** and **384** (Table **6.1**).[183] It was shown that acidic catalysis is the best conditions to convert anilides **381** and **382** to *N*-acylindole **383** and **384**. The *C*-terminal amides of pyroglutamic

acid amides have been possibly hydrolysed to afford the corresponding carboxylic acids.[184] Therefore, the development of a convertible isonitrile is necessary to facilitate a selective cleavage of the resulted *C*-terminal amide bond to yield pyroglutamic acids analogues.



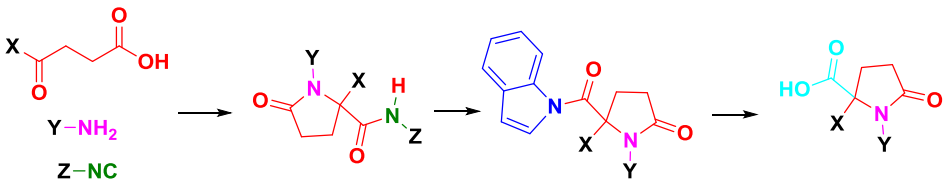
Scheme 72: Synthesis of pyroglutamic acid analogues **385** and **386**.

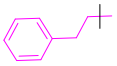
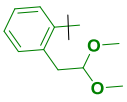
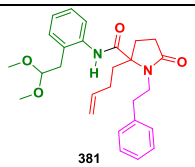
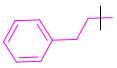
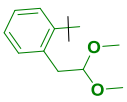
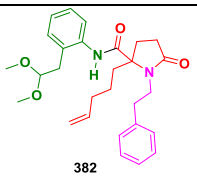
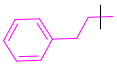
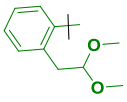
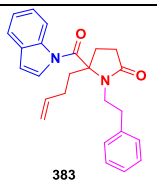
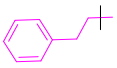
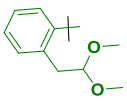
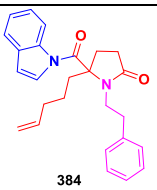
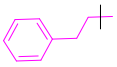
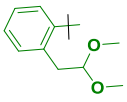
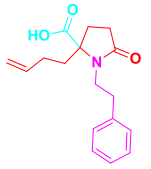
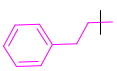
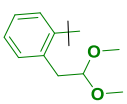
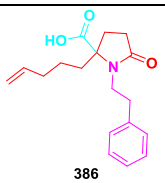
The mechanism of reaction was proposed that *N*-acylindoles **383** and **384** can be produced in excellent yield despite the sterically hindered position of the anilides **381** and **382**. The *N*-acylindoles **383** and **384** were treated with Cs_2CO_3 in DMF/H_2O (1:1) to yield the pyroglutamic acid analogues **385** and **386** (Scheme **73**).[185]



Scheme 73: Proposed mechanism of conversion Ugi products to *N*-acylindole derivatives.

Table 6.1. Synthesis of pyroglutamic acid analogues.



Entry	X	Y	Z	Product	Yield[a]
1 (Ugi product)				 **381**	75%
2 (Ugi product)				 **382**	60%
3 (*N*-Acylindole)				 **383**	82%
4 (*N*-Acylindole)				 **384**	70%
5 (Pyroglutamic acid analogue)				 **385**	85%
6 (Pyroglutamic acid analogue)				 **386**	80%

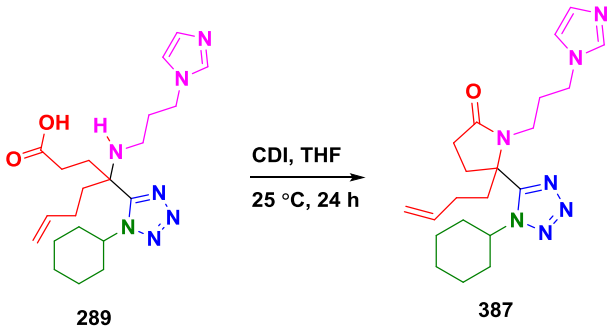
[a] yield of isolated product

Table **6.1** shows that Ugi products **381** and **382** (Table **6.1**, entries **1**-**2**) were synthesised from different ketoacids in moderate to good yields. The yield of the conversion of anilides **381** and **382** to *N*-acylindole **383** and **384** (Table **6.1**, entries **3**-**4**) was also affected by ketoacids,

giving 82% and 70% respectively. Good yields were observed for pyroglutamic acid analogues **385** and **386** (Table **6.1**, entries **4-6**).

6.1.2. Synthesis of lactam-tetrazoles derivatives

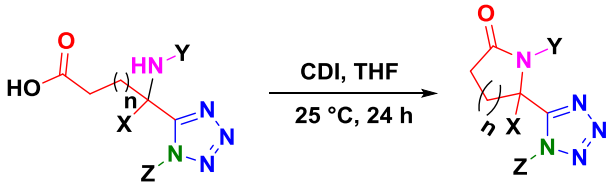
The functionalization of the synthesised Ugi-tetrazoles (**289-298**, **Chapter 3**) in a one-pot procedure can lead to *γ*-, *δ*- and *ε*-lactam-tetrazole products. The model reaction was selected from compound **289** which was treated with the coupling reagent 1,1-carbonyldiimidazole (CDI). The desired lactam-tetrazole product **387** was collected in good yield after 24 h at room temperature (Scheme **74**).

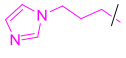
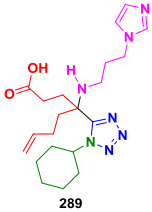
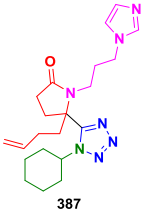


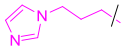
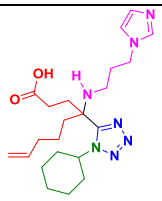
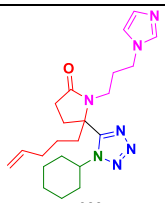
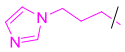
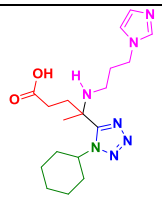
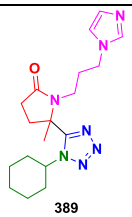
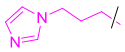
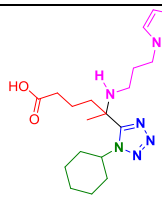
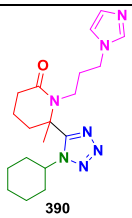
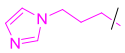
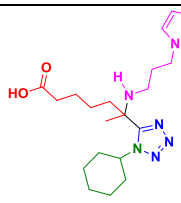
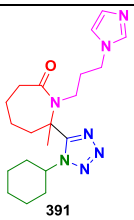
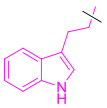
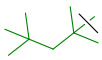
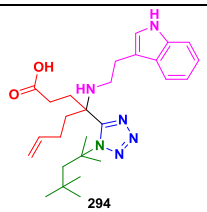
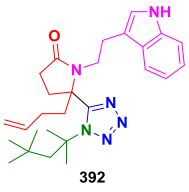
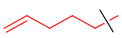
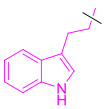
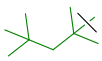
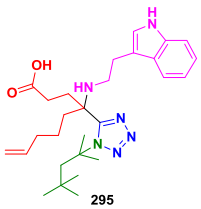
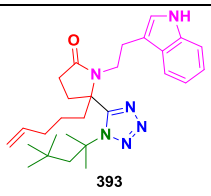
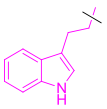
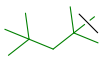
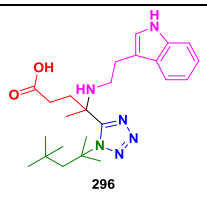
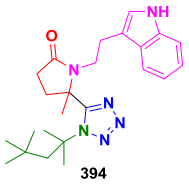
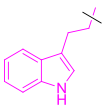
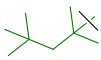
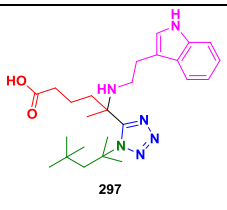
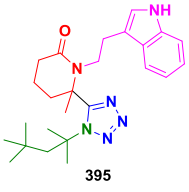
Scheme 74: The model reaction for synthesis of lactam-tetrazole products **387**.

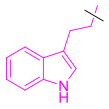
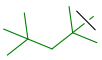
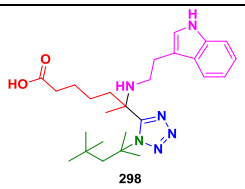
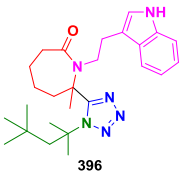
Therefore, the coupling reagent 1,1-carbonyldiimidazole (CDI) was used to activate Ugi-tetrazoles to afford lactam-tetrazoles (Table **6.2**). Different keto acids as bifunctional reagents, primary amines, isocyanides and $TMSN_3$ were applied with the easy expansion methodology to afford 5-, 6-, and 7-membered rings.

Table 6.2. Synthesis of *γ*-, *δ*- and *ε*-lactam tetrazoles.



Entry	n	X	Y	Z	Ugi-tetrazoles	Lactam tetrazoles	yield[a]
1	1						70%

2	1				 290	 388	50%
3	1	CH_3			 291	 389	80%
4	2	CH_3			 292	 390	60%
5	3	CH_3			 293	 391	20%
6	1				 294	 392	55%
7	1				 295	 393	40%
8	1	CH_3			 296	 394	62%
9	2	CH_3			 297	 395	55%

10	3	CH_3					23%

[a] yield of isolated product

The table **6.2** shows the functionalization of Ugi-tetrazoles to furnish *γ*-, *δ*- and *ε*-lactam-tetrazoles. Lactam-tetrazoles where synthesised from different keto acids, amines and isocyanides using (1,1-carbonyldiimidazole (CDI)) as efficient catalyst. It was observed that the yield of functionalization depends on the ring size of cyclic amides (Lactam). The lactam-tetrazole **389** (Table **6.2**, entries **3**) was obtained in 80%, lactam-tetrazole **391** (Table **6.2**, entries **5**) under the same conditions was obtained in 20%. Also, it was assumed that the yield of functionalization depends on the nature of the amine. The majority of lactam-tetrazoles (**387**-**391**) (Table **6.2**, entries **1**-**5**) were synthesised from amino pyrazole obtained in higher yields than lactam-tetrazoles (**392**-**396**) (Table **6.2**, entries **6**-**10**) which were synthesised from amino indole.

6.1.3. Diones derivatives

Dione substructure is found in some synthetic compounds and natural products which exhibit a wide range of biological activities. Compound **397** [186] which has been synthesised using five steps starting from L-proline. It provides broad-spectrum anticonvulsant activities in treating maximal electroshock seizure (MES), minimal colonic seizure (6Hz) and subcutaneous metrazole (scMET) animal models of Epilepsy. Pyrrolo-quinoxaline **398** which possess anti-phlogistic and antiviral activities was synthesised by three steps from 3-aroylpyrrolo[1,2-*a*]quinoxaline-1,2,4-trione, 2-methylquinoxaline and quinaldine.[187] Pteridinone **399** has been synthesized using a multistep synthetic route and studied as polo-like kinase inhibitors for treatment of neurodegenerative diseases[188] (Figure **32**).

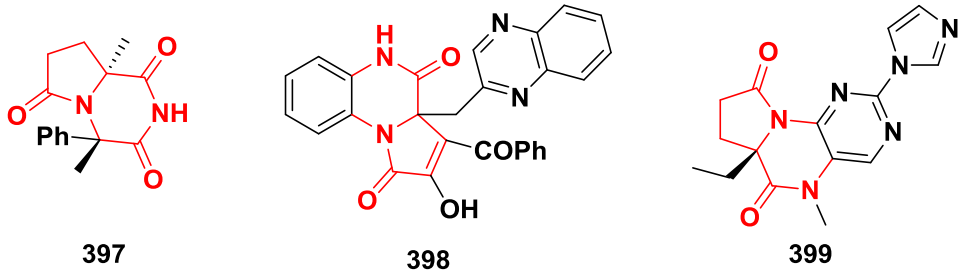


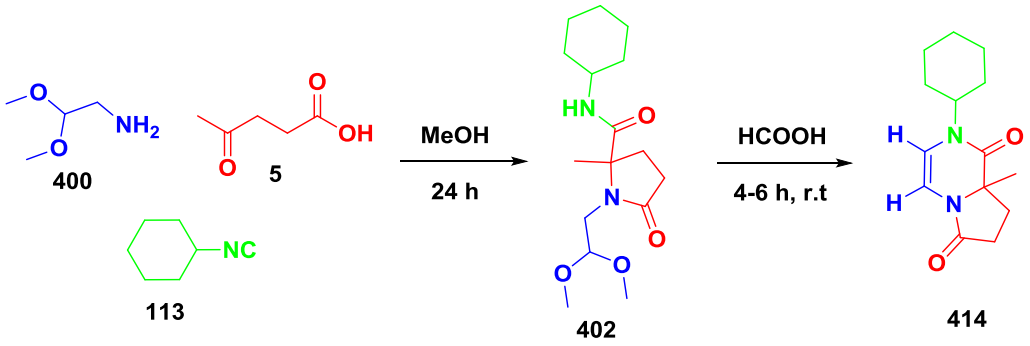
Figure 32: Some diones substructure have been used to clinical evaluation.

6.1.3.1. Synthesis of diones derivatives

The keto acids as bifunctional component can react with different amines and isocyanide to afford Ugi product. The Ugi products can be subsequently cyclized to obtain diverse heterocyclic compounds.[89] Keto acids were employed to be a bifunctional starting material in Ugi reaction and they could yield varied ring sizes diones.[189] The dione features privileged moiety in medicinal chemistry, therefore flexible synthetic methods are in high demand.

In this work, bifunctional keto acids were exploited in the synthesis of Ugi products from aminoacetaldehyde dimethylacetal **400** as a bifunctional amine. The amine would be subsequently undergone a cyclization reaction to afford the highly substituted heterocyclic diones.[190]The Ugi products bearing additional functional groups can be selectively functionalised to produce diverse cyclization manifolds. The major benefit of combining multicomponent reaction with post-transformation is that it facilitates access to diverse functionalized heterocyclic scaffolds in few synthetic steps.[191]

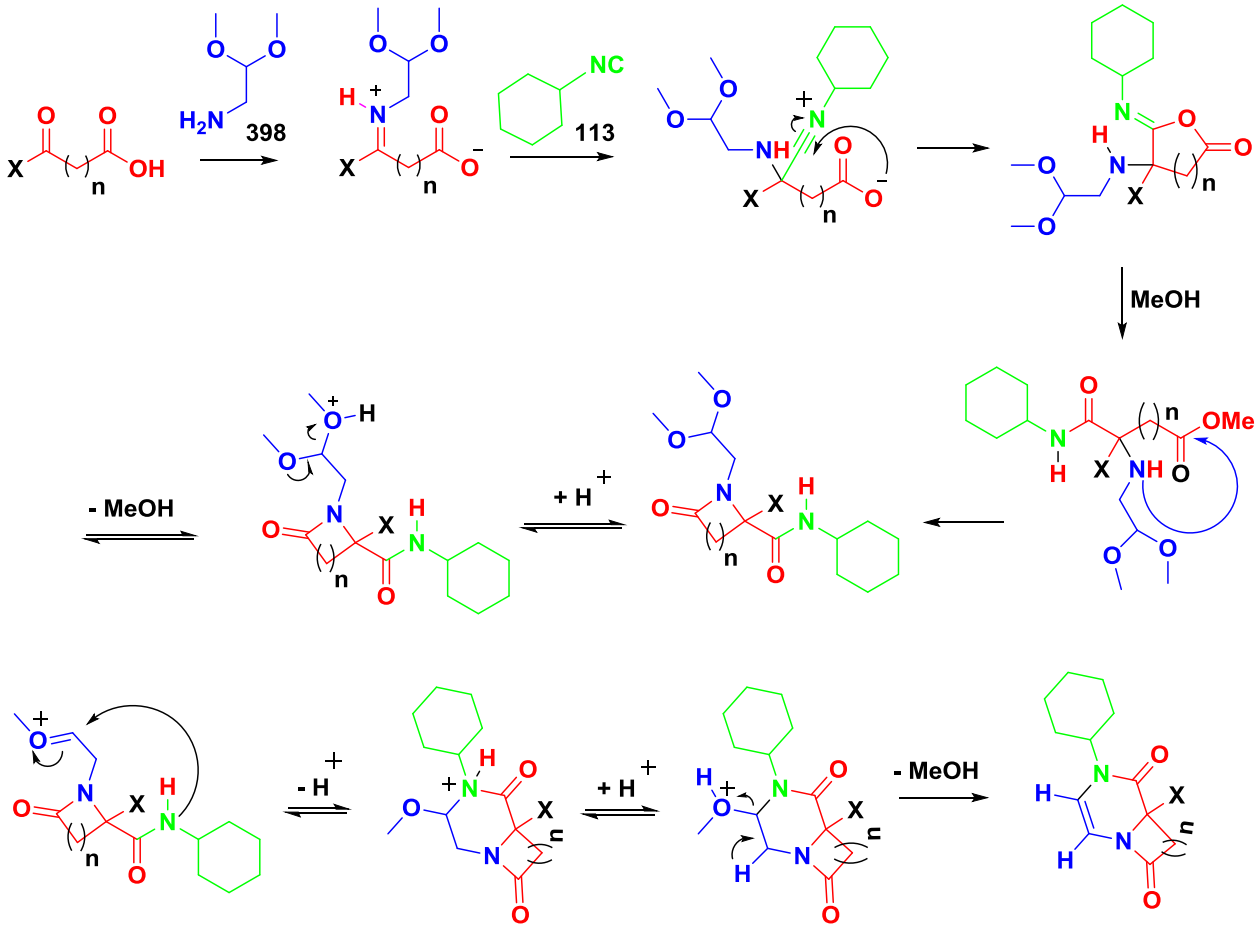
The model reaction was selected from compound **402** (Ugi product) which was treated with formic acid. The desired dione product **414** collected in good yield at room temperature for 6 h (Scheme **75**).



Scheme 75: The model reaction for synthesis of dione product **414**.

The ring closure process comprises methanesulfonic acid and formic acid which was investigated to optimize sequential reaction. The scope of optimization was carried out by employing different isocyanides and formic acid. The ring formation at room temperature is an efficient approach for more reactive Ugi products. The methanesulfonic acid was also used for cyclization of Ugi products which were derived from cyclohexylisocyanide **113**, but the yield was very low. The structural assignment can be confirmed *via* the presence of olefinic protons at δ 5.70-6.50 ppm according to literature.[192] This approach is suitable to provide high functionalized compounds such as Ugi products involving sequential reactions with

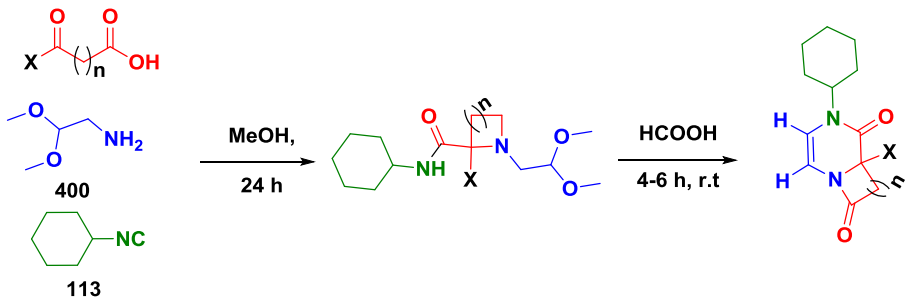
further post-transformation reactions for instance cyclization and nucleophilic addition[193] (Scheme **76**).



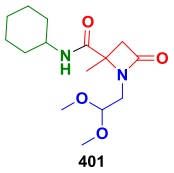
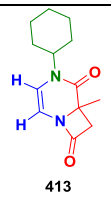
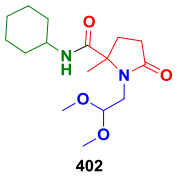
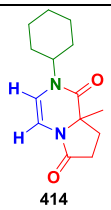
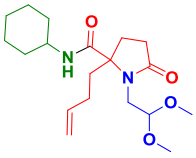
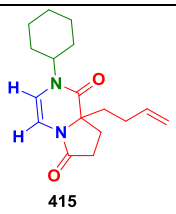
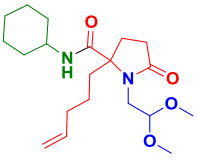
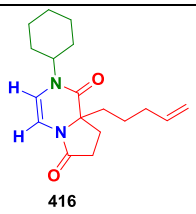
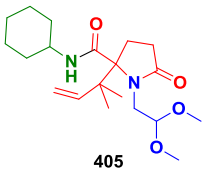
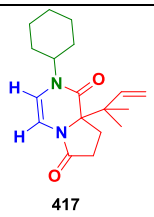
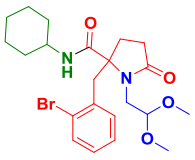
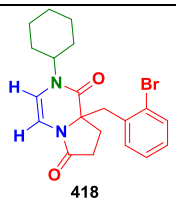
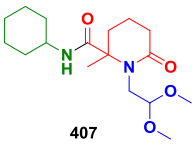
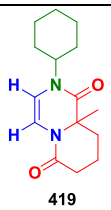
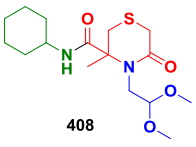
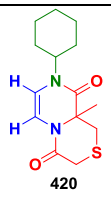
Scheme 76: The proposed mechanism for synthesis of diones derivatives **413-424**.

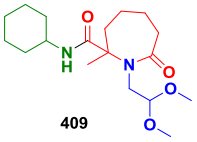
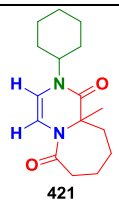
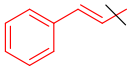
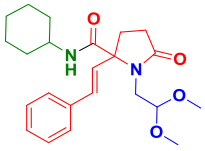
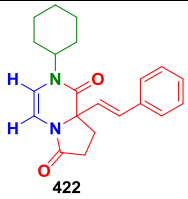
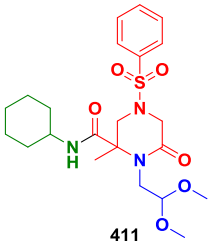
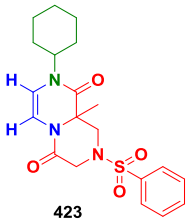
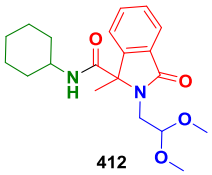
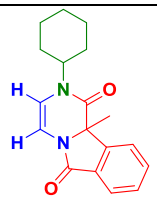
Subsequent cyclization reactions were carried out of the Ugi products (**401-412**) to synthesise diverse diones scaffolds in table **6.3**.

Table 6.3: Synthesis of Ugi products and their diones derivatives.



Entry	n	X	Ugi products	Yield[a]	Dione products	Yield[a]

1	1	CH_3	 401	65%	 413	75%
2	2	CH_3	 402	60%	 414	85%
3	2		 403	40%	 415	60%
4	2		 404	30%	 416	55%
5	2		 405	35%	 417	60%
6	2		 406	48%	 418	77%
7	3	CH_3	 407	70%	 419	75%
8	$(CH_2)_2S$	CH_3	 408	68%	 420	80%

9	4	CH_3	 409	40%	 421	60%
10	2		 410	35%	 422	55%
11	$CH_2PhNSO_2CH_2$	CH_3	 411	60%	 423	82%
12	Ph	CH_3	 412	65%	 424	80%

[a] yield of isolated product

From table **6.3**, it can be concluded that the Ugi products (**401**, **402**, **407**, **408**, **411** and **412**) were synthesised from different keto acids containing terminal methyl group in moderate yields 65%, 60%, 70%, 68%, 60% and 65% respectively. Lower yields were noted for other Ugi products from varied groups of terminal keto acids. The post-transformations of Ugi products above were carried out under mild conditions with moderate to very good yields. The structure of diones **414** and **423** was assigned and confirmed by X-ray crystallography (Figure **33**).

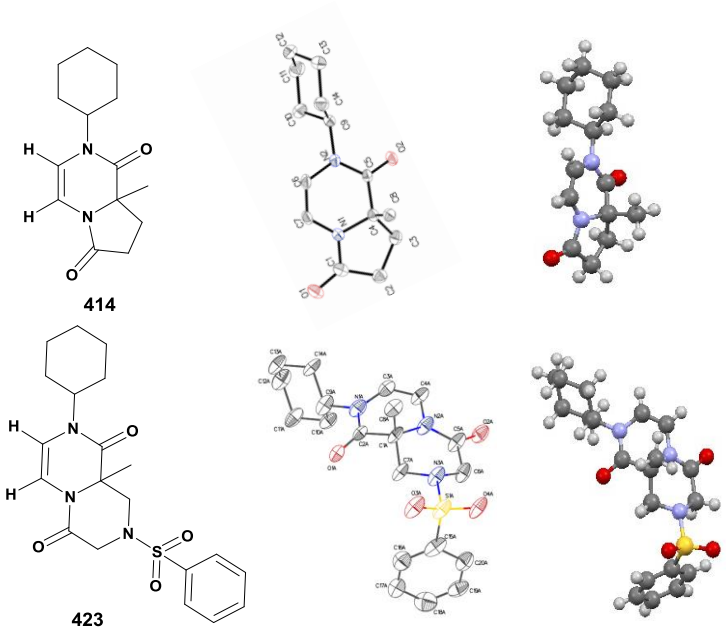


Figure 33: X-ray of crystal structure of diones **414** and **423**.

6.1.4. Pyrrole-substructures

Pyrrole-based agents have been employed as drugs for a few decades. Recently, re-emergence of novel pyrrole derivatives in the anti-infective field showed that they have broad-spectrum antimicrobial,[194] antitubercular,[195] antiviral, [196] antimalarial,[197] anticancer, [198] anti-inflammatory[199] and cytotoxic activities.[200]

The linker of the *N*-acylhydazone or semicarbazone between pyrrole moiety and the core of all these molecules showed that pyrrole-substructure possesses biological activities. Sunitinib **425** has been evaluated as an antitumor agent,[201] **426** is an effective inhibitor against CDK2[202] and compound **427** is a multi-kinase inhibitor [203] (Figure **34**).

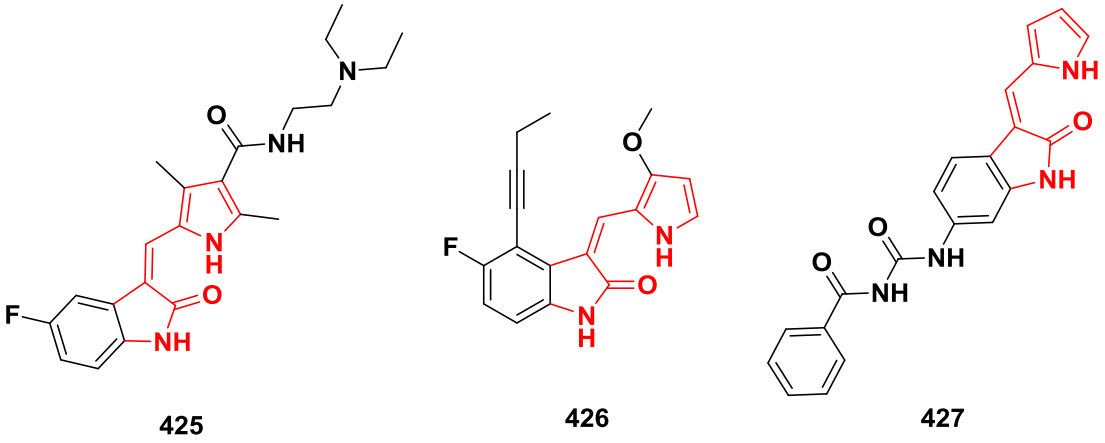
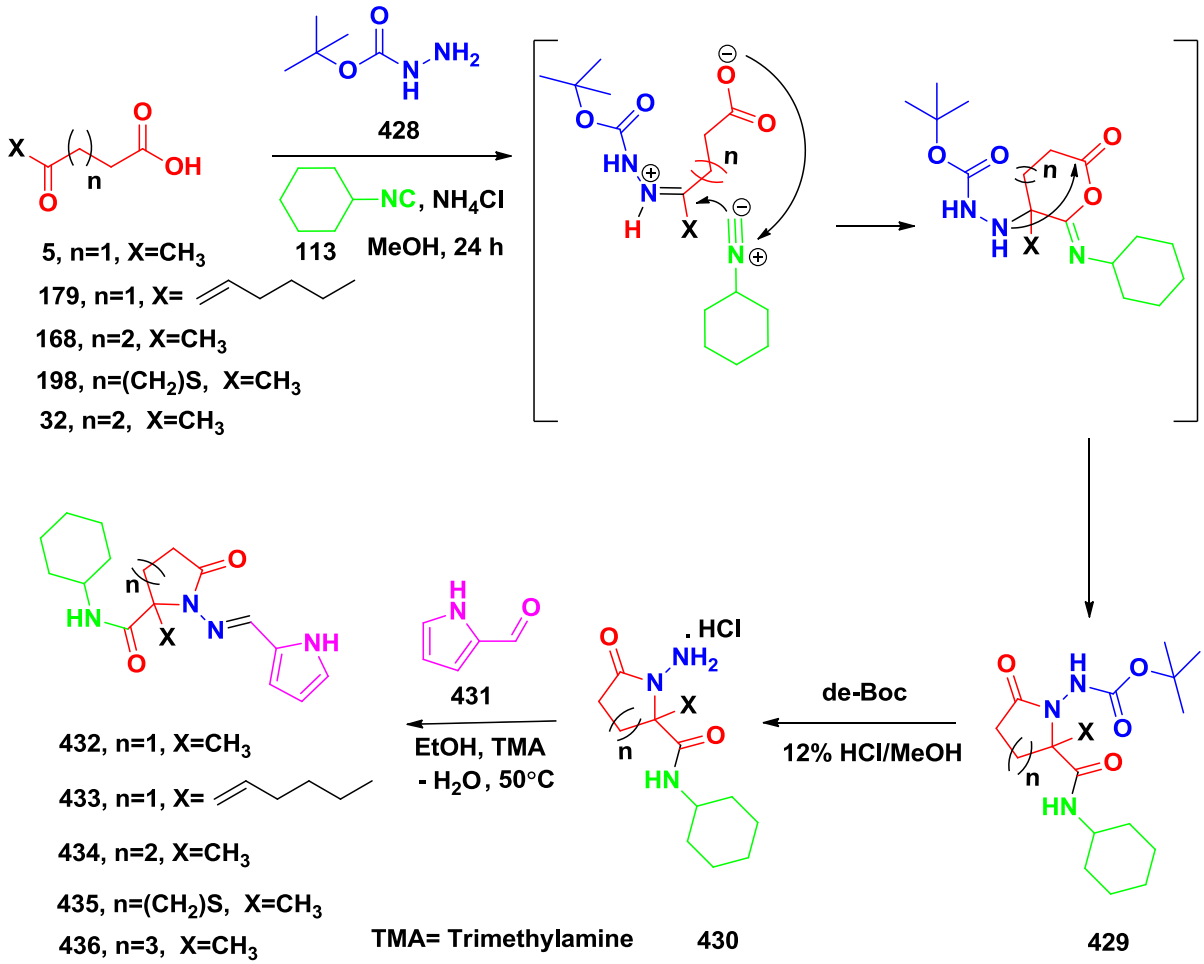


Figure 34: Some pharmacological pyrrole-indolinone derivatives.

This encouraged us to pursue a new efficient strategy involving a terminal unsubstituted *N*-acylhydrazine or a semicarbazide configuration which was connected with pyrrole *via* a straightforward hydrazine (semicarbazone) route. The area of the hydrazino-Ugi reaction provides a number of set compounds containing a hydrazine moiety.

6.1.4.1. Synthesis of pyrrole-oxopyrrolidine derivatives

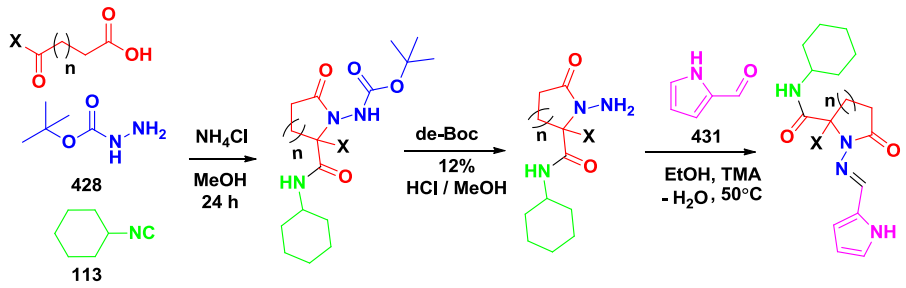
A set of decorated *N*-amino *γ*-, *δ*-lactams with a pyrrole moiety was synthesised using ketoacids-based multicomponent Ugi reaction and evaluated for their biological activity. Exploitation of the Ugi reaction includes various ketoacids (**5**, **179**, **168**, **198** and **32**), Boc-protected hydrazine **428** and cyclohexylisocyanide **113** furnished *N*-aminolactams **429** after purification. The removal of the protecting group in **430** would release a reactive *N*-acylhydrazine nitrogen atom in **430** which can be subsequently functionalised with pyrrole-aldehyde **431** to form structurally diverse pyrrole-oxopyrrolidine derivatives **432-436** (Scheme **77**).

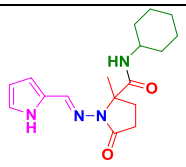
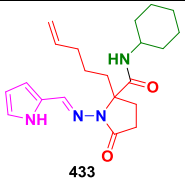
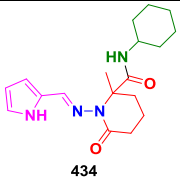
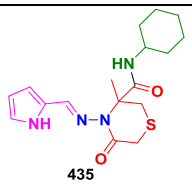


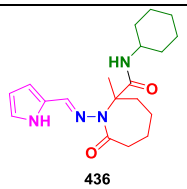
Scheme 77: Synthesis of some pyrrole-oxopyrrolidine derivatives from ketoacids.

Equimolar amounts of starting materials were reacted together in aqueous methanol containing ammonium chloride as a mild acidic catalyst. The hydrazino-Ugi reactions were completed within 24-48 h and the reactions afforded the desired *N*-Boc-amino-lactams **429** according to TLC and ^{1}HNMR data of the crude products. The Boc group in **429** was removed under mild conditions and the hydrochloride salts **430** were reacted with pyrrole-aldehyde **431** in the presence of anhydrous trimethylamine to afford the desired racemic hydrazones (**432-436**) in moderate to good yields (Table **6.4**). The pharmacological pyrrole moiety and cyclohexylisocyanide **113** can be affixed onto a novel cyclic amides scaffold (**432-436**), such that these scaffolds are potentially devoid of the labile hydrazine linkage which is considered as a potential source for toxic metabolites.

Table **6.4**: Synthesis of pyrrole-oxopyrrolidine derivatives (**432-436**).



Entry	n	X	Pyrrol-oxopyrrolidine	Yield[a]
1	1	CH_3	 432	76%
2	1		 433	70%
3	2	CH_3	 434	67%
4	$(CH_2)S$	CH_3	 435	65%

5	3	CH_3	 436	45%

[a] yield of isolated product

From table **6.4**, it can be seen pyrrole-oxopyrrolidine derivatives **432-436** (Table **6.4**, entries **1**-**5**) were synthesised from different keto acids in ranging yield 45%-76%. It was found that the yields of products (**432-436**) influence with size-member ring (cyclic amide) of resulted products. The good yields were obtained for compounds (five-membered ring) **432** and **433** (Table **6.4**, entries **1** and **2**), with moderate yields of compounds (six-membered ring) **434** and **435** (Table **6.4**, entries **3** and **4**).

6.1.5. Pyrrolo-pyridine derivatives

Pyrrolo-pyridine is a heterocyclic-fused system containing both pyrrole and pyridine. Compounds having the pyrrolo-pyridine structure have been studied because these compounds possess a wide range of biological activities. Pyrrolo[3,2-c]pyridin-4-one **437** was identified as selective and potent for ATP-competitive PLK1 inhibitors.[204] New pyrrolo-pyridine derivative **438** showed an excellent anti-proliferative activity toward a number of human tumour cell lines as potential DNA interactive drugs.[205] Archaeosine **439** and Queuosine **440** are clustered as 7-deazapurine nucleosides that are isolated from tRNA of eukaryotic organisms and prokaryotic respectively. These nucleosides behave as antibiotics due to their biological activities.[206] Novel pyrrolo-pyridines derivative **441** was evaluated as vicinal diary motif of tumour necrosis factor-TNF inhibitors.[207] Novel pyrrol[3,2-c]pyridine-4(5H)-one **442** derivative has been used as protein tyrosine kinase inhibitor.[208] PH-089 **443** and TAK-441 **444** are analogue molecules of pyrrolo-pyridines derivatives that possess a wide range of biological activities including DP receptor antagonists, CK1γ inhibitors, MK2 inhibitors, CB2 agonists, PLK1 inhibitors, ATP competition and CDc7 kinase inhibitors [209],[210] (Figure **35**).

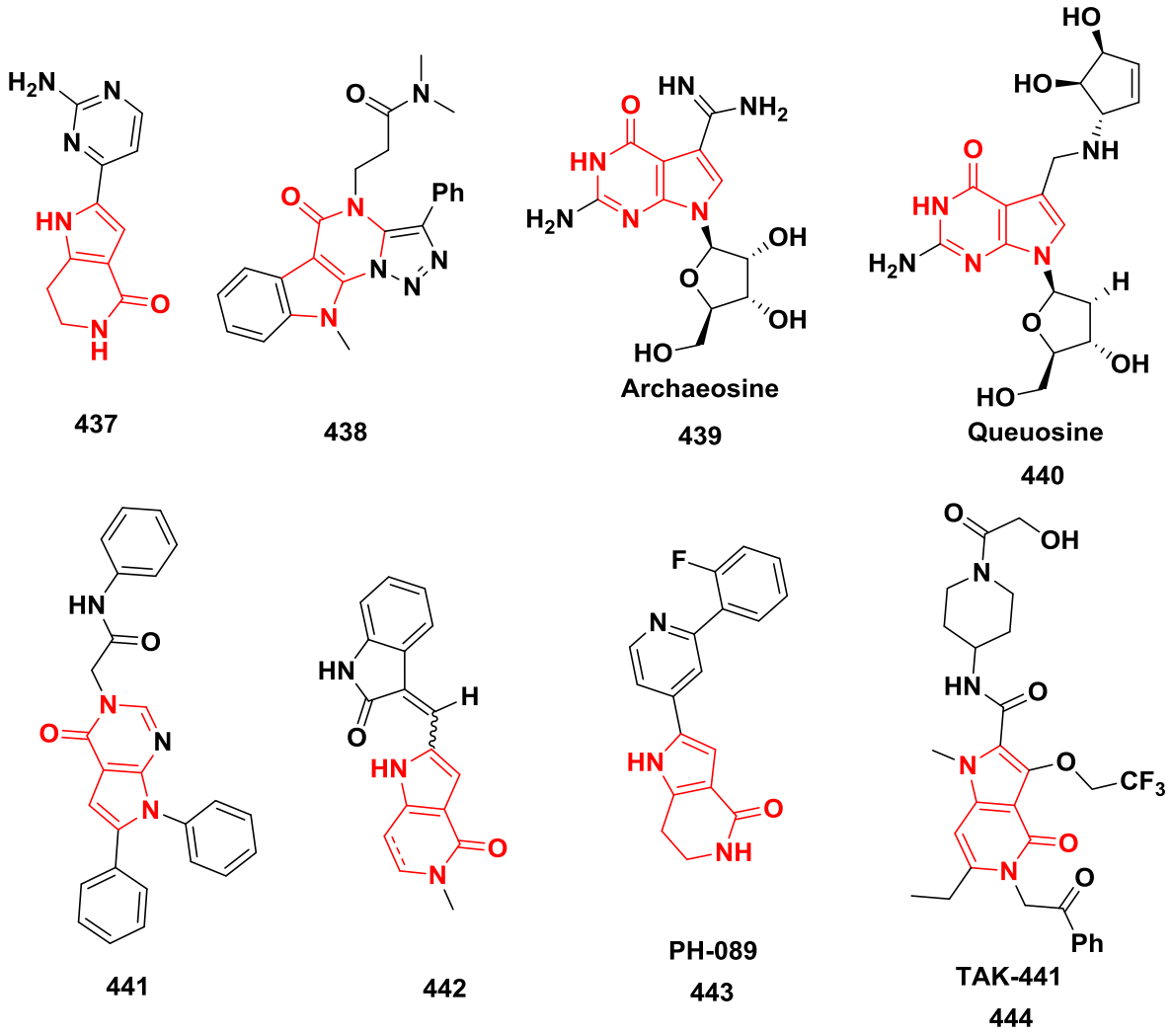
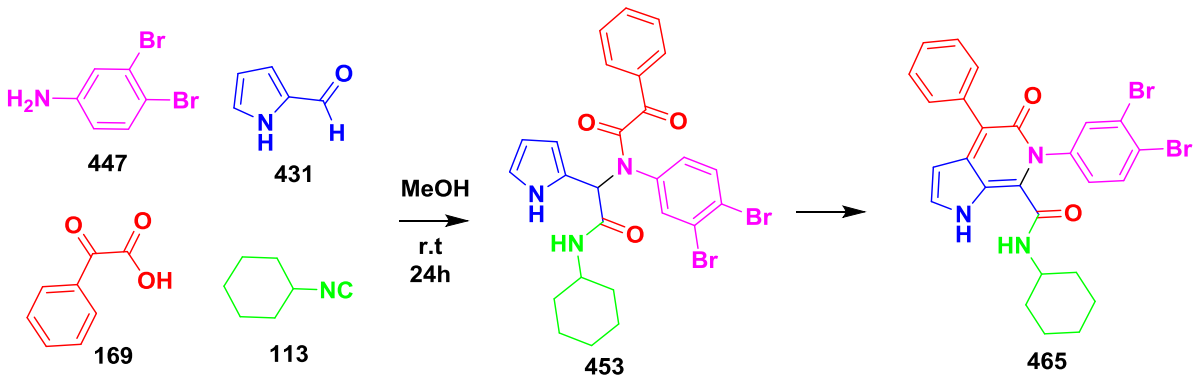


Figure 35: Some compounds containing pyrrolo-pyridine possess pharmacological features.

6.1.5.1. Synthesis of pyrrolo-pyridine derivatives

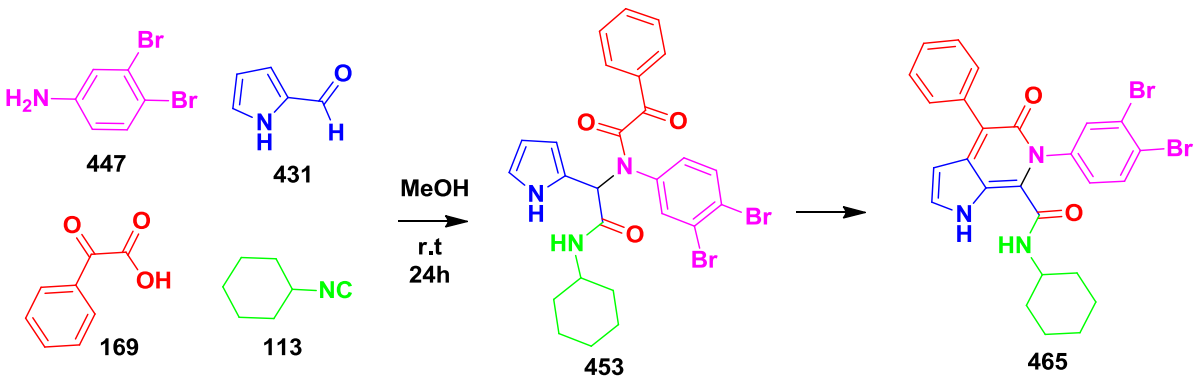
Although pyrrolo-pyridine derivatives have a broad range of pharmaceutical properties, limited research have been conducted to develop efficient methodologies for the synthesis of these compounds. Therefore, in this project, a mild and an efficient one-pot reaction was developed for synthesis of novel heterocyclic compounds having the pyrrolo-pyridine core as an extension of post-transformation of Ugi products. The procedure includes simply synthesis of Ugi adducts (Ugi 4C) then followed by post-transformation of Ugi adducts under acidic conditions to furnish pyrrolo-pyridine derivatives (Scheme **76**). The model reaction was conducted using 2-oxo-2-phenyl acetic acid **169**, 3,4-dibromoaniline **447**, cyclohexylisocyanide **113** and pyrrole-aldehyde **431** (Scheme **78**).



Scheme 78: The model reaction for synthesis of pyrrolo-pyridine **465**.

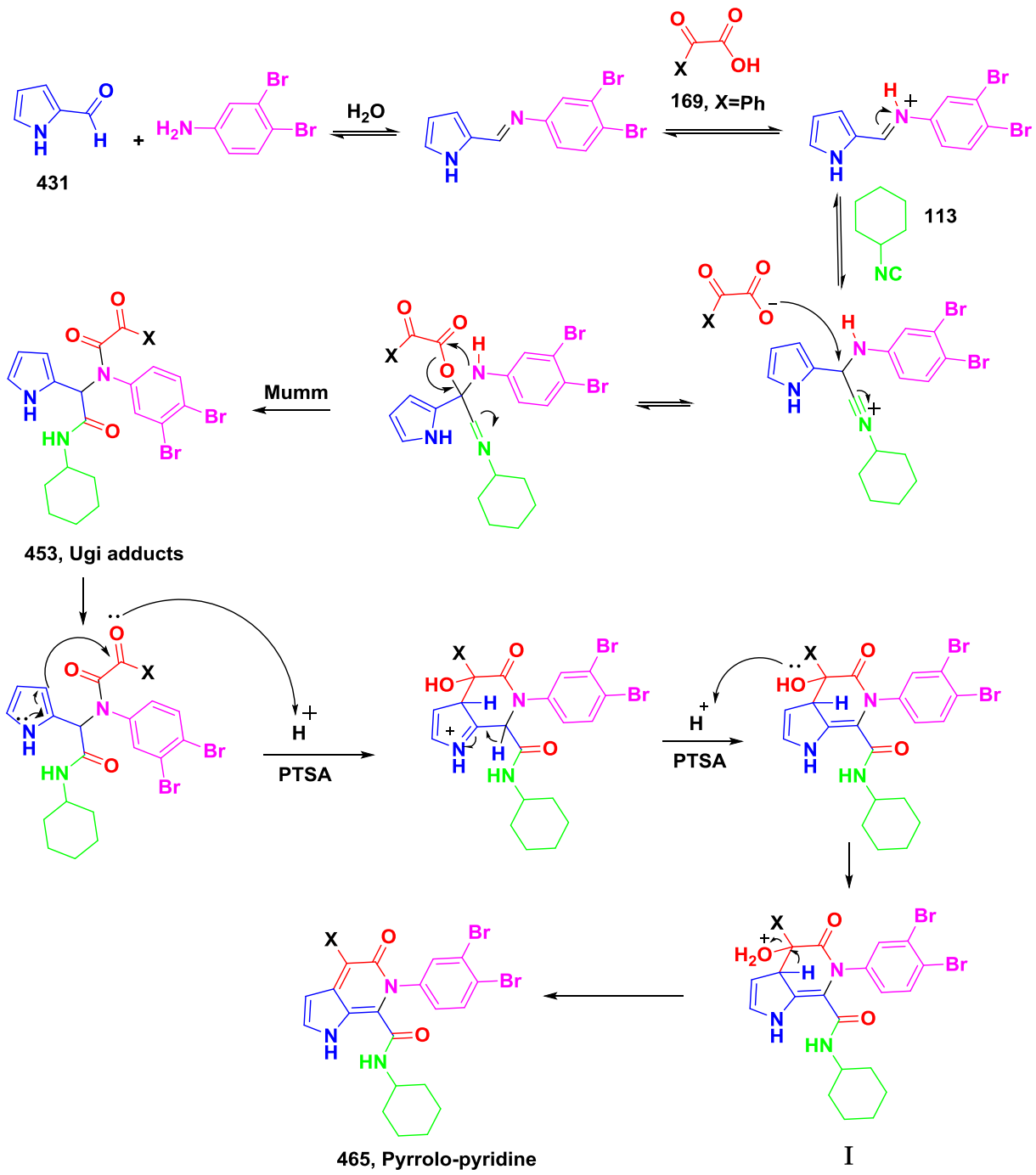
It was found that when the Ugi reactions were carried out in methanol at room temperature for 24 h, high yield of the Ugi adducts was afforded. In order to synthesise pyrrolo-pyridine derivatives, the Ugi adduct was then subjected to cyclization reactions under basic or acidic reaction conditions. The model reaction was firstly conducted in the presence of a base such as K_2CO_3, Na_2CO_3 or NaOH in methanol at 60 ºC for 24 h (Table **6**. **5**, entries **1-4**). The desired product was not obtained but when the reaction was subjected to range of acidic conditions the desired product was afforded. The Ugi adduct was subjected in the presence of TBACl and acetic acid with yields of 15% and 55% obtained respectively (Table **6**. **5**, entries **5** and **6**). In the presence of *p*-toluene sulfonic acid (PTSA) as an acidic catalyst in methanol at 60 ºC for 2 h, the yield of product was 80% (Table **6**. **5**, entry **8**). The conditions were optimized for using PTSA as efficient catalyst for cyclisation reactions. In the presence of PTSA (20 mol%), the yield of product decrease to 61% (Table **6**. **5**, entry **7**). From the optimized conditions it was found that PTSA (40 mol%) is the best amount of catalyst to acquire the condensation reaction of the Ugi adduct with highest yield of 80%.

Table 6.5: The optimized conditions for synthesis of pyrrolo-pyridine **465**.



Entry	Catalyst	Solvent	Temperature	Time	yield %
1	K_2CO_3	methanol	60ºC	24 h	-
2	Na_2CO_3	methanol	60ºC	24 h	-
3	NaOH	methanol	60ºC	12 h	-
4	NaOH	methanol	60ºC	24 h	-
5	TBACl	methanol	60ºC	24 h	15
6	AcOH	methanol	60ºC	8 h	55
7	PTSA(20mol%)	methanol	60ºC	2h	61
8	PTSA(40mol%)	methanol	60ºC	2h	80
9	PTSA(50mol%)	methanol	60ºC	2h	56

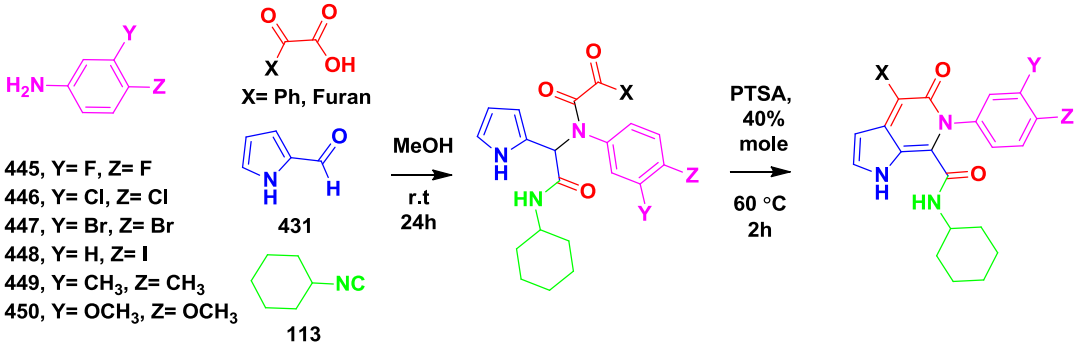
The proposed mechanism for the formation of Ugi adduct (**453**) and pyrrolo-pyridine derivative (**465**) is depicted in Scheme **79**. The possible route for the formation of Ugi products is followed according to Ugi strategy[64]. The Ugi adduct (**453**) formed as illustrated in Scheme **78** *via* Ugi reaction. It was then activated by subjecting to PTSA catalyst. Initially, the *α*-keto group of the Ugi adduct was protonated and the pyrrole ring has undergone electrophilic addition to generate the intermediate **I**. Under the acidic condition the intermediate **I** undergoes dehydration step to yield pyrrolo-pyridine derivatives.

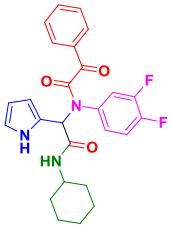
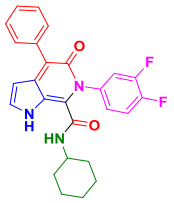
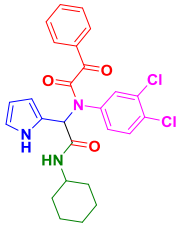
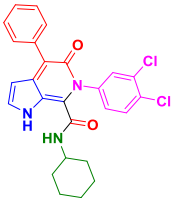
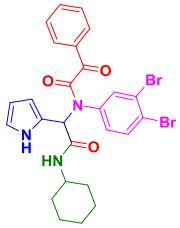
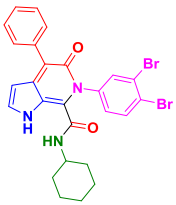
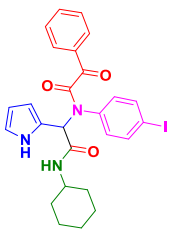
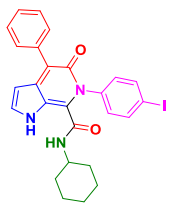


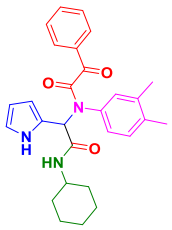
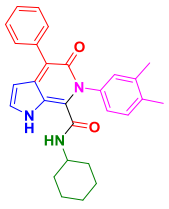
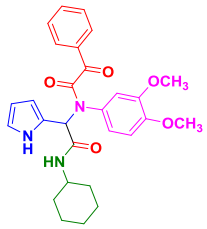
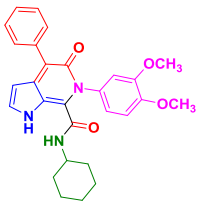
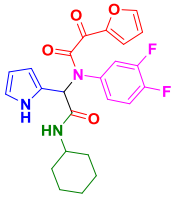
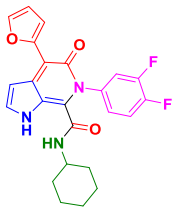
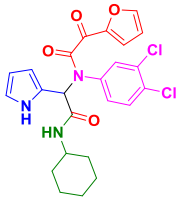
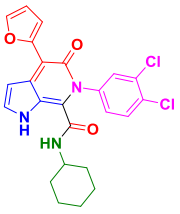
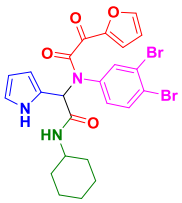
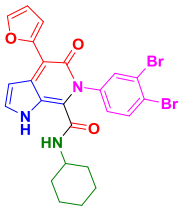
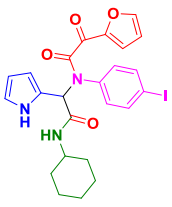
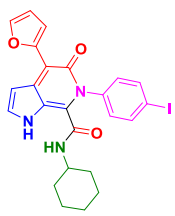
Scheme 79: The mechanism for the synthesis of pyrrolo-pyridine derivatives.

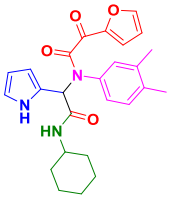
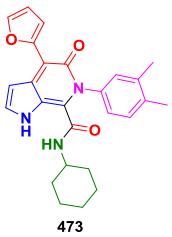
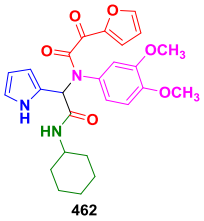
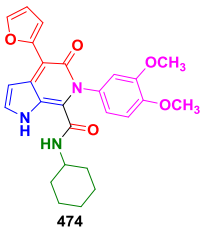
Under the optimised conditions, the reaction was further applied for a variety of Ugi adducts that were synthesized by various anilines, 2-oxo-2-phenylacetic acid **169**, 2-(furan-2-yl)-2-oxoacetic acid **201**, cyclohexylisocyanide **113** and pyrrole-aldehyde **431**. In all cases, the Ugi multicomponent reactions and their post-condensations proceeded smoothly, providing the corresponding Ugi adducts and pyrrolo-pyridine derivatives in moderate to good yields (51–80%) as summarized in Table **6.6**.

Table 6.6: The Ugi adducts and their pyrrolo-pyridine derivatives.



Entry	X	Y	Z	Ugi adducts	Yield[a]	Pyrrolo-pyridine	Yield[a]
1		F	F	 451	59%	 463	64%
2		Cl	Cl	 452	81%	 464	78%
3		Br	Br	 453	85%	 465	80%
4		H	I	 454	66%	 466	77%

5		CH_3	CH_3	 455	72%	 467	65%
6		OCH_3	OCH_3	 456	55%	 468	59%
7		F	F	 457	59%	 469	55%
8		Cl	Cl	 458	80%	 470	60%
9		Br	Br	 459	80%	 471	65%
10		H	I	 460	56%	 472	55%

11		CH_3	CH_3	 461	65%	 473	67%
12		OCH_3	OCH_3	 462	45%	 474	51%

[a] yield of isolated product

Of the Ugi products, compounds **452**, **453**, **458** and **459** (Table **6**. **6**, entries **2**, **3**, **8** and **9**) were synthesised overnight at room temperature in good yields after purification by chromatography. The other Ugi products were synthesised under the same conditions with yield ranging between 45%-72%. Pyrrolo-pyridine derivatives **463-474** (Table **6**. **6**, entries **1-12**) were successfully synthesised under acidic catalysis at 60 ºC for 2 h. The results obtained suggest that the yield of the conversion from Ugi products to their pyrrolo-pyridine derivatives is affected by the terminal group of ketoacids and di-substituted group at aniline part. Therefore, the yield of pyrrolo-pyridine derivatives (**463-474**) was observed to give moderate to good yields.

Chapter 7. Anti-prion lead discovery

7.1. A general overview of drug discovery and development process

In the past most drugs were discovered either by identifying the active ingredient from traditional remedies or by serendipitous discovery. As technology and science advanced, modern drug discovery has been much more rational and benefited from the human genome project and increased understanding of the underlying molecular mechanisms of many diseases.[211] Drug discovery and development are a lengthy and capital intensive process. Generally, it takes about £1 billion and between 12-15 years to bring a drug to market from a concept. The process involves target identification, lead discovery, lead optimisation, preclinical and clinical phases (Figure **36**).

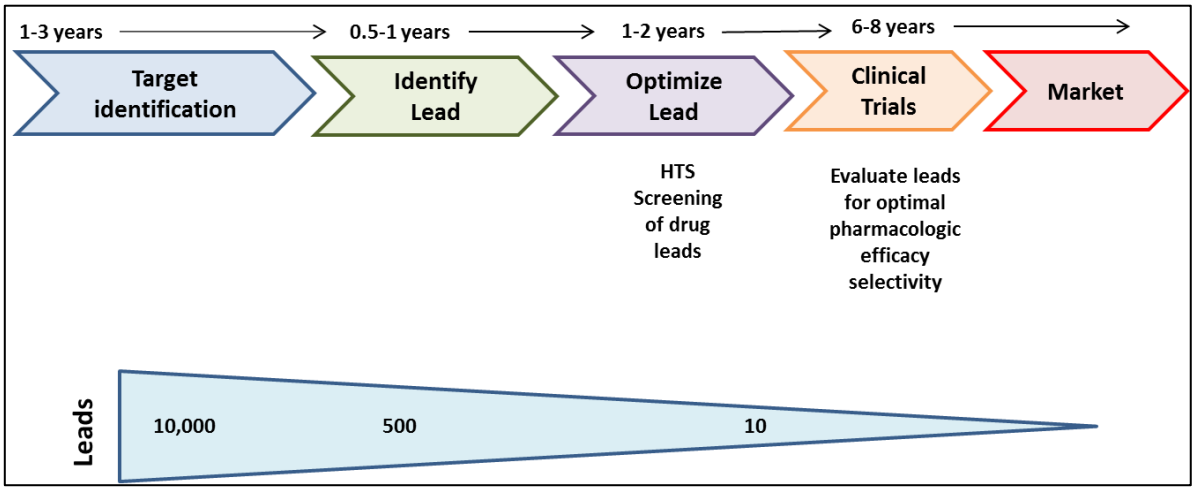


Figure 36: Shows drug discovery process.[212]

Drugs usually act on either cellular or genetic macromolecules such as proteins or nucleotides in the body known as targets, which are believed to be associated with disease. Therefore, the first step in drug discovery and development process is to study the disease and identify a specific drug target so that the appropriate screening assays can be developed for screening chemical libraries.

Lead discovery involves screening chemical libraries using the assays developed so that the initial hits can be identified. The chemical libraries have been used by pharmaceutical companies typically contain several million small molecules from organic synthesis or compounds isolated from plants and other natural products. Therefore, high throughput screening (HTS) is designed to handle the large number of compounds. The initial hits have to go through a hit-to-lead evaluation process before lead compounds are selected and then taken to full optimisation stage. Lead optimisation involves iterative cycles of design, synthesis and evaluation of the focused libraries of the lead compounds. Through this, a

detailed structure-activity relationship is established and both pharmacodynamics and pharmacokinetics profile of the lead compounds are improved from which the drug candidates are selected for preclinical studies. Preclinical development also is named preclinical studies or nonclinical studies in drug discovery. These studies take place before clinical trials in human to make sure that drugs have desired efficacy and safe to use in human. Finally, Clinical trials consist of Phase I, II, and III stages. Phase I tests the safety of the drug at different doses; Phase II is small scale trials for drug efficacy; Phase III is a large scale, double-blind randomised trial to ensure the drug has the right efficacy and safety profile for human use. A new drug can then be filed for clinical use after successful completion of Phase II clinical trial.[213]

Medicinal chemistry plays an important role in drug discovery development process. To determine if a chemical compound has certain pharmacological or biological activity, a balanced profile of chemical and physical properties that would make it drug-like, for which rules have been developed. The most famous and commonly used rule is Lipinski's rule of five, a rule of thumb to evaluate if a particular drug is likely to be orally active. The details of Lipinski's rule or 'rule of five' can be described as follows: [214]

1. The molecular weight of the compound is no greater than **500** Daltons (Ideally prefer to be **350 – 400**).

2. Lipophilicity of the compound (as calculated by the **LogP** from equation $\mathbf{log\ P = log10\ \frac{[solute]organic}{[solute]\ aqueous}}$) is no greater than **5** (Ideally should be **1 – 2.5**). Lipophilicity is defined as the ratio of the concentration of a compound in octanol to its concentration in water using the equation shown above. It represents the solubility of a compound in fats, lipids, oils, and non-polar solvents. For ionisable compounds **Log *D*** at a given pH is commonly used.

3. The compound has no more than five hydrogen-bond donors. Hydrogen bonding refers to an attractive force from a hydrogen atom covalently bonded to an electronegative atom (donor) and an electronegative atom (acceptor). The hydrogen bonds can be between two molecules (intermolecular) or exist within molecules (intramolecular). These bonds are very important for determining the interaction with its target protein.[215]

4. The compound has no more than ten hydrogen-bond acceptors.

The first, third and fourth rules can be directly calculated from a structure of compound. The Log *P* can be computationally predicted or experimentally determined. Other properties such as aqueous solubility, plasma-protein binding, permeability and rate of metabolism can be

affected by the LogP. A low LogP indicates that the compound is very hydrophilic (less lipophilic) therefore can pass through membranes of cell by diffusion to reach the drug target.[216] It should be mentioned that some compounds such as natural products do not follow the Lipinski's rule. These compounds can be transported across the cellular membranes *via* active transporter or specific protein carriers.

7.2. An overview of prion diseases

Transmissible spongiform encephalopathies (TSEs), also known as prion diseases, are a group of fatal neurodegenerative diseases which affect a wide variety of mammalian species. These diseases are potentially classified as inherited, sporadic and iatrogenic diseases. Humans prion diseases include Creutzfeldt-Jakob disease (CJD)[217] CJD (vCJD), [218] familial fatal insomnia (FFI), [219] Kura and Gerstmann-Straussler-Scheinker syndrome (GSS).[220] Animals TSEs disease include bovine spongiform encephalopathy (BSE) in cattle [221], scrapie in sheep [222] and chronic wasting disease (CWD) in moose and deer .[223]TSEs are developed slowly over a period of months or even years. [224] In 1982, Stanley Prusiner suggested that the neuropathology of the disorders is caused by non-nucleic acid-containing transmissible particles. He named these transmissible particles 'proteinaceous infectious particles' or 'prion' and thus TSEs also became known as prion diseases.[225]

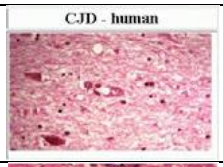
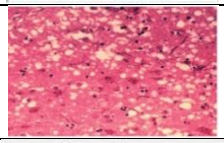
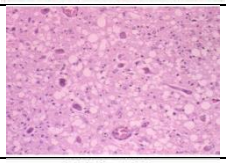
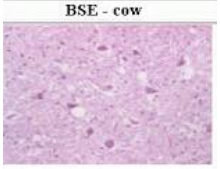
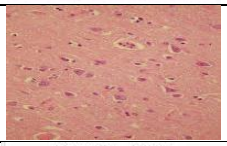
TSEs such as scrapie and BSE are usually spread through by infections. The cattle epidemic has claimed approximately 200000 BSE-affected animals and caused the slaughter of huge numbers of healthy animals in potentially infected herds.[226] BSE was reported to pass onto humans by the intake of BSE contaminated food.[227] The Creutzfeldt-Jakob disease (CJD) caused *via* dietary exposure to BSE prion is one of the most fatal prion diseases recently discovered in human. It was recorded that the average annual mortality from CJD type between 1990-2011 in England, Scotland and Wales were 0.99, 1.08 and 1.20 per million people per year respectively.

By the end of 2011, 176 cases in the United Kingdom were infected by vCJD and all of these cases were fatal. vCJD accounts for 15% of CJD cases. The rest, 85% of CJD occurs as sporadic and inheritable forms in nature. The hallmark of all prion diseases involves the post-translational conversion of a normal cellular protein (PrP^{C}) to an abnormal isoform (PrP^{Sc}).

Prion diseases have been categorized into three forms: First, sporadic form which has no known cause; the second is familial form that is caused by genetic mutations and the third is transmissible form where the disease can be passed on from one individual to the other.[225]

The abnormal prion isomer PrP^{Sc} can be transmitted by using contaminated growth hormone and from infected cadavers.[228] Table **7.1** shows some types of prion diseases and hosts.

Table 7.1: Some types of prion diseases in humans and animals.[229]

Prion disease	Host	Mechanism of pathogenesis	Histological characteristics
Familial CJD	Humans	Germline mutations in the PRNP	
GSS	Humans	Germline mutations in the PRNP	
Kuru	Humans	Infected through ritualistic cannibalism	
CWD	Deer, Elk	Infection	
BSE	Cattle	Infection or sporadic	
EUE	Kudu, Nyala	Infection with prion-contaminated MBM	
Scrapie	Sheep, goat	Infection with prions from sheep or cattle	

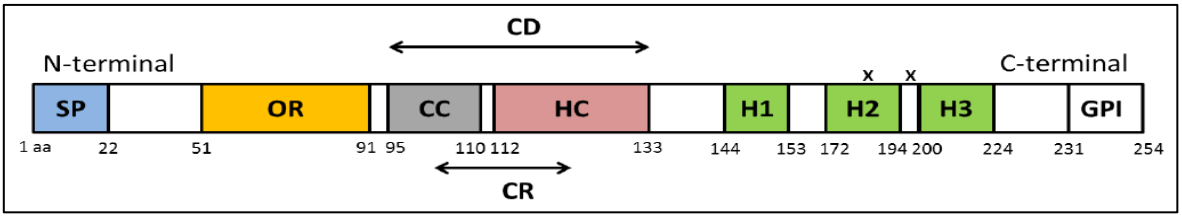
7.3. The prion protein (PrP^{C})

The prion protein, PrP^{C}, is widely expressed in the human body, situated on the extracellular side of cell membranes, especially the central nervous system and exists as a glycosolated cell-surface protein in neurons,[230] peripheral blood vessels, bone marrow and many other cells of the body.[231] The function of PrP^{C} is on the upkeep of white matter in the brain, response to oxidative stress and formation of neurons.

7.3.1. The structure of prion protein (PrP^C)

The structure and function of normal isoform protein (PrP^C) has attracted much attention in the past ten years to gain better understanding of how the normal isoform (PrP^C) transforms into the abnormal form protein (PrP^{Sc}) leading to a family of neurodegenerative diseases including scrapie, BSE, CJD and CWD etc. The physiochemical properties of (PrP^C) and (PrP^{Sc}) are different even thought that the primary structures of both proteins are the same. PrP^C has been observed as a globular protein that is soluble and can be easily broken down by proteinase K while, PrP^{Sc} is insoluble, possesses high resistance to proteinase K and forms amyloids.

NMR and X-ray crystallography studies showed that the structure of PrP^C has distinct domains: an unstructured, flexible N-terminal domain, a globular C-terminal domain and a glycosylphosphatidyl-isositol (GPI) anchor [232] (Figure **37**). The *N*-terminal domain (amino acids **23-110**) contains five octorepeats (**AA51-91**) and the *C*-terminal domain (**AA111-230**) contains two glycosylation sites (**Asn181, Asn197**).[233] The glycosylphosphatidyl-isositol anchor is connected to a serine residue (**Ser 230**) and is responsible for attaching PrP^C to the cell membrane.[234]



The human prion protein. SP indicates the signal peptide. OR referes to the octapeptide repeats. H1 to H3 denote the three α- helices. CD shows the central domain and CR denotes the central region that contains charged clusters (CC) and the hydrophobic core (HC). The glycosylphosphatidylinostol (GPI) anchor is abbreviated GPI. The region of crosses represents the glycosylation residues at amino acids 180 and 196.

Figure 37: The prion protein of human cells.[235]

GPI anchor is responsible to anchor the PrP^C to the detergent-rich domain of the membrane. The PrP^C is a cellular membrane protein and it has two trans-membrane isoforms that are identified as C trans-transmembrane-PrP (^{Ctm}PrP) and *N* trans-transmembrane-PrP (^{Ntm}PrP) based on their sequential orientation in relation to the lumen of the endoplasmic reticulum (ER). The *N*-trans-transmembrane-PrP (^{Ntm}PrP) possesses the opposite orientation while, the C trans-transmembrane PrP (^{Ctm}PrP) possesses its own COOH-terminus in the endoplasmic reticulum (ER).[236] Both isoforms are involved in the conformational conversion.

7.3.2. Normal physiological function of PrP^C

The exact role of PrP^C in a cell is still unclear. The prion protein is encoded by *PRNP* gene which have been shown in birds,[237] reptiles, [238] and amphibians.[239] A comparison analysis of forty mammalian *PRNP* genes indicated that high degree of conservation between species, assuming that its encoded protein PrP^C has an important physiological function.[229] The residues of glycine in the glycine rich region of the hydrophobic core of PrP^C have also been exhibited to be efficiently conserved across fifteen mammalian species.[240] The study of a PrP^C knockout mice model may help to explain the function of the normal isoform (PrP^C) protein.[241] The resistance of the PrP^C mouse knockout to the infection by prions and their incapability to duplicate prion is the most striking character of the PrP^C null mice. Other functions of PrP^C have been suggested from mouse models include its roles in sleep regulation by managing oxidative stress *via* copper binding, mitochondrial activities, superoxide dismutase (SOD), immune system function, neuroprotection, neuronal excitability, informing behaviour and stem cell biology.[241] Further studies in prion knockout mice have also showed a wide range of effects including disruption of olfactory behaviour,[242] tooth development,[243] iron transport,[244] glucose tolerance[245]and reformation of adult muscle tissue.[246] An autoimmune disease in experimental autoimmune encephalomyelitis (EAE) model showed to be more severe in PrP^C knockout mice.[247] It seems that the function of PrP^C as a negative regulator of T-cell receptor signalling is critical subject to the development of EAE. A critical role for PrP^C has been suggested in maintaining the integrity of the CNS in times of stress. Knockout mice were exhibited to gain lower levels of anxiety under artificially generated stressful conditions and the results suggested that PrP^C also has a role in managing the response to stress at a systems level.[248]

The other function of cellular PrP^C is that it promotes the survival of neuronal and non-neuronal cells. The PrP^C has been found to have a cytoprotective function by reducing the apoptotic rate after exposure to apoptotic stimuli.[249] It was suggested that PrP^C protects against oxidative stress, possibly through its capacity to bind copper.[250]*In vitro* studies of rat pheochromocytoma cells showed that those resisted to oxidative stress or copper toxicity have higher levels of PrP^C.[251]There is also general views, which are widely supported, that PrP^C is beneficial protein in synapse regions and is possibly involved in synapse formation.[252]The important function of PrP^C in the synapses has been assumed on the basis of its copper binding ability, and it is suggested that it can significantly modulate synaptic activity and neuronal excitability.[235]PrP^C may be involved in synaptic transmission process

via helping neurotransmitter release, controlling the Ca^{+2} flux, participating in altering expression of neurotransmitter receptors and long term potentiation.[253]

After exposure to apoptotic stimuli, it was found that PrP^{C} has a cytoprotective role.[249] This role is thought to come from the copper binding to PrP^{C} which modulates neuronal synaptic and excitability activity. In other words, the PrP^{C} is involved in synaptic transmission *via* assisting neuro-transmitter release, changing the expression level of neurotransmitter receptors and the controlling the flow of Ca^{+2}.[253] Furthermore, it has been found that the PrP^{C} is involved in the immune cells such as dendritic cells and the adaptive immune organelle, T lymphocytes.[254] The role of PrP^{C} is also to modify the responses of immune cell precursor and to promote the formation of the T-cell receptor (TCR) complex.[255]

7.4. Prion protein and diseases

7.4.1. Protein misfolding diseases

All prion diseases are caused by protein misfolding. In these diseases, the prion protein is found to be highly aggregated and forms ordered fibrils (cross-β spine or amyloid). Protein misfolding is a common cellular event where misfolded proteins are eliminated *via* the endoplasmic reticulum-combined degradation (ERAD) route. Notably, the diagnosis of the prion disease is dependent on the accumulation of the misfolded isomer of PrP^{C} which is known as PrP^{Sc}.[256] The insolubility of PrP^{Sc} under physiological conditions has made its characterisation extremely difficult. The structure of PrP^{Sc} amyloid remains unknown. Negative-stain electron-microscopy on two-dimensional crystals of N-terminally truncated PrP^{Sc} and a miniprion have been used to make models of PrP^{Sc}.[257] FTIR studies and CD spectroscopy, it suggested the secondary structure of PrP^{Sc} is significantly different to that of the PrP^{C}[258], with 30% α-helical character and 42% β-sheet (Figure **38**).

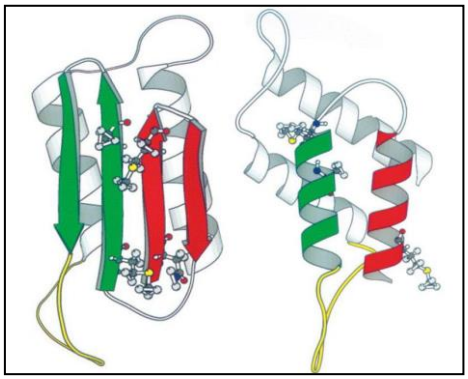


Figure 38: The normal cellular of PrP^{C} (**right**) and the abnormal isoform of PrP^{Sc} (**left**).[259]

7.4.2. **The protein-only hypothesis**

The infectious nature of prion diseases was firstly discovered when a herd of sheep were accidently vaccinated with a vaccine contaminated with the scrapie agent and 10% of the herd went on to develop scrapie.[260] Scrapie was successfully transmitted then subsequently to mice.[261] An infectious way for the human disease (Kuru) was also identified in the late 1960s *via* the demonstration of the disease which could transfer from humans to monkeys. [262] The infectious agent was suggested to be a kind of slow virus due to the long incubation times but the finding that the infectious agent was resistant to methods known to destroy nucleic acids cast doubt on this theory.[263] There was an early hypothesis that the infectious molecule may be a protein in the late 1960s but this was not discovered further until the term 'prion' was coined by Stanley Prusiner in 1982.[225] He suggested that the infectious agent was a 'proteinaceous infectious particle', also named as the protein-only hypothesis.

The protein-only hypothesis was proposed based on a fact that protease-K resistant PrP^{C} corresponds directly to infectivity.[264] This abnormal protein PrP^{C} was termed PrP^{Sc}, due to its association with scrapie, and the protease-resistant core was named PrP^{res} or $PrP^{27\text{-}30}$ due to its molecular size. Both isoforms of the protein were shown to be encoded by the *PRNP* gene. The direct connection between the infectious agent, PrP^{Sc}, and the normal cellular form of the protein[265] , PrP^{C}, is evident from the fact that transgenic mice which did not express the PrP^{C} were resistant to infection with PrP^{Sc} reinforcing the importance of the normal cellular isoform in disease pathogenesis. Moreover, discovering that the infectivity can be propagated in mouse neuroblastoma cells vaccinated with both brain homogenate and PrP^{Sc} derived from infected brains and spleens gave further evidence to support the theory[266].

The experiment demonstrating that PrP^{Sc} was able to induce the conversion of PrP^{C} in a cell free system[267] was key evidence to support the protein-only hypothesis, and these initial investigations have been carried out using Protein Misfolding Cyclic Amplification (PMCA) assay.[268] The infectious prions which can be formed *in vitro* are an essential piece of evidence that is required to support the protein-only hypothesis. Synthetic prions that were proved to induce neurodegenerative disease in mice that overexpressed PrP^{C} were firstly furnished in 2004.[269] A further study employed PMCA to produce prions from an initial mix of PrP^{C} and PrP^{Sc} and these prions were able to initiate neurodegenerative disease in some wild animals.[270] The prions produced in the presence of phosphatidylethanolamine and RNA being around 1 million fold more infective than 'protein-only' prions demonstrating the importance of co-factors.[271] The function of co-factors is not believed to negate the

hypothesis of protein-only as the main mode of disease transmission is still protein based. It has been recently suggested that the definition of the term 'prion' should be extended to a wider range of neurodegenerative diseases even though some of them are not typically thought of as infective.[272] It has therefore been proposed that the definition of prion should be updated to proteinaceous-nucleating particle to surround the pathological events which occur in non-infectious protein opathies and place all these conditions under the umbrella of the prion subject.

7.4.3. The conversion of PrP^{C} to PrP^{Sc}

A variety of hypotheses have been suggested on how PrP^{C} converts to PrP^{Sc}. One of these suggested that it is a virus which causes this conversion as initially proposed for scrapie.[273] Later when the scrapie was found to have no connection could with any viruses, it was proposed that the disease comes from proteinaceous infectious particles. The infectious particles are inactivation resistant due to the post-translational conformational change of PrP^{C} to PrP^{Sc} and the abnormal protein (PrP^{Sc}) can be detected when an individual is infected with a prion disease (Figure **39**).

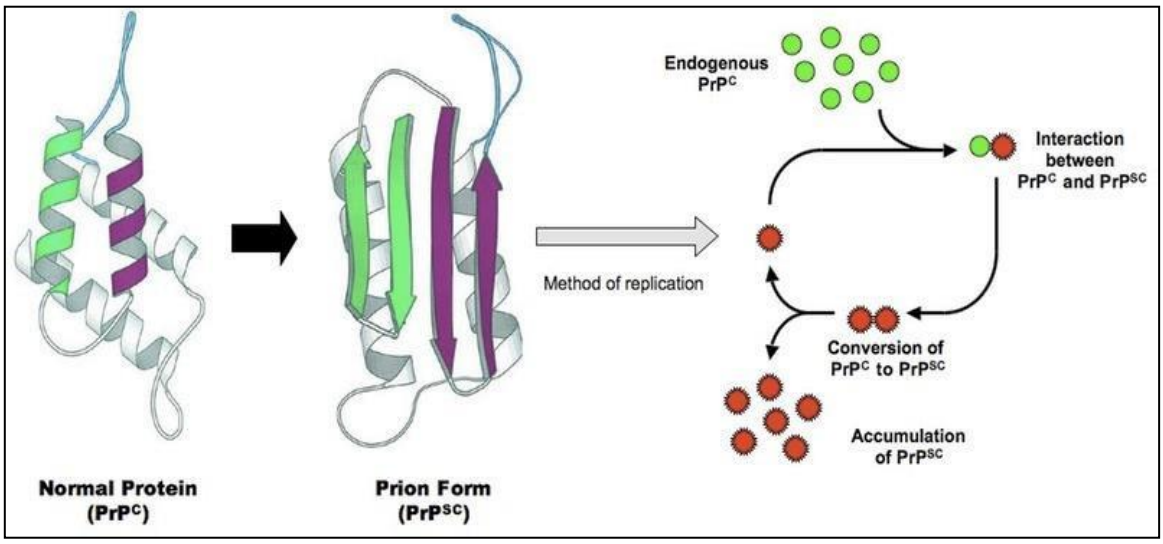


Figure 39: The conversion of the normal isoform (PrP^{C}) to the abnormal isoform (PrP^{Sc}).[274]

This conversion involves the structural change of α-helices into β-sheets.[235] The normal isoform (PrP^{C}) consist of 43% α-helices with no β-sheet structures, whereas the abnormal isoform (PrP^{Sc}) consist of 20% α-helices and 34% β-sheets due to two out of four the PrP^{C} helices plus extended residues being transformed to β-sheets.[275],[276] The precise structure of

PrP^{Sc} has not been completely determined due to its insolubility. However, the high content of β-sheet in PrP^{Sc} produce protease resistant aggregates within the brain. Proposed structural models of PrP^{Sc} and shown in Figure **40**.[277] The β-helix model (Figure **40a**) was generated from electron crystallography data.[278] The β-spiral model is proposed as shown in Figure **40b** which comes from the molecular dynamic simulations.[279] The assembly of the β-helix and the β-spiral models is thought to occur within the unfolded *N*-terminal region of PrP^{C}, however the α-helices is a preserved characteristic of both PrP^{C} and PrP^{Sc}. Figure **40c** shows β-sheet model as an extended register where a complete refolding of the protein occurs. In addition, this model has been suggested by using hydrogen-deuterium exchange coupled mass spectrometry (HX-MS) of brain-derived PrP^{Sc}[280] or recPrP.[281]

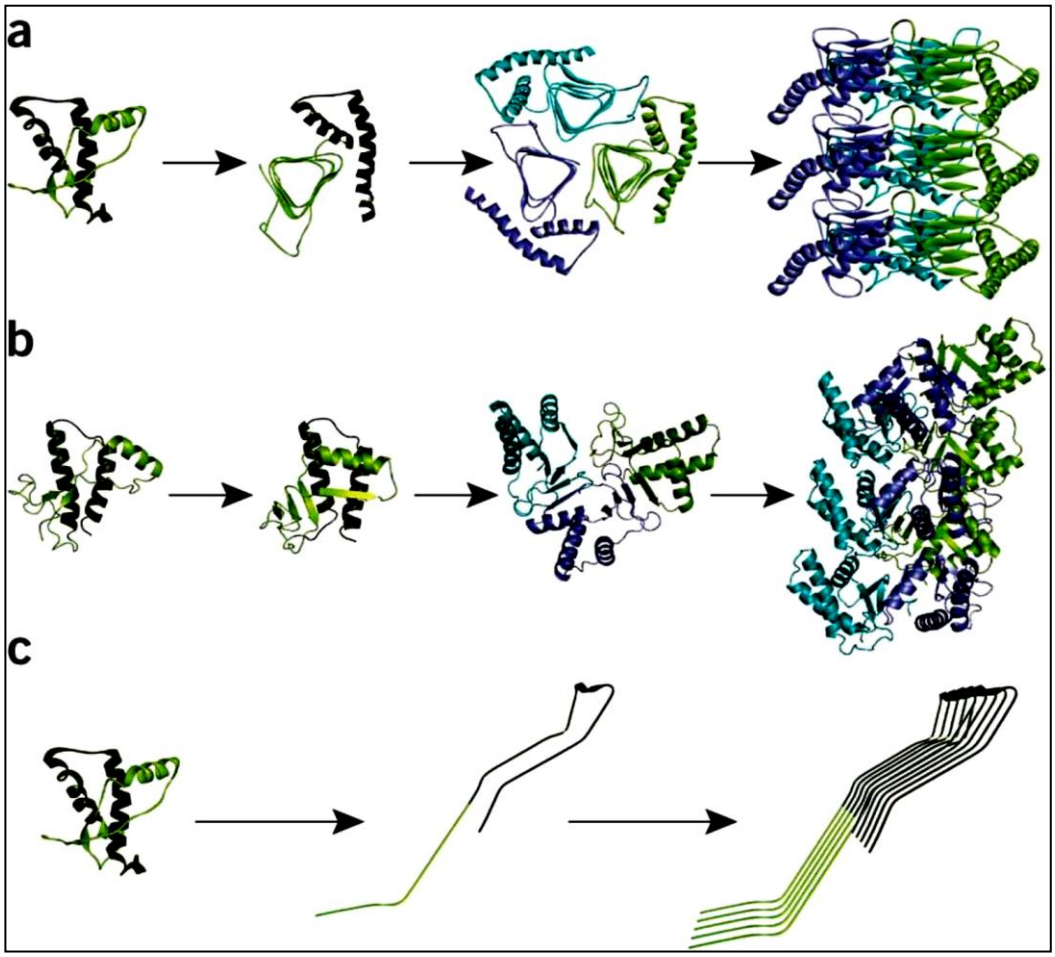


Figure 40: Schematic representation of various models for the suggested structure of PrP^{Sc}.[277]

The interaction between PrP^{C} and PrP^{Sc} occurs from an initial contact and this interaction drives to the further conversion of native PrP^{C} to PrP^{Sc}. It is believed to be the main

pathogenic event which leads to neurodegeneration, although the mechanism of transformation of PrP^{C} to PrP^{Sc} is yet to be fully understood[241] and two theories have been proposed. Misfolding of endogenous host PrP^{C} can be caused by PrP^{Sc}: the first model is the template directed refolding and the second model is the non-catalytic nucleated polymerisation seeding model.[221] Figure **41a** shows the template directed model which suggests that the formation of PrP^{Sc} begins by a catalytic cascade involving PrP^{C}. It is thought that this process would spontaneously occur and be assisted by chaperone activity and energy.

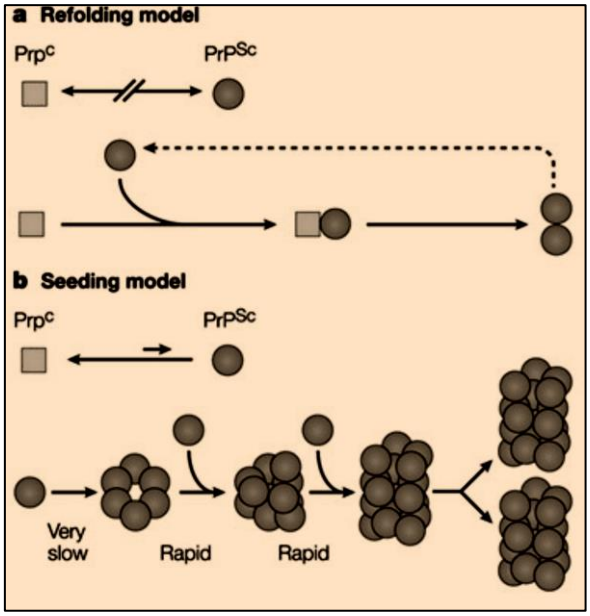


Figure 41: Models for the conversion of PrP^{C} to PrP^{Sc}, **a**: the template directed model, **b** non-catalytic nucleated polymerisation model.[282]

The second model is also named the seeding model which includes the thermodynamic control of a conformational change. Conversion of PrP^{C} to PrP^{Sc} and initial aggregation are the rate limiting step (Figure **41b**). The aggregation process is substantial for both transformation and in supporting the nucleation theory which includes a lag phase.[283] The conversion of PrP^{sc} from PrP^{c} is an autocatalytic process and only a small amount of PrP^{Sc} is needed for formation of extended aggregates of PrP^{Sc} (Figure **42**).

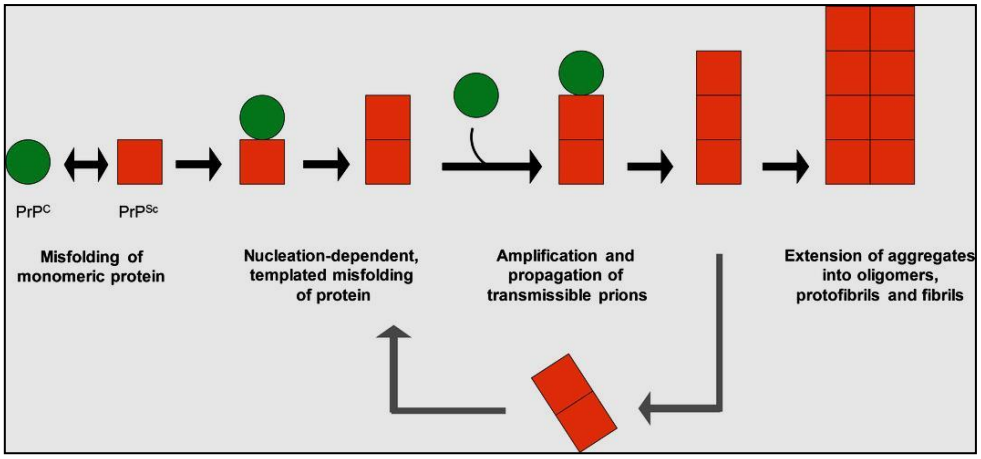


Figure 42: The Mechanism for PrP^{Sc} seeding.[284]

7.5. Therapeutic strategies and development

Both PrP^{C} and PrP^{Sc} can be targeted by therapeutic agents and anti-prion effects have been measured using three different assays models. In the *in vitro* assay model, PrP^{C} and PrP^{Sc} are incubated under specific conditions to enable the measurement of the cell-free conversion of normal prion PrP^{C} to its abnormal isoform PrP^{Sc}.[267] In this methodology PrP^{C} from the crude brain homogenates was mixed with PrP^{Sc} seeds and misfolding cyclic amplification was performed resulting in PrP^{Sc} in higher yield.[285] The second methodology is also an *in vitro* assay which uses cellular infectious phenotypic prions models where prions can be propagated by infected cell cultures. Prion infection in most cases does not display any visible morphological or pathological changes to the cell cultures.[286] Chemical libraries can be screened for their abilities in reducing the propagation and PrP^{Sc} production.[287] In the third methodology, is the *in vivo* model which involves the animals that are infected with prion diseases and this model is commonly used to investigate the therapeutic effects of potential therapeutics in the whole organism.[288]

Although a variety of compounds have been investigated using these assays, only two have been taken to clinical trials, pentosane polysulphate (PPS) and quinacrine. PPS trials used interventricular administration and an increased survival rate was seen when compared with in over quinacrine.[289] PPS treatment has been reported to stop neuro-pathological alteration in the brain. It is important to develop therapeutics to treat the prion disease after the appearance of symptoms. This treatment includes stopping the neurotoxic effects of the infection, inhibiting PrP^{Sc} formation, promoting the recovery of lost functions and destabilising existing PrP^{Sc}.[290] Recently, the dissociation of toxic PrP^{Sc} from infectivity suggested that unidentified toxic oligomeric species could be significant targets.[291]

7.5.1. **Clinical studies of existing drugs** (***in vivo***)

Anti-prion compounds are effective *via* different mechanisms. The PrP^{C} and/or PrP^{Sc} can be maintained by compounds which bind one or both isoforms and preventing the conversion or the aggregation. Limitation of available PrP^{C}, either from knocking it out or from altering composition in the cell membrane to remove it from the cell surface caused removing the PrP^{C} as a substrate for conversion. A small number of anti-prion therapeutics has been reported to exhibit some activity against prion diseases and their structures can be seen in Figure **43**.

Clinical trials for selected compounds have had a little success even for the early stages of the disease even though they showed modest delay on disease onset or death in pre-clinical *in vivo* studies. PERK inhibitors were shown to be a class of promising compounds in treating infected animals.[292]The role of these compounds reverses the shutdown of global protein formation caused by activation of the PERK/eIF-2α branch of the unfolded protein response (UPR), therefore preventing neurodegeneration and restoring formation of synaptic proteins.

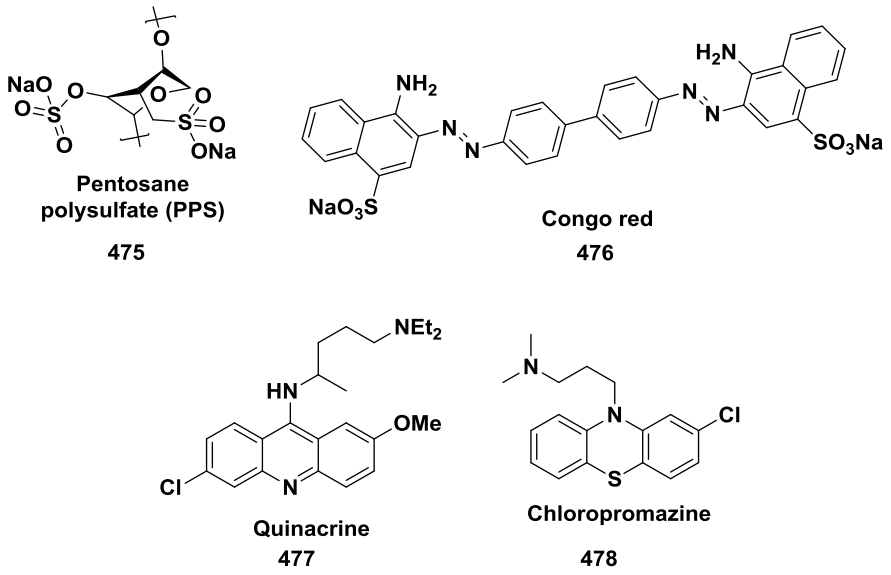


Figure 43: Structure of known anti-prion therapeutics in clinical studies.

Pentosane polysulfate (PPS) **475** was initially shown to have anti-prion activities in prion infected all culture studies.[293] The PPS **475** is a polyanionic compound and is able to bind to PrP^{C} *in vitro*, but only effective *in vivo* when administered soon after infection and before the onset of neuro-invasion. The PPS **475** can block the accumulation of PrP^{C} in scrapie-infected neuroblastoma cells and lengthen the incubation period of scrapie in mice after peripheral inoculation.[294] Also, PPS **475** has shown a prolonged survival time in clinical trials by minimising the available concentration of PrP^{C}, hence reducing the conversion.[295] Congo

red **476** is an azo dye compound that is able to stack extensively and it has a similar mechanism to the PPS **475**.[296] Congo red **476** and its analogues have been widely studied in order to improve their anti-prion activities and investigate the structure-activity relationship (SAR).[297],[298] Congo red **476** has been reported to work by binding to and stabilising PrP^{C}, by Surface Plasmon Resonance (SPR).[299] It was observed that Congo red **476** decreases the *β*-sheet content in rhPrR, increases the susceptibility to PK digestion [300], stabilises the structure, renders PrP^{Sc} resistance to denaturation, and reduces the potential for further conversion.[301] Quinacrine **477** is a tricyclic derivative of acridine has been tested in clinical trial further development. The quinacrine **477** can cross the blood-brain barrier (BBB) and has an EC_{50} lower than chlorpromazine **478**. Quinacrine **477** was reported to interact with PrP^{C} and hamper *de novo* PrP^{Sc} formation *in vitro* however, it was ineffective is *in vivo* studies.[302] Quinacrine **477** can accumulate in brain tissue and displays some anti-prion activity *in vitro*. Pharmacokinetic studies carried out in sheep and found that unbound extracellular concentration of quinacrine **477** too low to have real effects.[303] It was hypothesised from human clinical trial data that inadequate plasma quinacrine concentration was the reason for poor clinical outcome.[304]

7.5.2. **Pre-clinical studies of selected compounds**

A small number of anti-prion therapeutics has also been reported to exhibit some efficacy against prion diseases in animal studies and their structures are shown in Figure **44**. The tricyclic moiety in acridine **479** and phenothiazine **480** has been extensively studied in pre-clinical studies as anti-prion agents. [305]

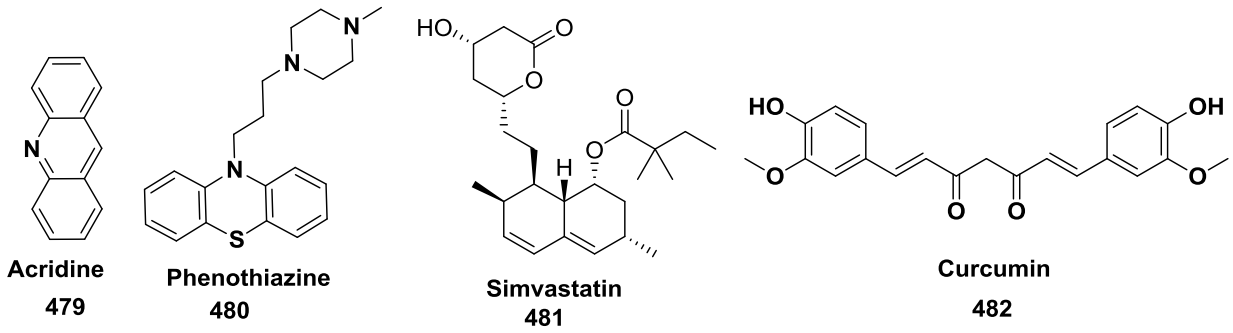


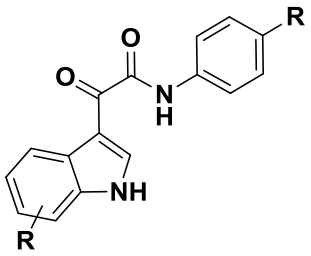
Figure 44: Structure of some anti-prion therapeutics in pre-clinical studies in animals.

Simvastatin **481** is a cholesterolic compound that can interfere the construction of lipid rafts which are important for PrP^{C} cell surface localization and play a vital role for decreasing of

cellular cholesterol and reducing the availability of PrP^{C} for conversion to PrP^{Sc}. Furthermore, the simvastatin **481** was shown to sensitize cells to quinacrine **477**, with an EC_{50} tenfold lower than in the absence of simvastatin.[306] Curcumin **482** which is derived from turmeric is well known anti-oxidative and anti-inflammatory agents and has exhibited anti-prion activity in several cell lines and mouse models because it can bind to both PrP^{Sc} oligomers and fibrils.[307],[308] DMSO has been shown to reduce accumulation of PrP^{Sc} and delay disease onset in mice when managed at an evaluated dose of 250 mg per day.[309] A high affinity was noted between a cationic porphyrin and rhPrP as anion using SPR. The resonance of light scattering and circular dichroism have been suggested to be useful factor in a therapeutic field.[310]

7.5.3. New lead discovery and screening assays (*in vitro*)

There is a wide range of screening assays *in vitro* which can be used to assess the activity of potential drug compounds. Cell based assay is a classic *in vitro* experiments and this assay can be used to assess the effects of a compound against the whole cell rather than just the isolated cellular components and to allow some properties such as toxicity to be determined.[311]Various different types of cells line have been subsequently shown to propagate PrP^{Sc} including pheochromocytoma cells, hypothalamic GT1 cells, cholinergic neuronal cells, hippocampal derived cells and cells come from the peripheral nervous system such as Schwann cells and non-neuronal cells such as fibroblasts microglia.[312] Furthermore, epithelial cells (neuroglial cells) isolated from rabbits and neuronal primary cultures isolated from mice have been developed to over express ovine PrP^{C} and are susceptible to scrapie infection.[313], [314] Cell culture models were initially used to show that PrP^{C} was the precursor to PrP^{Sc} and also demonstrate that the PrP^{C} endocytic pathway is crucial for the production of the abnormal isoform.[315] Later, they were adopted as the most common methodology to screen chemical libraries against prion infection cells (uninfected) when are exposed to an inoculum, the generation of *de novo* PrP^{Sc} can be measured by western order blotting. The inhibitory effects of compounds can be easily seen. The indole-moiety was shown to be active against prion diseases. Recently, a series of compounds (lead discovery) have been screened to evaluate their *in vitro* anti-prion activity. Indole-3-glyoxylamide moiety **483** was found to have low nanomolar EC_{50} values against a prion-infected cell line (SMB) (Figure **45**).[316],[317] Further investigations are required to allow these compounds for clinical use in treating prion disease.



Indole-3-glyoxylamide
483

Figure 45: Lead discovery as anti-prion therapeutics *in vitro*.

7.6. The cellular model used in this project (SMB cell line)

Cell culture models can be employed to investigate different aspects of prion diseases including various biochemical and physicochemical properties of normal prion PrP^{C} and abnormal prion PrP^{Sc}. These models have also been widely used for screening drugs. Initially, the cell culture was used to show that the normal prion PrP^{C} was the precursor to the abnormal prion PrP^{Sc} and also to demonstrate that the endocytic pathway of PrP^{C} is necessary for the formation of the abnormal isoform PrP^{Sc}.[318],[315] The establishment of a cell culture model supporting prion replication was firstly reported in 1970 using SMB cell line (A line of non-neuronal cells) which was isolated from the brain of a mouse infected with Chandler scrapie.[319] The models of homologous cell line indicated that PrP^{Sc} from the same species was used as the inoculum, whereas the models of heterologous cell line contained a mismatch between PrP^{C} cell line and the origin of the PrP^{Sc} inoculum.[320] The SMB cells can be treated and then infected again with other strains because in comparison with other cells they are susceptible to different prion strains individually or in combination.[320] The cell assay possesses high throughput screening capabilities and 24 compounds can be screened in three different concentrations in triplicate in a 96 well plate. The cells line is dosed with different concertation of compounds and then assessed by immunoblotting to assess the compounds which reduce the levels of PrP^{Sc} production.[312]

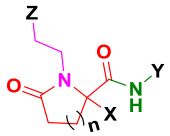
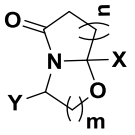
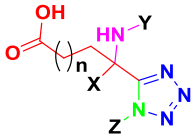
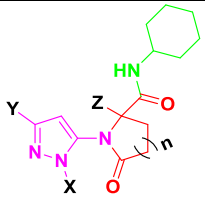
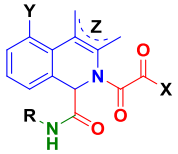
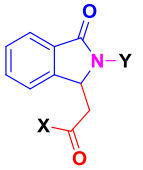
In this work, the SMB cell line assay was used to assess the effectiveness of all synthesised compounds as potential anti-prion therapeutics. The assay was used to evaluate the anti-prion activity and the cytotoxicity of the synthesised compounds. The SMB cell lines are exposed to different concentration of the compounds for 5 days before the concentration of PrP^{Sc} in the SMB cells are calculated relative to an untreated control. For an active hit, a dose-response study is carried out and an EC_{50} value was obtained. Compounds were considered to

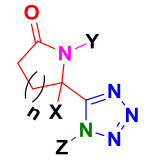
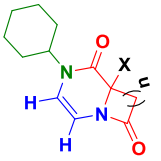
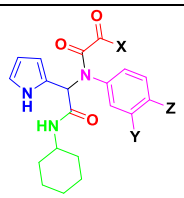
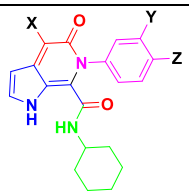
be active if the levels of PrP^{Sc} are reduced to less than 70% of that of the untreated control after **5** days, exposure.

7.7. **Aims of this project**

The aim of this part of the project is to screen and evaluate the anti-prion activity of all compounds synthesised in this work. In total, 112 compounds were screened (see their structures and screening data). There are ten different structural families amongst these 112 compounds (Table **7.2**).

Table 7.2: Generic structure of different libraries was screened.

Generic structure	No. compounds
	**226-241**
	**258-274**
	**289-298**
	**313-321**
	**333-340**
	**358-367**

	**387-396**
	**413-424**
	**451-462**
	**463-474**

The rest of the chapter outlines the detailed investigations of compounds in Ugi adducts family (**451-462**) because only compounds in this family showed apparent anti-prion activities.

7.8. Anti-prion results and discussions

7.8.1. Anti-prion activities in SMB cell line assay

The screening of the Ugi products (**451-462**) was carried out using the cell line (SMB) to determine their anti-prion activity and these details was then used to determine the SAR. The evaluation of ability of the Ugi products (**451-462**) to reduce PrP^{Sc} production in the SMB cells was initially carried out at concentrations of 1 µM, 10 µM and 20 µM respectively. A cell viability assay was carried out in tandem to ensure that hit compounds were a truly positive hit, not false positives and their anti-prion activity is not related to cytotoxicity. MTT assay was used to assess cell viability, hence the toxicity of the tested compounds. In MTT assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide **484** (MTT) stains the nucleus of viable cells. This stain was converted to a purple colour during an addition of hydrochloric acid (1M) in isopropanol that can be quantified spectroscopically in a plate reader. MTT assays were carried out to determine any cytotoxic compound by measuring the activity of living cells using mitochondrial dehydrogenases. The water-soluble MTT was

converted to an insoluble purple formazan **485** by the enzyme in viable cells that can be solubilised by acids and intensity of colour can be measured (Figure **46**).[321]

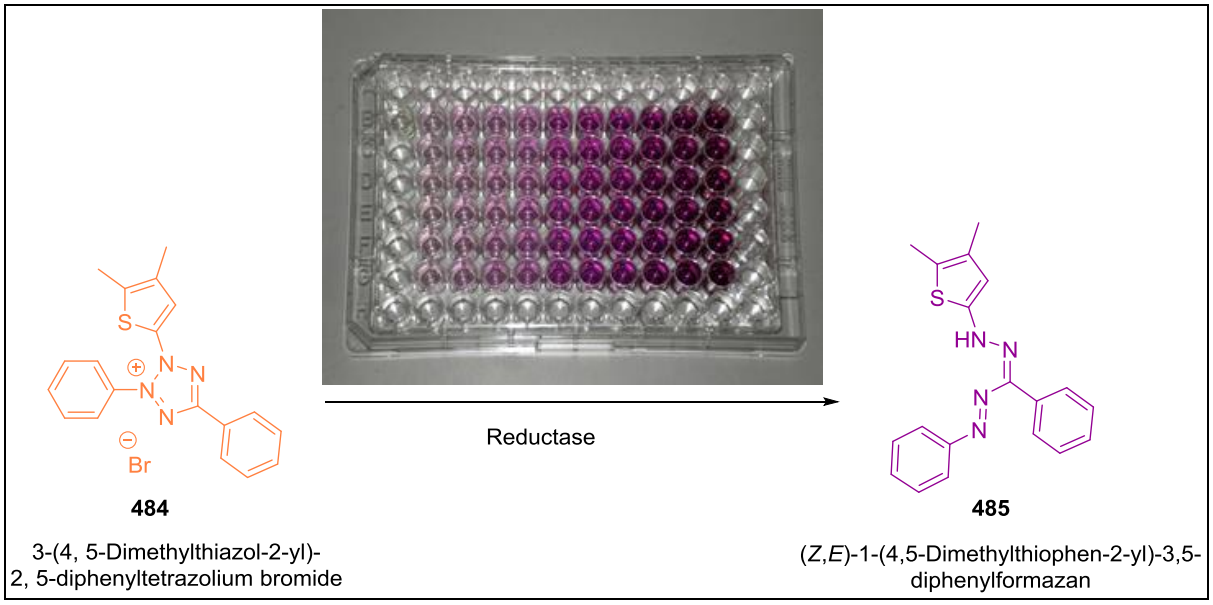


Figure 46: Mechanism of MTT assay (Colorimetric assay).

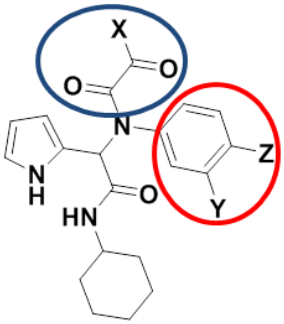
The amount of PrP^{Sc} in the cell lysate was determined using dot blot analysis and the protocol of this dot blot was optimised from a developed protocol which reported by Rudyk *et al.*[322] Positive and negative controls were involved in all screening cells. Curcumin was selected as a positive control as it has been shown to have anti-prion effects in most of cell lines studied.[308] 0.5% (v/v) DMSO was used as a negative control which has no impact on cell viability or PrP^{Sc} levels (data not shown because it shows no effects on PrP^{Sc} production). Stock solutions for all the Ugi products (**451-462**) were prepared in DMSO and concentrations for both tested compounds and controls were kept at 0.5% (v/v) to ensure that screening was conducted under same experimental conditions and effects of compounds can be normalised against the controls. Once the initial hit compounds were identified a full dose-response to determine their EC_{50} value using study for each compound was carried out nonlinear regression method. The EC_{50} value is a measure of the anti-prion activity of the compound and the term is known as half maximal effective concentration (EC_{50}) which refers to the concentration of a drug at which 50% of its effect is measured. This measurement refers to the halfway point between the baseline (no effective point) and the maximum effect after a specified exposure time.

7.8.2. Screening results

A series of heterocyclic compounds were synthesised using different techniques such as flow chemistry and microwave synthesis as described in **Chapter 9**. A majority of these compounds were successfully screened by **Dr. Jennifer Louth** using the scrapie mouse brain (SMB) cell line in a high throughput anti-prion screening assay, aiming to find active hits that reduce the production of PrP^{Sc} of SMB cells as described (**Chapter 9.15**). The data was obtained and the structure-activity relationship of these compounds was investigated. All clog P values were calculated using Chem Draw ultra **15.0**.

7.8.2.1. Initial screen results

The results from the initial screen of Ugi products were grouped into libraries based on the chemical scaffolds as shown in Figure **47**.



Library 1, X= Phenyl	Library 2, X= Furan
451= Y= F, Z= F	457= Y= F, Z= F
452= Y= Cl, Z= Cl	458= Y= Cl, Z= Cl
453= Y= Br, Z= Br	459= Y= Br, Z= Br
454= Y= H, Z= I	460= Y= H, Z= I
455= Y= CH_3, Z= CH_3	461= Y= CH_3, Z= CH_3
456= Y= OCH_3, Z= OCH_3	462= Y= OCH_3, Z= OCH_3

Figure 47: General structure of Ugi adducts libraries (**1** and **2**).

The Ugi products (**451-462**) were screened were shown to be non-toxic at screening concentration of 1μM by the MTT assay. Some compounds are only toxic at the highest concentration suggesting that all libraries **1** and **2** in the initial screen, therefore Ugi products with a di-carbonyl motif are tolerated by the SMB cells. An example of the data obtained for the initial screens is shown in Figure **48**.

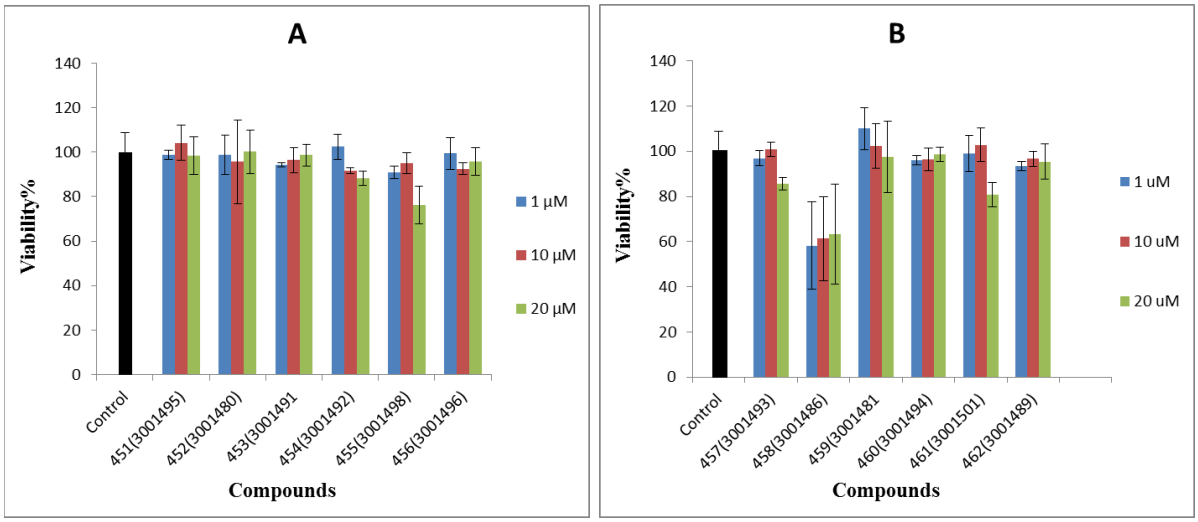


Figure 48: Initial screen data (MTT cell viability data) for Ugi adducts libraries (**A** library **1**, **B** library **2)**.

From MTT assay data seen in Figure **47**, each compound form library **1** and library **2** was screened in triplicate at 1 µM, 10 µM and 20 µM. All initial screen results were suggested and expressed as a percentage of the PrP^{Sc} signal relative to the untreated control (0.5% DMSO). The results of all initial screens were measured as a percentage of the PrP^{Sc} signal relative to the negative control 0.5% DMSO. The concentration of the positive and the negative controls used are 10 µM for curcumin and 0.5% for DMSO respectively. Each compound was screened in triplicate at 1 µM, 10 µM and 20 µM. These values are used to calculate the amount of PrP^{Sc} remaining after treatment as a percentage of the negative control.

7.8.2.2. EC_{50} activity screening

From dot blots (Figure **49**, **A**, **B**, **C** and **D**) it can be seen that all compounds in library **1** (Screening.no 3001495, **451**), (Screening.no 3001480, **452**), (Screening.no 3001491, **453**), (Screening.no 3001492, **454**), displayed reasonable anti-prion activities; similarly, except compounds (Screening.no 3001498, **455**) and (Screening.no 3001496, **456**). All compounds in library **2** also showed good anti-prion activities except compounds (Screening.no 3001501, **461**) and (Screening.no 3001489, **462**). These compounds were treated with 0.5% DMSO as negative control and with 10 µM curcumin as positive control. Each compound was tested at six concentrations in ascending order with the lowest one at the top and highest one at the bottom as shown in Figure **49** (**A**, **B**, **C** and **D**). These compounds were then taken forward in full dose response studies.

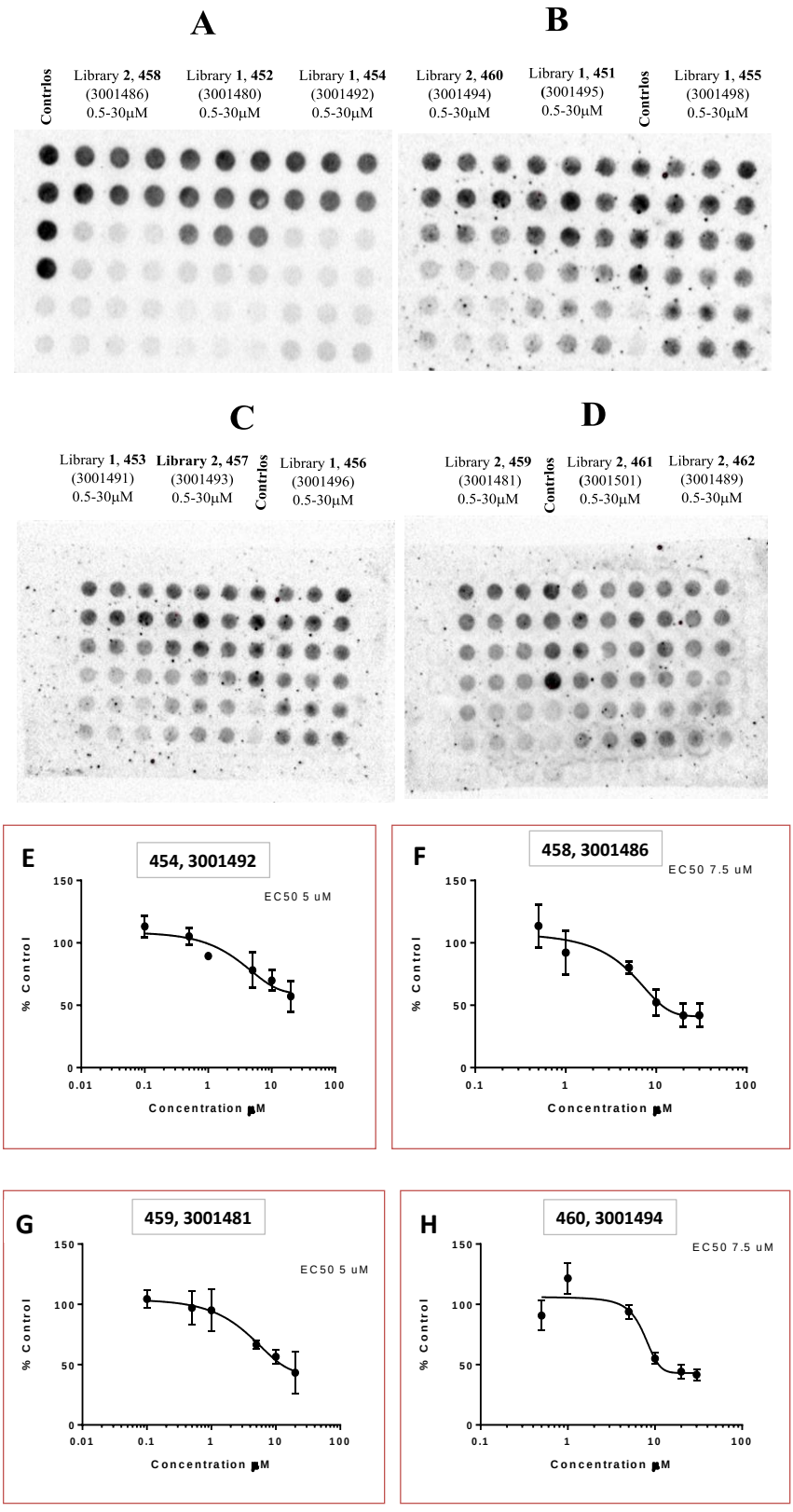


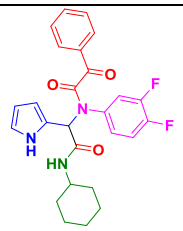
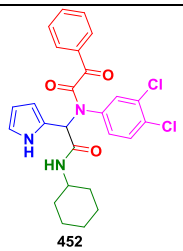
Figure 49: **A**, **B**, **C** and **D** Example of dot blots were taken from the EC_{50} screens on all Ugi adducts library **1**(**451**-**456**) and library **2**(**457**-**462**). **E**, **F**, **G** and **H** example of the dose-response curve and EC_{50} values for active adducts: from library **1**, **E** (Screening.no 3001492, **454**), from library **2**, **F** (Screening.no 3001486, **458**), **G** (Screening.no 3001481, **459**) and **H** (Screening.no 3001494, **460**).

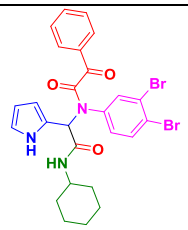
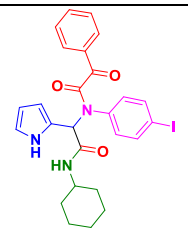
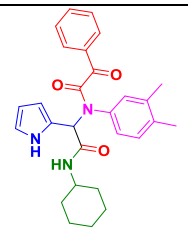
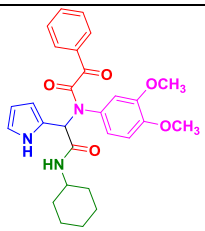
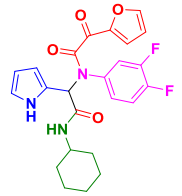
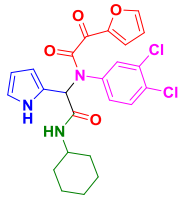
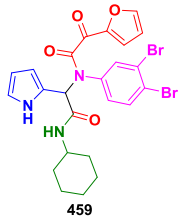
The EC_{50} value was obtained for each hit compound. An example of data obtained for the EC_{50} studies shown in Figure **49** (**E**, **F**, **G** and **H**). The example of presented data focuses on four Ugi adducts library **1 (**Figure **49**, **E** (Screening no 3001492, **454**)) and library **2** (Figure **49**, **F**, **G** and **H** (Screening.no 3001486, **458**), (Screening.no 3001481, **459**) and (Screening.no 3001494, **460**).

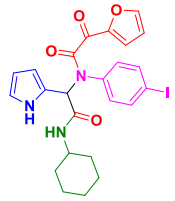
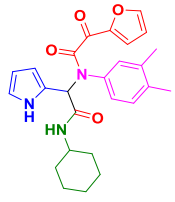
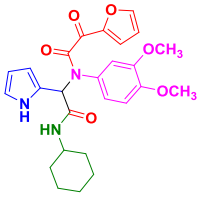
A non-linear regression was applied for the dose response curve to determine the value of EC_{50} after the data were plotted on a log axis using GraphPad Prism 5.0 shown in Figure **49**. The screening of all compounds was repeated at least three times (each in triplicate) to confirm the value of EC_{50} and the data are calculated as average with the standard deviation in μM. These values were in good agreement with the literature which validates the screening protocol. From Figure **49** (**E**, **F**, **G** and **H**) it can be seen that **454** (Screening no 3001492, EC_{50} 5.00±0.04 μM), **458** (Screening.no 3001486, EC_{50} 7.50±0.13 μM), **459** (Screening.no 3001481, EC_{50} 5.00±0.50 μM) and **460** (Screening.no 3001494, EC_{50} 7.50±0.16 μM) all have low micromolar EC_{50} and the most effective compounds are in library **1** and **2** with X equals to phenyl or furan, Y and Z equal to the substituted aniline ring (Figure **47** and Table **7.3**).

In addition to EC_{50} data, molecular weight (MW), calculated LogP (cLogP), total polar surface area (tPSA) and electronic substituent constant were presented for each active compound to help to correlate these properties with the EC_{50}.

Table 7.3: The screening results for Ugi adducts (**451-462**) and EC_{50} results.

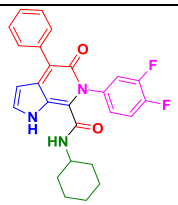
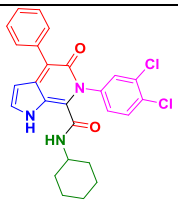
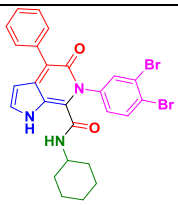
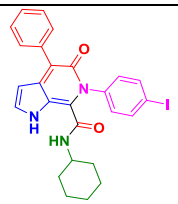
	Ugi adducts	Screening No.	MW	cLogP	tPSA	Electronic constant	EC_{50} [μM][a]
Library 1	 451	3001495	465	3.76	78.51	0.399	5.00±0.12
	 452	3001480	497	4.56	78.51	0.60	7.50±0.09

	 453	3001491	585	5.10	78.51	0.625	7.00±1.10
	 454	3001492	555	4.80	78.51	0.629	5.00±0.04
	 455	3001498	457	4.41	78.51	-0.239	inactive
	 456	3001496	489	3.19	96.97	-0.153	inactive
Library 2	 457	3001493	455	2.37	87.74	0.399	7.00±0.17
	 458	3001486	487	3.17	87.74	0.60	7.50±0.13
	 459	3001481	575	3.71	87.74	0.625	5.00±0.50

	 460	3001494	545	3.41	87.74	0.629	7.50±0.16
	 461	3001501	447	3.03	87.74	-0.239	inactive
	 462	3001489	479	1.80	106.20	-0.153	inactive

[a]: Values represent at least two experiments, each performed in triplicate.

Table 7.4: The screening results of the extension compounds (**463-474**) derived from of the Ugi adducts (**451-462**) show no anti-prion activity.

	Ext. products	Screening No.	MW	Anti-prion activity
Library 3	 463	3001502	447	inactive
	 464	3001479	479	inactive
	 465	3001482	567	inactive
	 466	3001497	537	inactive

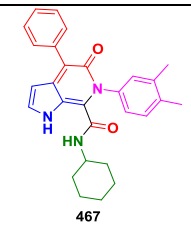
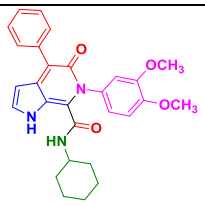
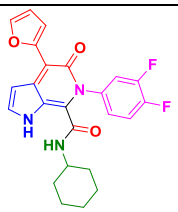
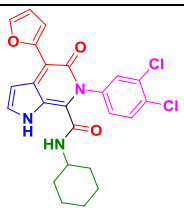
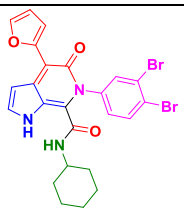
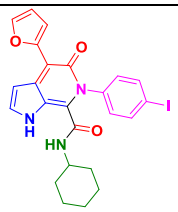
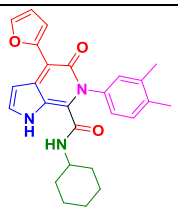
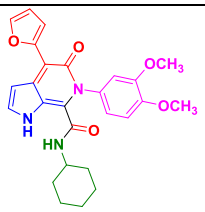
	 467	3001499	439	inactive
	 468	3001490	471	inactive
Library 4	 469	3001483	436	inactive
	 470	3001488	469	inactive
	 471	3001487	556	inactive
	 472	3001485	527	inactive
	 473	3001484	429	inactive
	 474	3001503	461	inactive

Table **7.4** shows the screening results of libraries **3** and **4** derived from post-Ugi cyclisation products of Ugi adducts libraries **1** and **2**. The screening data indicated that all compounds in libraries **3** and **4** showed complete loss of anti-prion activity.

7.8.2.3. Structure activity relationship (SAR) discussion

Some the Ugi adducts (**451-454**, library **1**) and (**457-460**, library **2**) derived from di-substituted aniline displayed good anti-prion activity, while those (**463-474**, libraries **3** and **4**) derived from post-Ugi transformation of libraries **1** and **2** were all inactive. It is noted that inserting electron withdrawing group such as halogen results in an increase in activity while electron donating substitution such as in compounds **455** (Screening no 3001498) and **456** (Screening no 3001496) in library **1** and **461** (Screening no 3001501) and **462** (Screening no 3001489) in library **2** reduced activity. It seemed that tPSA has no effects on anti-prion activities although active compounds in library **1** have the same tPSA values and so do the compounds in library **2**. The high values of tPSA for **456** and **462** are thought to be due to di-OCH_3 substitution on the aniline ring. The clogP values of Ugi adducts (**451-462,** libraries **1** and **2**) are all less than **5** which meet the Lipinski's rule. All compounds except the compounds **455**, **456**, **461** and **462** are inactive. The effect of cLogP on the EC_{50} is less obvious. Compounds containing two electron donating groups (CH_3 and OCH_3) on anilines at *meta*-and *para*-positions were inactive.

The screening data revealed that the post-Ugi cyclisation products (**463-474**, libraries **3** and **4**) derived from the Ugi adducts (**451-462**, libraries **1** and **2**) show no anti-prion activity at all (Table **7.4**). It may be that the functionalization of the carbonyl group kills the anti-prion activities. Di-substituted benzene ring fused to a pyrrole ring and di-carbonyl groups of keto acids have been found to be important to anti-prion activities. The compounds bearing a *meta*- and *para*-substituent on the aniline (red circled) were most potent in the SMB cell line system. The Ugi adducts (**451-462**, libraries **1** and **2**) consisted of substituted anilines attached to di-carbonyl aryl *via* a CN linker and an unsubstituted pyrrol *via* a CN linker (blue circled) (Figure **50**).

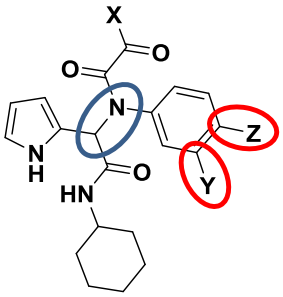


Figure **50**: Figure demonstrating *meta*- and *para*-substitutions on the aniline.

SAR study focuses on how physicochemical properties of Ugi adducts (**451-462**, libraries **1** and **2**) affect their biological activity. Figure **51** shows that how molecular weight (MW), calculated partition coefficient (cLogP), topological polar surface area (tPSA) and electronic substitution constant affect the anti-prion activities.

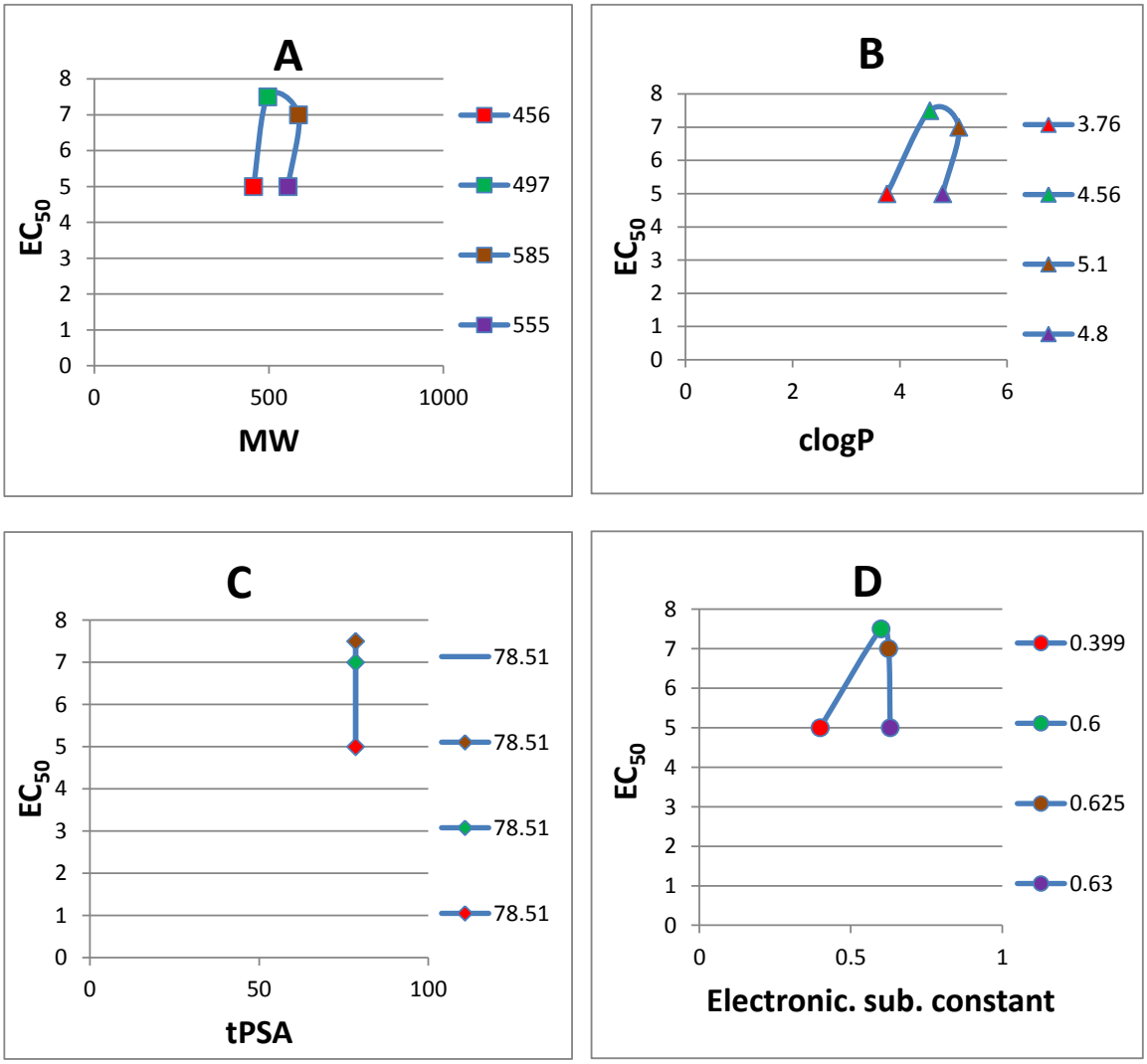


Figure 51: Shows SAR study for active Ugi adducts (**451-454**, libraries **1**).

From Figure **51** (**A**, **B**, **C** and **D**), it can be seen that relationships between physical properties and the activity (EC_{50}) of Ugi adducts (**451-454**, libraries **1**). Figure **51** (**A**) shows a response (EC_{50})-molecular weight (MW) curve was sigmoidal, although the portion between compounds **451** and **452** of maximal response (EC_{50}) approximates to a straight line. Partition coefficient or lipophilic efficiency (cLogP) refers to compound-lipophilicity efficiency which is used in drug discovery to evaluate the quality of scope compounds, such as permeability through biological membranes, linking with potency, solubility, lack of selectivity and to estimate drug-like. Plotting cLogP against (EC_{50}) (Figure **51**, **B**) for a range of active Ugi adducts (**451-454**, library **1**) indicates to correlation the partition coefficient (cLogP) and activity (EC_{50}). To assess the role of the topological polar surface area (tPSA) for each compounds on the potency (EC_{50}), tPSA values for each compounds were calculated and correlated them with (EC_{50}) values Figure **50** (**C**). It was observed from table **7.3** that (tPSA) values are similar for all active Ugi adducts (**451-454**, libraries **1**) and these values do not affect on the anti-prion activity. Electronic substitution constant is one of influential physicochemical properties which broadly affects on drug-like properties. It seems from Figure **51** (**D**) that obtained from plot between calculated electronic substitution constant of Ugi adducts (**451-454**, libraries **1**) and (EC_{50}) values, significant positive correlations was observed.

Figure **52** (**A**, **B**, **C** and **D**) also shows approximately similar effect of some physical properties of Ugi adducts (**457-460**, library **2**) such as: molecular weight (MW), hydrophobicity (clogP), topological polar surface area (tPSA) and electronic substitution constant on the anti-prion activities. Thus, the influence of the di-substitutions at the *meta*- and *para*- aniline ring seems to be more efficient effect towards anti-prion activities.

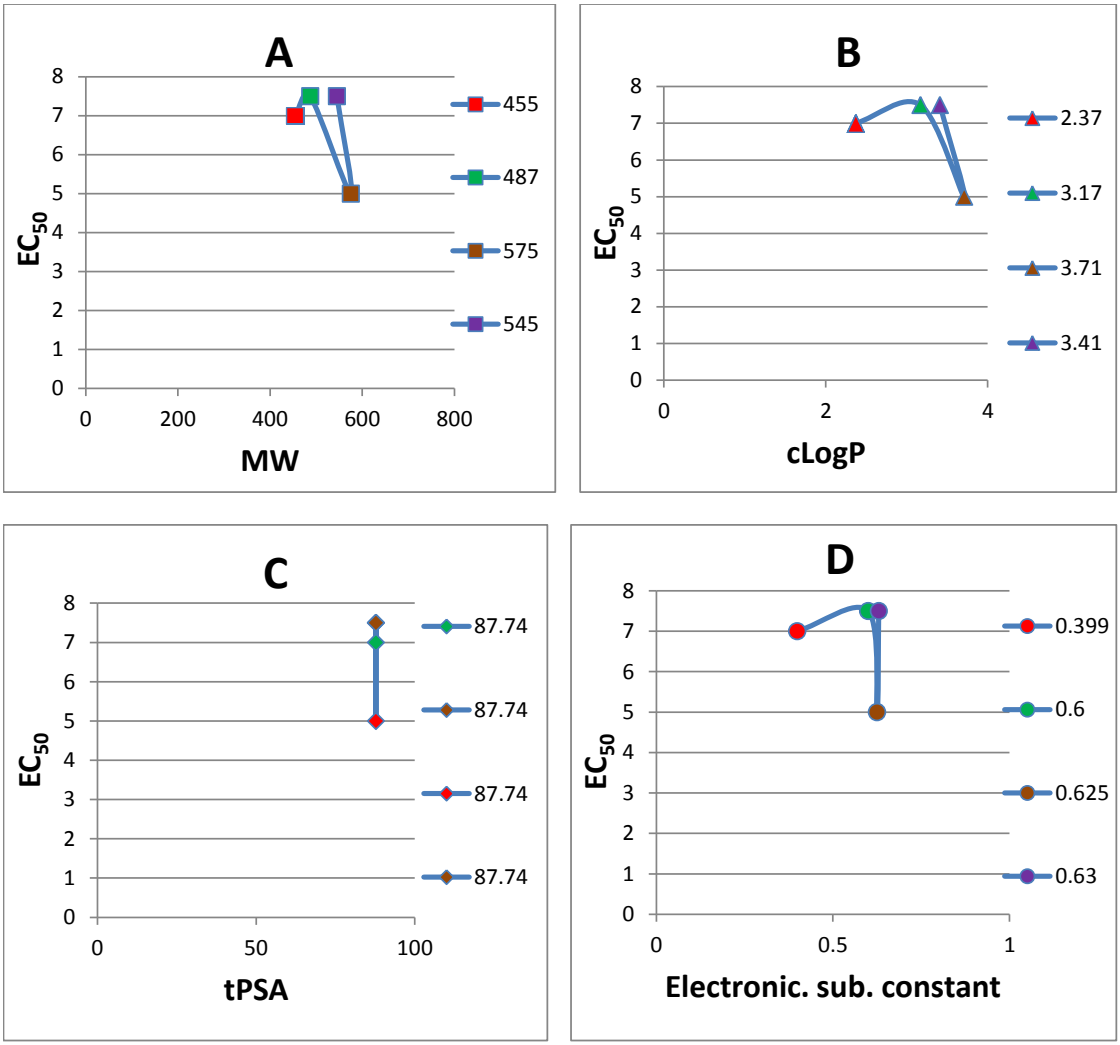


Figure 52: Shows SAR study for active Ugi adducts (**457-460**, libraries **2**).

It is interesting to see that post-Ugi cyclisation product, pyrrolo-pyridine derivatives (**463-474**, libraries **3** and **4**) which are derived from Ugi adducts (**451-462**, libraries **1**and **2**) were all inactive. Although some compounds showed weak activity in the initial screening, but these compounds did not display any activities in the EC_{50} studies so they were classed as inactive. It seems that removing one of the carbonyl groups or functionalizing the carbonyl groups makes the pyrrolo-pyridine core reduce in anti-prion activity (Figure **53**).

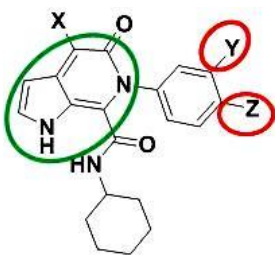


Figure 53: Shows functionalizing of the carbonyl group to make pyrrolo-pyridine core.

It can be seen that corresponding post-Ugi cyclisation products were not tolerated in terms of cell line activity due to the combined effect of reducing the number of rotating bonds after the cyclisation (See **Chapter six**, section **6.1.5.1** for mechanism of cyclisation) with the removal of one of the carbonyl groups adjacent anilines resulted in a total loss of anti-prion activity.

In conclusions, it can be seen that the majority of Ugi adducts (**451-462**, libraries **1** and **2**) with di-substituted anilines have good anti-prion activities. Strikingly, the removal of one of the carbonyl groups *via* post-Ugi cyclisation diminishes anti-prion activity compared to the same precursor Ugi adducts (**451-462**, libraries **1** and **2**). An interesting observation was noted that the total removal of PrP^{Sc} was not observed with any tested compounds. Even at the highest concentrations of the most potent compounds, 5 to 20% of the PrP^{Sc} remained. As seen in dot blot presented in Figure **49** (**A**, **B**, **C** and **D**) and faint spots were still visible on the dot blots. The initial screen data shows the residual PrP^{Sc} which remained after treatment. While this could be due to the limitation of assay itself (background and selection of antibodies, etc), it also raises a question of whether the cells still retain infective. It is possible to see that PrP^{Sc} levels have been reduced to such a degree that accumulation to pre-treatment stages would be prevented by normal cellular processes. Further work is required to show the long term effects of treatment on PrP^{Sc} levels, and also to assess whether the cells are still infectious. The reduction in levels of PrP^{Sc} and the activity of the chosen compounds should be reproduced using western blotting to validate the dot blot protocol used in this application. Nevertheless, data presented should furnish some insight into new direction of lead discovery against prion disease.

Chapter 8. Conclusion and future work

8.1. Conclusions

Three starting materials or more can be combined in one pot in multicomponent reactions (MCR) to give a product which contains most of atoms in the reagents. The multicomponent reactions such as famous Ugi MCR usually involves several steps in equilibrium followed by one irreversible step. This is important to allow a wide range of diversities to be introduced in one step. The MCRs studies in this thesis use ketoacids, amines and isocyanides as basic building blocks to synthesise a wide range of bioactive heterocyclic compounds. Ketoacids are beneficial starting materials which have been used in literatures as bifunctional substrates for the synthesis of various heterocyclic compounds, but its applications in MCR is relatively under explore. Isocyanides are the other important components in MCRs. They can act as both nucleophiles and electrophiles which make them the most wide used reagent in MCRs. In Ugi reaction, the terminal carbon of isocyanides undergoes electrophilic reactions to generate an electrophile which can be then trapped by another nucleophile. The combination of ketoacids, isocyanides and a wide range of amines allowed a large variety of Ugi-based MCRs and post transformation reactions to take place to produce a wide range of heterocyclic compounds.

Cyclic amides are widely used scaffolds in medicinal industry and attracted much attention in organic synthesis. The synthesis of complex cyclic amides has been explored using multicomponent reactions in this thesis. Approach for preparing a variety of cyclic amides such as pyrrolidin-2-ones and piperidin-2-ones from different isocyanide, amines and different keto acids has been developed using batch reactions. This method was utilized to synthesise novel 5-membered, 6-membered and 7-membered cyclic amides in yields ranging from 35-56%. Attempts to synthesise of azepane-2-ones (seven-member) proved successful which was carried out in high dilution, while the attempts to use the same method in the synthesis of azocane-2-carboxamide (eight-member) proved unsuccessful, with a complex mixture of side-products observed. The reaction was potentially hindered by the demand to generate a nine-membered intermediate which are both enthalpically and entropically disfavoured.

Meyers' lactamization is a bis-electrophile-bis-nucleophile reaction which was used to synthesise of bi-and tri-cyclic lactams using various ketoacids and different amino alcohols. Pyridinium *p*-toluenesulfonate (PPTS) was used as catalyst in Meyers' reaction to control the stereoselectivity of outcome of bi-and tri-cyclic amides (lactams).

Keto acids as bifunctional precursors were tethered with primary amines, isocyanides and trimethylsilyl azide reagent to produce the Ugi-tetrazole products in low yields. Flow chemistry was efficiently utilized to improve the productivities of the Ugi-tetrazole products in moderated yields. Microwave (MW) irradiation as conventional technology was exploited for synthesis of some pyrazole-oxopyrrolidine derivatives with satisfactory yields, short time and solvent free conditions; however the same reaction would require three days to afford these compounds in low yields.

Isoquinoline derivatives have a wide range of biological activities. The synthesis of their derivatives explored using Ugi reaction where isoqunoline was used as an amine component which reacts with α-ketoacids and isocyanides to allow a wide range of isoqunoline derivatives. These molecules may contribute to promote the functionalization of heterocyclic scaffolds with medicinal properties due to the fact that isoquinoline substructure is found in some natural product and synthetic bioactive heterocyclic compounds. An efficient multi-component cascade involves decarboxylative lactamization reaction in water as a green solvent was utilized for synthesis of a series of isoindolin-1-one derivatives in good yields. In this protocol, it was found that tetrabutyl ammonium bromide salt (TBAB) plays an important role to promote multi-component reactions.

The multicomponent reactions and subsequent cyclization reactions are considered as a powerful stratagem for the synthesis of various heterocyclic scaffolds. The multicomponent reactions are utilized to form adducts carrying varied functional groups which can then be selectively cyclized to yield more complex heterocyclic compounds. A central goal of further expansion is to provide stereochemical control over the reaction and prepare structurally diverse compounds for evaluation of their biological activities. Further expansion could be performed using different cyclization methods based on the properties ketoacids as bifunctional component leading to diverse spiro-heterocycles compounds. A convertible isocyanide in a sequential cyclization strategy of Ugi products including intra- or intermolecular attack from a variety of nucleophiles with a catalytic base to form the pyroglutamic acid analogues was investigated. A versatile and robust strategy coupling the Ugi-tetrazole adducts with ambient post-condensation modifications allowing ring-closure to variety of pharmacologically relevant scaffolds. The expansion of the Ugi-tetrazole products to form 5, 6 and 7-membered rings was subsequently investigated and reported that 1,1′-carbonyldiimidazole is convenient reagent for intramolecular amide coupling with good yields. Further cyclization reactions of multicomponent products were exploited to synthesise novel diones derivatives in yields ranging from 55-85%.

The Ugi and post-transformation reactions include various ketoacids with Boc-protected hydrazine was successfully employed for the synthesis of structurally diverse of pyrrol-oxopyrrolidine derivatives in order to evaluate their biological activity. Pyrrolo-pyridine substructures possess a high range of pharmacological properties, therefore the post-transformation reactions of the Ugi products under a mild condition were successfully utilized to synthesise a series of novel heterocyclic compounds. All synthesized compounds were screened as anti-prion therapeutics, a series of Ugi adducts (**451**-**454** and **457**-**460**) show as promising anti-prion agents and the structure-activity relationship (SAR) of these compounds was investigated.

8.2. Future work

Further reactions during the use of convertible isocyanide would furnish pyroglutamic acid analogue (Scheme **80**). The Ugi reaction was used to afford of 5-membered, 6-membered and 7-membered cyclic lactams then further post-transformation of these cyclic lactams would lead to the natural product. During my PhD studies, a similar multicomponent reaction was explored for the core of amathaspiramide alkaloid **487** (Figure **54**).

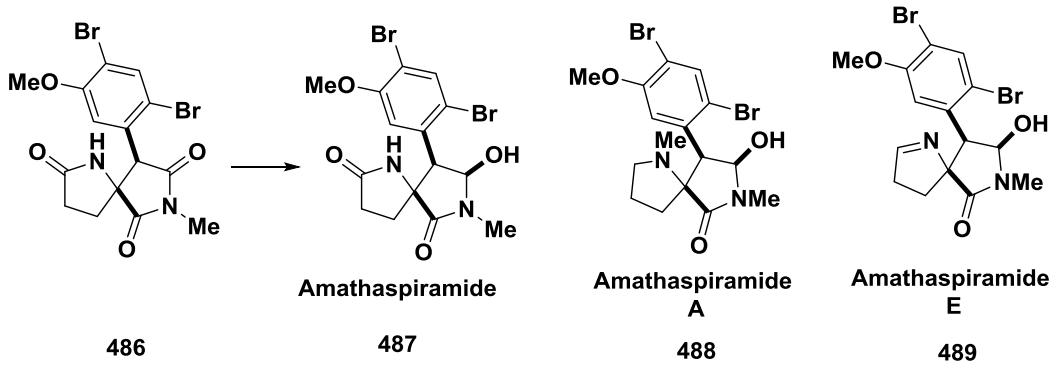
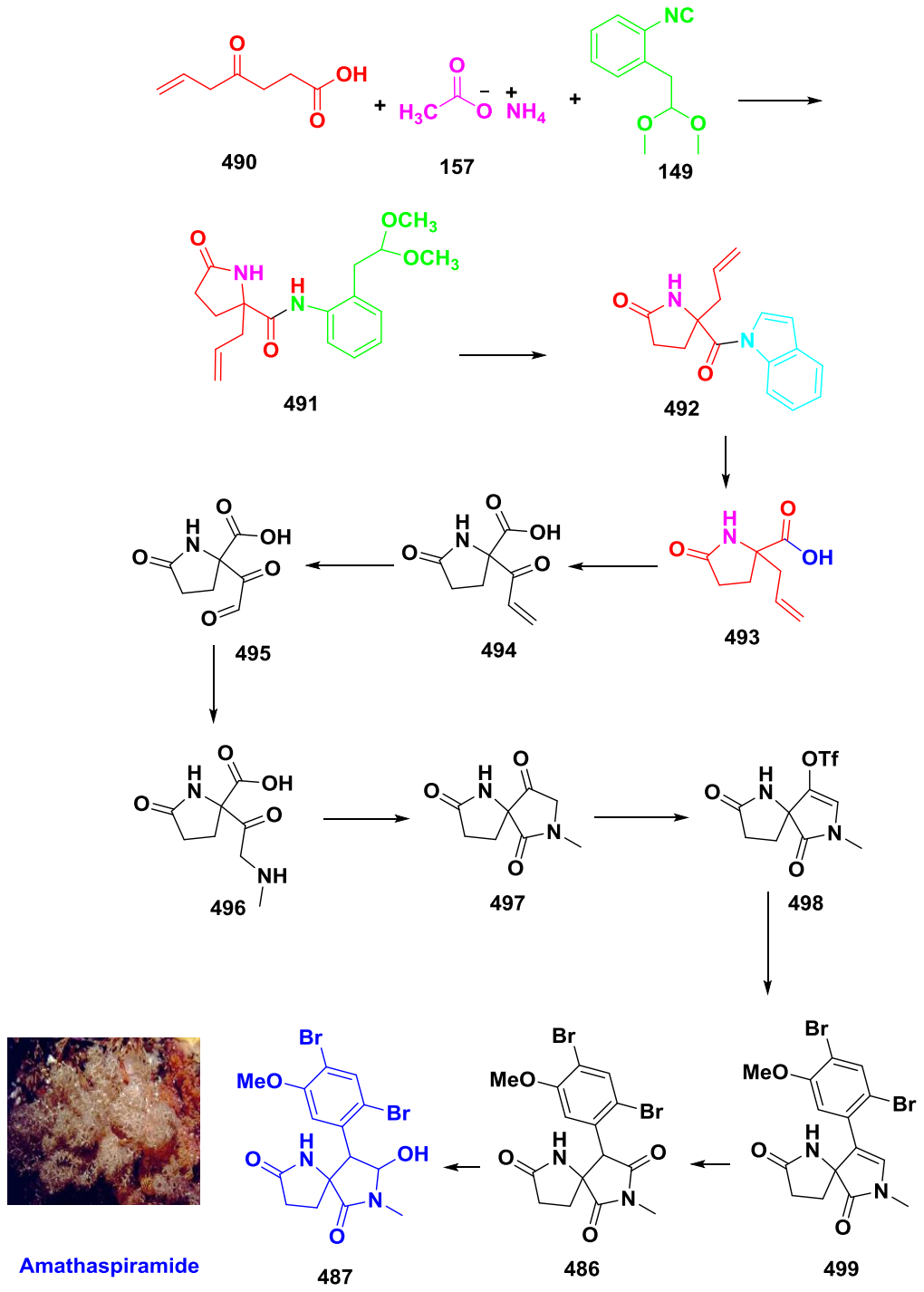


Figure 54: Intermediate **486** as a precursor key to amathaspiramide **487**.

Retrosynthetic analysis showed that an intermediate **486** might be formed from an Ugi reaction using convertible isocyanide **149**, keto acid **490** and ammonium acetate **157** (Scheme **80**). It has been believed that large amount of the keto acid **490** would be firstly needed due to the many steps that are required to utilize this keto acid **490** as essential component in multicomponent reactions. Multicomponent reactions could be then exploited as a beneficial route to reach amathaspiramides as natural product (Scheme **80**). Amathaspiramide **487** is one dibrominated alkaloids which has attracted attentions of pharmaceutical chemists. The common structure of these alkaloids is featured by a novel aza-spirobicyclic that consists three contiguous chiral centres in an amino group which is connected with a quaternary

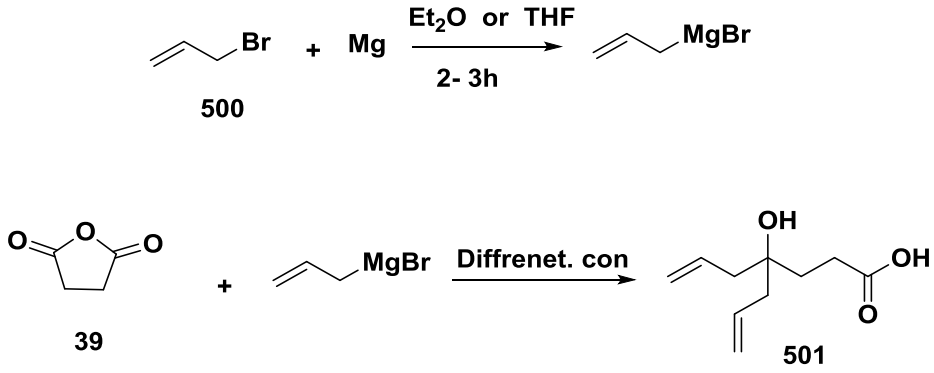
carbon centre, 11,13-dibrominated aromatic ring and a cyclic hemiaminal moiety. Amathaspiramides **A** (**488**) displays an important biological activity such as anti-microbial and antiviral (Figure **54**). Amathaspiramide **E** (**489**) has shown a potent antiviral activity, also against Poliovirus, Gram-positive bacterium bacillus subtilis and fungus.[323] Hence, it is necessary to look for a suitable method to produce keto acid **490**.



Scheme 80: A proposed route for amathaspiramide depends on keto acid **490** as an essential component in multicomponent reactions.[324]

8.2.1. **Experimental trails for synthesis of 4-oxoheptenoic acid 490**.

Many experiments (Table **8.1**) have been carried out for synthesis of 4-oxoheptenoic acid **490** using allyl magnesium bromide and succinic anhydride **55** with different conditions according to the literature. ^{1}HNMR and mass spectrometry analysis confirmed that the formation of multiple addition alcohol **501** in high quantity is the cause for failure in the preparation of the keto acid **490** (Scheme **81**).



Scheme 81: Potential an alcohol **501** as byproduct from Grignard reagent

Another attempt (Table **8.1**) was carried out using tetramethyl ethylene diamine (TMEDA) as bidentate ligand for (-)-sparteine which was used by Sintani in similar reaction.[325] It was believed that it might be possible to employ similar nitrogen bidentate ligand in order to reduce the reactivity of the allylmagnesium bromide *via* transforming TMEDA into a cluster of higher reactivity than the more stable hexamer (Figure **55**).

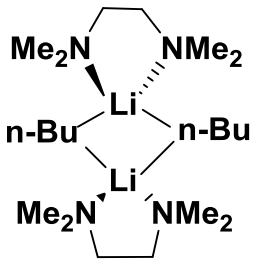


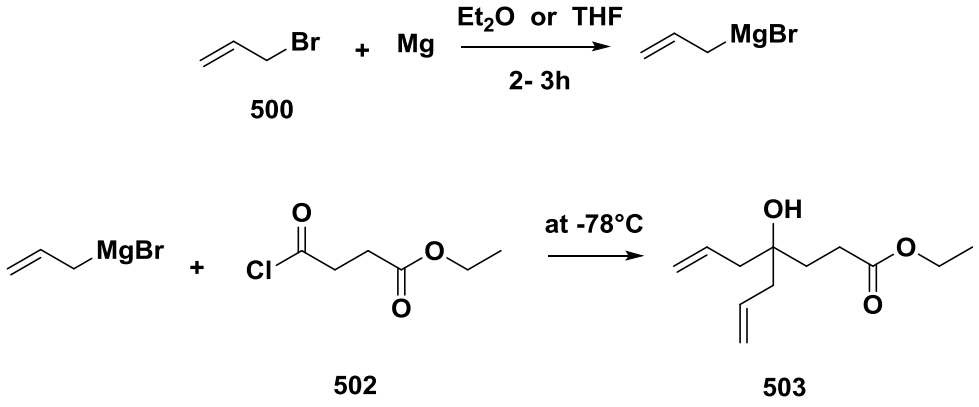
Figure 55: n-BuLi-tetramethylethylenediamine dimer.[326]

Unfortunately the free keto acid **490** cannot be produced using TMEDA to suppress the reactivity of Grignard reagents by forming complex[326].

Table 8.1: Different conditions were employed to synthesise keto acid **490**.

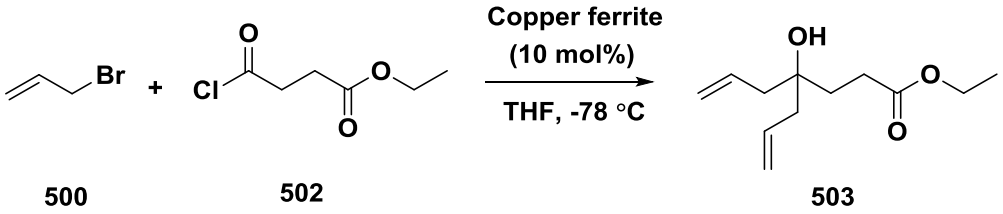
Entry	Method	Solvent	Reagents	Catalyst	Temperature	Outcome
1	Batch	THF	Succinic anhydride	CuI	-78 ºC	-
2	Batch	Et_2O, THF	Succinic anhydride	TMEDS, CuI	-20 ºC	-
3	Batch	Et_2O, THF	Succinic anhydride	TMEDS, CuI	-40 ºC	-
4	Batch	THF	Succinic anhydride	TMEDS, CuI	-78 ºC	-
5	Batch	THF	Succinic anhydride	TMEDS, CuI	-100 ºC	-
6	Batch	Et_2O, HMPA	Succinic anhydride	CuI	-78 ºC	-
7	Batch	THF	Ethyl succinyl chloride	Copper ferrite	-78 ºC	-
8	Batch	THF	Ethyl succinyl chloride	Zn dust	-78 ºC	-
9	Batch	Et_2O	Ethyl succinyl chloride	Diallylcuprate	-40 ºC	-
10	Batch	Et_2O	Ethyl succinyl chloride	Diallylcuprate	-78 ºC	-
11	Flow machine	Et_2O	Ethyl succinyl chloride	Diallylcuprate	-70 ºC	-
12	Batch	Et_2O	Ethyl succinyl chloride	Diallylcadmium	-78 ºC	-
13	Flow machine	Et_2O	Ethyl succinyl chloride	Diallylcadmium	-70ºC	-
14	Flow machine	Et_2O, THF	Succinic anhydride	CuI	-70ºC	-

Further attempts were carried out to find a convenient method for preparing keto acid **490**. Recently, some publications have concerned on using low temperature Grignard reactions to form some ketones. Some of publications reported that many ketones could be prepared from acid chlorides at low temperature in the existence of Grignard reagents.[327] Therefore, it was hoped that this procedure could be applied to ethyl succinyl chloride **502** under different conditions, but unfortunately the product was an ester alcohol **503** (Scheme **82**), not the desired ketoacid **490**.



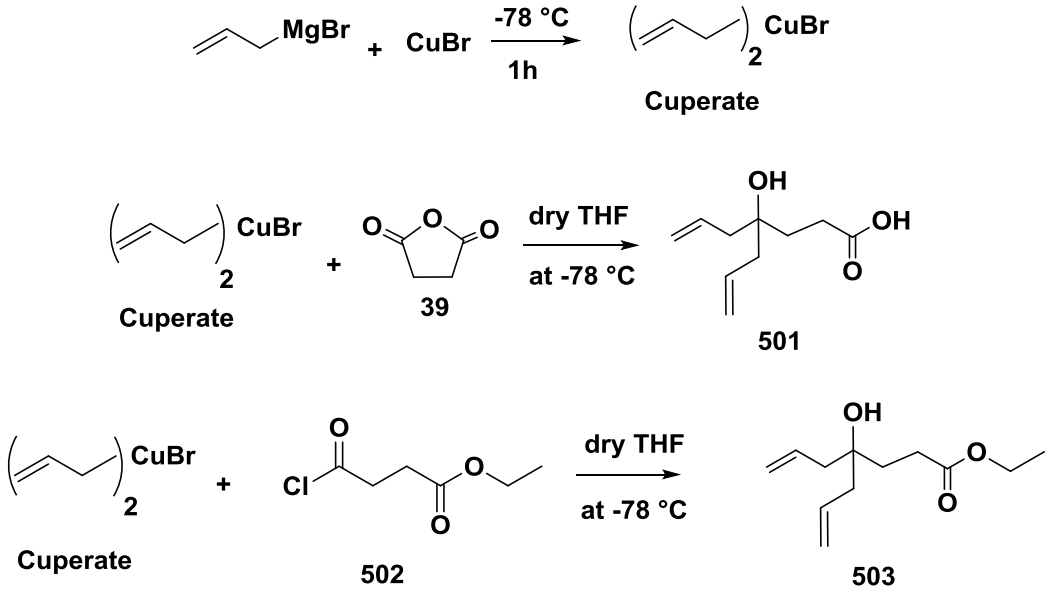
Scheme 82: A potential synthetic route for ester keto acid **490**.

Another article mentioned that copper ferrite nano material can be used as reusable catalyst in allylation of acid chlorides.[328] Unfortunately, after workup the pure compound was also an ester alcohol **503** (Scheme **83**).



Scheme 83: A proposed way to synthesise keto acid **490** by using copper nano ferrite.

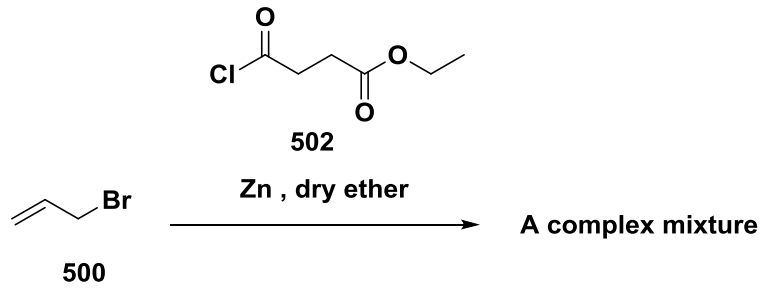
Regioselective reaction between organocuprate reagent and terminal vinyl carbon was reported.[329],[330] The organocopper reagent attacks regioselectively acid chloride to yield keto compounds and this approach was explored in the synthesis of 4-oxohept-6-enoic acid **490**. Unfortunately reaction produced almost exclusively the corresponding alcohol **501** and **503** (Scheme **84**).



Scheme 84: Utilizing bromomagnesium divinylcuprate for synthesis of keto acid **490**.

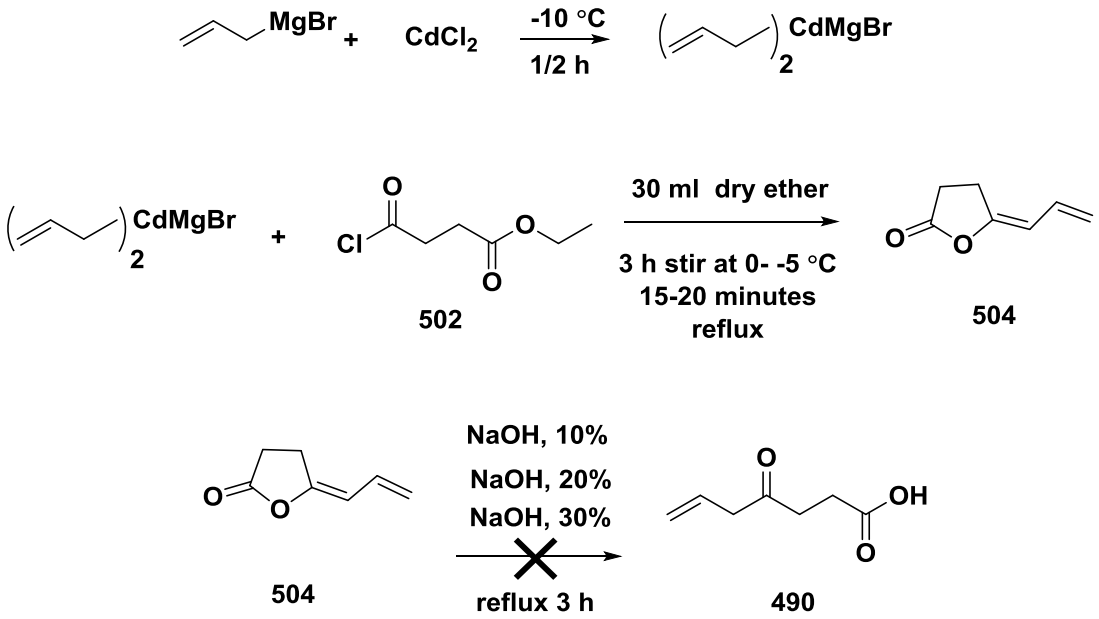
Transition metals are highly versatile and have a wide range of applications in synthetic organic chemistry. Although zinc reagents has been used, they suffers from poor regiospecificity.[331] An extended range of acid chlorides was successfully converted to the corresponding allylic ketones by the same procedure.[332] Therefore, the reaction is summarized to be commonly applicable to acid chlorides. Allyl bromide **500** and ethyl

succinyl chloride **502** were reacted with dust zinc (Scheme **85**), but this methodology also failed to afford 4-oxohept-6-enoic acid **490**.



Scheme 85: A synthetic route for using of zinc reagent.

The final attempt was achieved using the organocadmium reagent which reacts regioselectively with acid chloride [333], this approach was also used in the synthesis of 4-oxohept-6-enoic acid **490**. Unfortunately, the reaction gave lactone **504** and three attempts were carried out for the hydrolysis lactone **504** to keto acid **490**, but all failed (Scheme **86**).



Scheme 86: Utilizing of bromomagnesium divinylcadmium for keto acid **490**.

In summary, the synthesis of the ketoacid **490** should be further investigated so that full synthesis of amathaspiramides can be synthesised using convertible isocyanide in Ugi-based MCRs.

Chapter 9. Experimental procedures

9.1. Experimental procedures

All Flash column chromatography was carried out on 230-400 mesh silica gel unless stated. Thin-layer chromatographs (TLC) were performed on aluminium-backed plates pre-coated with silica, which were visualised using either ultraviolet light, or potassium permanganate as an alkaline aqueous solution.

Reduction reactions were performed using H-cube reactor (ThalesNano system), which was supplied with a 10 % Pd/C cartridge. Flow reactions were carried out using a Vapourtec E-series machine. All microwave reactions were accomplished using Smith Creator™ Optimiser EXP reactor, using Smith Process Vials™.

HPLC analysis was achieved using two types of columns first: Phenomenex lux cellulose-1 3μm column (4.6 mm × 250mm); 20% IPA in hexane, over 25 min; UV detection at 228 nm; second: Waters Xbridge a C18 5 μm column (4.6 mm × 250mm); 5−95% MeCN/ aqueous TFA (0.1%) over 20 min, hold 10 min; UV detection at 215 nm. Optical rotations were recorded on an Optical activity Ltd. Specific rotations were measured to the nearest 0.1 degrees using Automatic Polarimeter at 589 nm and the concentrations were given to the nearest 0.1 units of 10 mg/ml.

NMR spectra were recorded on a Bruker AV-400 and a Bruker AV-1400 model. Chemical shifts were recorded in parts per million (ppm) with the resonance of solvents chloroform-d and dimethyl sulfoxide-d_6 as an internal standard (^{1}H NMR: $CHCl_3$: δ 7.27 ppm and DMSO-d_6: δ 2.50 ppm; ^{13}C NMR: $CDCl_3$: δ 77.0 ppm and DMSO-d_6: δ 39.52 ppm).

High-resolution mass spectrometry (HRMS) was recorded for accurate mass analysis using a Micro Mass LCT operating in Electrospray mode (ES).

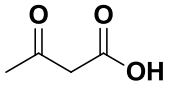
Infra-red spectra were recorded on a Perkin Elmer Paragon 100 FTIR spectrophotometer, the recording absorbance was taken between 4000-700 cm^{-1} and only those absorptions suitable to recognise functional groups are reported. Melting points were reported using a Gallenkamp melting point apparatus and are uncorrected.

Reagents and solvents were used either as purchased from commercial sources or when necessary, purified by standard laboratory techniques published in Purification of Laboratory Chemicals by Perrin, Armarego. Dry solvents were acquired from an in-house Grubbs solvent system.

All reagents and materials for anti-prion activity *in vitro* include: Reagents for cells line (SMB) screening: Medium 199, New born Calf Serum (NBCS), Foetal Bovine Serum (FBS), Penicillin-Streptomycin, Tryp-LE dissociation solution, Hanks balanced salt solution (HBSS), RIPA lysis buffer, Benzonase, Bradford reagent, Bovine serum albumin (BSA), Thiazolyl blue tetrazolium bromide (MTT), Nitrocellulose membrane, Proteinase K (PK), Phenylmethylsulfonyl fluoride (PMSF), Guanadine thiocyanate, Skimmed milk powder, 8H4 anti-prion monoclonal antibody, ECL detection reagents and HRP-tagged IgG secondary antibody. Filter Units, P10 tips, P200 tips, P1000 tips, T75 tissue culture treated flasks, 96 well tissue culture treated plates, TBS (10 x stock), Tween 20 and Transparencies were obtained from appropriate commercial sources (Sigma, Fisher, Greiner Bio-One, Biolegend and Geneflow). The visualization process was performed using the ChemiDoc instrument in a dark room.

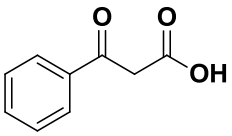
9.2. Experimental procedures for synthesis of keto acids

9.2.1. 3-Oxobutanoicacid 2 [334]



To ethyl acetoacetate **7** (4.0 g, 30 mmol), 1N sodium hydroxide solution (30 mL, 30 mmol) was added and the solution was stirred for 24 hours at room temperature. After that the solution was washed with diethyl ether (50 mL), the aqueous solution was cooled with an ice bath and acidified with concentrated hydrochloric acid until to pH = 2. The mixture was further extracted with ethyl acetate (3 x 150 mL). The combined organic layers were dried with magnesium sulphate, filtered and concentrated under reduced pressure at 5-10 ºC over 6 hours. Purification was achieved by flash column chromatography (ethyl acetate: petroleum ether 4: 6, R_f = 0.3) to yield ketoacid **2** as a pale oil (3.2 g, 80% yield); υ_{max} (ATR/cm^{-1})= 3335, 2935, 1798, 1720, 1613; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 3.55 (2H, s, CH_2), 2.33 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 201.7 (*C*=O), 171.7 (*C*=O), 48.8 (*C*H$_2$), 30.4 (*C*H$_3$).

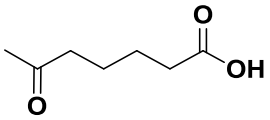
9.2.2. 3-Oxo-3-phenylpropanoic acid 3



To ethyl benzoylacetate **171** (5.76 g, 30 mmol), 1M sodium hydroxide solution (30 mL, 30 mmol) was added and the solution was stirred for 24 hours. After that the solution was

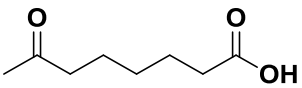
washed with diethyl ether (50 mL), the aqueous solution was cooled with an ice bath and acidified *via* concentrated hydrochloric acid until to pH = 2. The mixture was further extracted with ethyl acetate (3 x 150 mL). The combined organic layers were dried with magnesium sulphate, filtered and concentrated under reduced pressure at 5-10 ºC over 6 hours. Purification was achieved by column chromatography (ethyl acetate: petroleum ether 4: 6, R_f = 0.4) to yield ketoacid **3** as a pale solid (4.6 g, 80% yield); υ_{max} (ATR/cm^{-1})= 3549, 3060, 1681, 1600; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.06-7.45 (5H, m, ArC*H*), 4.11 (2H, s, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176.9 (*C*=O), 171.4 (*C*=O), 134.7 (Ar*C*H), 129 (Ar*C*H), 128.7 (Ar*C*H), 126.4 (Ar*C*H), 43.7 (CH_2).

9.2.3. 6-Oxo-heptanoic acid 32[26]



2-Acetylcyclopentanone **31** (1.26 g, 10 mmol) was dissolved in H_2O (10 mL) and $FeCl_3$ (0.16 g, 10 mol%) was added with closed-vessel conditions. The reaction mixture was stirred for 18 h at 80 °C. The reaction mixture was extracted with DCM (50 mL) and the combined organic phases were washed with distilled water (2 x 50 mL). The organic phase was dried with magnesium sulphate, filtered and concentrated under reduced pressure. Purification was achieved by column chromatography (ethyl acetate: petroleum ether 3:7, R_f = 0.3) to afford ketoacid **32** as a pale yellowish oil (0.94 g, 75%); υ_{max}(ATR/cm^{-1})= 3441, 2948, 2873, 1704, 1415; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 2.49 (2H, t, *J* = 6.8 Hz, CH_2), 2.39 (2H, t, *J* = 7 Hz, CH_2), 2.16 (3H, s, CH_3), 1.67-1.63 (4H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 208.6 (*C*=O), 178.5 (*C*=O), 43.3 (CH_2), 33.6 (CH_2), 30.0 (CH_3), 24.1 (CH_2), 23.2 (CH_2); HRMS (ESI$^+$) found 145.0859 $C_7H_{12}O_3H^+$ requires 145.0851.

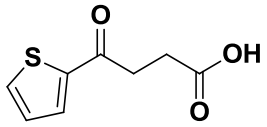
9.2.4. 7-Oxo-8-enoic acid 34[29]



To a mixture of 2-acetylcyclohexanone **33** (1.0 g, 10 mmol) and H_2O (10 mL), $Fe(OTf)_3$ (0.25 g, 0.5 mmol) was added. The reaction mixture was vigorously stirred at 80 °C for 12 hours; the progress of the reaction was monitored by TLC. The reaction mixture was warmed to keep room temperature and was then treated with ethyl acetate (200 mL). The mixture was then washed with water (200 mL) and brine solution (200 mL). The organic combined layers were dried with magnesium sulphate, filtered and concentrated under reduced pressure.

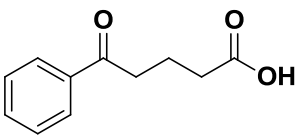
Purification was achieved by column chromatography (diethyl ether: petroleum ether 4:6, R_f = 0.3) to afford ketoacid **34** as a colourless oil (0.85 g, 85%); υ_{max}(ATR/cm^{-1})= 3196, 2948, 2871, 1702, 1410; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 2.46 (2H, t, J = 7.4 Hz, CH_2), 2.37 (2H, t, J = 7.5 Hz, CH_2), 2.16 (3H, s, CH_3), 1.70-1.57 (4H, m, CH_2), 1.40-1.32 (2H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 209.0 (*C*=O), 177.4 (*C*=O), 43.5 (*C*H_2), 33.8 (*C*H_2), 30.0 (*C*H_2), 28.5 (*C*H_3), 24.5 (*C*H_2), 23.4 (*C*H_2); HRMS (ESI$^-$) found 157.0874 $C_8H_{14}O_3H^+$ requires 157.0870.

9.2.5. 4-Oxo-4-(thiophen-2-yl)butanoic acid 173[335]



To a solution of succinic anhydride **39** (5.9 g, 59 mmol) in dry dichloromethane (250 mL), anhydrous $AlCl_3$ (9.5 g, 71 mmol) was added at -5 ° C under N_2. The reaction mixture was stirred for 30 min. After that thiophene **172** (5.0 g, 59 mmol) was dissolved in dichloromethane (50 mL) and added dropwise over a period of 60 minutes. The reaction mixture was then stirred for 4 hours at room temperature. Water (150 mL) was added and the mixture was extracted with dichloromethane (3 x150 mL). The organic combined layers were dried with magnesium sulphate, filtered and concentrated under reduced pressure. Purification was achieved by column chromatography (dichloromethane: methanol 9:1, R_f = 0.18) to yield ketoacid **173** as a pale yellow solid (3.5 g, 60%); m.p = 73-76 °C (literature, m.p = 72-82 °C); υ_{max}(ATR/cm^{-1})= 3009, 2818, 2597, 1698, 1655, 1506; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H = 12.19 (1H, s, O*H*), 8.01-7.98 (2H, m, C_4H_3S), 7.25 (1H, t, J = 4.4 Hz, C_4H_3S), 3.20 (2H, t, J = 6.4 Hz, CH_2), 2.57 (2H, t, J = 6.5 Hz, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C = 191.6 (*C*=O), 173.7 (*C*=O), 143.4 (C_3H_3*C*S), 134.5 (C_3H_3*C*S), 133.2 (C_3H_3*C*S), 128.7 (C_3H_3*C*S), 33.5 (*C*H_2), 27.75 (*C*H_2); HRMS (ESI$^+$) found 185.0267 $C_8H_8O_3SH^+$ requires 185.0267.

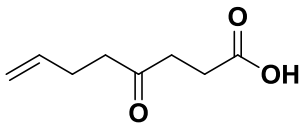
9.2.6. 4-Benzoylbutyric acid 175 [336]



To a solution of glutaric anhydride **174** (3.86 g, 33.9 mmol) in dry benzene (25 mL) with a vigorously stirred, a suspension of anhydrous aluminium chloride (9.96 g, 74.7 mmol) in dry benzene (10 mL) was added at 0 °C. The reaction mixture was stirred for approximately 20 minutes with the internal temperature kept below 20 °C. The obtained thick dark brown was

then stirred at room temperature for additional 12 hours. The reaction mixture was carefully quenched at -5 °C by addition water (20 mL) followed by concentrated sulphuric acid (7 mL). The mixture was further extracted with chloroform (3 x 50 mL) and the organic combined layers were dried with magnesium sulphate, filtered and concentrated under reduced pressure at room temperature to form a pale orange solid. The crude keto acid was then recrystallized from boiling ethyl acetate to furnish ketoacid **175** as a white solid (2.81 g, 73%); m.p = 125-129 °C; υ_{max} (ATR/cm^{-1})= 3058, 2965, 2944, 2914, 2881, 1688, 1672, 1594, 1571; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.0-7.97 (2H, m, ArC*H*), 7.61-7.57 (1H, m, ArC*H*), 7.50-7.47 (2H, m, ArC*H*), 3.11 (2H, t, *J* = 7.2 Hz, CH_2), 2.53 (2H, t, *J* = 7.1 Hz, CH_2), 2.11 (2H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 199.4 (*C*=O), 178 (*C*=O), 136.8 (Ar*C*H), 133 (Ar*C*H), 128.7 (Ar*C*H), 128.0 (Ar*C*H), 37.3 (*C*H$_2$), 32.9 (*C*H$_2$), 19.0 (*C*H$_2$); HRMS (ESI$^+$) found 193.0860 $C_{11}H_{12}O_3H^+$ requires 145.0859.

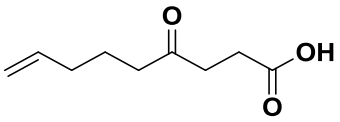
9.2.7. 4-Oxooct-7-enoic acid 178 [337]



4-Bromo-1-butene **176** (2.0 mL, 16.8 mmol) in dry tetrahydrofuran (20 mL) was added dropwise by cannula over a period 1 hour to a vigorously stirring suspension of magnesium (0.534 g, 22.0 mmol) in dry tetrahydrofuran (20 mL) under N_2. The Grignard reagent solution formed was immediately transferred dropwise by cannula to a solution of succinic anhydride **39** (1.69 g, 16.8 mmol) and copper iodide (0.09 g, 0.49 mmol) in dry tetrahydrofuran (40 mL) at -20 ° C. After the addition was completed, the mixture of reaction was warmed to 0 °C and stirred for 3 hours. The reaction mixture was quenched with aqueous hydrochloric acid (2 M, 40 mL) and concentrated under reduced pressure. The organic layer was extracted with dichloromethane (2 × 100 mL). The combined organic layers were then washed with sodium hydroxide (2 M, 2 × 100 mL). The aqueous combined layers were re-extracted with dichloromethane (3 × 100 mL) after acidification with concentrated hydrochloric acid until pH = 2. The organic combined layers were dried with magnesium sulphate, filtered and concentrated under reduced pressure. Purification was achieved by column chromatography (ethyl acetate: acetic acid: petroleum ether 3: 0.05: 6.95, R_f = 0.4) to yield ketoacid **178** as a pale yellow viscous oil (0.27 g, 50%); υ_{max} (ATR/cm^{-1})= 3078, 2983, 2918, 2668, 2568, 1711; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 5.87-5.74 (1H, m, C*H*), 5.16-4.98 (2H, m, CH_2), 2.76-2.72 (2H, m, CH_2), 2.70-2.62 (2H, m, CH_2), 2.58 (2H, t, *J* = 7.4 Hz, CH_2), 2.43-2.34

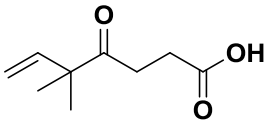
(2H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 208 (*C*=O), 178.3 (*C*=O), 136.9 (*C*H), 115.4 (*C*H_2), 41.8 (*C*H_2), 36.9 (*C*H_2), 29.0 (*C*H_2), 27.7 (*C*H_2); HRMS (ESI$^-$) found 155.0716 $C_8H_{12}O_3$ requires 155.0714.

9.2.8. 4-Oxonon-8-enoic acid 179[338]



4-Oxonon-8-enoic acid **179** was synthesized analogously to 4-oxooct-7-enoic acid **178**. To a suspension of magnesium (1.0 g, 41 mmol) in dry tetrahydrofuran (10 mL) in a round-bottom flask equipped with a rapidly stirring, 5-bromo-1-pentene **177** (2.1 mL, 18.1 mmol) in dry tetrahydrofuran (10 mL) was added over 1 hour under N_2. The resulted Grignard reagent was immediately transferred dropwise *via* cannula to a solution of succinic anhydride **39** (1.81 g, 18.1 mmol) in dry tetrahydrofuran (20 mL) with keeping the temperature below -10 °C. After the addition was completed, the reaction mixture was stirred at 0 °C for 3 hours. The reaction mixture was then quenched with aqueous hydrochloric acid (2 M, 25 mL) and concentrated under reduced pressure. The organic layer was extracted with dichloromethane (2 × 25 mL). The combined organic layers were washed with sodium hydroxide (2 M, 2 × 25 mL). The combined aqueous layers were re-extracted with dichloromethane (3 × 25 mL) after acidification with concentrated hydrochloric acid (2 M, 25 mL). The organic combined layers were dried with magnesium sulphate, filtered and concentrated under reduced pressure. Purification was achieved by column chromatography (ethyl acetate: acetic acid: petroleum ether 10: 0.5: 89.5, R_f = 0.4) to give ketoacid **179** as a pale oil (0.58 g, 58%); υ_{max}(ATR/cm^{-1})= 3084, 2979, 2940, 1699; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 5.83-5.73 (1H, m, C*H*), 5.06-4.98 (2H, m, CH_2), 2.73 (2H, t, *J* = 6.2 Hz, CH_2), 2.66-2.63 (2H, m, CH_2), 2.48 (2H, t, *J* = 7.5 Hz, CH_2), 2.11-2.05 (2H, m, CH_2), 1.72-1.70 (2H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 208.7 (*C*=O), 178.5 (*C*=O), 137.9 (*C*H), 115.4 (*C*H_2), 41.8 (*C*H_2), 36.9 (*C*H_2), 33.0 (*C*H_2), 22.8 (*C*H_2), 27.8 (*C*H_2); HRMS (ESI$^-$) found 169.0876 $C_9H_{14}O_3$ requires 155.0870.

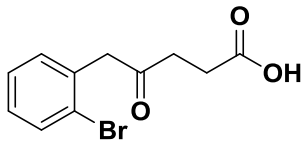
9.2.9. 5, 5-Dimethyl-4-oxohept-6-enoic acid 181 [339]



To a suspension of magnesium turnings (2.1 g, 85 mmol) in anhydrous diethyl ether (10 mL) a solution of 1-bromo-3-methyl-2-butene **180** (2 ml, 17 mmol) in anhydrous diethyl ether (17 mL) was slowly added using syringe pump over a period of 1h at room temperature under N_2.

The reaction mixture was additionally stirred at room temperature for 3 h. The resulted Grignard reagent was immediately added dropwise to a suspension of succinic anhydride **39** (1.0 g, 10 mmol) and copper iodide (0.29 g, 1.5 mmol) in dry tetrahydrofuran (20 mL) at -40 °C over a period of 1h. The reaction mixture was stirred at -40 °C, warmed to (-5 - 0 °C) for 3.5 h, quenched with HCl (2 M, 30 mL) at -5 °C and extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with brine (100 mL). The organic combined layer was dried with magnesium sulphate, filtered and concentrated under reduced pressure. Purification was achieved by column chromatography (ethyl acetate: petroleum ether 3:7, R_f = 0.25) to yield ketoacid **181** as a pale yellow oil (0.49 g, 49%); υ_{max} (ATR/cm^{-1})= 3092, 2977, 2918, 2669, 2570, 1703; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 5.95 (1H, dd, J = 17.6 Hz, 10.6 Hz, C*H*), 5.20-5.18 (2H, m, CH_2), 2.80 (2H, t, J = 6.6 Hz, CH_2), 2.59 (2H, t, J = 6.6 Hz, CH_2), 1.26 (6H, s, 2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 211.5 (*C*=O), 179 (*C*=O), 142.4 (*C*H), 114.6 (*C*H_2), 52.4 (*C*), 32.3 (*C*H_2), 28.2 (*C*H_2), 23.6 (*C*H_3); HRMS (ESI$^-$) found 169.0875 $C_9H_{14}O_3$ requires 169.0870.

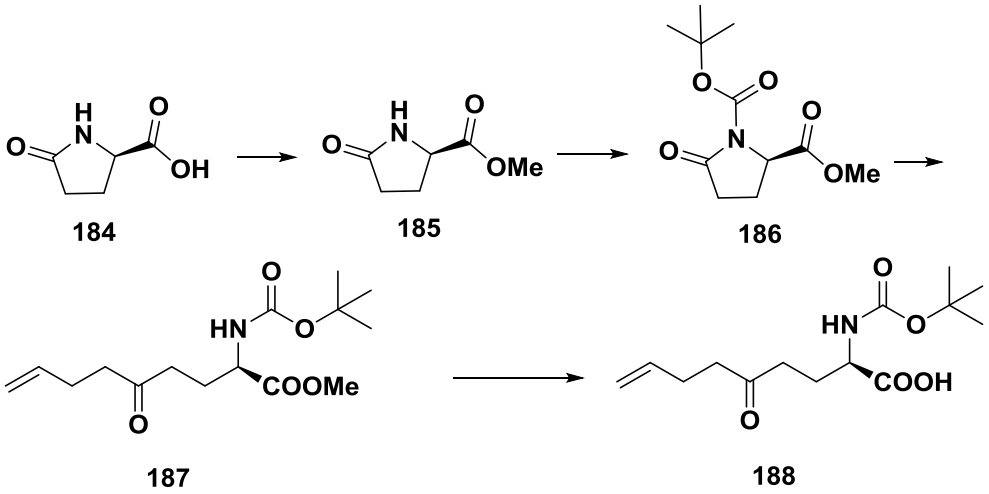
9.2.10. 5-(2-Bromophenyl)-4-oxopentanoic acid 183 [340]



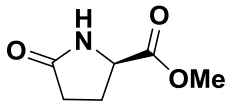
To a suspension of magnesium turnings (1.0 g, 41 mmol) in dry tetrahydrofuran (10 mL) in a three-necked round-bottom flask, 2-bromobenzyl bromide **182** (10.0 g, 41.1 mmol) in anhydrous diethyl ether (90 mL) was added with a gentle reflux for over 1 hour under N_2. The reaction mixture was refluxed for additional 3 h then allowed to warm to room temperature. The resulted Grignard reagent was immediately transferred dropwise *via* cannula to a solution of succinic anhydride **39** (2.74 g, 27 mmol) in dry tetrahydrofuran (150 mL) with keeping the temperature below -78 °C. After the addition was completed, the reaction mixture was then stirred at -78 °C for 4 hours. The reaction mixture was then quenched with H_2O (30 mL) and aqueous hydrochloric acid (2 M, 80 mL). The aqueous layer was then extracted with ethyl acetate (3 × 100 mL), the combined organic layers were washed with brine (100 mL). The organic layer was dried with magnesium sulphate, filtered and concentrated under reduced pressure. Purification was achieved by flash column chromatography (ethyl acetate: chloroform 4: 1, R_f = 0.2) to afford ketoacid **183** as a white solid (1.09 g, 40%); m.p = 87-89 ºC; υ_{max}(ATR/cm^{-1})= 3435, 3051, 2917, 1715, 1469; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.59 (1H, dd, J = 7.9 Hz, 2.2 Hz, ArC*H*), 7.32-7.24 (2H, m,

ArC*H*), 7.16 (1H, dd, *J* = 9.4 Hz, 3.9 Hz, ArC*H*), 3.9 (2H, s, CH_2), 2.9 (2H, t, *J* = 6.5 Hz, CH_2), 2.67 (2H, t, *J* = 6.5 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 205.0 (*C*=O), 178.7 (*C*=O), 134.4 (Ar*C*), 132.9 (Ar*C*H), 131.9 (Ar*C*H), 129.0 (Ar*C*H), 128.2 (Ar*C*H), 125.0 (Ar*C*), 50.0 (CH_2), 36.8 (CH_2), 27.9 (CH_2); HRMS (ESI^+) found 270.9968 $C_{11}H_{11}BrO_3$ requires 270.9964.

9.2.11. Synthesis of 2-((tert-butoxycarbonyl) amino)-5-oxonon-8-enoic acid 188

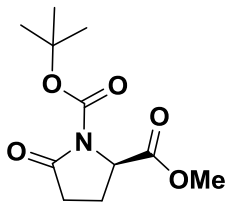


9.2.11.1. (*R*)-Methyl-5-oxopyrrolidine-2-carboxylate 185[341]



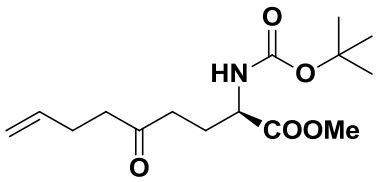
To a solution of *L*-pyrroglutamic acid **184** (5.0 g, 38.7 mmol) in dry methanol (30 mL), concentrated hydrochloric acid (100 µL, 35 %) was added and the solution was allowed to stirrer at room temperature for 24 h. Excess methanol was removed under vacuum to yield crude (*R*)-methyl-5-oxopyrrolidine-2-carboxylate. Purification was achieved by column chromatography (dichloromethane: methanol 9: 1, R_f = 0.4) to give (*R*)-methyl-5-oxopyrrolidine-2-carboxylate **185** as a pale oil (4.5 g, 90%); $[\alpha]_D^{25}$ = +8.5 (c= 1.0, CH_2Cl_2); υ_{max} (ATR/cm^{-1})= 3241, 3104, 2996, 2954, 1744; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 4.29 (1H, dd, *J* = 5 Hz, 2.5 Hz, C*H*), 3.79 (3H, s, OCH_3), 2.41-2.39 (4H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 179.0 (*C*=O), 172.7 (*C*=O), 55.6 (O*C*H$_3$), 52.6 (*C*H), 29.5 (CH_2), 24.7 (CH_2); HRMS (ESI^+) found 166.0479 $C_6H_9NO_3Na$ $[M + Na]^+$ requires 166.0482.

9.2.11.2. (*R*)-1-Tert-butyl-2-methyl-5-oxopyrrolidine-1,2-dicarboxylate 186 [342]



To a solution of (*R*)-methyl-5-oxopyrrolidine-2-carboxylate **185** (4.0 g, 27.9 mmol) in dichloromethane (25 mL), di-*tert*-butyl dicarbonate (9.6 g, 41.7 mmol), trimethylamine (2.89 g, 28.6 mmol) and 4-(dimethylamino) pyridine (DMAP) (0.34 g, 2.78 mmol) were added. The reaction mixture was stirred at room temperature for 24 h and then extracted with diethyl ether (3 × 80 mL). The combined organic layer was washed with an aqueous solution of potassium hydrogen sulphate (40 mL), aqueous solution of sodium carbonate (1M, 40 mL), brine (2 × 40 mL), dried with sodium sulphate and concentrated under reduced pressure. Purification was achieved by column chromatography (diethyl ether: petroleum ether 8:2, R_f = 0.6) to yield (*R*)-1-tert-butyl 2-methyl-5-oxopyrrolidine-1,2-dicarboxylate **186** as a white solid (3.2 g, 80%); m.p = 71-73 °C; $[\alpha]_D^{25}$ = + 49.7 (c= 1.0, CH_3OH); υ_{max} (ATR/cm^{-1})= 3467, 2983, 1795, 1746, 1714, 1457; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 4.63 (1H, dd, J = 5.5 Hz, 2.4 Hz, C*H*), 3.80 (3H, s, OCH_3), 2.71-2.61 (1H, m, CH_2), 2.55-2.47 (1H, m, CH_2), 2.39-2.28 (1H, m, CH_2), 2.09-2.01 (1H, m, CH_2), 1.51 (9H, s, 3CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 173.3 (*C*=O), 171.9 (*C*=O), 149.0 (*C*=O), 83.7 (*C*), 58.9 (*C*H), 52.6 (OCH_3), 31.2 (CH_2), 27.9 (CH_3), 21.50 (CH_2); HRMS (ESI^+) found 266.1000 $C_{11}H_{17}NO_5Na$ [M + Na]$^+$ requires 266.0999.

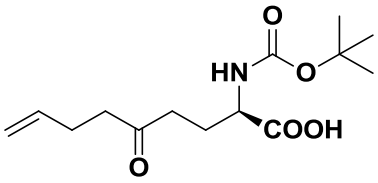
9.2.11.3. (*R*)-methyl 2-((tert-butoxycarbonyl)amino)-5-oxonon-8-enoate 187 [343]



A round-bottom two neck flask was charged with magnesium turnings (0.4 g, 16.5 mmol) and dry tetrahydrofuran (20 mL). To this suspension solution 4-bromo-1-butene **176** (0.20 mL, 1.98 mmol) was added with a rapidly stirring at room temperature under N_2. After 20 minutes, an additional portion of 4-bromo-1-butene **176** (0.64 mL, 6.3 mmol) was completed. The resulted yellow solution was then allowed to stirrer for additional 10 minutes and then transferred *via* cannula to a stirred solution of (*R*)-methyl 5-oxopyrrolidine-2-carboxylate **186** (1.0 g, 4.15 mmol) in dry tetrahydrofuran (25 mL) at -78 °C. The reaction mixture was stirred

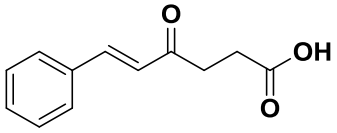
for 3 hours at -78 °C. A saturated ammonium chloride (10 mL) was added and the mixture was poured into a mixture of water (120 mL) and diethyl ether (20 mL). The aqueous layer was extracted with diethyl ether (3 x 40 mL). The combined layers were dried with magnesium sulphate, filtered and concentrated under reduced pressure. Purification was achieved by column chromatography (ethyl acetate: petroleum ether 1:2, R_f = 0.5) to provide methyl 2-((tert-butoxycarbonyl)amino)-5-oxonon-8-enoate **187** as a yellow oil (0.6 g, 60%); υ_{max} (ATR/cm^{-1})= 3363, 3084, 2977, 2935, 1743, 1639; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 5.86-5.76 (1H, m, C*H*), 5.10-4.98 (2H, m, CH_2), 4.32-4.26 (1H, brs, C*H*), 3.75 (3H, s, OCH_3), 2.61-2.45 (4H, m, CH_2), 2.39 (2H, dd, *J* = 7.1 Hz, 3.4 Hz, CH_2), 2.19-2.11 (1H, m, CH_2), 1.95-1.86 (1H, m, CH_2), 1.46 (9H, s, 3CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 209.0 (*C*=O), 173.0 (*C*=O), 155.0 (*C*=O), 137.0 (*C*H), 115.4 (*C*H_2), 77.4 (*C*), 53.0 (*C*H), 52.5 (O*C*H_3), 41.9 (*C*H_2), 38.6 (*C*H_2), 28.4 (*C*H_3), 27.8 (*C*H_2), 26.5 (*C*H_2); HRMS (ESI$^+$) found 322.2000 $C_{15}H_{25}NO_5Na$ [M + Na]$^+$ requires 322.2000.

9.2.11.4. (*R*)-2-((tert-butoxycarbonyl)amino)-5-oxonon-8-enoic acid 188



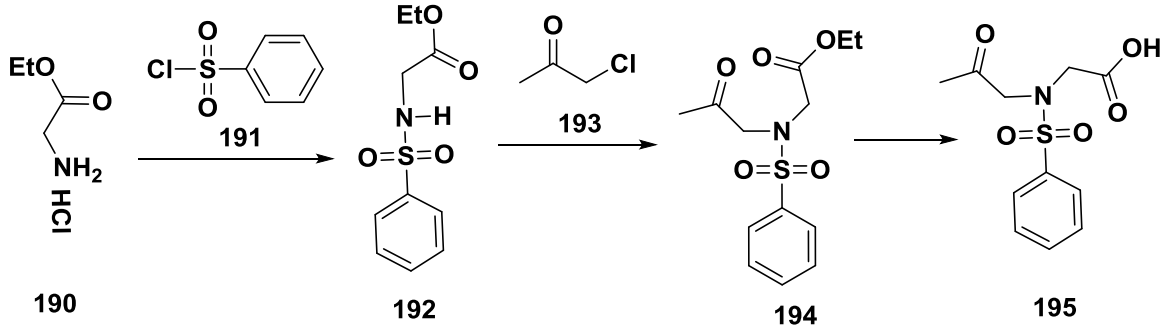
To methyl 2-((tert-butoxycarbonyl) amino)-5-oxonon-8-enoate **187** (0.4 g, 1.33 mmol), sodium hydroxide solution (3.0 mL, 3.0 mmol) was added then the solution was stirred for 24 hours. After that the solution was washed with diethyl ether (5 mL), the aqueous solution was cooled with an ice bath and acidified *via* concentrated hydrochloric acid until to pH= 2. The mixture was further extracted with ethyl acetate (3 x 15 mL). The organic combined layers were dried with magnesium sulphate, filtered and concentrated under reduced pressure. Purification was achieved by column chromatography (ethyl acetate: petroleum ether 4: 6, R_f = 0.3) to yield ketoacid **188** as a pale oil (0.32 g, 80% yield); $[\alpha]_D^{25}$ = +28.9 (c=1.0, CH_3OH); υ_{max} (ATR/cm^{-1})= 3341, 3078, 2980, 2928, 1714; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 5.86-5.77 (1H, m, C*H*), 5.07-4.98 (2H, m, CH_2), 4.28-4.25 (1H, brs, C*H*), 2.72-2.54 (4H, m, CH_2), 2.35 (2H, dd, *J* = 7.2 Hz, 3.3 Hz, CH_2), 2.24-2.15 (1H, m, CH_2), 2.01-1.95 (1H, m, CH_2), 1.46 (9H, s, 3CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 209.0 (*C*=O), 176.3 (*C*=O), 156.0 (*C*=O), 136.9 (*C*H), 115.5 (*C*H_2), 77.4 (*C*), 53.0 (*C*H), 41.9 (*C*H_2), 38.8 (*C*H_2), 28.3 (*C*H_3), 27.8 (*C*H_2), 26.2 (*C*H_2); HRMS (ESI$^+$) found 308.1473 $C_{15}H_{25}NO_5Na$ [M + Na]$^+$ requires 308.1468.

9.2.12. (*E*)-4-Oxo-6-phenylhex-5-enoic acid 189 [344]



To a solution of benzaldehyde **72** (1.06 g, 10 mmol) and 4-oxopentanoic acid **5** (1.16 g, 10 mmol) in dry benzene (30 mL), piperidine (0.13 mL, 1.25 mmol) and glacial acetic acid (0.5 mL, 7 mmol) were added. The reaction mixture was refluxed using a Dean–Stark apparatus until after 24h the starting material was no longer detectable by TLC (dichloromethane: methanol 9.5: 0.5). The reaction was then cooled to room temperature and evaporated under reduced pressure. The residue was then dissolved in ethyl acetate (25 mL), washed with HCl (2M, 2 x 20 mL), brine (40 mL), dried over anhydrous sodium sulphate and filtered. Purification was achieved by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.4) to afford ketoacid **189** as a yellow solid (0.47 g, 40%); m.p = 123-124 ºC; υ_{max}(ATR/cm^{-1})= 3202, 3058, 2916, 1709, 1652; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.43-7.63 (5H, m, ArC*H*), 7.41 (1H, d, *J* = 2.9 Hz, C*H*), 6.78 (1H, d, *J* = 16.3 Hz, C*H*), 3.03 (2H, t , *J* = 6.7 Hz, CH_2), 2.77 (2H, t , *J* = 6.7 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 198 (*C*=O), 178.9 (*C*=O), 143.3 (Ar*C*H), 134.4 (Ar*C*), 129 (Ar*C*H), 128.4 (Ar*C*H), 35.0 (*C*H_2), 28.0 (*C*H_2); HRMS (ESI$^-$) found 203.0717 $C_{12}H_{12}O_3$ requires 203.0714.

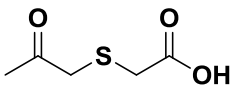
9.2.13. 2-(*N*-(2-Oxopropyl) phenylsulfonamido) acetic acid 195 [345]



Distilled triethylamine (8.5 mL, 0.06 mol) was added dropwise at -20 °C to a vigorously stirred suspension of glycine ethyl ester hydrochloride **190** (3.50 g, 0.025 mol) and benzene sulfonyl chloride **191** (4.50 g, 0.025 mol) in anhydrous THF (75 mL). The reaction mixture was heated at 50 °C for 5 h, then cooled to room temperature and filtered. The filter cake was thoroughly washed with more anhydrous THF (100 mL). The collected filtrate was washed and concentrated under reduced pressure. The residue was then crystallized from ethanol to

give ethyl *N*-sulfonyl glycinate **192** (85%, the compound at least 90% purity by ^{1}H NMR) which was used without further purification. To a solution of ethyl *N*-sulfonyl glycinate **192** in anhydrous acetonitrile (50 mL), chloroacetone **193** (1.70 mL, 0.022 mol), anhydrous potassium carbonate (3.45 g, 0.025 mol) and 18-crown-6 (13 mg) were added and the reaction mixture was refluxed with vigorous stirring for 3h. The reaction mixture was cooled, concentrated to a volume of about 5 mL, poured into 5% aqueous potassium chloride (125 mL) and the residue of mixture was stirred for 20 minutes. It was then extracted with chloroform (3 x 75 mL), the combined organic layers were washed with water (75 mL), brine (75 mL), dried over sodium sulphate, filtered and concentrated under reduced pressure to give ester **194**. An ester **194** was suspended in a solution of KOH (2%) in 50% ethanol/water (50 mL) and stirred at -50 °C for 3 h. The reaction mixture was concentrated to a volume of (12.5 mL) under reduced pressure. The mixture was acidified with HCl (2%) to pH = 3, washed with water, filtered, dried and the crude keto acid was purified by flash column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.3) to afford ketoacid **195** as a brown solid (1.9 g, 55%); m.p = 145-146 ºC; υ_{max}(ATR/cm^{-1})= 3293, 3063, 2929, 2854, 1730; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.84 (2H, d, *J* = 7.2 Hz, ArC*H*), 7.64 (2H, t, *J* = 6.9 Hz, ArC*H*), 7.55 (1H, t, *J* = 7.8 Hz, ArC*H*), 4.26 (2H, s, CH_2), 4.07 (2H, s, CH_2), 4.26 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 204.0 (*C*=O), 171.5 (*C*=O), 138.5 (Ar*C*), 133.4 (Ar*C*H), 129.4 (Ar*C*H), 127.4 (Ar*C*H), 57.5 (*C*H$_2$), 49.5 (*C*H$_2$), 27.0 (*C*H$_3$); HRMS (ESI$^+$) found 272.0590 $C_{11}H_{13}NO_5S$ requires 272.05987.

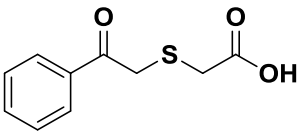
9.2.14. 2-((2-Oxopropyl)thio)acetic acid 198 [346]



2-Mercaptoacetic acid **196** (0.72 g, 8.0 mmol) was slowly added dropwise to a cooled solution of sodium hydroxide (0.32 g, 16 mmol) in water (300 mL) at 0 ºC. Chloroacetone **193** (0.65 mL, 8.0 mmol) was added slowly to the content of the flask and stirred for 5 h at 20 ºC. Later, the reaction mixture was then poured onto crushed ice and acidified with concentrated hydrochloric acid to pH = 2. The crude was extracted with dichloromethane (3 x 100 mL), dried, filtered and concentrated under reduced pressure. Purification was achieved by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.2) to afford ketoacid **198** as a yellow oil (0.54 g, 76%); υ_{max}(ATR/cm^{-1})= 3510, 2925, 2662, 1710; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 3.48 (2H, s, CH_2), 3.31 (2H, s, CH_2), 2.32 (3H, s, CH_3); ^{13}C NMR (100

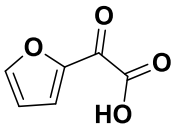
MHz, $CDCl_3$) δ_C = 203.5 (*C*=O), 175.4 (*C*=O), 42.0 (*C*H_2), 33.0 (*C*H_2), 28.5 (*C*H_3); HRMS (ESI^+) found 149.0269 $C_5H_8O_3S$ requires 149.0267.

9.2.15. 2-((2-Oxo-2-phenylethyl)thio)acetic acid 199 [346]



2-Mercaptoacetic acid **196** (1.4 g, 16 mmol) was slowly added dropwise to a cooled solution of sodium hydroxide (0.64 g, 32 mmol) in water (300 mL) at 0 ºC. Phenacyl bromide **197** (3.18 g, 16 mmol) was added slowly to the content of the flask and stirred for 5 h at 20 ºC. Later, the reaction mixture was then poured on to crushed ice and acidified with concentrated hydrochloric acid to pH= 2. The crude was extracted with dichloromethane (3 x 100 mL), dried, filtered, concentrated under reduced pressure. Purification was achieved by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.3) to ketoacid **199** (0.90 g, 68%) as a brown solid, m.p = 90-92 ºC; υ_{max}(ATR/cm^{-1})= 3350, 2895, 1732, 1681, 1527; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.99 (2H, d, *J* = 7.4 Hz, ArC*H*), 7.63 (1H, t, *J* = 7.5 Hz, ArC*H*), 7.51 (2H, t, *J* = 7.6 Hz, ArC*H*), 4.0 (2H, s, CH_2), 3.41 (2H, s, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 194.5 (*C*=O), 174.4 (*C*=O), 135 (Ar*C*), 133.9 (Ar*C*H), 128.8 (Ar*C*H), 128.5 (Ar*C*H), 37.8 (*C*H_2), 33.3 (*C*H_2); HRMS (ESI^+) found 210.0251 $C_{10}H_{10}O_3S$ requires 210.0249.

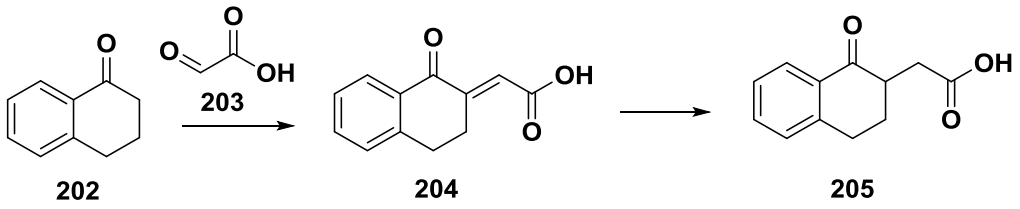
9.2.16. 2-(Furan-2-yl)-2-oxoacetic acid 201[347]



A dried round-bottom was charged with 1-(furan-2-yl) ethanone **200** (2.2 g, 20 mmol), selenium dioxide SeO_2 (3.34 g, 30 mmol) and anhydrous pyridine (10 mL). The reaction mixture was rapidly stirred under N_2 at 110 ºC for 1 h, the mixture was then stirred at 90 ºC for an additional 5 h. The precipitated mixture (containing selenium) was filtered through a Buchner funnel and the residue was thoroughly washed with ethyl acetate (500 mL). The organic filtrate was extracted with HCl (1M, 400 mL) and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (3 x 300 mL) and the organic layers were combined, treated with NaOH (1M, 400 mL) and the aqueous layer was separated. The organic layer was washed with water (500 mL) and the combined aqueous layers were acidified using HCl (1M) to pH = 1.5. The mixture was then extracted with ethyl acetate (3 x 400 mL) and the organic combined layers were dried with magnesium sulphate, filtered and

concentrated under reduced pressure. The crude furylglyoxylic acid was purified by flash column chromatography (ethyl acetate: petroleum ether 8: 2, R_f = 0.2) to yield ketoacid **201** as a grey solid (1.43 g, 65%), m.p = 92-95 ºC; υ_{max} (ATR/cm^{-1})= 3488, 3145, 1734, 1675, 1666; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.75 (1H, s, O*H*), 8.22 (1H, d, *J* = 3.8 Hz, C4*H*$_3$O), 7.89 (1H, d, *J* = 2.2 Hz, C4*H*$_3$O), 6.12 (1H, dd, *J* = 3.8 Hz, 1.7 Hz, C4*H*$_3$O); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 169.7 (*C*=O), 158.7 (*C*=O), 151.4 (*C4*H$_3$O), 148.4 (*C4*H$_3$O), 131.0 (*C4*H$_3$O), 129.0 (*C4*H$_3$O).

9.2.17. 2-(1-Oxo-1,2,3,4-tetrahydronaphthalen-2-yl) acetic acid 205 [112]

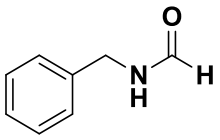


A mixture of *α*-tetralone **202** (1.05 g), glyoxylic acid **203** (1.0 g) and sulfuric acid 90% (0.3 mL) was mixed in dioxane (15 mL) and refluxed for 1h. Reaction mixture was then evaporated under reduced pressure to afford (*E*)-2-(1-oxo-3,4-dihydronaphthalen-2(1H)-ylidene) acetic acid **204**; ^{1}HNMR (DMSO-d$_6$) δ_H = 7.87 (1H, dd, *J* = 7.7 Hz, 2.1 Hz, ArC*H*), 7.58 (1H, t, *J* = 7.5 Hz, ArC*H*), 7.3-7.4 (2H, m, ArC*H*), 6.6 (1H, s, C*H*), 3.3 (2H, t, *J* = 6.5Hz, C*H*$_2$), 2.96 (2H, t, *J* = 6.0 Hz, C*H*$_2$). (*E*)-2-(1-Oxo-3,4-dihydronaphthalen-2(1H)-ylidene) acetic acid **204** (1.3 g), zinc dust (1.35 g), acetic acid (15 mL) and water (5 mL) were refluxed for 1h. The solvent was evaporated and the residual solid was dried reduced pressure in presence of NaOH to eliminate all traces of acetic acid. After trituration in methanol, the insoluble was then filtrated off. Methanol was removed and the solid was diluted in a mixture of water/ethyl acetate to remove salt. The organic layer was evaporated to yield ketoacid **205** as a grey solid (0.78 g, 60%); m.p = 97-99 ºC; υ_{max} (ATR/cm^{-1})= 3433, 3061, 2936, 1774, 1705, 1681; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H = 12.3 (1H, s, O*H*), 7.86 (1H, d, *J* = 7 Hz, ArC*H*), 7.54 (1H, td, *J* = 8.6 Hz, 3.7 Hz, ArC*H*), 7.34 (2H, t, *J* = 7.5 Hz, ArC*H*), 2.71 (1H, dd, *J* = 16.5 Hz, 5.8 Hz, C*H*), 2.5 (1H, dd, *J* = 16.6 Hz, 6.3 Hz, C*H*), 2.01 (1H, dtd, *J* = 13 Hz, 4.5 Hz, C*H*), 2.90-3.15 (4H, m, 2C*H*$_2$); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ_C = 198.7 (*C*=O), 173.9 (*C*=O), 144.7 (Ar*C*), 133.9 (Ar*C*H), 132.3 (Ar*C*), 130.2 (Ar*C*H), 129.5 (Ar*C*H), 127.0 (Ar*C*H), 44.7 (*C*H), 35.0 (*C*H$_2$), 29.0 (*C*H$_2$), 23.4 (*C*H$_2$); HRMS (ESI$^+$) found 205.0861 $C_{12}H_{12}O_3$ requires 205.0859.

9.3. Synthesis of isocyanides

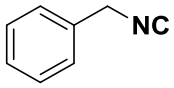
9.3.1. Synthesis of (isocyanomethyl) benzene 59

9.3.1.1. Synthesis of *N*-benzylformamide 208 [348]



A mixture of benzyl amine **206** (1.07 g, 10 mmol), methyl formate **207** (2.5 mL, 40 mmol) and 1,5,7-triazabicyclo [4.4.0] dec-5-ene (0.14 g, 1 mmol) was dissolved in toluene (100 mL) and stirred at reflux overnight. The solvent was removed under reduced pressure and crude compound was purified by column chromatography (ethyl acetate: petroleum ether 1:1, R_f = 0.6) to yield *N*-benzylformamide **208** as a white solid (0.80 g, 75%); m.p = 62-64 ^{o}C; υ_{max} (ATR/cm^{-1}) = 3276, 3058, 3029, 2876, 1649, 1535; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.30 (1H, s, C*H*O), 7.42-7.27 (5H, m, ArC*H*), 5.79 (1H, brs, N*H*), 4.52 (2H, d, *J* = 5.9 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 160.9 (*C*=O), 137.5 (Ar*C*H), 129.0 (Ar*C*), 128.9 (Ar*C*H), 127.9 (Ar*C*H), 42.2 (*C*H_2); HRMS (ES) found 136.0697 $C_8H_9NOH^+$ requires 136.0700.

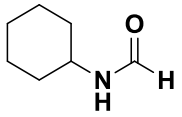
9.3.1.2. Synthesis of (isocyanomethyl) benzene 59[349]



To a cooled solution of *N*-benzylformamide **208** (0.67 g, 5 mmol) and triethylamine (1.5 mL, 11 mmol) in dry dichloromethane (50 mL), a solution of phosphorus (V) oxychloride (0.5 mL, 5.5 mmol) in dry dichloromethane (50 mL) was added dropwise at 0 ^{o}C. The reaction mixture was warmed to room temperature with stirring until complete by TLC (approximately 20 minutes). After that the reaction mixture was diluted with dichloromethane (150 mL) and saturated sodium bicarbonate solution (100 mL). The aqueous layer was extracted with dichloromethane (2 × 100 mL). The organic combined layers were dried with sodium sulphate, filtered and concentrated under reduced pressure. Purification was achieved by flash column chromatography (ethyl acetate: petroleum ether 1: 9, R_f = 0.6) to yield isocyanomethyl benzene **59** as a brown oil (0.23 g, 35%); υ_{max}(ATR/cm^{-1})= 3090, 3065, 3035, 2150, 1500, 1455, 1440; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.45-7.33 (5H, m, ArC*H*), 4.68 (2H, s, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 137.0 (Ar*C*), 129.0 (Ar*C*H), 128.5 (Ar*C*H), 126.6 (Ar*C*H), 45.6 (*C*H_2); HRMS (ES) found 118.0602 $C_8H_7NH^+$ requires 118.0604.

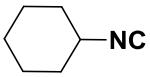
9.3.2. Synthesis of cyclohexylisocyanide 113

9.3.2.1. Synthesis of *N*-cyclohexylformamide 210[348]



A mixture of cyclohexylamine **209** (1.0 g, 10 mmol), methyl formate **207** (2.5 mL, 40 mmol) and 1,5,7-triazabicyclo [4.4.0] dec-5-ene (0.14 g, 1 mmol) was dissolved in toluene (100 mL) and stirred at reflux overnight. The solvent was removed under reduced pressure and crude compound was purified by column chromatography (ethyl acetate: petroleum ether 1:2, R_f = 0.3) to yield *N*-cyclohexylformamide **210** as a white oil (0.85 g, 85%); υ_{max} (ATR/cm^{-1}) = 3276, 2930, 1661, 1540; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.08 (1H, s, C*H*O), 6.09 (1H, brs, N*H*), 3.88-3.75 (1H, m, C*H*), 1.88-1.22 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 162.9 (*C*=O), 45.2 (*C*H), 32.5 (*C*H_2), 25.2 (*C*H_2), 24.5 (*C*H_2); HRMS (ES) found 128.0996 $C_7H_{13}NOH^+$ requires 128.0994.

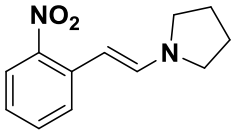
9.3.2.2. Synthesis of cyclohexylisocyanide 113



To a cooled solution of *N*-cyclohexylformamide **210** (0.63 g, 5 mmol) and triethylamine (1.5 mL, 11 mmol) in drydichloromethane (50 mL), a solution of phosphorus (V) oxychloride (0.5 mL, 5.5 mmol) in drydichloromethane (50 mL) was added dropwise at 0 ^{o}C. The reaction mixture was warmed to room temperature with stirring until complete by TLC (approximately 20 minutes). After that the reaction mixture was diluted with dichloromethane (150 mL) and saturated sodium bicarbonate solution (100 mL). The aqueous layer was extracted with dichloromethane (2 × 100 mL). The organic combined layers were dried with sodium sulphate, filtered and concentrated under reduced pressure. Purification was achieved by column chromatography (ethyl acetate: petroleum ether 1: 10, R_f = 0.7) to yield cyclohexylisocyanide **113** as a white oil (0.41 g, 65%); υ_{max} (ATR/cm^{-1}) = 3090, 3065, 2155; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 3.66-3.56 (1H, m, C*H*), 1.92-1.26 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 154.4 (*C*), 52.3 (*C*H), 33.7 (*C*H_2), 25.7 (*C*H_2), 23.5 (*C*H_2); HRMS (ES) found 110.0966 $C_7H_{11}NH^+$ requires 110.0964.

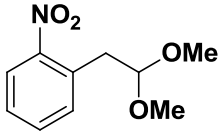
9.3.3. Synthesis of convertible isocyanide 149

9.3.3.1. 1-[(*E*)-2-(2-Nitrophenyl)vinyl]pyrrolidine 214[97]



A solution of 2-nitrotoluene **211** (5.0 g, 36.2 mmol), dimethylformamide diethyl acetal **212** (5.1 g, 42 mmol) and pyrrolidine **213** (3.0 g, 42.2 mmol) was refluxed in DMF (25 ml) for 5 hours, during that time the reaction mixture turned dark. ^{1}HNMR analysis of a reaction aliquot exhibited a 3:1 ratio of 2-nitrotoluene to desired product **214**. Therefore, the temperature of reaction was increased to 80 ºC and stirring was continued for 19 hours, after that time ^{1}HNMR analysis showed no 2-nitrotoluene remained. The resulted dark red compound was evaporated to dryness on a rotary evaporator and this crude was poured in water (50 mL). The solution was further extracted with ethyl acetate (5 × 200 mL). The organic combined layers were dried with sodium sulphate, filtered and concentrated under reduced pressure. Purification was achieved by column chromatography (ethyl acetate: petroleum ether 1:4, R_f = 0.28) to yield enamine **214** as a red oil (4.5 g, 70%); υ_{max} (ATR/cm^{-1})= 3010, 1615, 1510, 1345, 982; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.01-7.79 (1H, m, ArC*H*), 7.87-7.84 (1H, m, ArC*H*), 7.54-7.33 (2H, m, ArC*H*), 6.93 (1H, d, *J* = 12.9 Hz, C*H*), 5.85 (1H, d, *J* = 13.5 Hz, C*H*), 3.41-3.35 (4H, m, 2CH_2), 2.00-1.95 (4H, m, 2CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 140.0 (Ar*C*), 133.0 (Ar*C*H), 132.7 (Ar*C*H), 128.9 (Ar*C*), 126.8 (Ar*C*H), 125.5 (Ar*C*H), 122.0 (*C*H), 91.0 (*C*H), 49.2 (*C*H_2), 25.3 (*C*H_2); HRMS (ES) found 219.1100 $C_{12}H_{14}N_2O_2H^+$ requires 219.1110.

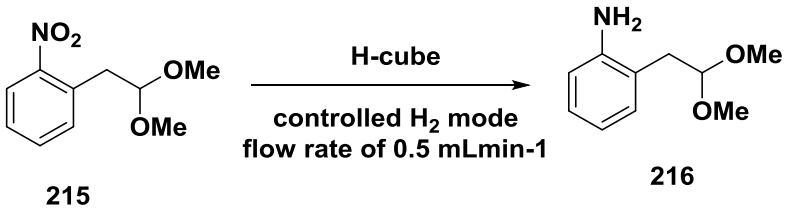
9.3.3.2. (2,2-Dimethoxyethyl)-2-nitrobenzene 215 [350]



A solution of enamine **214** (4.5 g, 4.45 mmol) in methanol (40 mL) was added to a solution of *para*-toluenesulfonic acid (4.70 g, 24.5 mmol) in methanol (25 mL). The reaction mixture was heated to reflux for 12 hours until TLC detected full conversation. The mixture of reaction was allowed to cooling. Sodium carbonate (0.7 g, 6.3 mmol) was carefully added and the reaction mixture was additionally stirred for 15 minutes. Excess methanol was removed under vacuum and the reaction mixture was separated between toluene (20 mL) and water (20 mL). The aqueous layer was then extracted with toluene (3 × 50 mL). The organic combined layers were dried with sodium sulphate, filtered and concentrated under reduced

pressure. Purification was achieved by column chromatography (ethyl acetate: petroleum ether 1:4, R_f = 0.24) to yield **215** as an orange oil (2.58 g, 60%); υ_{max} (ATR/cm^{-1}) = 1510, 1355, 1120, 980; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.91-7.87 (1H, m, ArC*H*), 7.57-7.52 (1H, m, ArC*H*), 7.45-7.38 (2H, m, ArC*H*), 4.59 (1H, t, *J* = 5.4 Hz, C*H*), 3.38 (6H, s, 2OCH_3), 3.24 (2H, d, *J* = 5.5 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 150.0 (Ar*C*), 133.7 (Ar*C*), 132.6 (Ar*C*H), 131.6 (Ar*C*H), 127.6 (Ar*C*H), 124.5 (Ar*C*H), 104.6 (*C*H), 54.4 (O*C*H$_3$), 37.0 (*C*H$_2$); HRMS (EI$^+$) of $C_{10}H_{13}NO_4Na$ found 234.0732 (M+Na$^+$) requires 234.0742.

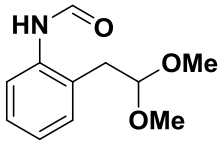
9.3.3.3. 2-(2,2-Dimethoxyethyl)aniline 216 [350]



215 **216**

The H-cube reactor of ThalesNano system was fitted with a 10 % Pd/C cartridge; a hydrogen mode was primed at 1 mLmin^{-1} using methanol for 5 minutes. A 0.1 M solution of **215** (0.42 g, 2 mmol) in 40 mL methanol was prepared and passed through the H-cube reactor at a rate of 0.5 mLmin^{-1}, which was supplied with hydrogen pressure of 80 bar and a reactor temperature of 80 ^{o}C. The output stream the reactor was collected and solvent was then removed under reduced pressure to yield a 5: 1 mixture of **216** and **215** respectively. Purification was achieved by column chromatography (ethyl acetate: petroleum ether 1:4, R_f = 0.35) to yield **216** as a yellow oil (0.33 g, 80%); υ_{max} (ATR/cm^{-1})= 3430, 1121, 980; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.10-7.06 (2H, m, Ar*C*H), 6.78-6.69 (2H, m, Ar*C*H), 4.52 (1H, t, *J* = 5.34 Hz, C*H*), 4.08 (2H, brs, NH_2), 3.42 (6H, s, 2OCH_3), 2.89 (2H, d, *J* = 5.5 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 146.0 (Ar*C*), 131.3 (Ar*C*H), 127.8 (Ar*C*H), 122.4 (Ar*C*) 118.7 (Ar*C*H), 116.3 (Ar*C*H), 106.6 (*C*H), 54.0 (O*C*H$_3$), 36.5 (*C*H$_2$); HRMS (ES) (MH$^+$) found 182.1176 $C_{10}H_{15}NO_2H^+$ requires 182.1776.

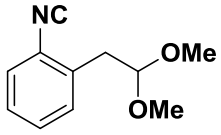
9.3.3.4. *N*-(2-(2,2-Dimethoxyethyl)phenyl)formamide 218[351]



To a solution of hexamethyldisilazane (4.6 mL, 22.0 mmol) in THF (40 mL) n-butyl lithium (25 mL, 1.0 M in hexanes) was added at 0 ^{o}C. After 30 minutes, a solution of **216** (2.0 g, 11.0

mmol) in THF (40 mL) was added, then followed by ethyl formate **217** (1.4 mL, 16.6 mmol). The solution was further refluxed for 18 hours until TLC (diethyl ether: petroleum ether 1:1) detected full conversion. A saturated ammonium chloride (100 mL) was added and the reaction mixture was extracted with ethyl acetate (3 × 50 mL). The organic combined layers were dried with sodium sulphate, filtered and concentrated under reduced pressure. Purification was achieved by flash column chromatography (ethyl acetate: petroleum ether 3:7, R_f = 0.5) to yield **218** as a brown oil (1.0 g, 50%); υ_{max} (ATR/cm^{-1})= 1650, 1116, 995; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.79 (1H, s, C*H*O), 8.52-8.44 (1H, m, ArC*H*), 7.94 (1H, d, *J* = 8.04 Hz, ArC*H*), 7.74-7.71 (1H, m, ArC*H*), 7.56-7.54 (1H, m, ArC*H*), 4.48-4.40 (1H, m, C*H*), 3.44 (3H, s, OCH_3), 3.41 (3H, s, OCH_3), 2.96 (2H, d, *J* = 6.0 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 130.2 (Ar*C*), 129.5 (Ar*C*H), 127.9 (Ar*C*H), 126.4 (Ar*C*H), 123.2 (Ar*C*H), 102.9 (Ar*C*), 100.5 (*C*H), 55.0 (O*C*H$_3$), 36.2 (*C*H$_2$); HRMS (EI$^+$) of $C_{11}H_{15}NO_3Na$ found 232.0943 (M+Na$^+$) requires 232.0950.

9.3.3.5. 1-(2,2-Dimethoxyethyl)-2-isocyanobenzene 149 [351]

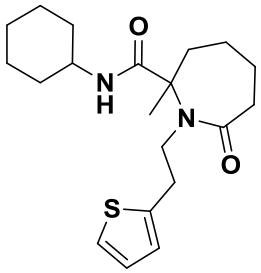


To a solution of **218** (1.2 g, 5.8 mmol) dissolved in dry THF (20 mL), triethylamine (2.0 mL, 14.6 mmol) was added and the solution was cooled to -60 º C with an ethanol/dry ice bath. Phosphorus(V) oxychloride (0.8 mL, 8.6 mmol) was added dropwise, subsequently the solution was allowed to warm to room temperature and stirred until TLC (petroleum ether: diethyl ether 1:1) detected full conversion (approximately 1 hour). The reaction mixture was poured into ice water (150 mL) then extracted with diethyl ether (3 × 100 mL). The organic combined layers were washed with saturated sodium chloride solution (100 mL). The organic combined layers were dried with sodium sulphate, filtered and concentrated under reduced pressure. Purification was achieved by flash column chromatography (ethyl acetate: petroleum ether 3:7, R_f = 0.6) to yield **149** as a brown oil (0.86 g, 77%); υ_{max} (ATR/cm^{-1})= 2987, 2853, 2922, 2834, 2120, 1490; ^{1}HNMR (400 MHz, $CDCl_3$) δ_H = 7.37-7.34 (2H, m, ArC*H*), 7.28-7.23 (2H, m, ArC*H*), 4.62 (1H, t, *J* = 4.9 Hz, C*H*), 3.4 (6H, s, 2OCH_3), 3.2 (2H, d, *J* = 5.3 Hz, CH_2); ^{13}CNMR (100 MHz, $CDCl_3$) δ_C= 131.4 (Ar*C*), 129.4 (Ar*C*H), 127.5 (Ar*C*H), 126.9 (Ar*C*H), 124.0 (Ar*C*H), 103.8 (Ar*C*), 100.4 (*C*H), 54.0 (O*C*H$_3$), 36.0 (*C*H$_2$); HRMS (ES$^+$) found 192.1027 $C_{11}H_{13}NO_2H^+$ requires 192.0925.

9.4. Synthesis of cyclic amides[103]

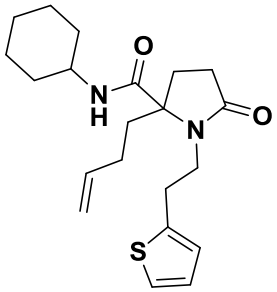
To a solution of keto-acid (1.0 mmol) in methanol (5 mL) at room temperature, amine (1.25 mmol) was added at once and the mixture was stirred for 40 minutes to ensure imine formation. An isocyanide (1.0 mmol) was added at once and the reaction mixture was stirred at room temperature for 48-72 hours. The excess methanol was evaporated under reduced pressure and the residue was dissolved in dichloromethane (10 mL). The reaction was worked up by quenching once with hydrochloric acid (10%, 10 mL) to ensure removal of the most unreacted amine. The organic layer was then washed twice with sodium hydroxide (5 M, 50 mL) to ensure the removal of the most unreacted keto-acid. The organic combined layer was dried with magnesium sulphate, filtered and concentrated under reduced pressure. The crude cyclic amides were purified by column chromatography to obtain the titled cyclic amide.

9.4.1. *N*-Cyclohexyl-2-methyl-7-oxo-1-(2-(thiophen-2-yl) ethyl) azepane-2-carboxamide 226



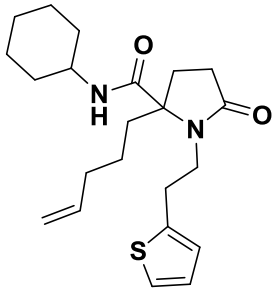
Isolated as a pale oil, following method above using 6-oxoheptenoic acid **32** (0.36 g, 2.5 mmol), 2-(thiophen-2-yl) ethanamine **224** (0.39 g, 3.12 mmol) and cyclohexylisocyanide **113** (0.27 g, 2.5 mmol) for 72 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.26) to yield amide **226** (0.14 g, 40% yield); υ_{max} (ATR/cm^{-1})= 3332, 2993, 2930, 2853, 1629, 1521; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H= 7.18 (1H, d, J = 2.2 Hz, C_4H_3S), 6.98 (1H, dd, J = 4.9 Hz, 2.8 Hz, C_4H_3S), 6.89 (1H, d, J = 2.2 Hz, C_4H_3S), 3.99-3.92 (1H, m, C*H*), 3.81-3.72 (2H, m, CH_2), 3.52-3.43 (2H, m, CH_2), 3.23-3.13 (2H, m, CH_2), 3.10-3.03 (2H, m, CH_2), 2.34-1.69 (10H, m, 5CH_2), 1.62 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 177.0 (*C*=O), 173.3 (*C*=O), 140.9 (C_3H_2SC), 127.0 (C_3H_2SCH), 125.4 (C_3H_2SCH), 123.9 (C_3H_2SCH), 65.5 (*C*), 48.4 (*C*H), 47.0 (*C*H$_2$), 38.5 (*C*H$_2$), 37.4 (*C*H$_2$), 32.9 (*C*H$_2$), 29.9 (*C*H$_2$), 28.7 (*C*H$_2$), 25.4 (*C*H$_2$), 24.9 (*C*H$_2$), 24.8 (*C*H$_2$), 22.0 (*C*H$_3$); HRMS (ESI$^+$) (M+H$^+$) found 363.2100 $C_{20}H_{30}N_2O_2S$ requires 363.2101.

9.4.2. 2-(But-3-en-1-yl)-*N*-cyclohexyl-5-oxo-1-(2-(thiophen-2-yl) ethyl) pyrrolidine-2-carboxamide 228



Isolated as a pale oil, following method above using 4-oxooct-7-enoic acid **178** (0.10 g, 0.64 mmol), 2-(thiophen-2-yl) ethanamine **224** (0.10 g, 0.80 mmol) and cyclohexylisocyanide **113** (0.07 g, 0.64 mmol) for 72 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.4) to yield amide **228** (0.06 g, 55% yield); υ_{max} (ATR/cm^{-1})= 3328, 3078, 2931, 2853, 1665, 1526; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.18 (1H, d, J = 6.4 Hz, C_4H_3S), 6.96 (1H, dd, J = 5.2 Hz, 2.4 Hz, C_4H_3S), 6.89 (1H, d, J = 6.5 Hz, C_4H_3S), 5.83-5.79 (1H, m, C*H*), 5.11-5.02 (2H, m, CH_2), 3.81-3.70 (1H, m, C*H*), 3.47-3.38 (2H, m, CH_2), 3.25-3.19 (2H, m, CH_2), 2.51-2.45 (2H, m, CH_2), 2.23-2.12 (4H, m, 2CH_2), 1.91-1.83 (2H, m, CH_2), 1.75-1.18 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C= 176.5 (*C*=O), 172.3 (*C*=O), 140.7 (*C*H), 137.0 (C_3H_2SC), 127.0 (C_3H_2SCH), 125.5 (C_3H_2SCH), 124.0 (C_3H_2SCH), 115.5 (CH_2), 70.2 (*C*), 48.7 (*C*H), 44 (CH_2), 33.6 (CH_2), 33 (CH_2), 32.5 (CH_2), 29.8 (CH_2), 29.3 (CH_2), 28.5 (CH_2), 27.7 (CH_2), 25.4 (CH_2); HRMS (ESI$^+$) (M+H$^+$) found 375.2098 $C_{21}H_{30}N_2O_2S$ requires 375.2101.

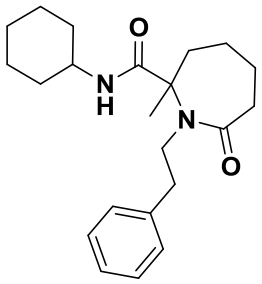
9.4.3. ***N*-Cyclohexyl-5-oxo-2-(pent-4-en-1-yl)-1-(2-(thiophen-2-yl) ethyl) pyrrolidine-2-carboxamide 229**



Isolated as a brown oil, following method above using 4-oxonon-8-enoic acid **179** (0.10 g, 0.588 mmol), 2-(thiophen-2-yl) ethanamine **224** (0.094 g, 0.74 mmol) and cyclohexylisocyanide **113** (0.07 g, 0.59 mmol) for 72 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.5) to yield amide **229** (0.039 g, 39% yield); υ_{max} (ATR/cm^{-1})= 3318, 3077, 2926, 2856, 1674, 1528, 1444; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.18 (1H, d, J = 5.1 Hz, C_4H_3S), 6.96 (1H, dd, J = 4.8 Hz, 2.3 Hz,

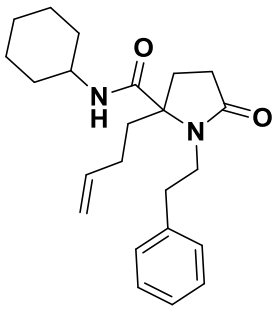
C_4H_3S), 6.88 (1H, d, J = 5.1 Hz, C_4H_3S), 5.84-5.74 (1H, m, C*H*), 5.07-5.00 (2H, m, CH_2), 3.79-3.71 (1H, m, C*H*), 3.48-3.32 (2H, m, CH_2), 3.28-3.11 (2H, m, CH_2), 2.51-2.40 (2H, m, CH_2), 2.21-2.05 (4H, m, CH_2), 1.88-184 (2H, m, CH_2), 1.55-1.46 (2H, m, CH_2), 1.52-1.42 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176.5 (*C*=O), 172.5 (*C*=O), 140.7 (*C*H), 136.7 (C_3H_2SC), 127.0 (C_3H_2SCH), 125.5 (C_3H_2SCH), 124.0 (C_3H_2SCH), 115.7 (*C*H_2), 70.4 (*C*), 48.7 (*C*H), 44.1 (*C*H_2), 33.9 (*C*H_2), 33.7 (*C*H_2), 32.9 (*C*H_2), 29.9 (*C*H_2), 29.5 (*C*H_2), 28.5 (*C*H_2), 25.4 (*C*H_2), 24.9 (*C*H_2), 22.8 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 389.2258 $C_{22}H_{32}N_2O_2S$ requires 389.2257.

9.4.4. ***N*-Cyclohexyl-2-methyl-7-oxo-1-phenethylazepane-2-carboxamide 230**



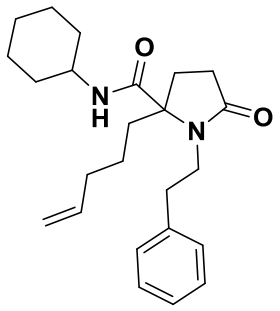
Isolated as a colourless oil, following method above using 6-oxoheptenoic acid **32** (0.36 g, 2.5 mmol), 2-phenylethanamine **225** (0.37 g, 3.12 mmol) and cyclohexylisocyanide **113** (0.27 g, 2.5 mmol) for 48 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.3) to yield amide **230** (0.12 g, 35% yield); υ_{max} (ATR/cm^{-1})= 3328, 3065, 3026, 2931, 2853, 1623, 1522; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H= 7.35-7.23 (5H, m, ArC*H*), 3.92-3.72 (1H, m, C*H*), 3.52-3.43 (2H, m, CH_2), 2.99-2.82 (4H, m, 2CH_2), 2.67-2.62 (4H, m, 2CH_2), 2.35-1.72 (10H, m, 5CH_2), 1.62 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176.9 (*C*=O), 173.4 (*C*=O), 138.8 (Ar*C*), 128.8 (Ar*C*H), 128.7 (Ar*C*H), 126.6 (Ar*C*H), 65.5 (*C*), 48.4 (*C*H), 46.9 (*C*H_2), 38.6 (*C*H_2), 37.5 (*C*H_2), 36.0 (*C*H_2), 32.9 (*C*H_2), 28.8 (*C*H_2), 25.5 (*C*H_2), 24.8 (*C*H_2), 24.8 (*C*H_2), 22.0 (*C*H_3); HRMS (ESI$^+$) (M+H$^+$) found 357.2542 $C_{22}H_{32}N_2O_2$ requires 357.2537.

9.4.5. **2-(But-3-en-1-yl)-*N*-cyclohexyl-5-oxo-1-phenethylpyrrolidine-2-carboxamide 232**



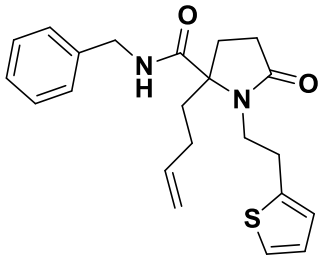
Isolated as a colourless oil, following method above using 4-oxooct-7-enoic acid **178** (0.10 g, 0.64 mmol), 2-phenylethanamine **225** (0.09 g, 0.80 mmol) and cyclohexylisocyanide **113** (0.07 g, 0.64 mmol) for 72 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.5) to yield amide **232** (0.04 g, 40 % yield); v_{max} (ATR/cm^{-1})= 3311, 3073, 3024, 2930, 2856, 1660, 1528; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H= 7.35-7.25 (5H, m, ArC*H*), 5.83-5.82 (1H, m, C*H*), 5.11-5.01 (2H, m, CH_2), 3.81-3.72 (1H, m, C*H*), 3.46-3.25 (2H, m, CH_2), 3.06-2.88 (2H, m, CH_2), 2.51-2.46 (4H, m, 2CH_2), 2.30-2.13 (4H, m, CH_2), 1.91-1.85 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C= 176.4 (*C*=O), 172.5 (*C*=O), 138.5 (*C*H), 137 (Ar*C*), 128.8 (Ar*C*H), 128.8 (Ar*C*H), 126.8 (Ar*C*H), 115.5 (*C*H_2), 70.3 (*C*), 48.6 (*C*H), 44 (*C*H_2), 34.5 (*C*H_2), 33.4 (*C*H_2), 33.1 (*C*H_2), 32.8 (*C*H_2), 29.8 (*C*H_2), 29.3 (*C*H_2), 27.7 (*C*H_2), 24.9 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 369.2536 $C_{23}H_{32}N_2O_2$ requires 369.2537.

9.4.6. ***N*-Cyclohexyl-5-oxo-2-(pent-4-en-1-yl)-1-phenethylpyrrolidine-2-carboxamide 233**



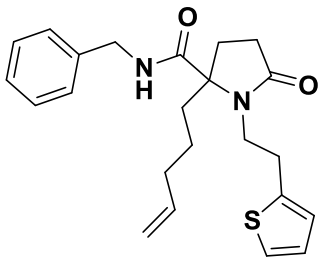
Isolated as a colourless oil, following method above using 4-oxonon-8-enoic acid **179** (0.1 g, 0.58 mmol), 2-phenylethanamine **225** (0.09 g, 0.73 mmol) and cyclohexylisocyanide **113** (0.064 g, 0.588 mmol) for 48 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 4: 6, R_f = 0.5) to yield amide **233** (0.045 g, 45% yield); υ_{max} (ATR/cm^{-1})= 3325, 3059, 3024, 2937, 2856, 1664, 1524, 1493; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.34-7.22 (5H, m, ArC*H*), 5.84-5.75 (1H, m, C*H*), 5.07-4.98 (2H, m, CH_2), 3.79-3.72 (1H, m, C*H*), 3.42-3.25 (2H, m, CH_2), 3.03-2.87 (2H, m, CH_2), 2.51-2.38 (2H, m, CH_2), 2.20-2.06 (4H, m, CH_2), 1.89-185 (2H, m, CH_2), 1.56-1.46 (2H, m, CH_2), 1.55-1.44 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176.5 (*C*=O), 172.7 (*C*=O), 138.7 (*C*H), 137.8 (Ar*C*), 128.8 (Ar*C*H), 128.6 (Ar*C*H), 126.7 (Ar*C*H), 115.5 (*C*H_2), 70.2 (*C*), 48.7 (*C*H), 44 (*C*H_2), 34.5 (*C*H_2), 33.9 (*C*H_2), 33.8 (*C*H_2), 33.0 (*C*H_2), 32.9 (*C*H_2), 29.9 (*C*H_2), 25.4 (*C*H_2), 24.9 (*C*H_2), 24.9 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 383.2693 $C_{24}H_{34}N_2O_2$ requires 383.2693.

9.4.7. ***N*-Benzyl-2-(but-3-en-1-yl)-5-oxo-1-(2-(thiophen-2-yl) ethyl) pyrrolidine-2-carboxamide 234**



Isolated as a brown oil, following method above using 4-oxooct-7-enoic acid **178** (0.10 g, 0.64 mmol), 2-(thiophen-2-yl) ethanamine **224** (0.10 g, 0.80 mmol) and isocyanomethyl benzene **59** (0.07 g, 0.64 mmol) for 48 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 7: 3, R_f = 0.3) to yield amide **234** (0.06 g, 62% yield); υ_{max} (ATR/cm^{-1})= 3344, 3165, 2981, 2945, 2834, 1688, 1520; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.45-7.31 (5H, m, ArC*H*), 7.22 (1H, d, *J* = 5.2 Hz, C_4H_3S), 6.9 (1H, dd, *J* = 6.3 Hz, 2.8 Hz, C_4H_3S), 6.83 (1H, d, *J* = 5.2 Hz, C_4H_3S), 5.81-5.79 (1H, m, C*H*), 5.21-5.09 (2H, m, CH_2), 4.41 (2H, $_S$, CH_2), 3.52-3.45 (2H, m, CH_2), 2.84-2.71 (2H, m, CH_2), 2.45-2.31 (2H, m, CH_2), 2.26-2.22 (2H, m, CH_2), 2.23-2.12 (2H, m, CH_2), 1.82-1.78 (2H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 174.5 (*C*=O), 169.3 (*C*=O), 139.1 (*C*H), 137.8 (Ar*C*), 136.9 (C_3H_2SC), 129.5 (C_3H_2SCH), 128.0 (Ar*C*H), 127.8 (C_3H_2SCH), 125.8 (Ar*C*H), 125.2 (Ar*C*H), 124.0 (C_3H_2SCH), 116.5 (*C*H_2), 69.2 (*C*), 45.8 (*C*H_2), 43.0 (*C*H_2), 33.2 (*C*H_2), 32.8 (*C*H_2), 32.5 (*C*H_2), 29.8 (*C*H_2), 22.5 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 383.1770 $C_{22}H_{26}N_2O_2S$ requires 383.1768.

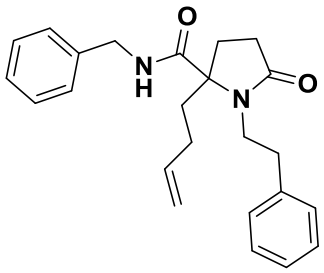
9.4.8. ***N*-Benzyl-5-oxo-2-(pent-4-en-1-yl)-1-(2-(thiophen-2-yl) ethyl) pyrrolidine-2-carboxamide 235**



Isolated as a brown oil, following method above using 4-oxooct-7-enoic acid **179** (0.11 g, 0.64 mmol), 2-(thiophen-2-yl) ethanamine **224** (0.10 g, 0.80 mmol) and isocyanomethyl benzene **59** (0.07 g, 0.64 mmol) for 48 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 7: 3, R_f = 0.4) to yield amide **235** (0.055 g,

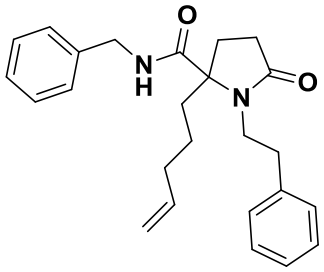
55% yield); υ_{max} (ATR/cm^{-1})= 3345, 3095, 2982, 2945, 2836, 1698, 1521; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.55-7.37 (5H, m, ArC*H*), 7.23 (1H, d, *J* = 5.2 Hz, C_4H_3S), 6.87 (1H, dd, *J* = 7.1 Hz, 3.2 Hz, C_4H_3S), 6.8 (1H, d, *J* = 5.1 Hz, C_4H_3S), 5.81-5.78 (1H, m, C*H*), 5.21-5.12 (2H, m, CH_2), 4.4 (2H, s, CH_2), 3.52-3.75 (2H, m, CH_2), 2.84-2.71 (2H, m, CH_2), 2.45-2.33 (2H, m, CH_2), 2.27-2.24 (2H, m, CH_2), 2.23-2.11 (2H, m, CH_2), 1.99-1.87 (2H, m, CH_2), 1.82-1.78 (2H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 173.5 (*C*=O), 169.4 (*C*=O), 139.4 (*C*H), 138.1 (Ar*C*), 137.0 (C_3H_2SC), 130.1 (C_3H_2SCH), 128.0 (Ar*C*H), 127.8 (C_3H_2SCH), 125.8 (Ar*C*H), 125.2 (Ar*C*H), 124.0 (C_3H_2SCH), 115.5 (*C*H_2), 69.2 (*C*), 45.7 (*C*H_2), 43.4 (*C*H_2), 33.3 (*C*H_2), 32.9 (*C*H_2), 32.4 (*C*H_2), 29.8 (*C*H_2), 28.3 (*C*H_2), 22.8 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 397.1984 $C_{23}H_{28}N_2O_2S$ requires 397.1982.

9.4.9. ***N*-Benzyl-2-(but-3-en-1-yl)-5-oxo-1-phenethylpyrrolidine-2-carboxamide 236**



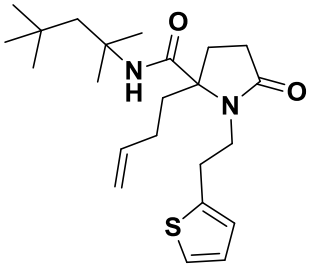
Isolated as a yellow oil, following method above using 4-oxooct-7-enoic acid **178** (0.10 g, 0.64 mmol), 2-phenylethanamine **225** (0.09 g, 0.80 mmol) and isocyanomethyl benzene **59** (0.07 g, 0.64 mmol) for 48 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 7: 3, R_f = 0.4) to yield amide **236** (0.045 g, 50% yield); υ_{max} (ATR/cm^{-1})= 3334, 3109, 2988, 2935, 1688, 1520; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.40-7.23 (10H, m, ArC*H*), 5.82-5.76 (1H, m, C*H*), 5.18-5.12 (2H, m, CH_2), 4.4 (2H, s, CH_2), 3.50-3.43 (2H, m, CH_2), 2.75-2.69 (2H, m, CH_2), 2.35-2.30 (2H, m, CH_2), 2.28-2.25 (2H, m, CH_2), 2.23-2.16 (2H, m, CH_2), 1.88-1.79 (2H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 173.4 (*C*=O), 168.5 (*C*=O), 139.8 (Ar*C*), 139.1 (*C*H), 137.4 (Ar*C*), 128.6 (Ar*C*H), 128.2 (Ar*C*H), 127.4 (Ar*C*H), 126.8 (Ar*C*H), 126.2 (Ar*C*H), 125.2 (Ar*C*H), 115.4 (*C*H_2), 70.2 (*C*), 44.8 (*C*H_2), 43.1 (*C*H_2), 33.1 (*C*H_2), 32.5 (*C*H_2), 29.8 (*C*H_2), 28.2 (*C*H_2), 23.0 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 377.2214 $C_{24}H_{28}N_2O_2$ requires 377.2210.

9.4.10. ***N*-Benzyl-5-oxo-2-(pent-4-en-1-yl)-1-phenethylpyrrolidine-2-carboxamide 237**



Isolated as a yellow oil, following method above using 4-oxooct-7-enoic acid **179** (0.11 g, 0.64 mmol), 2-phenylethanamine **225** (0.09 g, 0.80 mmol) and isocyanomethyl benzene **59** (0.07 g, 0.64 mmol) for 48 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 7: 3, R_f = 0.4) to yield amide **237** (0.055 g, 50% yield); υ_{max} (ATR/cm^{-1})= 3341, 3109, 2981, 2945, 1697, 1533; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.52-7.34 (10H, m, ArC*H*), 5.8-5.74 (1H, m, C*H*), 5.19-5.12 (2H, m, CH_2), 4.43 (2H, $_S$, CH_2), 3.51-3.42 (2H, m, CH_2), 2.78-2.72 (2H, m, CH_2), 2.39-2.32 (2H, m, CH_2), 2.29-2.25 (2H, m, CH_2), 2.22-2.17 (4H, m, 2CH_2), 1.89-1.80 (2H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 174.5 (*C*=O), 169.9 (*C*=O), 139.8 (Ar*C*), 139 (*C*H), 137.5 (Ar*C*), 129.2 (Ar*C*H), 128.6 (Ar*C*H), 127.7 (Ar*C*H), 126.9 (Ar*C*H), 126.2 (Ar*C*H), 125.4 (Ar*C*H), 115.1 (*C*H$_2$), 70 (*C*), 44.4 (*C*H$_2$), 43.5 (*C*H$_2$), 33.1 (*C*H$_2$), 32.6 (*C*H$_2$), 32.2 (*C*H$_2$), 29.9 (*C*H$_2$), 28.6 (*C*H$_2$), 23.2 (*C*H$_2$); HRMS (ESI$^+$) (M+H$^+$) found 391.2314 $C_{23}H_{20}N_2O_2$ requires 391.2311.

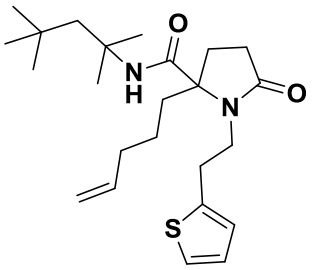
9.4.11. 2-(But-3-en-1-yl)-5-oxo-1-(2-(thiophen-2-yl) ethyl)-*N*-(2,4,4-trimethylpentan-2-yl) pyrrolidine-2-carboxamide 238



Isolated as a pale oil, following method above using 4-oxooct-7-enoic acid **178** (0.156 g, 1.0 mmol), 2-(thiophen-2-yl) ethanamine **226** (0.158 g, 1.25 mmol) and 2-isocyano-2, 4, 4-trimethylpentane **223** (0.139 g, 1.0 mmol) for 48 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 4: 6, R_f = 0.3) to yield amide **238** (0.087 g, 56% yield); υ_{max} (ATR/cm^{-1})= 3342, 3077, 2954, 2867, 1667, 1521, 1479; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.14 (1H, d, *J* = 4.2 Hz, C_4H_3S), 6.9 (1H, dd, *J* = 5.4 Hz, 2.7 Hz, C_4H_3S), 6.86 (1H, d, *J* = 4.2 Hz, C_4H_3S), 5.83-5.73 (1H, m, C*H*), 5.07-4.98 (2H, m, CH_2), 3.25-3.12 (2H, m, CH_2), 2.46-2.40 (2H, m, CH_2), 2.22-2.02 (4H, m, CH_2), 1.81-1.72 (2H, m, CH_2), 1.72 (6H, s, 2CH_3) 1.67 (2H, s, CH_2), 0.99 (9H, s, 3CH_3); ^{13}C NMR (100 MHz,

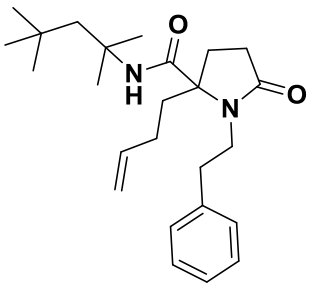
CDCl3) δ_C = 176.3 (*C*=O), 172.0 (*C*=O), 140.7 (*C*H), 137.0 (C_3H_2SC), 127.1 (C_3H_2SCH), 125.4 (C_3H_2SCH), 123.9 (C_3H_2SCH), 115.5 (CH_2) 70.5 (*C*), 55.8 (CH_2), 52.4 (*C*), 44.0 (*C*), 33.8 (CH_2), 31.7 (CH_2), 31.5 ($3CH_3$), 30.0 (CH_2), 29.8 (CH_2), 29.0 ($2CH_3$), 28.9 (CH_2), 27.7 (CH_2); HRMS (ESI^+) ($M+H^+$) found 405.2573 $C_{23}H_{36}N_2O_2S$ requires 405.2570.

9.4.12. 5-Oxo-2-(pent-4-en-1-yl)-1-(2-(thiophen-2-yl) ethyl)-*N*-(2,4,4-trimethylpentan-2-yl) pyrrolidine-2-carboxamide 239



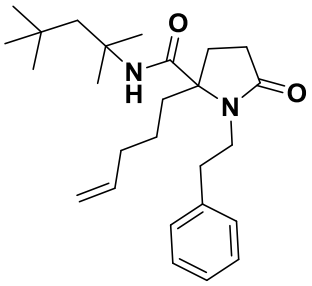
Isolated as a pale oil, following method above using 4-oxonon-8-enoic acid **179** (0.1 g, 0.588 mmol), 2-(thiophen-2-yl) ethanamine **224** (0.09 g, 0.73 mmol) and 2-isocyano-2, 4, 4-trimethylpentane **223** (0.081 g, 0.588 mmol) for 72 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.4) to yield amide **239** (0.035 g, 35% yield); υ_{max} (ATR/cm^{-1})= 3339, 3073, 2951, 2867, 1671, 1528, 1447; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.18 (1H, d, *J* = 6.4 Hz, C_4H_3S), 6.97 (1H, dd, *J* = 4.9 Hz, 2.85 Hz, C_4H_3S), 6.90 (1H, d, *J* = 6.4 Hz, C_4H_3S), 5.84-5.74 (1H, m, C*H*), 5.07-4.99 (2H, m, CH_2), 3.28-3.15 (2H, m, CH_2), 2.48-2.43 (2H, m, CH_2), 2.14-2.01 (4H, m, CH_2), 1.77-1.71 (4H, m, $2CH_2$), 1.69-1.60 (2H, m, CH_2), 1.45 (6H, s, $2CH_3$), 1.40 (2H, s, CH_2), 1.02 (9H, s, $3CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176.4 (*C*=O), 172.2 (*C*=O), 140.8 (*C*H), 137.7 (C_3H_2SC), 127 (C_3H_2SCH), 125.4 (C_3H_2SCH), 123.9 (C_3H_2SCH), 115.7 (CH_2), 70.7 (*C*), 55.8 (CH_2), 52.6 (*C*), 44.0 (*C*), 37.8 (CH_2), 34.0 (CH_2), 31.7 ($3CH_3$) 31.6 (CH_2) 29.8 (CH_2), 29.2 (CH_2), 29.0 ($2CH_3$), 28.5 (CH_2), 22.9 (CH_2); HRMS (ESI^+) ($M+H^+$) found 419.2726 $C_{24}H_{38}N_2O_2S$ requires 419.2727.

9.4.13. 2-(But-3-en-1-yl)-5-oxo-1-phenethyl-*N*-(2,4,4-trimethylpentan-2-yl) pyrrolidine-2-carboxamide 240



Isolated as a colourless oil, following method above using 4-oxooct-7-enoic acid **178** (0.156 g, 1.0 mmol), 2-phenylethanamine **225** (0.151 g, 1.25 mmol) and 2-isocyano-2, 4, 4-trimethylpentane **223** (0.139 g, 1.0 mmol) for 48 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 4: 6, R_f = 0.4) to yield amide **240** as (0.07 g, 45% yield); υ_{max} (ATR/cm^{-1})= 3335, 3070, 3028, 2951, 2870, 1671, 1524, 1451; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.30-7.23 (5H, m, ArC*H*), 5.84-5.74 (1H, m, C*H*), 5.08-4.98 (2H, m, CH_2), 3.04-2.88 (2H, m, CH_2), 2.47-2.42 (2H, m, CH_2), 2.23-2.06 (4H, m, CH_2), 1.99-1.90 (4H, m, 2CH_2), 1.73 (6H, s, 2CH_3), 1.66 (2H, s, CH_2), 0.99 (9H, s, 3CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176.2 (*C*=O), 172.0 (*C*=O), 138.7 (*C*H), 137 (Ar*C*), 128.8 (Ar*C*H), 128.5 (Ar*C*H), 126.7 (Ar*C*H), 115.4 (*C*H_2), 70.5 (*C*), 55.8 (*C*H_2), 52.5 (*C*), 44.5 (*C*), 34.6 (*C*H_2), 33.7 (*C*H_2), 31.7 (3*C*H_3), 31.6 (*C*H_2), 29.0 (2*C*H_3), 28.8 (*C*H_2), 28.2 (*C*H_2), 27.7 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 399.3005 $C_{25}H_{38}N_2O_2$ requires 399.3006.

9.4.14. 5-Oxo-2-(pent-4-en-1-yl)-1-phenethyl-*N*-(2,4,4-trimethylpentan-2-yl) pyrrolidine-2-carboxamide 235

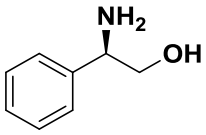


Isolated as a colourless oil, following method above using 4-oxonon-8-enoic acid **179** (0.1 g, 0.588 mmol), 2-phenylethanamine **225** (0.09 g, 0.73 mmol) and 2-isocyano-2, 4, 4-trimethylpentane **223** (0.081 g, 0.588 mmol) for 72h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3 : 7, R_f = 0.5) to yield amide **241** (0.04 g, 40% yield); υ_{max} (ATR/cm^{-1})= 3337, 3065, 3026, 2951, 2866, 1668, 1603, 1522; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.35-7.25 (5H, m, ArC*H*), 5.83-5.73 (1H, m, C*H*), 5.06-4.98 (2H, m, CH_2), 3.04-2.91 (2H, m, CH_2), 2.48-2.43 (2H, m, CH_2), 2.22-2.05 (4H, m, CH_2), 1.78-1.74 (4H, m, 2CH_2), 1.45 (6H, s, 2CH_3), 1.39 (2H, s, CH_2), 1.35-1.25 (2H, m, CH_2), 1.01 (9H, s, 3CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176.3 (*C*=O), 172.4 (*C*=O), 138.7 (*C*H), 137.8 (Ar*C*), 128.8 (Ar*C*H), 128.7 (Ar*C*H), 126.7 (Ar*C*H), 115.6 (*C*H_2) 70.8 (*C*), 55.8 (*C*H_2), 52.7 (*C*), 44.2 (*C*H_2), 34.6 (*C*H_2), 34 (*C*), 33.9 (*C*H_2), 31.7 (3*C*H_3), 31.6 (*C*H_2), 29.9 (*C*H_2), 29.2 (2*C*H_3), 28.1 (*C*H_2), 22.8 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 413.3163 $C_{26}H_{40}N_2O_2$ requires 413.3163.

9.5. Synthesis of bi- and tri-cyclic amides (Lactams)

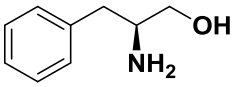
9.5.1. Synthesis of amino alcohols [352]

9.5.1.1. (*R*)-2-Amino-2-phenylethanol 255



3.78 g, 100 mmole of sodium borohydride was added to a suspension of *D*−(−)-*α*-phenylglycine **253** (5.61 g, 41.0 mmole) in dry THF (100 mL) under argon atmosphere and cooled the mixture to -5 ºC. Iodine (10.7 g, 41.0 mmole) in dry THF (100 mL) was added dropwise to the mixture over 1 h. The reaction mixture was heated under reflux for 18 h and the reaction was monitored by TLC (TLC 10% methanol in chloroform). After completion of the reaction, the reaction mixture was allowed to cool at room temperature and methanol (60 mL) was added until the white sticky solution went clear. The solution was concentrated and potassium hydroxide (20%, 100 mL) was added with stirring for 4 h. The aqueous layer was extracted with CH_2Cl_2 (3 × 100) and the combined organic layers was dried over sodium sulphate and concentrated under reduced pressure at below 50 ºC to furnished (*R*)-2-amino-2-phenylethanol **255** as a pale yellow solid (4.48 g, 80%); m.p = 69-70 ºC; $[\alpha]_D^{24}$ = -32.0 (c = 0.75, 1M HCl); υ_{max} (ATR/cm^{-1})= 3354, 3275, 2916, 2860, 1662, 1596, 1490; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.38-7.28 (5H, m, ArC*H*), 4.07 (1H, dd, *J* = 8.4 Hz, 4.5 Hz, C*H*), 3.8 (1H, dd, *J* = 10.5 Hz, 4.4 Hz, C*H*), 3.55 (1H, dd, *J* = 10.1 Hz, 7.5 Hz, C*H*), 2.22 (3H, brs, O*H* & N*H*$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 141.5 (Ar*C*), 127.2 (Ar*C*H), 126.0 (Ar*C*H), 123.9 (Ar*C*H), 68.5 (*C*H_2), 57.2 (*C*H); HRMS (ESI^+) ($M+H^+$) found 137.088 $C_8H_{11}NO$ requires 137.0798.

9.5.1.2. (*S*)-2-Amino-3-phenylpropan-1-ol 256



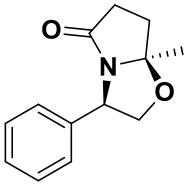
3.78 g, 100 mmole of sodium borohydride was added to a suspension of *L*-phenylalanine **254** (6.19 g, 41.0 mmole) in dry THF (100 mL) under argon atmosphere and cool the mixture to -5 ºC. Iodine (10.7 g, 41.0 mmole) in dry THF (100 mL) was added drop wise to the mixture over 1h. The reaction was heated under reflux for 18 h and the reaction was monitored by TLC (TLC 10% methanol in chloroform). After completion of the reaction, the reaction mixture was allowed to cool to room temperature and methanol (60 mL) was added until the white sticky solution went clear. The solution was concentrated and potassium hydroxide

(20%, 100 mL) was added with stirring for 4 h. The aqueous layer was extracted with CH_2Cl_2 (3 × 100) and the combined organic layers was dried over sodium sulphate and concentrated under reduced pressure at below 50 ºC to furnished (*S*)-2-amino-3-phenylpropan-1-ol **256** as a pale yellow solid (5.25 g, 85%); m.p = 94-95 ºC; $[\alpha]_D^{22}$ = −22.9 (c= 1.2, 1M HCl); υ_{max} (ATR/cm^{-1})= 3351, 3064, 2923, 2857, 1583, 1494, 1451; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.34-7.20 (5H, m, ArC*H*), 3.66 (1H, dd, *J* = 10.6 Hz, 3.4 Hz, C*H*), 3.41 (1H, dd, *J* = 11.2 Hz, 4.2 Hz, C*H*), 3.17-3.10 (1H, m, C*H*), 2.81 (1H, dd, *J* = 13.6 Hz, 5.2 Hz, C*H*), 2.54 (1H, dd, *J* = 13.8 Hz, 7.0 Hz, C*H*), 2.11 (3H, brs, O*H* & N*H*$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 140.8 (Ar*C*), 128.2 (Ar*C*H), 126.9 (Ar*C*H), 125.5 (Ar*C*H), 66.5 (*C*H$_2$), 56.2 (*C*H), 41.2 (*C*H$_2$); HRMS (ESI$^+$) (M+H$^+$) found 151.0102 $C_8H_{11}NO$ requires 150.0098.

9.5.2. General Synthesis of bi- and tricyclic amides (Lactams)

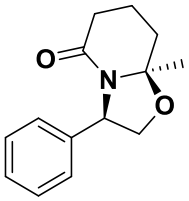
A mixture of amino alcohols (3.75 mmol), pyridinium *p*-toluenesulfonate (PPTS) (0.188 g, 0.75 mmol) and keto acids (3.75 mmol) in dry toluene (70 mL) was refluxed in a Dean-Stark apparatus for overnight. The reaction mixture was allowed to cool at room temperature and the obtained precipitate was filtrated through celite. After solvent was evaporated, the crude product was collected as a pale to yellow oil. The oil crudes were purified by flash column chromatography to obtain the titled hydropyrrolo derivatives.

9.5.2.1. (3*R*,7a*R*)-7a-Methyl-3-phenyltetra hydropyrrolo[2,1-b]oxazol-5 (6H)-one 258



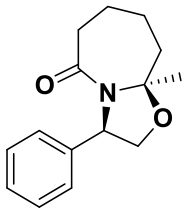
Isolated as a white solid, following method above using (*R*)-2-amino-2-phenylethanol **255** (0.51 g, 3.75 mmol), PPTS (0.188 g, 0.75 mmol) and 4-oxopentanoic acid **5** (0. 43 g, 3.75 mmol) for 24 h. The crude compound was purified by flash column chromatography (ethyl acetate: petroleum ether 4: 6, R_f = 0.3) to yield **258** (0.36 g, 86%); m.p = 128-131ºC; $[\alpha]_D^{22}$ = −23.3, (c= 1.0 in $CHCl_3$); υ_{max} (ATR/cm^{-1})= 3068, 3027, 2995, 2890, 1714, 1580, 1492; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.35-7.24 (5H, m, ArC*H*), 5.16 (1H, t, *J* = 7.5 Hz, C*H*), 4.61 (1H, dd, *J* = 17.2 Hz, 4.7 Hz, C*H*), 4.09 (1H, dd, *J* = 19.6 Hz, 8.7 Hz, C*H*), 2.58 (1H, t, *J* = 8.4 Hz, C*H*), 2.45-2.39 (1H, m, C*H*), 2.30-2.25 (2H, m, C*H*$_2$), 1.45 (3H, s, C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 178.9 (*C*=O), 140.9 (Ar*C*), 128.7 (Ar*C*H), 127.4 (Ar*C*H), 125.5 (Ar*C*H), 109.6 (*C*), 73.3 (*C*H$_2$), 57.6 (*C*H), 33.9 (*C*H$_2$), 33.1 (*C*H$_2$), 24.2 (*C*H$_3$); HRMS (ESI$^+$) (M+H$^+$) found 218.1178 $C_{13}H_{15}NO_2$ requires 218.1176.

9.5.2.2. (3*R*, 8a*R*)-8a-Methyl-3-phenyltetrahydro-2H-oxazolo[3,2-a]pyridin-5 (3H)-one 259



Isolated as a white solid, following method above using (*R*)-2-amino-2-phenylethanol **255** (0.51 g, 3.75 mmol), PPTS (0.188 g, 0.75 mmol) and 5-oxohexanoic acid **168** (0.48 g, 3.75 mmol) for 24 h. The crude compound was purified by flash column chromatography (ethyl acetate: petroleum ether 4: 6, R_f = 0.4) to yield **259** (0.37g, 78%); m.p = 136-138 ºC; $[\alpha]_D^{22}$ = −37.9, (c= 1.0 in $CHCl_3$); υ_{max} (ATR/cm^{-1})= 3059, 3030, 2983, 2884, 1649, 1492, 1449; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.34-7.28 (5H, m, ArC*H*), 5.37 (1H, t, *J* = 8.2 Hz, C*H*), 4.52 (1H, t, *J* = 8.8 Hz, C*H*), 3.95 (1H, dd, *J* = 8.8 Hz, 7.7 Hz, C*H*), 2.61 (1H, dd, *J* = 18.4 Hz, 3.5 Hz, C*H*), 2.47-2.36 (1H, m, C*H*), 2.20 (1H, dd, *J* = 12.5 Hz, 3.2 Hz, C*H*), 2.04-1.83 (2H, m, CH_2), 1.71 (1H, dd, *J* = 17.3 Hz, 6.5 Hz, C*H*), 1.45 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 172.9 (*C*=O), 140.8 (Ar*C*), 127.7 (Ar*C*H), 126.4 (Ar*C*H), 124.5 (Ar*C*H), 96.5 (*C*), 70.2 (*C*H_2), 56.6 (*C*H), 33.7 (*C*H_2), 31.2 (*C*H_2), 22.7 (*C*H_3), 17.7 (*C*H_2); HRMS (ESI^+) ($M+H^+$) found 232.1333 $C_{14}H_{17}NO_2$ requires 232.1332.

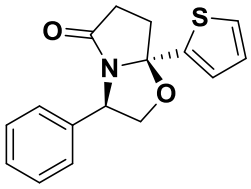
9.5.2.3. (3*R*, 9a*R*)-9a-Methyl-3-phenylhexahydrooxazolo[3,2-a]azepin-5 (6H)-one 260



Isolated as a white solid, following method above using (*R*)-2-amino-2-phenylethanol **255** (0.51 g, 3.75 mmol), PPTS (0.188 g, 0.75 mmol) and 6-oxoheptanoic acid **32** (0.54 g, 3.75 mmol) for 24 h. The crude compound was purified by flash column chromatography (ethyl acetate: petroleum ether 7: 3, R_f = 0.4) to yield **360** (0.37 g, 70%); m.p = 121-123 ºC; $[\alpha]_D^{22}$ = −20.5, (c= 1.0 in $CHCl_3$); υ_{max} (ATR/cm^{-1})= 3065, 3030, 2951, 2855, 1658, 1629, 1492; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.38-7.27 (5H, m, ArC*H*), 5.26 (1H, t, *J* = 9.1 Hz, C*H*), 4.67 (1H, dd, *J* = 10.0 Hz, 8.4 Hz, C*H*), 4.14 (1H, dd, *J* = 13.3 Hz, 7.8 Hz, C*H*), 2.78 (1H, dd, *J* = 8.5 Hz, 4.3 Hz, C*H*), 2.62 (1H, dd, *J* = 8.2 Hz, 4.1 Hz, C*H*), 2.53 (2H, t, *J* = 7.5 Hz, CH_2), 2.12 (3H, s, CH_3), 1.94-1.85 (4H, m, 2CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 169.9 (*C*=O),

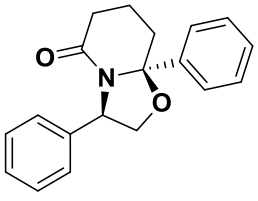
140.2 (Ar*C*), 126.7 (Ar*C*H), 125.4 (Ar*C*H), 124.5 (Ar*C*H), 120.2 (*C*), 74.2 (*C*H_2), 68.6 (*C*H), 42.7 (*C*H_2), 34.9 (*C*H_2), 25.2 (*C*H_2), 21.9 (*C*H_2), 17.1 (*C*H_3); HRMS (ESI^+) ($M+H^+$) found 246.1493 $C_{15}H_{19}NO_2$ requires 246.1489.

9.5.2.4. (3*R*,7a*S*)-3-Phenyl-7a-(thiophen-2-yl)tetrahydropyrrolo[2,1-b]oxazol-5 (6H)-one 261



Isolated as a pale solid, following method above using (*R*)-2-amino-2-phenylethanol **255** (0.51 g, 3.75 mmol), PPTS (0.188 g, 0.75 mmol) and 4-oxo-4-(thiophen-2-yl) butanoic acid **173** (0.69 g, 3.75 mmol) for 24 h. The crude compound was purified by flash column chromatography (ethyl acetate: petroleum ether 7: 3, R_f = 0.3) to yield **261** (0.41 g, 60%); m.p = 134-135 ºC; $[\alpha]_D^{22}$ = −18.6, (c= 1.0 in $CHCl_3$); υ_{max} (ATR/cm^{-1})= 3071, 3033, 2992, 2887, 1714, 1492; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.30-7.23 (5H, m, ArC*H*), 7.19 (1H, t, *J* = 4.3 Hz, C_4H_3S), 7.04 (1H, dd, *J* = 3.5 Hz, 1.2 Hz, C_4H_3S), 6.93 (1H, dd, *J* = 4.9 Hz, 3.6 Hz, C_4H_3S), 5.16 (1H, t, *J* = 8.2 Hz, C*H*), 4.76 (1H, dd, *J* = 8.8 Hz, 7.9 Hz, C*H*), 4.13 (1H, dd, *J* = 8.8 Hz, 7.0 Hz, C*H*), 3.12-3.00 (1H, m, C*H*), 2.70-2.61 (2H, m, CH_2), 2.56-2.61 (1H, m, C*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 179.3 (*C*=O), 145.9 (Ar*C*), 138.2 (C_3H_2SC), 125.7 (Ar*C*H), 124.6 (Ar*C*H), 123.5 (Ar*C*H), 122.9 (C_3H_2SCH), 122.2 (C_3H_2SCH), 121.9 (C_3H_2SCH), 102.2 (*C*), 75.6 (*C*H_2), 58.7 (*C*H), 37.3 (*C*H_2), 32.9 (*C*H_2); HRMS (ESI^+) ($M+H^+$) found 286.0900 $C_{16}H_{15}NO_2S$ requires 286.0896.

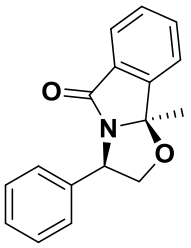
9.5.2.5. (3*R*,8a*S*)-3, 8a-Diphenyltetrahydro-2H-oxazolo[3,2-a]pyridin-5 (3H)-one 262



Isolated as a white solid, following method above using (*R*)-2-amino-2-phenylethanol **255** (0.51g, 3.75 mmol), PPTS (0.188 g, 0.75 mmol) and 4-benzoylbutyric acid **175** (0.72 g, 3.75 mmol) for 24 h. The crude compound was purified by flash column chromatography (ethyl acetate: petroleum ether 4: 6, R_f = 0.3) to yield **262** (0.51, 72%); m.p = 137-139 ºC; $[\alpha]_D^{22}$ = −15.3, (c= 1.0 in $CHCl_3$); υ_{max} (ATR/cm^{-1})= 3065, 3030, 2951, 2855, 1658, 1629, 1492; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.52-7.37 (5H, m, ArC*H*), 7.22-7.12 (5H, m, ArC*H*), 5.31

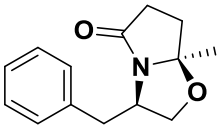
(1H, t, *J* = 8.8 Hz, C*H*), 4.43 (1H, dd, *J* = 9.1 Hz, 8.2 Hz, C*H*), 3.66 (1H, t, *J* = 9.4 Hz, C*H*), 2.69 (1H, dd, *J* = 18.7 Hz, 7.1 Hz, C*H*), 2.55-2.43 (1H, m, C*H*), 2.27 (1H, dd, *J* = 12.5 Hz, 2.9 Hz, C*H*), 2.03-1.95 (1H, m, C*H*), 1.84-1.77 (1H, m, C*H*), 1.71-1.58 (1H, m, C*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 170.9 (*C*=O), 141.2 (Ar*C*), 136.2 (Ar*C*), 126.8 (Ar*C*H), 125.4 (Ar*C*H), 125.0 (Ar*C*H), 124.5 (Ar*C*H), 124.3 (Ar*C*H), 98.2 (*C*), 70.2 (*C*H_2), 61.6 (*C*H), 38.7 (*C*H_2), 31.8 (*C*H_2), 15.4 (*C*H_2); HRMS (ESI^+) ($M+H^+$) found 294.1489 $C_{19}H_{19}NO_2$ requires 294.1489.

9.5.2.6. (3*R*,9b*R*)-9b-Methyl-3-phenyl-2,3-dihydrooxazolo[2,3-a]isoindol-5 (9bH)-one 263



Isolated as a white solid, following method above using (*R*)-2-amino-2-phenylethanol **255** (0.51 g, 3.75 mmol), PPTS (0.188 g, 0.75 mmol) and 2-acetylbenzoic acid **170** (0.61 g, 3.75 mmol) for 24 h. The crude compound was purified by flash column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.4) to yield **263** (0.56 g, 92%); m.p = 131-132 ºC; $[\alpha]_D^{22}$ = −26.0, (c= 1.0 in $CHCl_3$); υ_{max} (ATR/cm^{-1})= 3082, 3062, 2986, 2884, 1714, 1615, 1498; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.84 (2H, d, *J* = 7.44 Hz, ArC*H*), 7.68-7.53 (5H, m, ArC*H*), 7.42-7.29 (2H, m, ArC*H*), 5.34 (1H, t, *J* = 7.6 Hz, C*H*), 4.83 (1H, dd, *J* = 16.6 Hz, 8.2 Hz, C*H*), 4.38 (1H, dd, *J* = 16.1Hz, 7.9 Hz, C*H*), 1.75 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 172.6 (*C*=O), 145.6 (Ar*C*), 138.9 (Ar*C*), 132.2 (Ar*C*H), 129.4 (Ar*C*), 126.5 (Ar*C*H), 125.5 (Ar*C*H), 124.4 (Ar*C*H), 124.0 (Ar*C*H), 123.4 (Ar*C*H), 122.1 (Ar*C*H), 101.2 (*C*), 75.9 (*C*H_2), 59.9 (*C*H), 21.3 (*C*H_3); HRMS (ESI^+) ($M+H^+$) found 266.1176 $C_{17}H_{15}NO_2$ requires 266.1176.

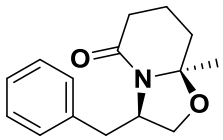
9.5.2.7. (3*R*, 7a*R*)-3-Benzyl-7a-methyltetrahydropyrrolo[2, 1-b]oxazol-5 (6H)-one 264



Isolated as a white solid, following method above using (*S*)-2-amino-3-phenylpropan-1-ol **256** (0.56 g, 3.75 mmol), PPTS (0.188 g, 0.75 mmol) and 4-oxopentanoic acid **5** (0. 43 g, 3.75 mmol) for 24 h. The crude compound was purified by flash column chromatography

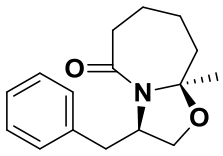
(ethyl acetate: petroleum ether 4: 6, R_f = 0.2) to yield **264** (0.38 g, 90%); m.p = 133-135 ºC; $[\alpha]_D^{22}$ = −27.2, (c= 1.0 in $CHCl_3$); υ_{max} (ATR/cm^{-1})= 3085, 3059, 2983, 2873, 1708, 1495, 1454; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.34-7.24 (5H, m, ArC*H*), 4.32 (1H, dd, *J* = 15.3 Hz, 6.4 Hz, C*H*), 4.07 (1H, dd, *J* = 8.8 Hz, 7.3 Hz, C*H*), 3.91 (1H, dd, *J* = 8.9 Hz, 6.4 Hz, C*H*), 3.14 (1H, dd, *J* = 13.7 Hz, 5.4 Hz, C*H*), 2.85-2.72 (2H, m, CH_2), 2.52-2.44 (1H, m, C*H*), 2.22-2.11 (2H, m, CH_2), 1.45 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C= 177.9 (*C*=O), 136.9 (Ar*C*), 127.7 (Ar*C*H), 126.7 (Ar*C*H), 124.7 (Ar*C*H), 100.6 (*C*), 72.3 (*C*H_2), 62.61 (*C*H), 41.8 (*C*H_2), 35.8 (*C*H_2), 32.3 (*C*H_2), 22.3 (*C*H_3); HRMS (ESI^+) ($M+H^+$) found 232.1334 $C_{14}H_{17}NO_2$ requires 232.1332.

9.5.2.8. (3*R*,8a*R*)-3-Benzyl-8a-methyltetrahydro-2H-oxazolo[3, 2-a]pyridin-5 (3H)-one 265



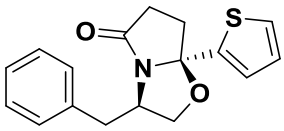
Isolated as a white solid, following method above using (*S*)-2-amino-3-phenylpropan-1-ol **256** (0.56 g, 3.75 mmol), PPTS (0.188 g, 0.75 mmol) and 5-oxohexanoic acid **168** (0.48 g, 3.75 mmol) for 24 h. The crude compound was purified by flash column chromatography (ethyl acetate: petroleum ether 4: 6, R_f = 0.3) to yield **265** (0.38 g, 80%) as a white solid; m.p = 74-75 ºC; $[\alpha]_D^{22}$ = −17.0, (c= 1.0 in $CHCl_3$); υ_{max} (ATR/cm^{-1})= 3084, 3064, 2989, 2880, 1642, 1490, 1454; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.32-7.21 (5H, m, ArC*H*), 4.44 (1H, dd, *J* = 17.9 Hz, 3.8 Hz, C*H*), 3.97 (1H, dd, *J* = 9.1 Hz, 8.0 Hz, C*H*), 3.83 (1H, t, *J* = 8.6 Hz, C*H*), 3.45 (1H, dd, *J* = 13.2 Hz, 3.5 Hz, C*H*), 2.76 (1H, dd, *J* = 13.2 Hz, 9.9 Hz, C*H*), 2.53 (1H, dd, *J* = 18.3 Hz, 6.7 Hz, C*H*), 2.41-2.32 (1H, m, C*H*), 2.10-2.05 (1H, m, C*H*), 1.97-1.89 (1H, m, C*H*), 1.85-1.73 (1H, m, C*H*), 1.56 (1H, dd, *J* = 17.6 Hz, 6.6 Hz, C*H*), 1.29 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 172.3 (*C*=O), 135.5 (Ar*C*), 128.7 (Ar*C*H), 125.8 (Ar*C*H), 122.3 (Ar*C*H), 93.6 (*C*), 68.8 (*C*H_2), 55.8 (*C*H), 39.8 (*C*H_2), 36.4 (*C*H_2), 30.9 (*C*H_2), 23.7 (*C*H_3), 17.5 (*C*H_2); HRMS (ESI^+) ($M+H^+$) found 246.1492 $C_{15}H_{19}NO_2$ requires 246.1489.

9.5.2.9. (3*R*,9a*R*)-3-Benzyl-9a-methylhexahydrooxazolo[3,2-a]azepin-5 (6H)-one 266



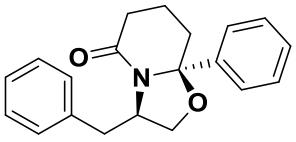
Isolated as a white solid, following method above using (*S*)-2-amino-3-phenylpropan-1-ol **256** (0.56 g, 3.75 mmol), PPTS (0.188 g, 0.75 mmol) and 6-oxoheptanoic acid **32** (0.54 g, 3.75 mmol) for 24 h. The crude compound was purified by flash column chromatography (ethyl acetate: petroleum ether 4: 6, R_f = 0.4) to yield **266** (0.35 g, 65%); m.p = 69-72 ºC; $[\alpha]_D^{22}$ = −13.2, (c= 1.0 in $CHCl_3$); υ_{max} (ATR/cm^{-1})= 3051, 2982, 2906, 1662, 1606, 1421; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.33-7.23 (5H, m, ArC*H*), 4.45 (1H, dd, *J* = 16.2 Hz, 6.9 Hz, C*H*), 4.19 (1H, t, *J* = 8.9 Hz, C*H*), 4.03 (1H, dd, *J* = 8.2 Hz, 7.5 Hz, C*H*), 3.23 (1H, dd, *J* = 13.6 Hz, 4.5 Hz, C*H*), 2.74-2.61 (4H, m, 2CH_2), 2.49 (2H, t, *J* = 7.2 Hz, CH_2), 2.06 (3H, s, CH_3), 1.87 (2H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 166.0 (*C*=O), 138.5 (Ar*C*), 126.8 (Ar*C*H), 125.9 (Ar*C*H), 123.3 (Ar*C*H), 72.6 (*C*), 69.2 (*C*H_2), 67.8 (*C*H), 43.9 (*C*H_2), 41.2 (*C*H_2), 36.0 (*C*H_2), 22.0 (*C*H_2), 15.8 (*C*H_3); HRMS (ESI^+) ($M+H^+$) found 260.1647 $C_{16}H_{21}NO_2$ requires 260.1645.

9.5.2.10. (3*R*,7a*S*)-3-Benzyl-7a-(thiophen-2-yl)tetrahydropyrrolo[2,1-b]oxazol-5 (6H)-one 267



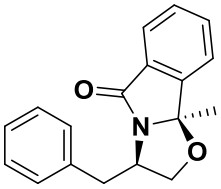
Isolated as a yellow solid, following method above using (*S*)-2-amino-3-phenylpropan-1-ol **256** (0.56 g, 3.75 mmol), PPTS (0.188 g, 0.75 mmol) and 4-oxo-4-(thiophen-2-yl) butanoic acid **173** (0.69 g, 3.75 mmol) for 24 h. The crude compound was purified by flash column chromatography (ethyl acetate: petroleum ether 4: 6, R_f = 0.4) to yield **267** (0.41 g, 60%); m.p = 129-131ºC; $[\alpha]_D^{22}$ = −40.0, (c= 1.0 in $CHCl_3$); υ_{max}(ATR/cm^{-1})= 3091, 3028, 2992, 2877, 1711, 1494; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H= 7.35-7.30 (5H, m, ArC*H*), 7.16 (1H, t, *J* = 7.2 Hz, C_4H_3S), 7.11(1H, dd, *J* = 3.5 Hz, 1.2 Hz, C_4H_3S), 7.04 (1H, dd, *J* = 4.9 Hz, 1.48 Hz, C_4H_3S), 4.40 (1H, dd, *J* = 16.2 Hz, 6.8 Hz, C*H*), 4.20 (1H, dd, *J* = 8.6 Hz, 7.5 Hz, C*H*), 3.82 (1H, t, *J* = 8.0 Hz, C*H*), 3.11 (1H, dd, *J* = 13.6 Hz, 6.0 Hz, C*H*), 2.96-2.87 (1H, m, CH_2), 2.61-2.40 (4H, m, 2CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 177.4 (*C*=O), 142.2 (C_3H_2SC), 135.7 (Ar*C*), 126.9 (Ar*C*H), 125.8 (Ar*C*H), 124.6 (Ar*C*H), 123.5 (C_3H_2SCH), 122.4 (C_3H_2SCH), 122.2 (C_3H_2SCH), 99.9 (*C*), 73.8 (*C*H_2), 58.0 (*C*H), 40.0 (*C*H_2), 35.9 (*C*H_2), 32.7 (*C*H_2); HRMS (ESI^+) ($M+H^+$) found 300.1055 $C_{17}H_{17}NO_2S$ requires 300.1053.

9.5.2.11. (3*R*,7a*S*)-3-Benzyl-7a-phenyltetrahydropyrrolo[2,1-b]oxazol-5 (6H)-one 268



Isolated as a white solid, following method above using (*S*)-2-amino-3-phenylpropan-1-ol **256** (0.56 g, 3.75 mmol), PPTS (0.188 g, 0.75 mmol) and 4-benzoylbutyric acid **175** (0.72 g, 3.75 mmol) for 24 h. The crude compound was purified by flash column chromatography (ethyl acetate: petroleum ether 4 : 6, R_f = 0.2) to yield **268** (0.65 g, 91%); m.p = 139-140 ºC; $[\alpha]_D^{22}$ = −15.6, (c= 1.0 in $CHCl_3$); υ_{max} (ATR/cm^{-1})= 3087, 3064, 2952, 2873, 1645, 1497, 1461; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.49-7.40 (5H, m, ArC*H*), 7.25-7.15 (5H, m, ArC*H*), 4.55-4.48 (1H, m, C*H*), 3.93 (1H, dd, *J* = 8.9 Hz, 7.7 Hz, C*H*), 3.62 (1H, dd, *J* = 12.9 Hz, 3.6 Hz, C*H*), 3.33 (1H, t, *J* = 8.9 Hz, C*H*), 2.67 (1H, dd, *J* = 18.6 Hz, 6.8 Hz, C*H*), 2.51-2.42 (1H, m, C*H*), 2.22 (1H, t, *J* = 11.9 Hz, C*H*), 1.96-1.88 (1H, m, C*H*), 1.81-1.73 (1H, m, C*H*), 1.60-1.48 (2H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 171.3 (*C*=O), 142.2 (Ar*C*), 137.8 (Ar*C*), 126.8 (Ar*C*H), 126.3 (Ar*C*H), 125.4 (Ar*C*H), 125.3 (Ar*C*H), 125.0 (Ar*C*H), 124.3 (Ar*C*H), 96.9 (*C*), 69.1 (*C*H_2), 58.8 (*C*H), 39.7 (*C*H_2), 33.5 (*C*H_2), 31.0 (*C*H_2), 17.9 (*C*H_2); HRMS (ESI^+) ($M+H^+$) found 308.1647 $C_{20}H_{21}NO_2$ requires 308.1645.

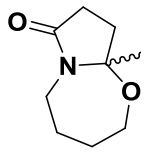
9.5.2.12. (3*R*,9b*R*)-3-Benzyl-9b-methyl-2,3-dihydrooxazolo[2,3-a]isoindol-5 (9bH)-one 269



Isolated as a white solid, following method above using (*S*)-2-amino-3-phenylpropan-1-ol **256** (0.56 g, 3.75 mmol), PPTS (0.188 g, 0.75 mmol) and 2-acetylbenzoic acid **170** (0.61 g, 3.75 mmol) for 24 h. The crude compound was purified by flash column chromatography (ethyl acetate: petroleum ether 4: 6, R_f = 0.3) to yield **269** (0.56 g, 92%); m.p = 144-145 ºC; $[\alpha]D^{22}$ = −19.0, (c= 1.0 in $CHCl_3$); υ_{max} (ATR/cm^{-1})= 3091, 3028, 2989, 2880, 1711, 1612, 1464; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.77 (1H, d, *J* = 7.3 Hz, ArC*H*), 7.39 (1H, t, *J* = 7.4 Hz, ArC*H*), 7.55-7.51 (2H, m, ArC*H*), 7.38-7.25 (5H, m, ArC*H*), 4.47 (1H, dd, *J* = 14.1 Hz, 7.0 Hz, C*H*), 4.41 (1H, dd, *J* = 14.0 Hz, 6.9 Hz, C*H*), 4.30 (1H, t, *J* = 8.1 Hz, C*H*), 3.29 (1H, dd, *J* = 13.8 Hz, 5.8 Hz, C*H*), 3.03 (1H, dd, *J* = 13.7 Hz, 8.6 Hz, C*H*), 1.71 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 175.8 (*C*=O), 146.0 (Ar*C*), 137.8 (Ar*C*), 132.2 (Ar*C*H), 130.2 (Ar*C*), 126.8 (Ar*C*H), 125.9 (Ar*C*H), 124.9 (Ar*C*H), 124.1 (Ar*C*H), 123.7 (Ar*C*H), 122.8

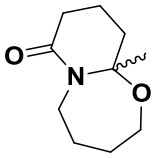
(Ar*C*H), 99.0 (*C*), 74.7 (*C*H_2), 55.7 (*C*H), 40.2 (*C*H_2), 23.0 (*C*H_3); HRMS (ESI^+) ($M+H^+$) found 280.1334 $C_{18}H_{17}NO_2$ requires 280.1332.

9.5.2.13. 9a-Methylhexahydropyrrolo[2,1-b][1,3]oxazepin-7 (8H)-one 270



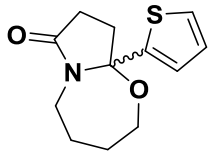
Isolated as a pale solid, following method above using 4-aminobutan-1-ol **257** (0.34 g, 3.75 mmol), PPTS (0.188 g, 0.75 mmol) and 4-oxopentanoic acid **5** (0.43 g, 3.75 mmol) for 24 h. The crude compound was purified by flash column chromatography (ethyl acetate: petroleum ether 8: 2, R_f = 0.2) to yield **270** (0.22 g, 65%); υ_{max} (ATR/cm^{-1})= 2983, 2945, 2877, 1678, 1444, 1413; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 3.92 (1H, dd, *J* = 13.9 Hz, 7.7 Hz, C*H*), 3.69 (1H, dd, *J* = 13.9 Hz, 4.6 Hz, C*H*), 3.39 (1H, t, *J* = 11.6 Hz, C*H*), 2.77 (1H, dd, *J* = 12.9 Hz, 5.8 Hz, C*H*), 2.50-2.34 (2H, m, CH_2), 2.10-1.95 (2H, m, CH_2), 1.71-1.64 (4H, m, 2CH_2), 1.39 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 172.6 (*C*=O), 96.6 (*C*), 61.7 (*C*H_2), 36.7 (*C*H_2), 28.5 (*C*H_2), 27.1 (*C*H_2), 25.9 (*C*H_2), 25.2 (*C*H_2), 24.0 (*C*H_3); HRMS (ESI^+) ($M+H^+$) found 170.1178 $C_9H_{15}NO_2$ requires 170.1178.

9.5.2.14. 10a-Methylhexahydro-2H-pyrido[2,1-b][1,3]oxazepin-7(3H)-one 271



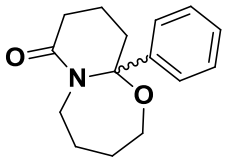
Isolated as a pale oil, following method above using 4-aminobutan-1-ol **257** (0.34 g, 3.75 mmol), PPTS (0.188 g, 0.75 mmol) and 5-oxohexanoic acid **168** (0.48 g, 3.75 mmol) for 24 h. The crude compound was purified by flash column chromatography (ethyl acetate: petroleum ether 7: 3, R_f = 0.2) to yield **271** (0.20 g, 60 %); υ_{max} (ATR/cm^{-1})= 2956, 2873, 1625, 1448, 1398; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 4.15 (1H, dd, *J* = 13.4 Hz, 7.7 Hz, C*H*), 3.70 (1H, dd, *J* = 11.4 Hz, 5.0 Hz, C*H*), 3.59-3.53 (1H, m, C*H*), 2.97 (1H, dd, *J* = 12.1 Hz, 5.9 Hz, C*H*), 2.51-2.31 (2H, m, CH_2), 1.87-1.80 (2H, m, CH_2), 1.80-1.64 (2H, m, 2CH_2), 1.63-1.55 (4H, m, 2CH_2), 1.45 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 170.9 (*C*=O), 89.5 (*C*), 62.5 (*C*H_2), 40.8 (*C*H_2), 33.2 (*C*H_2), 32.0 (*C*H_2), 29.6 (*C*H_2), 26.9 (*C*H_2), 25.7 (*C*H_3), 17.7 (*C*H_2); HRMS (ESI^+) ($M+H^+$) found 184.1334 $C_{10}H_{17}NO_2$ requires 184.1332.

9.5.2.15. 9a-(Thiophen-2-yl)hexahydropyrrolo[2,1-b][1,3]oxazepin-7 (8H)-one 272



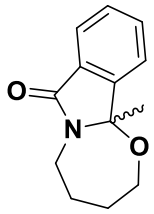
Isolated as a brown solid, following method above using 4-aminobutan-1-ol **257** (0.34 g, 3.75 mmol), PPTS (0.188 g, 0.75 mmol) and 4-oxo-4-(thiophen-2-yl) butanoic acid **173** (0.69 g, 3.75 mmol) for 24 h.The crude compound was purified by flash column chromatography (ethyl acetate: petroleum ether 8: 2, R_f = 0.3) to yield **272** (0.13 g, 40%); m.p = 70-72 ºC; υ_{max} (ATR/cm^{-1})= 2945, 2928, 2870, 1692, 1440; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.29 (1H, d, *J* = 5.1 Hz, C_4H_3S), 6.98 (1H, t, *J* = 4.3 Hz, C_4H_3S), 6.90 (1H, d, *J* = 3.5 Hz, C_4H_3S), 3.95 (1H, t, *J* = 13.0 Hz, C*H*), 3.58 (1H, dd, *J* = 11.5 Hz, 7.0 Hz, C*H*), 3.22 (1H, t, *J* = 12.8 Hz, C*H*), 2.78 (1H, dd, *J* = 12.9 Hz, 6.0 Hz, C*H*), 2.61-2.57 (2H, m, CH_2), 2.47-2.30 (2H, m, CH_2), 1.80-1.59 (4H, m, 2CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 173.7 (*C*=O), 126.7 (C_3H_2SC), 125.5 (C_3H_2SCH), 122.9 (C_3H_2SCH), 121.9 (C_3H_2SCH), 95.3 (*C*), 63.6 (*C*H$_2$), 39.8 (*C*H$_2$), 33.9 (*C*H$_2$), 30.3 (*C*H$_2$), 30.9 (*C*H$_2$), 26.3 (*C*H$_2$); HRMS (ESI$^+$) (M+H$^+$) found 238.0899 $C_{12}H_{15}NO_2S$ requires 238.0896.

9.5.2.16. 10a-Phenylhexahydro-2H-pyrido[2,1-b][1,3]oxazepin-7 (3H)-one 273



Isolated as a white solid, following method above using 4-aminobutan-1-ol **257** (0.34 g, 3.75 mmol), PPTS (0.188 g, 0.75 mmol) and 4-benzoylbutyric acid **175** (0.72 g, 3.75 mmol) for 24 h. The crude compound was purified by flash column chromatography (ethyl acetate: petroleum ether 8: 2, R_f = 0.2) to yield **273** (0.15 g, 45%); m.p = 111-113 ºC; υ_{max} (ATR/cm^{-1})= 3087, 3032, 2949, 2877, 1636, 1492, 1444; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.40-7.32 (5H, m, ArC*H*), 4.21 (1H, d, *J* = 13.0 Hz, C*H*), 3.94 (1H, dd, *J* = 12.6 Hz, 2.3 Hz, C*H*), 3.79 (1H, dd, *J* = 15.3 Hz, 4.6 Hz, C*H*), 2.70-2.55 (1H, m, C*H*), 2.46 (1H, dd, *J* = 18.3 Hz, 6.1 Hz, C*H*), 2.12 (1H, dd, *J* = 16.9 Hz, 6.7 Hz, C*H*), 2.61-1.96 (2H, m, CH_2), 1.73-1.62 (4H, m, 2CH_2), 1.52-1.40 (2H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 171.3 (*C*=O), 143.2 (Ar*C*), 128.3 (Ar*C*H), 127.9 (Ar*C*H), 126.2 (Ar*C*H), 93.4 (*C*), 63.2 (*C*H$_2$), 43.0 (*C*H$_2$), 35.4 (*C*H$_2$), 32.1 (*C*H$_2$), 29.5 (*C*H$_2$), 25.7 (*C*H$_2$), 16.2 (*C*H$_2$); HRMS (ESI$^+$) (M+H$^+$) found 246.1486 $C_{15}H_{19}NO_2$ requires 246.1489.

9.5.2.17. 11b-Methyl-2,3,4,5-tetrahydro-[1,3]oxazepino[2,3-a]isoindol-7(11bH)-one 274



Isolated as a white solid, following method above using 4-aminobutan-1-o **257** (0.34 g, 3.75 mmol), PPTS (0.188 g, 0.75 mmol) and 2-acetylbenzoic acid **170** (0.61 g, 3.75 mmol) for 24 h. The crude compound was purified by flash column chromatography (ethyl acetate: petroleum ether 8: 2, R_f = 0.2) to yield **274** (0.23 g, 70%); m.p = 106-107 ºC; υ_{max}(ATR/cm^{-1})= 3052, 2942, 2870, 1698, 1609, 1464; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.78 (1H, d, *J* = 7.4 Hz, ArC*H*), 7.57 (1H, t, *J* = 7.4 Hz, ArC*H*), 7.51-7.46 (2H, m, ArC*H*), 4.14 (1H, dd, *J* = 14.0 Hz, 7.2 Hz, C*H*), 3.52 (1H, dd, *J* = 12.8 Hz, 5.7 Hz, C*H*), 3.16-3.03 (1H, m, C*H*), 1.74-1.68 (4H, m, 2CH_2), 1.63 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 171.0 (*C*=O), 148.1 (Ar*C*), 133.9 (Ar*C*), 132.4 (Ar*C*H), 129.8 (Ar*C*H), 123.9 (Ar*C*H), 122.9 (Ar*C*H), 92.0 (*C*), 62.8 (*C*H_2), 39.9 (*C*H_2), 30.0 (*C*H_2), 24.8 (*C*H_2), 24.1 (*C*H_3); HRMS (ESI$^+$) (M+H$^+$) found 218.1179 $C_{13}H_{15}NO_2$ requires 218.1176.

9.6. Synthesis of Ugi-tetrazoles

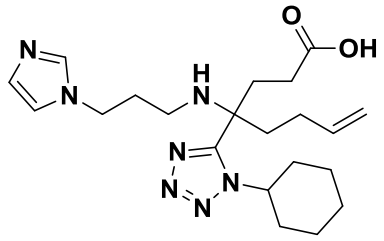
9.6.1. A. General synthesis of Ugi-tetrazoles (Method A, batch conditions)

To a solution of keto-acids (1.0 mmol), primary amine (1.0 mmol) and isocyanides (1.0 mmol) in methanol (5 mL) at room temperature, $TMSN_3$ (1.0 mmol) was added at once and the reaction mixture was stirred at room temperature for 24 hours. The reduced pressure was used to remove the excess methanol and purification was achieved by column chromatography to obtain the titled Ugi-tetrazoles.

9.6.1. B. General synthesis of Ugi-tetrazoles (Method B, flow reactions)

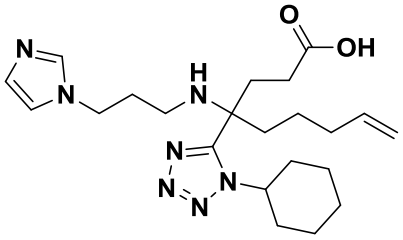
The flow reactor was configured according to Figure **24**. A back-pressure regulator was fitted to allow that the reaction temperature exceed to the boiling point of solvents used can be achieved. After the flow system was premixed by isopropnol at least 30 minutes prior to use. Two 2 mL reactant solutions were prepared; the solutions were simultaneously and automatically injected. A pump **A** was charged keto acid (1.0 M, 1.0 equiv.), primary amine (1.0 M, 1.0 equiv.) and isocyanide (1.0 M, 1.0 equiv.) in methanol, and input to pump **B** was charged with trimethylsilicon azide (1.0 M, 1.0 equiv.). The reagents were assembled in the reactor coil at range temperatures (40-100 °C) for different residence times with a flow rate of 0.4 mL/min, producing in a residence time of 20 minutes. The reaction mixture was then collected and purified by column chromatography.

9.6.1. 4-((3-(1H-Imidazol-1-yl) propyl) amino)-4-(1-cyclohexyl-1H-tetrazol-5-yl) oct-7-enoic acid 289



Isolated as a viscous pale oil, following **method A** using 4-oxooct-7-enoic acid **178** (0.15 g, 1.0 mmol), 1-(3-aminopropyl) imidazole **286** (0.12 g, 1 mmol), cyclohexylisocyanide **113** (0.10 g, 1.0 mmol) and $TMSN_3$ **288** (0.13 mL, 1.0 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9: 1, R_f = 0.5) to yield Ugi-tetrazole **289** (0.05 g, 36% yield); following **method B**, (0.09 g, 62% yield); υ_{max} (ATR/cm^{-1})= 3291, 2932, 2852, 2150, 1647, 1539; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.60 (1H, s, C_3N_2H), 7.10 (1H, d, J = 12.2 Hz, C_3N_2H), 6.99 (1H, d, J = 10.9 Hz, C_3N_2H), 5.85-5.74 (1H, m, C*H*), 5.57 (1H, d, J = 8.1 Hz, N*H*), 5.08-4.97 (2H, m, CH_2), 4.04 (2H, t, J = 7.2 Hz, CH_2), 3.18 (2H, t, J = 7.5 Hz, CH_2), 2.76-2.33 (2H, m, CH_2), 2.20-2.09 (4H, m, 2CH_2), 1.94-1.85 (2H, m, CH_2), 1.79-1.62 (2H, m, CH_2), 1.42-1.06 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176.5 (*C*=O), 172.0 (N_4C), 136.9 (*C*H), 136.7 (C_3N_2CH), 129.1 (C_3N_2CH), 118.8 (C_3N_2CH), 115.8 (*C*H_2), 69.8 (*C*), 48.8 (*C*H), 45.2 (*C*H_2), 41.9 (*C*H_2), 38.9 (*C*H_2), 33.5 (*C*H_2), 33.0 (*C*H_2), 29.8 (*C*H_2), 28.9 (*C*H_2), 27.7 (*C*H_2), 25.4 (*C*H_2), 24.8 (*C*H_2); HRMS (ESI^+) ($M+H^+$) found 416.2776 $C_{21}H_{33}N_7O_2$ requires 416.2768.

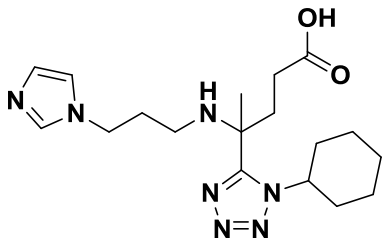
9.6.2. 4-((3-(1H-Imidazol-1-yl) propyl) amino)-4-(1-cyclohexyl-1H-tetrazol-5-yl) non-8-enoic acid 290



Isolated as a viscous pale oil, following **method A** using 4-oxonon-8-enoic acid **179** (0.17 g, 1.0 mmol), 1-(3-aminopropyl) imidazole **286** (0.12 g, 1.0 mmol), cyclohexylisocyanide **113** (0.10 g, 1.0 mmol) and $TMSN_3$ **288** (0.13 mL, 1 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9: 1, R_f = 0.4) to yield Ugi-tetrazole **290** (0.051 g, 30% yield); following **method B**, (0.1 g, 58% yield); υ_{max} (ATR/cm^{-1})= 3297,

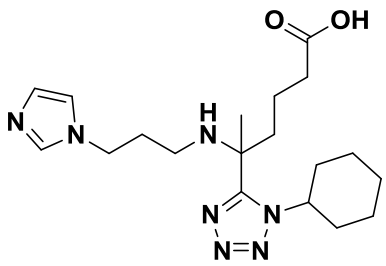
2935, 2855, 2150, 1664, 1530; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.58 (1H, s, C_3N_2H), 7.08 (1H, d, J = 11.2 Hz, C_3N_2H), 6.97 (1H, d, J = 11.9 Hz, C_3N_2H), 5.83-5.71 (1H, m, C*H*), 5.54 (1H, d, J = 8.2 Hz, N*H*), 5.07-4.97 (2H, m, CH_2), 4.16-3.97 (2H, m, CH_2), 3.23-3.10 (2H, m, CH_2), 2.55-2.36 (2H, m, CH_2), 2.19-1.93 (4H, m, 2CH_2), 1.73-1.63 (2H, m, CH_2), 1.49-1.05 (12H, m, 6CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176.5 (*C*=O), 172.2 (N_4C), 137.5 (*C*H), 136.9 (C_3N_2CH), 129.2 (C_3N_2CH), 118.8 (C_3N_2CH), 115.9 (*C*H_2), 69.9 (*C*), 48.7 (*C*H), 45.2 (*C*H_2), 41.9 (*C*H_2), 38.9 (*C*H_2), 33.8 (*C*H_2), 33.7 (*C*H_2), 29.9 (*C*H_2), 29.7 (*C*H_2), 29.1 (*C*H_2), 25.4 (*C*H_2), 24.8 (*C*H_2), 22.7 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 430.1766 $C_{22}H_{35}N_7O_2$ requires 430.1744.

9.6.3. 4-((3-(1H-Imidazol-1-yl) propyl) amino)-4-(1-cyclohexyl-1H-tetrazol-5-yl) pentanoic acid 291



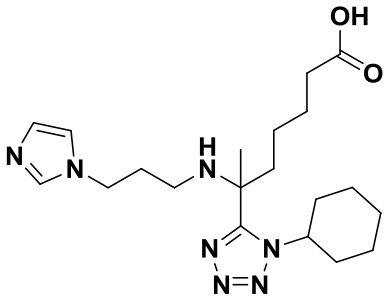
Isolated as a viscous yellow oil, following **method A** using 4-oxopentanoic acid **5** (0.11 g, 1.0 mmol), 1-(3-aminopropyl) imidazole **286** (0.12 g, 1.0 mmol), cyclohexylisocyanide **113** (0.10 g, 1.0 mmol) and $TMSN_3$ **288** (0.13 mL, 1.0 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9: 1, R_f = 0.6) to yield Ugi-tetrazole **291** (0.042 g, 42% yield); following **method B**, (0.084 g, 77% yield); υ_{max} (ATR/cm^{-1})= 3441, 2939, 2855, 1654, 1536; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.71 (1H, s, C_3N_2H), 7.12 (1H, d, J = 9.9 Hz, C_3N_2H), 7.03 (1H, d, J = 7.4 Hz, C_3N_2H), 5.53 (1H, s, N*H*), 4.08 (1H, m, C*H*), 3.75-3.51 (2H, m, CH_2), 3.37-3.09 (2H, m, CH_2), 2.56-2.45 (2H, m, CH_2), 2.28-2.14 (4H, m, 2CH_2), 2.02-1.12 (10H, m, 5CH_2), 1.50 (3H, s, CH_3); ^{13}CNMR (100 MHz, $CDCl_3$) δ_C = 176.4 (*C*=O), 175 (N_4C), 136.9 (C_3N_2CH), 129.1 (C_3N_2CH), 118.9 (C_3N_2CH), 67.1 (*C*), 48.8 (*C*H), 45.2 (*C*H_2), 38.4 (*C*H_2), 33.1 (*C*H_2), 30.5 (*C*H_2), 29.5 (*C*H_2), 26.4 (*C*H_2), 25.4 (*C*H_2), 24.8 (*C*H_2), 22.8 (*C*H_3); HRMS (ESI$^+$) (M+H$^+$) found 376.2460 $C_{18}H_{29}N_7O_2$ requires 376.2455.

9.6.4. 5-((3-(1H-Imidazol-1-yl) propyl)amino)-5-(1-cyclohexyl-1H-tetrazol-5-yl) hexanoic acid 292



Isolated as a viscous yellow oil, following **method A** using 5-oxohexanoic acid **168** (0.13 g, 1.0 mmol), 1-(3-aminopropyl) imidazole **286** (0.12g, 1.0 mmol), cyclohexylisocyanide **113** (0.10 g, 1.0 mmol) and $TMSN_3$ **288** (0.13 mL, 1.0 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9 : 1, R_f = 0.5) to yield Ugi-tetrazole **292** (0.046 g, 36% yield); following **method B**, (0.085 g, 66% yield); υ_{max} (ATR/cm^{-1})= 3136, 2939, 2855, 1704, 1640, 1530; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.92 (1H, s, C_3N_2H), 7.16 (1H, d, J = 11.4 Hz, C_3N_2H), 7.03 (1H, d, J = 7.9 Hz, C_3N_2H), 5.76 (1H, s, N*H*), 4.07-4.0 (1H, m, C*H*), 2.56-2.50 (4H, m, 2CH_2), 2.40 (2H, t, J = 7 Hz, CH_2), 2.18-2.45 (2H, t, J = 13 Hz, CH_2), 1.95-1.88 (4H, m, 2CH_2), 1.81-1.35 (10H, m, 5CH_2), 1.53 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 210 (*C*=O), 177.8 (C_3N_2*C*H), 172.3 (C_3N_2*C*H), 171.9 (C_3N_2*C*H), 66.5 (*C*), 48.9 (*C*H), 45.8 (*C*H_2), 42.8 (*C*H_2), 42.5 (*C*H_2), 36.2 (*C*H_2), 33.1 (*C*H_2), 32.5 (*C*H_2), 29.9 (*C*H_2), 25.4 (*C*H_2), 24.9 (*C*H_2), 18.7 (*C*H_3); HRMS (ESI^+) ($M+H^+$) found 390.2617 $C_{19}H_{31}N_7O_2$ requires 390.2612.

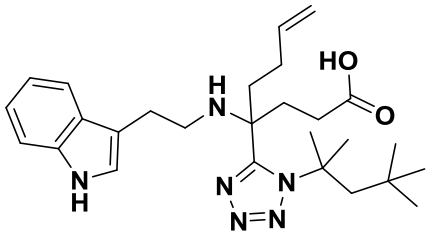
9.6.5. 6-((3-(1H-Imidazol-1-yl) propyl)amino)-6-(1-cyclohexyl-1H-tetrazol-5-yl) heptanoic acid 293



Isolated as a viscous yellow oil, following **method A** using 6-oxo-heptanoic acid **32** (0.14 g, 1.0 mmol), 1-(3-aminopropyl) imidazole **286** (0.12 g, 1.0 mmol), cyclohexylisocyanide **113** (0.10 g, 1.0 mmol) and $TMSN_3$ **288** (0.13 mL, 1.0 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9: 1, R_f = 0.3) to yield Ugi-tetrazole **293** (0.028 g, 20% yield); following **method B**, (0.06 g, 45% yield); υ_{max} (ATR/cm^{-1})= 3314, 3125, 3052, 2939, 2863, 1712, 1509; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.64 (1H, s, C_3N_2H), 7.10 (1H, d, J = 7.4 Hz, C_3N_2H), 6.91 (1H, d, J = 7.8 Hz, C_3N_2H), 5.15 (1H, s, N*H*), 4.08-3.96 (1H, m, C*H*), 3.60-2.99 (6H, m, 3CH_2), 2.31 (2H, t, J = 7.4 Hz, CH_2), 2.00-1.97 (6H, m,

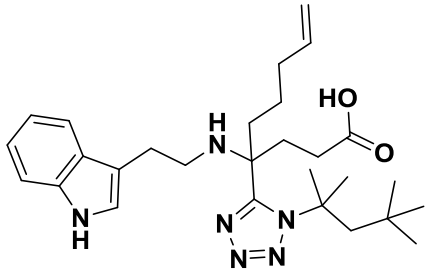
3CH_2), 1.60 (3H, s, CH_3), 1.38-1.19 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 173.8 (*C*=O), 156.7 (N_4C), 136.8 (C_3N_2CH), 129.2 (C_3N_2CH), 118.9 (C_3N_2CH), 58.8 (*C*), 56.4 (*C*H), 51.9 (*C*H_2), 45.2 (*C*H_2), 40.4 (*C*H_2), 39.8 (*C*H_2), 33.7 (*C*H_2), 33.5 (*C*H_2), 25.7 (*C*H_2), 25.7 (*C*H_2), 24.9 (*C*H_2), 24.4 (*C*H_2), 23.3 (*C*H_3); HRMS (ESI$^+$)(M+H$^+$) found 404.2767 $C_{20}H_{33}N_7O_2$ requires 404.2768.

9.6.6. 4-((2-(1H-Indol-3-yl) ethyl)amino)-4-(1-(2,4,4-trimethylpentan-2-yl)-1H-tetrazol-5-yl)oct-7-enoic acid 294



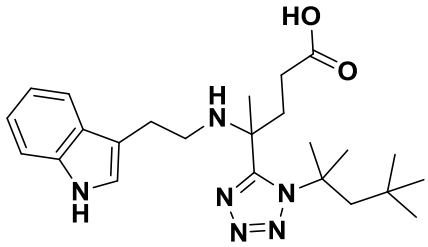
Isolated as a viscous yellow oil, following **method A** using 4-oxooct-7-enoic acid **178** (0.15 g, 1.0 mmol), 1,3-(2-aminoethyl) indole **287** (0.16 g, 1.0 mmol), 1,1,3,3-tetramethylbutyl isocyanide **223** (0.14 g, 1.0 mmol) and $TMSN_3$ **288** (0.13 mL, 1.0 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9: 1, R_f = 0.3) to yield Ugi-tetrazole **294** (0.037 g, 25% yield); following **method B**, (0.1 g, 67% yield); υ_{max} (ATR/cm^{-1})= 3304, 3080, 2945, 2870, 1709, 1643, 1519; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.62 (1H, s, C_8H_5NH), 8.20 (1H, brs, N*H*), 7.72 (1H, d, *J* = 7.8 Hz, ArC*H*), 7.39 (1H, d, *J* = 8.1 Hz, ArC*H*), 7.22 (1H, t, *J* = 7.3 Hz, ArC*H*), 7.15 (1H, t, *J* = 7.2 Hz, ArC*H*), 5.87-5.77 (1H, m, C*H*), 5.57 (1H, s, N*H*), 5.08-4.99 (2H, m, CH_2), 3.58-3.42 (2H, m, CH_2), 3.23-3.05 (4H, m, 2CH_2), 2.50 (2H, t, *J* = 8.90 Hz, CH_2), 2.30-2.11 (4H, m, 2CH_2), 1.79 (6H, s, 2CH_3), 1.40-1.34 (2H, s, CH_2), 0.97 (9H, s, 3CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176.5 (*C*=O), 172.4 (N_4C), 140.5 (*C*H), 137.1 (ArC*H*), 122.2 (Ar*C*H), 119.5 (Ar*C*H), 118.9 (Ar*C*H), 115.4 (Ar*C*), 112.8 (*C*H_2), 111.3 (Ar*C*H), 70.7 (*C*), 62.8 (*C*), 55.7 (*C*H_2), 54.3 (*C*H_2), 42.9 (*C*H), 33.4 (*C*), 31.5 (*C*H_2), 30.6 (2*C*H_3), 29.9 (*C*H_2), 28.9 (*C*H_2), 27.9 (3*C*H_3), 24.4 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 481.3296 $C_{27}H_{40}N_6O_2$ requires 481.3286.

9.6.7. 4-((2-(1H-Indol-3-yl) ethyl)amino)-4-(1-(2,4,4-trimethylpentan-2-yl)-1H-tetrazol-5-yl)non-8-enoic acid 295



Isolated as a viscous yellow oil, following **method A** using 4-oxonon-8-enoic acid **179** (0.17 g, 1.0 mmol), 1,3-(2-aminoethyl) indole **287** (0.16 g, 1.0 mmol), 1,1,3,3-tetramethylbutyl isocyanide **223** (0.14 g, 1.0 mmol) and $TMSN_3$ **288** (0.13 mL, 1.0 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9: 1, R_f = 0.3) to yield Ugi-tetrazole **295** (0.03 g, 22% yield); following **method B**, (0.09 g, 53% yield); υ_{max} (ATR/cm^{-1})= 3266, 3052, 2956, 2866, 1674, 1519; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.64 (1H, s, C_8H_5NH), 8.54 (1H, brs, N*H*), 7.71 (1H, d, *J* = 7.7 Hz, ArC*H*), 7.38 (1H, d, *J* = 7.2 Hz, ArC*H*), 7.20 (1H, t, *J* = 7.2 Hz, ArC*H*), 7.13 (1H, t, *J* = 5.8 Hz, ArC*H*), 5.82-5.71 (1H, m, C*H*), 5.05-4.96 (2H, m, CH_2), 3.48 (2H, t, *J* = 9.5 Hz, CH_2), 3.21-3.07 (4H, m, $2CH_2$), 2.82-2.79 (2H, m, CH_2), 2.48 (2H, t, *J* = 9.9 Hz, CH_2), 2.23-2.06 (4H, m, $2CH_2$), 1.77 (6H, s, $2CH_3$), 1.40-1.34 (2H, s, CH_2), 0.96 (9H, s, $3CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176.6 (*C*=O), 172.6 (N_4C), 140.6 (*C*H), 137.7 (Ar*C*H), 136.4 (Ar*C*H), 127.3 (ArCH), 122.2 (Ar*C*H), 119.5 (Ar*C*), 118.9 (Ar*C*H), 115.5 (Ar*C*), 112.7 (ArC), 112.7 (CH_2), 70.8 (*C*), 62.9 (*C*), 55.7 (CH_2), 54.3 (CH_2), 42.8 (CH_2), 38.9 (*C*), 33.8 (CH_2), 31.5 ($2CH_3$), 29.9 (CH_2), 28.9 (CH_2), 27.9 ($3CH_3$), 24.4 (CH_2); HRMS (ESI^+) ($M+H^+$) found 495.3447 $C_{28}H_{42}N_6O_2$ requires 495.3442.

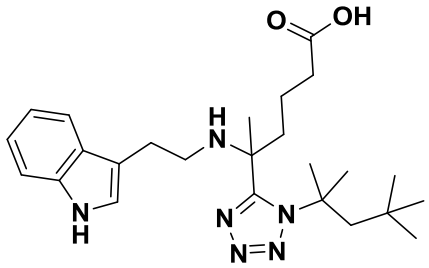
9.6.8. 4-((2-(1H-Indol-3-yl) ethyl)amino)-4-(1-(2,4,4-trimethylpentan-2-yl)-1H-tetrazol-5-yl)pentanoic acid 296



Isolated as a viscous yellow oil, following **method A** using 4-oxopentanoic acid **5** (0.11 g, 1.0 mmol), 1,3-(2-aminoethyl) indole **287** (0.16 g, 1.0 mmol), 1,1,3,3-tetramethylbutyl isocyanide **223** (0.14 g, 1.0 mmol) and $TMSN_3$ **288** (0.13 mL, 1.0 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9: 1, R_f = 0.5) to yield Ugi-tetrazole **296** (0.042 g, 39% yield); following **method B**, (0.08 g, 76% yield); υ_{max} (ATR/cm^{-1})= 3359, 3076, 2952, 2870, 1712, 1640; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.12 (1H, s, C_8H_5NH), 8.09 (1H, brs, N*H*), 7.69 (1H, d, *J* = 7.8 Hz, ArC*H*), 7.39 (1H, d, *J* = 8 Hz, ArC*H*), 7.22 (1H, t, *J* = 7.5 Hz, ArC*H*), 7.15 (1H, t, *J* = 7.5 Hz, ArC*H*), 3.72 (2H, t, *J* = 14.2 Hz, CH_2), 3.19 (2H, t, *J* = 14.3 Hz, CH_2), 2.47 (2H, t, *J* = 8.1 Hz, CH_2), 1.95 (2H, t, *J* = 11.4 Hz, CH_2) 1.54 (6H, s, $2CH_3$), 1.40 (2H, s, CH_2), 1.34 (3H, s, CH_3), 0.95 (9H, s, $3CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176 (*C*=O), 172.5 (N_4C), 136.3 (ArC), 137.8

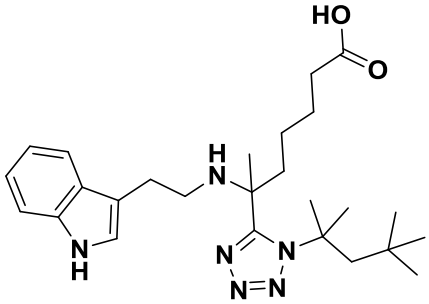
(ArC*H*), 127.3 (Ar*C*), 122.2 (Ar*C*H), 121.9 (Ar*C*H), 119.7 (Ar*C*H), 118.9 (Ar*C*H), 112.9 (Ar*C*), 70.8 (*C*), 68 (*C*), 42.4 (*C*H_2), 33.2 (*C*H_2), 31.5 (*C*), 31.5 (2*C*H_3), 30.9 (*C*H_3), 29.8 (*C*H_2), 29.2 (*C*H_2), 28.8 (3*C*H_3), 24.7 (*C*H_2); HRMS (ESI^+) ($M+H^+$) found 441.3000 $C_{24}H_{36}N_6O_2$ requires 441.3002.

9.6.9. 5-((2-(1H-Indol-3-yl) ethyl)amino)-5-(1-(2,4,4-trimethylpentan-2-yl)-1H-tetrazol-5-yl)hexanoic acid 297



Isolated as a viscous yellow oil, following **method A** using 5-oxohexanoic acid **164** (0.13 g, 1.0 mmol), 1,3-(2-aminoethyl) indole **287** (0.16 g, 1.0 mmol), 1,1,3,3-tetramethylbutyl isocyanide **223** (0.14 g, 1.0 mmol) and $TMSN_3$ **288** (0.13 mL, 1.0 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9 : 1, R_f = 0.3) to yield Ugi-tetrazole **297** (0.057 g, 44% yield); following **method B**, (0.078 g, 60% yield); υ_{max} (ATR/cm^{-1})= 3262, 3056, 2952, 2866, 1654, 1612, 1574; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.20 (1H, s, C_8H_5NH), 7.80 (1H, d, *J* = 7.8 Hz, ArC*H*), 7.38 (1H, d, *J* = 8.1 Hz, ArC*H*), 7.22 (1H, t, *J* = 6.9 Hz, ArC*H*), 7.15 (1H, t, *J* = 7.1 Hz, ArC*H*), 4.13-3.64 (2H, m, CH_2), 3.38-3.11 (2H, m, CH_2), 2.04-2.86 (4H, m, 2CH_2), 2.50 (2H, t, *J* = 5.89 Hz, CH_2), 1.65 (6H, s, 2CH_3), 1.42 (2H, s, CH_2), 1.37 (3H, s, CH_3), 0.98 (9H, s, 3CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 172.5 (*C*=O), 171.2 (N_4C), 136.3 (ArC), 127.8 (ArC*H*), 122.7 (Ar*C*), 121.9 (Ar*C*H), 121.9 (ArC*H*), 119.5 (ArC*H*), 119.4 (ArC*H*), 113.4 (Ar*C*), 66.8 (*C*), 55.8 (*C*), 53.3 (*C*), 46.9 (*C*H_2), 44.1 (*C*H_2), 36.4 (*C*), 32.8 (2*C*H_3), 31.5 (*C*H_3), 28.9 (*C*H_2), 27.7 (*C*H_2), 25 (3*C*H_3), 24.7 (*C*H_2), 19.9 (*C*H_2); HRMS (ESI^+) ($M+H^+$) found 455.3000 $C_{25}H_{38}N_7O$ requires 455.3000.

9.6.10. 6-((2-(1H-Indol-3-yl) ethyl)amino)-6-(1-(2,4,4-trimethylpentan-2-yl)-1H-tetrazol-5-yl)heptanoic acid 298

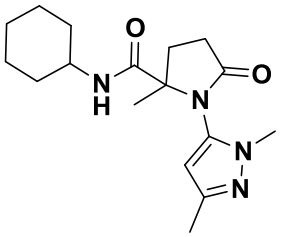


Isolated as a viscous yellow oil, following **method A** using 6-oxo-heptanoic acid **47** (0.14 g, 1.0 mmol), 1,3-(2-aminoethyl) indole **287** (0.16 g, 1.0 mmol), 1,1,3,3-tetramethylbutyl isocyanide **223** (0.14 g, 1.0 mmol) and $TMSN_3$ **288** (0.13 mL, 1.0 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9: 1, R_f = 0.2) to yield Ugi-tetrazole **298** (0.039 g, 28% yield); following **method B**, (0.06 g, 42% yield); υ_{max} (ATR/cm^{-1})= 3311, 3132, 2952, 2877, 1733, 1457; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.66 (1H, s, C_8H_5NH), 7.54 (1H, d, *J* = 7.7 Hz, ArC*H*), 7.34 (1H, d, *J* = 7.8 Hz, ArC*H*), 7.12 (1H, t, *J* = 7.6 Hz, ArC*H*), 7.04 (1H, t, *J* = 8.1Hz, ArC*H*), 3.0-2.93 (2H, m, CH_2), 2.90-2.85 (2H, m, CH_2), 2.41 (2H, t, *J* = 6.3 Hz, CH_2), 2.28 (2H, t, *J* = 6.4 Hz, CH_2), 2.20 (2H, t, *J* = 12 Hz, CH_2), 1.73 (6H, s, 2CH_3), 1.56 (2H, t, *J* = 3.5 Hz, CH_2), 1.49 (2H, s, CH_2), 1.37 (3H, s, CH_3), 0.92 (9H, s, 3CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176 (*C*=O), 172.5 (N_4C), 136.3 (ArC), 127.4 (ArC), 122.3 (Ar*C*H), 121.9 (ArC*H*), 119.7 (ArC*H*), 118.9 (Ar*C*H), 113 (Ar*C*), 111.2 (ArC*H*), 68 (*C*), 55.6 (*C*), 52.2 (*C*H_2), 42.4 (*C*H_2), 33.2 (*C*H_2), 31.9 (*C*), 31.6 (2*C*H_3), 31.5 (*C*H_3), 29.8 (*C*H_2), 28.8 (*C*H_2), 28.3 (3*C*H_3), 24.8 (*C*H_2), 22.9 (*C*H_2); HRMS (ESI^+)($M+H^+$) found 469.3297 $C_{26}H_{40}N_6O_2$ requires 469.3286.

9.7. **Synthesis of pyrazole-oxopyrrolidine derivatives using microwave techniques** [139]

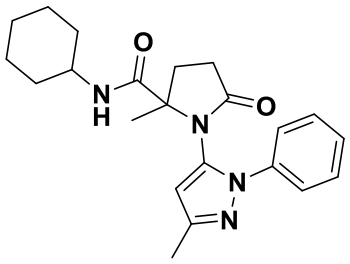
9.7.1. **General protocol**: All microwave reactions were performed in a capped 10 mL microwave-vessel (Borosilicate glass vial sealed) which was placed in a microwave cavity. The ketoacids (1.0 mmol), the amine (1.0 mmol) and the isocyanide (1.0 mmol) were mixed and continuously stirred. The pressure was set at 17 bar (Average of effective pressure= 4 bar) and the power at 75 W. The mixture reaction was heated at 100 ºC for 15 min. After the reaction vessel was cooled to ambient temperature, the crude products were dissolved in DCM (20 mL), washed with HCl (2M, 10 mL), sat. $NaHCO_3$ (10 mL) and dried over sodium sulphate. The residual mixture was concentrated and purified by column chromatography to obtain the titled pyrazole-oxopyrrolidine derivatives.

9.7.1.1. ***N*-Cyclohexyl-1-(1,3-dimethyl-1H-pyrazol-5-yl)-2-methyl-5-oxopyrrolidine-2-carboxamide 313**



Isolated as a yellow solid, following method above using 4-oxopentanoic acid **5** (0.11 g, 1.0 mmol), 5-amino-1,3-dimethylpyrazole **310** (0.11 g, 1.0 mmol) and cyclohexylisocyanide **113** (0.10 g, 1.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.4) to yield pyrazole-oxopyrrolidine **313** (0.07g, 72% yield); m.p = 120-122 ºC; υ_{max} (ATR/cm^{-1})= 3351, 2936, 2858, 1790, 1672; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 6.25 (1H, s, C_3N_2H), 3.80-3.72 (1H, m, C*H*), 2.60 (3H, s, CH_3), 2.59-2.45 (2H, m, CH_2), 1.96-1.87 (2H, m, CH_2), 1.92 (3H, s, CH_3), 1.77-1.24 (10H, m, 5CH_2), 1.42 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 175.6 (*C*=O), 171.0 (*C*=O), 156.2 (C_2N_2C), 144.9 (C_2N_2C), 85.8 (C_2N_2CH), 52.2 (*C*), 48.4 (*C*H), 33.1 (*C*H$_3$), 35.4 (*C*H$_2$), 32.9 (*C*H$_2$), 32.1 (*C*H$_3$), 28.3 (*C*H$_2$), 26.4 (*C*H$_2$), 25.4 (*C*H$_2$), 24.9 (*C*H$_3$); HRMS (ESI$^+$) (M+H$^+$) found 319.2197 $C_{17}H_{26}N_4O_2$ requires 319.2186.

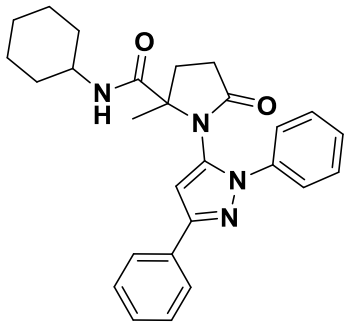
9.7.1.2. *N*-Cyclohexyl-2-methyl-1-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-5-oxopyrrolidine-2-carboxamide 314



Isolated as a yellow solid, following method above using 4-oxopentanoic acid **5** (0.11 g, 1.0 mmol), 5-amino-3-methyl-1-phenylpyrazole **311** (0.17 g, 1.0 mmol) and cyclohexylisocyanide **113** (0.10 g, 1.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.3) to yield pyrazole-oxopyrrolidine **314** (0.07g, 65% yield); m.p = 138-139 ºC; υ_{max} (ATR/cm^{-1})= 3351, 3051, 2976, 2934, 1705, 1660; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.50-7.35 (5H, m, ArC*H*), 6.12 (1H, s, C_3N_2H), 3.81-3.72 (1H, m, C*H*), 2.78-2.41 (2H, m, CH_2), 2.33 (3H, s, CH_3), 1.92-1.83 (2H, m, CH_2), 1.66 (3H, s, CH_3), 1.40-1.06 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 177.0 (*C*=O), 171.1(*C*=O), 149.4 (C_2N_2C), 138.9 (C_2N_2C), 134.5 (Ar*C*), 129.1 (Ar*C*H), 128.1 (Ar*C*H), 124.6 (Ar*C*H), 105.1 (C_2N_2CH), 68.9 (*C*), 48.9 (*C*H), 33.5 (*C*H$_2$),

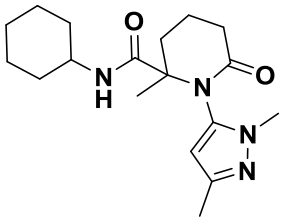
32.9 (*C*H_2), 30.4 (*C*H_2), 25.4 (*C*H_2), 24.8 (*C*H_2), 24.0 (*C*H_3), 14.1 (*C*H_3); HRMS (ESI$^+$) (M+H$^+$) found 381.2294 $C_{22}H_{28}N_4O_2$ requires 381.2285.

9.7.1.3. *N*-Cyclohexyl-1-(1,3-diphenyl-1H-pyrazol-5-yl)-2-methyl-5-oxopyrrolidine-2-carboxamide 315



Isolated as a yellow solid, following method above using 4-oxopentanoic acid **5** (0.11 g, 1.0 mmol), 5-amino-1,3-diphenyl-1H-pyrazole **312** (0.23 g, 1.0 mmol) and cyclohexylisocyanide **113** (0.10 g, 1.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.4) to yield pyrazole-oxopyrrolidine **315** (0.06 g, 55% yield); m.p = 223-224 ºC; υ_{max} (ATR/cm^{-1})= 3410, 2985, 2935, 2853, 1785, 1712; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.89 (2H, d, *J* = 7.1 Hz, ArC*H*), 7.58-7.33 (8H, m, ArC*H*), 6.64 (1H, s, C_3N_2H), 3.80-3.72 (1H, m, C*H*), 2.67-2.47 (2H, m, CH_2), 2.37-2.30 (2H, m, CH_2), 2.20-1.19 (10H, m, 5CH_2), 1.92 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 175.5 (*C*=O), 171.1 (*C*=O), 151.2 (C_2N_2C), 139.9 (C_2N_2C), 129.6 (Ar*C*H), 129.3 (Ar*C*H), 129.1 (Ar*C*), 128.6 (Ar*C*H), 128.2 (Ar*C*H), 128.1 (Ar*C*H), 128.0 (Ar*C*), 125.6 (Ar*C*H), 102.9 (C_2N_2CH), 85.8 (*C*), 48.4 (*C*H), 33.1 (*C*H_2), 32.9 (*C*H_2), 32.1 (*C*H_2), 28.2 (*C*H_2), 25.3 (*C*H_2), 24.8 (*C*H_3); HRMS (ESI$^+$) (M+H$^+$) found 443.2499 $C_{27}H_{30}N_4O_2$ requires 443.2482.

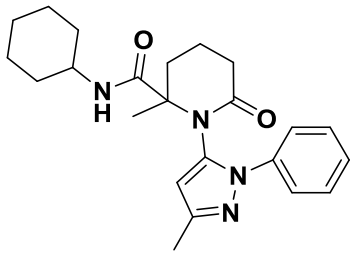
9.7.1.4. *N*-Cyclohexyl-1-(1,3-dimethyl-1H-pyrazol-5-yl)-2-methyl-6-oxopiperidine-2-carboxamide 316



Isolated as a yellow solid, following method above using 5-oxohexanoic acid **168** (0.13 g, 1.0 mmol), 5-amino-1,3-dimethylpyrazole **310** (0.11g, 1 mmol) and cyclohexylisocyanide **113** (0.10 g, 1.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.5) to yield pyrazole-oxopyrrolidine **316** (0.08 g, 67%

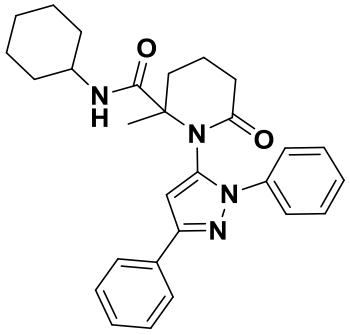
yield); m.p = 96-98 ºC; υ_{max} (ATR/cm^{-1})=3393, 2924, 2851, 1731, 1646; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 6.35 (1H, s, C_3N_2H), 3.81-3.72 (1H, m, C*H*), 2.63-2.41 (2H, m, CH_2), 1.92-1.86 (2H, m, CH_2), 1.80-1.73 (2H, m, CH_2), 1.58 (3H, s, CH_3), 1.45-0.92 (10H, m, 5CH_2), 1.28 (6H, s, 2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C = 174.7 (*C*=O), 170.1 (*C*=O), 155.2 (C_2N_2C), 143.9 (C_2N_2C), 85.7 (C_2N_2CH), 53.2 (*C*), 48.5 (*C*H), 33.2 (*C*H$_3$), 32.7 (*C*H$_2$), 31.9 (*C*H$_3$), 30.7 (*C*H$_2$), 29.7 (*C*H$_2$), 26.2 (*C*H$_2$), 25.4 (*C*H$_2$), 21.5 (*C*H$_2$), 17.1 (*C*H$_3$); HRMS (ESI$^+$) (M+H$^+$) found 333.2256 $C_{18}H_{28}N_4O_2$ requires 333.2249.

9.7.1.5. *N*-Cyclohexyl-2-methyl-1-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-6-oxopiperi di ne-2-carboxamide 317



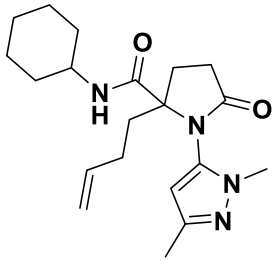
Isolated as a yellow solid, following method above using 5-oxohexanoic acid **168** (0.13 g, 1.0 mmol), 5-amino-3-methyl-1-phenylpyrazole **311** (0.17g, 1.0 mmol) and cyclohexylisocyanide **113** (0.10 g, 1.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.3) to yield pyrazole-oxopyrrolidine **317** (0.06 g, 50% yield); m.p = 133-134 ºC; υ_{max} (ATR/cm^{-1})= 3351, 2931, 2856, 1665, 1594; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.43-7.35 (5H, m, ArC*H*), 6.11 (1H, s, C_3N_2H), 3.91-3.81 (1H, m, C*H*), 2.67-2.37 (2H, m, CH_2), 2.34 (3H, s, CH_3), 2.18-1.92 (2H, m, CH_2), 1.83-1.16 (12H, m, 6CH_2), 1.08 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 173.0 (*C*=O), 171.4 (*C*=O), 149.2 (C_2N_2C), 138.8 (C_2N_2C), 137.6 (Ar*C*), 129.1 (Ar*C*H), 127.9 (Ar*C*H), 124.5 (Ar*C*H), 104.1 (C_2N_2CH), 67.8 (*C*), 48.2 (*C*H), 35.5 (*C*H$_2$), 33.2 (*C*H$_2$), 31.9 (*C*H$_2$), 25.4 (*C*H$_2$), 24.9 (*C*H$_2$), 23.8 (*C*H$_2$), 17.4 (*C*H$_3$) 14.1 (*C*H$_3$); HRMS (ESI$^+$) (M+H$^+$) found 395.2447 $C_{23}H_{30}N_4O_2$ requires 395.2442.

9.7.1.6. *N*-Cyclohexyl-1-(1,3-diphenyl-1H-pyrazol-5-yl)-2-methyl-6-oxopiperidine-2-carboxamide 318



Isolated as a yellow solid, following method above using 5-oxohexanoic acid **168** (0.13 g, 1.0 mmol), 5-amino-1,3-diphenyl-1H-pyrazole **312** (0.23 g, 1.0 mmol) and cyclohexylisocyanide **113** (0.10 g, 1.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.3) to yield pyrazole-oxopyrrolidine **318** (0.078 g, 60% yield); m.p = 155-156 ºC; υ_{max} (ATR/cm^{-1})= 3353, 2934, 2856, 1662, 1596; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.84 (2H, d, *J* = 7.2 Hz, ArC*H*), 7.54-7.34 (8H, m, ArC*H*), 6.65 (1H, s, C_3N_2H), 3.95-3.86 (1H, m, C*H*), 2.72-2.36 (2H, m, CH_2), 2.19-1.96 (2H, m, CH_2), 1.88-1.17 (12H, m, 6CH_2), 1.12 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 173.4 (*C*=O), 171.4 (*C*=O), 151.5 (C_2N_2C), 139.1 (C_2N_2C), 138.6 (Ar*C*H), 133.0 (Ar*C*H), 129.1 (Ar*C*), 128.2 (Ar*C*H), 128.2 (Ar*C*H), 125.6 (Ar*C*H), 124.7 (Ar*C*), 124.2 (Ar*C*H), 102.9 (C_2N_2CH), 67.8 (*C*), 48.2 (*C*H), 35.6 (*C*H_2), 33.2 (*C*H_2), 33.1 (*C*H_2), 29.7 (*C*H_2), 25.4 (*C*H_2), 24.8 (*C*H_2), 17.5 (*C*H_3); HRMS (ESI$^+$) (M+H$^+$) found 457.2605 $C_{28}H_{32}N_4O_2$ requires 457.2598.

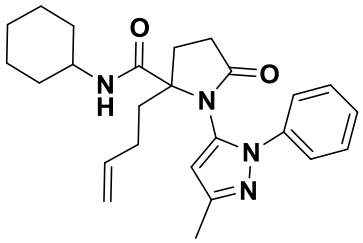
9.7.1.7. 2-(But-3-en-1-yl)-*N*-cyclohexyl-1-(1,3-dimethyl-1H-pyrazol-5-yl)-5-oxopyrrolidine-2-carboxamide 319



Isolated as a yellow viscous oil, following method above using 4-oxooct-7-enoic acid **178** (0.15 g, 1.0 mmol), 5-amino-1,3-dimethylpyrazole **310** (0.11 g, 1.0 mmol) and cyclohexylisocyanide **113** (0.10 g, 1.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.3) to yield pyrazole-oxopyrrolidine **319** (0.09 g, 65% yield); υ_{max} (ATR/cm^{-1})= 3424, 3351, 2931, 2853, 1783, 1660; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 6.29 (1H, s, C_3N_2H), 5.83-5.73 (1H, m, C*H*), 5.07-4.98 (2H, m, CH_2), 3.83-3.74 (1H, m, C*H*), 2.64-2.54 (2H, m, CH_2), 2.24-2.18 (4H, m,

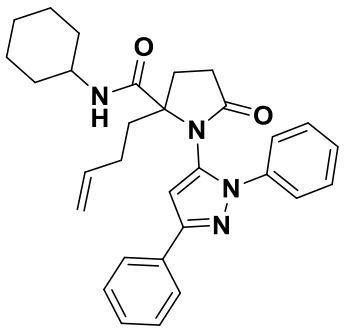
2CH_2), 2.09-1.16 (12H, m, 6CH_2), 1.58 (3H, s, CH_3), 1.27 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 175.7 (*C*=O), 171.3 (*C*=O), 148.3 (C_2N_2C), 137.1 (C_2N_2CH), 134.7 (*C*H), 114.2 (*C*H_2), 104.8 (C_2N_2CH), 70.9 (*C*), 48.5 (*C*H), 37.6 (*C*H_2), 33.1 (*C*H_2), 33.0 (*C*H_3), 31.3 (*C*H_2), 29.71 (*C*H_2), 28.1 (*C*H_2), 28.0 (*C*H_2), 25.4 (*C*H_2), 21.8 (*C*H_3); HRMS (ESI$^+$) (M+H$^+$) found 359.2488 $C_{20}H_{30}N_4O_2$ requires 359.2476.

9.7.1.8. 2-(But-3-en-1-yl)-*N*-cyclohexyl-1-(1-methyl-3-phenyl-1H-pyrazol-5-yl)-5-oxopyrrolidine-2-carboxamide 320



Isolated as a yellow viscous oil, following method above using 4-oxooct-7-enoic acid **178** (0.15 g, 1.0 mmol), 5-amino-3-methyl-1-phenylpyrazole **311** (0.17 g, 1.0 mmol) and cyclohexylisocyanide **113** (0.10 g, 1.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.3) to yield pyrazole-oxopyrrolidine **320** (0.08 g, 55% yield); υ_{max} (ATR/cm^{-1})= 3360, 3056, 2924, 1714, 1662; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.54-7.37 (5H, m, ArC*H*), 6.17 (1H, s, C_3N_2H), 5.56-5.49 (1H, m, C*H*), 4.93-4.81 (2H, m, CH_2), 3.81-3.71 (1H, m, C*H*), 2.74-2.39 (2H, m, CH_2), 2.37 (3H, s, CH_3), 1.86-1.77 (4H, m, 2CH_2), 1.73-1.08 (12H, m, 6CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 177.3 (*C*=O), 171.4 (*C*=O), 149.3 (C_2N_2C), 136.8 (C_2N_2C), 134.7 (*C*H), 129.2 (Ar*C*H), 128.2 (Ar*C*), 124.5 (Ar*C*H), 123.5 (Ar*C*H), 115.3 (*C*H_2), 105.9 (C_2N_2CH), 71.9 (*C*), 48.8 (*C*H), 35.5 (*C*H_2), 32.9 (*C*H_2), 32.7 (*C*H_2), 31.1 (*C*H_2), 29.9 (*C*H_2), 27.6 (*C*H_2), 25.8 (*C*H_2), 14.2 (*C*H_3); HRMS (ESI$^+$) (M+H$^+$) found 421.2605 $C_{25}H_{32}N_4O_2$ requires 421.2598.

9.7.1.9. 2-(But-3-en-1-yl)-*N*-cyclohexyl-1-(1,3-diphenyl-1H-pyrazol-5-yl)-5-oxopyrrolidine-2-carboxamide 321



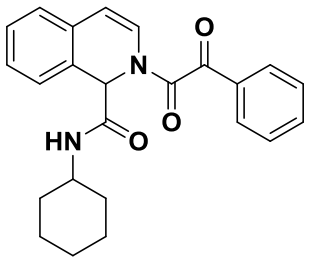
Isolated as a yellow solid, following method above using 4-oxooct-7-enoic acid **178** (0.15 g, 1.0 mmol), 5-amino-1,3-diphenyl-1H-pyrazole **312** (0.23 g, 1.0 mmol) and cyclohexylisocyanide **113** (0.10 g, 1.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.2) to yield pyrazole-oxopyrrolidine **321** (0.09 g, 60% yield); m.p = 95-96 ºC; υ_{max} (ATR/cm^{-1})= 3422, 3063, 2934, 1787, 1721; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.90-7.88 (2H, m, ArC*H*), 7.59-7.36 (8H, m, ArC*H*), 6.29 (1H, s, C_3N_2H), 5.83-5.63 (1H, m, C*H*), 5.07-4.83 (2H, m, CH_2), 3.83-3.75 (1H, m, C*H*), 2.71-2.54 (4H, m, 2CH_2), 2.22-1.98 (4H, m, 2CH_2), 1.77-1.16 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C = 175.2 (*C*=O), 172.4 (*C*=O), 149.5 (C_2N_2C), 140.1 (C_2N_2C), 136.8 (Ar*C*H), 135.9 (*C*H), 129.2 (Ar*C*), 129.1 (Ar*C*H), 128.6 (Ar*C*H), 128.5 (Ar*C*H), 128.2 (Ar*C*), 125.7 (Ar*C*H), 123.7 (Ar*C*H), 115 (*C*H_2), 114.8 (Ar*C*), 103.9 (C_2N_2CH), 88.9 (*C*), 48.5 (*C*H), 37.5 (*C*H_2), 33.1 (*C*H_2), 32.9 (*C*H_2), 31.3 (*C*H_2), 29.1 (*C*H_2), 28.1 (*C*H_2), 25.4 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 483.2733 $C_{30}H_{34}N_4O_2$ requires 483.2729.

9.8. Synthesis of isoquinoline derivatives from *α*-ketoacids

9.8.1. General procedure[353]

To a magnetically stirred solution of isoquinoline derivatives (1.40 mmol) and *α*-ketoacids (1.86 mmol) in dichloromethane (4 mL), isocyanides (1.86 mmol) was added and the reaction mixture was stirred for 48 h. After completion of the reaction, as indicated by TLC, the residual product was concentrated under reduced pressure and purified by column chromatography to furnish the corresponding isoquinoline derivatives.

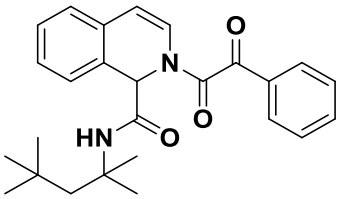
9.8.1.1. *N*-Cyclohexyl-2-(2-oxo-2-phenylacetyl)-1,2-dihydroisoquinoline-1-carbox amide 333



Isolated as a yellow solid, following method above using isoquinoline **331** (0.18 g, 1.40 mmol), 2-oxo-2-phenylacetic acid **169** (0.27 g, 1.86 mmol) and cyclohexylisocynaide **113** (0.20 g, 1.86 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.2) to yield isoquinoline derivative **333** (0.11 g, 65% yield); m.p = 123-124 ºC; υ_{max} (ATR/cm^{-1})= 3304, 2931, 2849, 1681, 1640; ^{1}H NMR (400

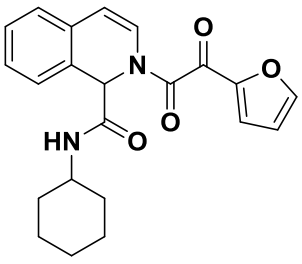
MHz, $CDCl_3$) δ_H = 8.40 (1H, s, N*H*), 7.71-7.50 (5H, m, ArC*H*), 7.48-7.42 (4H, m, ArC*H*), 6.99 (1H, s, ArC*H*), 6.85 (1H, d, *J* = 7.6 Hz, ArC*H*), 6.48 (1H, d, *J* = 7.6 Hz, ArC*H*), 3.77-3.68 (1H, m, C*H*), 2.06-1.06 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 191.1 (*C*=O), 162.3 (*C*=O), 158.9 (*C*=O), 137.1 (Ar*C*), 133.2 (Ar*C*), 132.9 (Ar*C*H), 132.7 (Ar*C*), 130.8 (Ar*C*H), 130.4 (Ar*C*H), 130.1 (Ar*C*H), 129.6 (Ar*C*H), 128.9 (Ar*C*H), 128.2 (Ar*C*H), 127.7 (Ar*C*H), 107.6 (Ar*C*H), 64.6 (*C*H), 48.6 (*C*H), 32.6 (*C*H_2), 29.4 (*C*H_2), 25.9 (*C*H_2); HRMS (ESI^+) ($M+H^+$) found 389.1867 $C_{24}H_{24}N_2O_3$ requires 389.1860.

9.8.1.2. 2-(2-Oxo-2-phenylacetyl)-*N*-(2,4,4-trimethylpentan-2-yl)-1,2-dihydro isoquino line-1-carboxamide 334



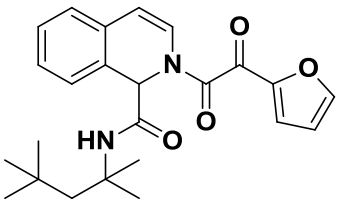
Isolated as a colourless solid, following method above using isoquinoline **331** (0.18 g, 1.40 mmol), 2-oxo-2-phenylacetic acid **169** (0.27 g, 1.86 mmol) and 2-isocyano-2,4,4-trimethylpentane **223** (0.25 g, 1.86 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.2) to yield isoquinoline derivative **334** (0.06 g, 80% yield); m.p = 119-121 ºC; υ_{max} (ATR/cm^{-1})= 3393, 2955, 2849, 1643, 1623; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.41 (1H, d, *J* = 7.9 Hz, ArC*H*), 7.67 (1H, t, *J* = 7.5 Hz, ArC*H*), 7.53-7.45 (5H, m, ArC*H*), 7.40-7.38 (4H, m, ArC*H*), 7.00 (1H, s, N*H*), 6.90 (1H, s, C*H*), 1.65 (2H, s, CH_2), 1.45 (6H, s, 2CH_3), 0.96 (9H, s, 3CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 192.1 (*C*=O), 162.6 (*C*=O), 158.6 (*C*=O), 137.0 (Ar*C*), 132.6 (Ar*C*), 131.6 (Ar*C*H), 130.3 (Ar*C*), 129.5 (Ar*C*H), 127.9 (Ar*C*H), 126.9 (Ar*C*H), 125.9 (Ar*C*H), 125.4 (Ar*C*H), 124.5 (Ar*C*H), 124.2 (Ar*C*H), 122.2 (Ar*C*H), 64.4 (*C*H), 55.4 (*C*H_2), 51.6 (*C*), 31.6 (*C*), 31.3 (3*C*H_3), 28.6 (2*C*H_2); HRMS (ESI^+) ($M+H^+$) found 419.2334 $C_{26}H_{30}N_2O_3$ requires 419.2329.

9.8.1.3. *N*-Cyclohexyl-2-(2-(furan-2-yl)-2-oxoacetyl)-1,2-dihydroisoquinoline-1-carbox amide 335



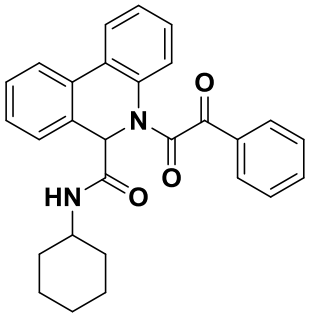
Isolated as a red solid, following method above using isoquinoline **331** (0.18 g, 1.40 mmol), 2-(furan-2-yl)-2-oxoacetic acid **201** (0.26 g, 1.86 mmol) and cyclohexylisocynaide **113** (0.20 g, 1.86 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.3) to yield isoquinoline derivative **335** (0.09 g, 50% yield); m.p = 118-119 ºC; υ_{max} (ATR/cm^{-1})= 3304, 3072, 2935, 2856, 1657, 1530; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.82 (1H, t, *J* = 4.1 Hz, C_4OH_3), 7.63 (1H, t, *J* = 2.0 Hz, C_4OH_3), 7.49 (1H, d, *J* = 3.6 Hz, C_4OH_3), 7.34-7.28 (3H, m, ArC*H*), 7.15 (1H, d, *J* = 3.1 Hz, ArC*H*), 6.64 (1H, dd, *J* = 3.7 Hz, 1.8 Hz, ArC*H*), 6.54 (1H, d, *J* = 1.9 Hz, ArC*H*), 5.86 (1H, s, C*H*), 3.82-3.74 (1H, m, C*H*), 1.91-1.21 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 190.1 (*C*=O), 167.6 (*C*=O), 148.9 (*C*=O), 142.9 (Ar*C*), 138.1 (Ar*C*), 130.6 (C_3OCH_3), 127.9 (C_3OCH_3), 127.8 (Ar*C*H), 127.4 (Ar*C*H), 121.1 (Ar*C*H), 121.1 (Ar*C*H), 112.4 (C_3OCH_3), 110.6 (Ar*C*H), 108.4 (C_3OCH_3), 70.1 (*C*H), 48.7 (*C*H), 32.7 (*C*H_2), 25.4 (*C*H_2), 24.6 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 379.1664 $C_{22}H_{22}N_2O_4$ requires 379.1660.

9.8.1.4. 2-(2-(Furan-2-yl)-2-oxoacetyl)-*N*-(2,4,4-trimethylpentan-2-yl)-1,2-dihydro isoquinoline-1-carboxamide 336



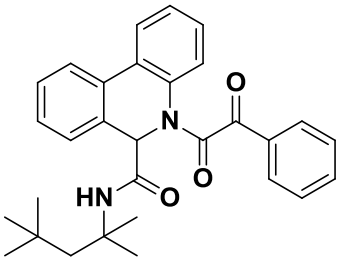
Isolated as a yellow solid, following method above using isoquinoline **331** (0.18 g, 1.40 mmol), 2-(furan-2-yl)-2-oxoacetic acid **201** (0.26 g, 1.86 mmol) and 2-isocyano-2,4,4-trimethylpentane **223** (0.25 g, 1.86 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3:7, R_f = 0.4) to yield isoquinoline derivative **336** (0.06 g, 36% yield); m.p = 129-130 ºC; υ_{max} (ATR/cm^{-1})= 3137, 2955, 2924, 2863, 1671, 1517; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.92 (1H, d, *J* = 4.9 Hz, C_4OH_3), 7.60 (1H, t, *J* = 2.0 Hz, C_4OH_3), 7.50-7.32 (4H, m, ArC*H*), 7.44 (1H, d, *J* = 3.6 Hz, ArC*H*), 6.54 (1H, dd, *J* = 3.8 Hz, 2.0 Hz, C_4OH_3), 6.63 (1H, d, *J* = 3.7 Hz, ArC*H*), 6.10 (1H, s, C*H*), 1.73 (2H, s, CH_2), 1.47 (6H, s, 2CH_3), 1.06 (9H, s, 3CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 189.1 (*C*=O), 164.8 (*C*=O), 149.9 (*C*=O), 143.9 (Ar*C*), 137.1 (Ar*C*), 132.1 (C_3OCH_3), 126.9 (C_3OCH_3), 126.8 (Ar*C*H), 122.4 (Ar*C*H), 121.1 (Ar*C*H), 117.5 (Ar*C*H), 116.4 (C_3OCH_3), 112.6 (Ar*C*H), 111.2 (C_3OCH_3), 65.4 (*C*H), 54.4 (*C*H_2), 52.6 (*C*), 32.2 (*C*), 31.5 (3*C*H_3), 28.2 (2*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 409.2012 $C_{24}H_{28}N_2O_4$ requires 409.2009.

9.8.1.5. *N*-Cyclohexyl-5-(2-oxo-2-phenylacetyl)-5,6-dihydrophenanthridine-6-carbox amide 337



Isolated as a yellow solid, following method above using phenanthridine **332** (0.25 g, 1.40 mmol), 2-oxo-2-phenylacetic acid **169** (0.27g, 1.86 mmol) and cyclohexylisocynaide **113** (0.20 g, 1.86 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 2: 8, R_f = 0.3) to yield phenanthridine derivative **337** (0.15 g, 60% yield); m.p = 201-202 ºC; υ_{max} (ATR/cm^{-1})= 3234, 2932, 2851, 1721, 1679; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.83-7.69 (5H, m, ArC*H*), 7.54-7.37 (4H, m, ArC*H*), 7.33-7.26 (2H, m, ArC*H*), 6.93-6.87 (2H, m, ArC*H*), 5.74 (1H, s, C*H*), 4.03-3.95 (1H, m, C*H*), 2.08-1.29 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 188.1 (*C*=O), 160.8 (*C*=O), 138.9 (*C*=O), 135.1 (Ar*C*), 134.3 (Ar*C*), 133.5 (Ar*C*H), 133.1 (Ar*C*), 131.2 (Ar*C*H), 130.4 (Ar*C*H), 129.6 (Ar*C*H), 129.2 (Ar*C*), 128.9 (Ar*C*H), 128.4 (Ar*C*), 127.7 (Ar*C*H), 127.3 (Ar*C*H), 127.2 (Ar*C*H), 126.9 (Ar*C*H), 123.2 (Ar*C*H), 122.1 (Ar*C*H), 52.8 (*C*H), 48.5 (*C*H), 32.7 (*C*H_2), 29.7 (*C*H_2), 25.4 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 439.2020 $C_{28}H_{26}N_2O_3$ requires 439.2016.

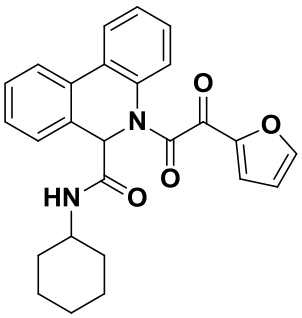
9.8.1.6. 5-(2-Oxo-2-phenylacetyl)-*N*-(2,4,4-trimethylpentan-2-yl)-5,6-dihydro phenan thridine-6-carboxamide 238



Isolated as a pale solid, following method above using phenanthridine **332** (0.25 g, 1.40 mmol), 2-oxo-2-phenylacetic acid **169** (0.27 g, 1.86 mmol) and 2-isocyano-2,4,4-trimethylpentane **223** (0.25 g, 1.86 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 2: 8, R_f = 0.2) to yield phenanthridine

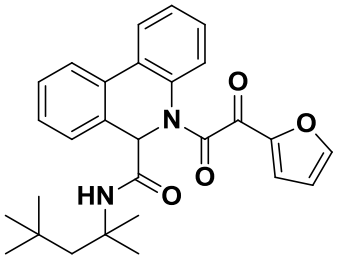
derivative **338** (0.11 g, 44% yield); m.p = 196-198 ºC; υ_{max}(ATR/cm^{-1})= 3056, 2980, 2856, 1763, 1622; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.50 (2H, d, *J* = 7.9 Hz, ArC*H*), 8.34 (2H, t, *J* = 6.8 Hz, ArC*H*), 7.86-7.43 (4H, m, ArC*H*), 7.40-7.32 (5H, m, ArC*H*), 6.28 (1H, s, C*H*), 1.66 (2H, s, CH_2), 1.36 (6H, s, 2CH_3), 1.01 (9H, s, 3CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 188.2 (*C*=O), 161.8 (*C*=O), 160.9 (*C*=O), 133.9 (Ar*C*), 133.4 (Ar*C*), 133.5 (Ar*C*H), 129.9 (Ar*C*), 129.1 (Ar*C*H), 128.8 (Ar*C*H), 128.4 (Ar*C*H), 128.2 (Ar*C*H), 128.1 (Ar*C*H), 124.3 (Ar*C*), 123.6 (Ar*C*H), 123.4 (Ar*C*H), 122.1 (Ar*C*), 121.9 (Ar*C*H), 120.2 (Ar*C*H), 116.1 (Ar*C*H), 63.6 (*C*H), 55.1 (*C*H_2), 52.1 (*C*), 31.5 (*C*), 31.6 (3*C*H_3), 29.7 (2*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 469.2489 $C_{30}H_{32}N_2O_3$ requires 469.2486.

9.8.1.7. *N*-Cyclohexyl-5-(2-(furan-2-yl)-2-oxoacetyl)-5,6-dihydrophenanthridine-6-carboxamide 339



Isolated as a red solid, following method above using phenanthridine **332** (0.25 g, 1.40 mmol), 2-(furan-2-yl)-2-oxoacetic acid **201** (0.26 g, 1.86 mmol) and cyclohexylisocynaide **113** (0.20 g, 1.86 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.3) to yield phenanthridine derivative **339** (0.11 g, 45% yield); m.p = 192-194 ºC; υ_{max} (ATR/cm^{-1})= 3234, 3062, 2915, 2852, 1622, 1527; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.62 (1H, t, *J* = 7.2 Hz, C_4OH_3), 8.22 (1H, t, *J* = 5.2 Hz, C_4OH_3), 7.77-7.63 (4H, m, ArC*H*), 7.55 (1H, d, *J* = 7.1 Hz, C_4OH_3), 7.51-7.45 (4H, m, ArC*H*), 6.41 (1H, s, C*H*), 3.83-3.77 (1H, m, C*H*), 1.93-1.23 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 192.1 (*C*=O), 166.2 (*C*=O), 153.4 (*C*=O), 144.1 (Ar*C*), 136.2 (Ar*C*), 132.7 (C_3OCH_3), 131.2 (Ar*C*), 129.9 (Ar*C*H), 128.8 (Ar*C*H), 128.0 (Ar*C*H), 127.6 (Ar*C*), 127.2 (Ar*C*H), 126.3 (C_3OCH_3), 125.6 (Ar*C*H), 124.2 (Ar*C*H), 122.8 (C_3OCH_3), 121.1 (C_3OCH_3), 120.9 (Ar*C*H), 69.3 (*C*H), 48.2 (*C*H), 32.3 (*C*H_2), 31.6 (*C*H_2), 25.4 (*C*H_2), 24.5 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 429.2644 $C_{26}H_{24}N_2O_4$ requires 429.2640.

9.8.1.8. 5-(2-(Furan-2-yl)-2-oxoacetyl)-*N*-(2,4,4-trimethylpentan-2-yl)-5,6-dihydro phenanthridine-6-carboxamide 340



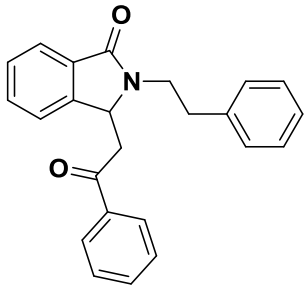
Isolated as a yellow solid, following method above using phenanthridine **332** (0.25 g, 1.40 mmol), 2-(furan-2-yl)-2-oxoacetic acid **201** (0.26 g, 1.86 mmol) and 2-isocyano-2, 4, 4-trimethylpentane **223** (0.25 g, 1.86 mmol).The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.4) to yield phenanthridine derivative **340** (0.10 g, 40% yield); m.p = 184-186 ºC; υ_{max} (ATR/cm^{-1})= 3304, 3042, 2927, 2833, 1712, 1628; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.79 (2H, t, *J* = 2.7 Hz, ArC*H*), 7.60 (2H, d, *J* = 1.9 Hz, ArC*H*), 7.49 (1H, d, *J* = 3.7 Hz, C_4OH_3), 7.43 (1H, d, *J* = 3.6 Hz, C_4OH_3), 7.41-7.12 (4H, m, ArC*H*), 6.6 (1H, dd, *J* = 3.2 Hz, 1.9 Hz, C_4OH_3), 6.0 (1H, s, C*H*), 1.74 (2H, s, CH_2), 1.47 (6H, s, $2CH_3$), 1.05 (9H, s, $3CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 177.8 (*C*=O), 168.9 (*C*=O), 159.7 (*C*=O), 157.3 (C_3OCH_3), 150.1 (C_3OCH_3), 149.0 (Ar*C*), 148.7 (Ar*C*), 147.7 (Ar*C*H), 135.8 (Ar*C*), 126.8 (Ar*C*H), 125.7 (Ar*C*), 124.2 (Ar*C*H), 122.9 (Ar*C*H), 120.6 (C_3OCH_3), 121.1 (Ar*C*H), 113.4 (Ar*C*H), 112.6 (C_3OCH_3), 112.2 (Ar*C*H), 86.3 (*C*H), 56.4 (CH_2), 52.8 (*C*), 31.6 (*C*), 31.4 ($3CH_3$), 28.5 ($2CH_2$); HRMS (ESI$^+$) ($M+H^+$) found 459.3320 $C_{28}H_{30}N_2O_4$ requires 459.3316.

9.9. Synthesis of isoindoline derivatives from *β*-ketoacids

9.9.1. General procedure

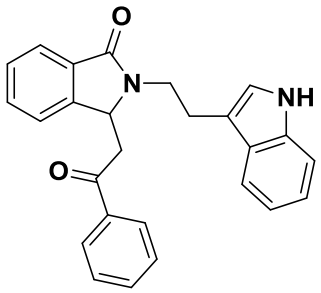
A conical flask (25 mL) was fitted with a reflux condenser and charged with tetrabutylammonium bromide salt (TBAB) (0.2 mmol, 20 mol %), 2-carboxybenzaldehyde (1.0 mmol), *β*-ketoacids (3.0 mmol), and amine (6.0 mmol) in water (3.0 mL). The reaction mixture was heated at 95 ºC for 18 h, cooled to r.t, extracted with ethyl acetate (3 × 20 mL). The organic combined layers were dried with magnesium sulphate, filtered and concentrated under reduced pressure. Purification was achieved by column chromatography to afford the corresponding isoindoline derivatives.

9.9.1.1. 3-(2-Oxo-2-phenylethyl)-2-phenethylisoindolin-1-one 358



Isolated as a yellow solid, following method above using tetrabutylammonium bromide salt (TBAB) (0.07 g, 0.2 mmol), 2-carboxybenzaldehyde **351** (0.15 g, 1.0 mmol), 3-oxo-3-phenylpropanoic acid **3** (0.49 g, 3.0 mmol) and 2-phenylethanamine **225** (0.73 g, 6.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 4: 6, R_f = 0.3) to yield isoindoline derivative **358** (0.12 g, 80% yield); m.p = 145-146 ºC; υ_{max} (ATR/cm^{-1})= 3057, 2933, 2861, 1681, 1596; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.93-7.87 (4H, m, ArC*H*), 7.64-7.40 (5H, m, ArC*H*), 7.30-7.21 (5H, m, ArC*H*), 4.14 (1H, dtd, *J* = 21.3 Hz, 4.4 Hz, C*H*), 3.45-3.36 (2H, m, CH_2), 3.29 (1H, dd, *J* = 17.6 Hz, 6.8 Hz, C*H*), 3.16 (1H, dd, *J* = 17.8 Hz, 6.9 Hz, C*H*), 3.09-2.98 (2H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 197.3 (*C*=O), 168.5 (*C*=O), 145.8 (Ar*C*), 138.9 (Ar*C*), 136.3 (Ar*C*), 133.9 (Ar*C*H), 132.1 (Ar*C*), 131.7 (Ar*C*H), 128.9 (Ar*C*H), 128.8 (Ar*C*H), 128.6 (Ar*C*H), 128.4 (Ar*C*H), 128.2 (Ar*C*H), 126.5 (Ar*C*H), 123.6 (Ar*C*H), 122.7 (Ar*C*H), 55.9 (*C*H_2), 42.9 (*C*H), 42.2 (*C*H_2), 34.5 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 356.1651 $C_{24}H_{21}NO_2$ requires 356.1645.

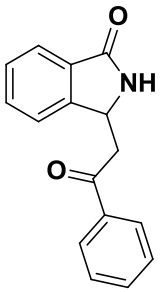
9.9.1.2. 2-(2-(1H-Indol-3-yl) ethyl)-3-(2-oxo-2-phenylethyl) isoindolin-1-one 359



Isolated as a red solid, following method above using tetrabutylammoniumbromide salt (TBAB) (0.07 g, 0.2 mmol), 2-carboxybenzaldehyde **351** (0.15 g, 1.0 mmol), 3-oxo-3-phenylpropanoic acid **3** (0.49 g, 3.0 mmol) and 3-(2-aminoethyl) indole **285** (0.96 g, 6.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 6 : 4, R_f = 0.4) to yield isoindoline derivative **359** (0.10 g, 68% yield); m.p = 108-109 ºC; υ_{max} (ATR/cm^{-1})= 3050, 2915, 2848, 1663, 1616; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.10 (1H, s, ArC*H*), 7.90-7.78 (4H, m, ArC*H*), 7.69 (1H, d, *J* = 7.8 Hz, ArC*H*), 7.60

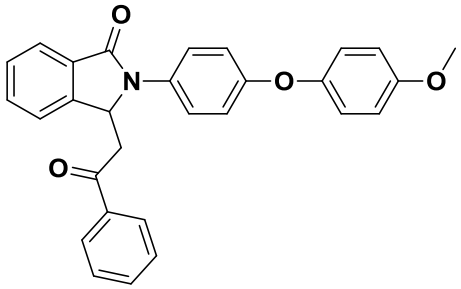
(1H, t, *J* = 7.4 Hz, ArC*H*), 7.51-7.4 (7H, m, ArC*H*), 5.31 (1H, dd, *J* = 7.4 Hz, 4.9 Hz, C*H*), 4.22 (1H, dd, *J* = 16.3 Hz, 7.0 Hz, C*H*), 3.57 (1H, dd, *J* = 16.1 Hz, 4.5 Hz, C*H*), 3.36 (1H, dd, *J* = 17.8 Hz, 5.0 Hz, C*H*), 3.21(1H, dd, *J* = 12.1 Hz, 3.6 Hz, C*H*), 3.05-2.99 (2H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 197.4 (*C*=O), 168.5 (*C*=O), 145.9 (Ar*C*), 136.2 (Ar*C*), 133.7 (Ar*C*), 132.1 (Ar*C*H), 131.6 (Ar*C*H), 128.7 (Ar*C*), 128.4 (Ar*C*H), 128.1 (Ar*C*), 127.4 (Ar*C*H), 123.5 (Ar*C*H), 122.8 (Ar*C*H), 122.3 (Ar*C*), 122.1 (Ar*C*H), 121.2 (Ar*C*H), 119.5 (Ar*C*H), 119.4 (Ar*C*H), 118.6 (Ar*C*H), 56.1 (*C*H_2), 41.9 (*C*H), 29.7 (*C*H_2), 24.2 (*C*H_2); HRMS (ESI^+) ($M+H^+$) found 395.1767 $C_{26}H_{22}N_2O_2$ requires 395.1754.

9.9.1.3. 3-(2-Oxo-2-phenylethyl) isoindolin-1-one 360



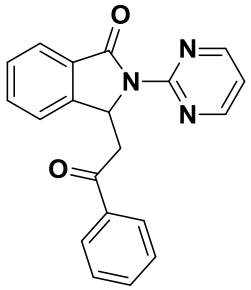
Isolated as a pale solid, following method above using tetrabutylammoniumbromide salt (TBAB) (0.07 g, 0.2 mmol), 2-carboxybenzaldehyde **351** (0.15 g, 1.0 mmol), 3-oxo-3-phenylpropanoic acid **3** (0.49 g, 3.0 mmol) and ammonium hydroxide **352** (32%, 2mL, 6.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 6 : 4, R_f = 0.4) to yield isoindoline derivative **360** (0.11 g, 75% yield); m.p = 160-161 ºC; υ_{max} (ATR/cm^{-1})= 3444, 3053, 2986, 1707, 1684; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.98 (2H, d, *J* = 7.1 Hz, ArC*H*), 7.91 (1H, d, *J* = 7.5 Hz, ArC*H*), 7.50-7.49 (6H, m, ArC*H*), 6.92 (1H, s, N*H*), 5.17 (1H, dd, *J* = 10.2 Hz, 2.9 Hz, C*H*), 3.75 (1H, dd, *J* = 18.1 Hz, 3.2 Hz, C*H*), 3.13 (1H, dd, *J* = 18.1 Hz, 10.2 Hz, C*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 197.9 (*C*=O), 169.9 (*C*=O), 146.5 (Ar*C*), 136.0 (Ar*C*), 133.9 (Ar*C*H), 132.0 (Ar*C*H), 128.8 (Ar*C*), 128.6 (Ar*C*H), 128.1 (Ar*C*H), 124.2 (Ar*C*H), 123.7 (Ar*C*H), 122.4 (Ar*C*H), 52.4 (*C*H_2), 44.1 (*C*H); HRMS (ESI^+) ($M+H^+$) found 252.1023 $C_{16}H_{13}NO_2$ requires 252.1019.

9.9.1.4. 2-(4-(4-Methoxyphenoxy) phenyl)-3-(2-oxo-2-phenylethyl) isoindolin-1-one 361



Isolated as a yellow solid, following method above using tetrabutylammoniumbromide salt (TBAB) (0.07 g, 0.2 mmol), 2-carboxybenzaldehyde **351** (0.15 g, 1.0 mmol), 3-oxo-3-phenylpropanoic acid **3** (0.49 g, 3.0 mmol) and 4-(4-methoxyphenoxy) aniline **353** (1.20 g, 6.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 4 : 6, R_f = 0.2) to yield isoindoline derivative **361** (0.11 g, 72% yield); m.p = 240-241 ºC; υ_{max} (ATR/cm^{-1})= 3053, 2986, 2836, 1766, 1684; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.24 (1H, d, *J* = 9.3 Hz, ArC*H*), 7.99-7.93 (4H, m, ArC*H*), 7.70-7.7.49 (6H, m, ArC*H*), 7.06-6.84 (6H, m, ArC*H*), 6.20 (1H, dd, *J* = 6.8 Hz, 3.1 Hz, C*H*), 3.85 (3H, s, OCH_3), 3.80 (1H, dd, *J* = 18.7 Hz, 8.9 Hz, C*H*), 3.42 (1H, dd, *J* = 17.9 Hz, 7.3 Hz, C*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 196.1 (*C*=O), 170.2 (*C*=O), 161.3 (Ar*C*), 149.8 (Ar*C*), 136.2 (Ar*C*), 134.3 (Ar*C*), 133.9 (Ar*C*H), 133.8 (Ar*C*H), 132.4 (Ar*C*), 129.5 (Ar*C*H), 128.9 (Ar*C*H), 128.8 (Ar*C*H), 127.7 (Ar*C*H), 125.9 (Ar*C*H), 125.3 (Ar*C*H), 123.9 (Ar*C*H), 122.8 (Ar*C*H), 121.4 (Ar*C*), 120.9 (Ar*C*H), 115.1 (Ar*C*), 55.7 (*C*H_2), 43.7 (*C*H), 41.2 (O*C*H_3); HRMS (ESI$^+$) (M+H$^+$) found 450.1698 $C_{29}H_{23}NO_4$ requires 450.1700.

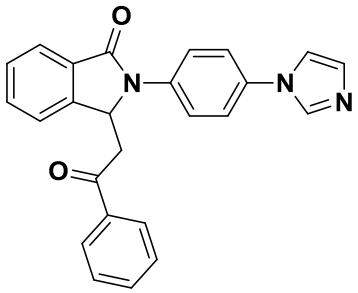
9.9.1.5. 3-(2-Oxo-2-phenylethyl)-2-(pyrimidin-2-yl) isoindolin-1-one 362



Isolated as a pale solid, following method above using tetrabutylammoniumbromide salt (TBAB) (0.07 g, 0.2 mmol), 2-carboxybenzaldehyde **351** (0.15 g, 1.0 mmol), 3-oxo-3-phenylpropanoic acid **3** (0.49 g, 3.0 mmol) and 2-aminopyrimidine **354** (0.59 g, 6.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 4 : 6, R_f = 0.4) to yield isoindoline derivative **362** (0.09 g, 65% yield), m.p = 190-191 ºC; υ_{max} (ATR/cm^{-1})= 3059, 2987, 2905, 1766, 1681; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.00-7.94 (3H, m, ArC*H*), 7.70-7.49 (9H, m, ArC*H*), 6.21 (1H, dd, *J* = 6.8 Hz, 2.9 Hz, C*H*), 3.81 (1H, dd, *J*

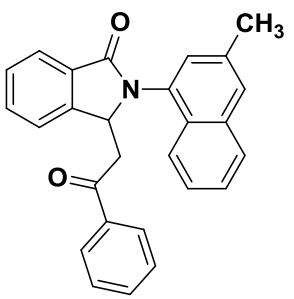
= 17.6 Hz, 5.7 Hz, C*H*), 3.42 (1H, dd, *J* = 17.6 Hz, 7.4 Hz, C*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 196.6 (*C*=O), 170.2 (*C*=O), 149.8 (Ar*C*), 136.9 (Ar*C*), 134.3 (Ar*C*H), 133.9 (Ar*C*H), 129.5 (Ar*C*), 129.2 (Ar*C*H), 128.8 (Ar*C*H), 128.2 (Ar*C*H), 126.3 (Ar*C*H), 125.9 (Ar*C*H), 125.8 (Ar*C*), 123.4 (Ar*C*H), 122.8 (Ar*C*H), 55.9 (CH_2), 43.7 (*C*H); HRMS (ESI^+) ($M+H^+$) found 330.1252 $C_{20}H_{15}N_3O_2$ requires 330.1249.

9.9.1.6. 2-(4-(1H-Imidazol-1-yl) phenyl)-3-(2-oxo-2-phenylethyl)isoindolin-1-one 363



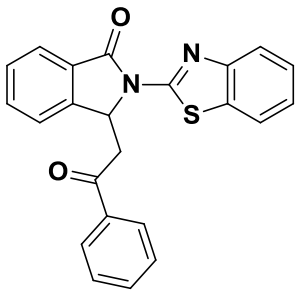
Isolated as a colourless solid, following method above using tetrabutylammoniumbromide salt (TBAB) (0.07 g, 0.2 mmol), 2-carboxybenzaldehyde **351** (0.15 g, 1.0 mmol), 3-oxo-3-phenylpropanoic acid **3** (0.49 g, 3.0 mmol) and 4-(1H-imidazol-1-yl) aniline **355** (0.96 g, 6.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 4: 6, R_f = 0.3) to yield isoindoline derivative **363** (0.11 g, 77% yield); m.p = 150-151 ºC; υ_{max} (ATR/cm^{-1})= 3062, 2983, 2912, 1766, 1684; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.99-7.94 (4H, m, ArC*H*), 7.83 (1H, d, *J* = 2.4 Hz, $C_3N_2H_3$), 7.70-7.60 (5H, m, ArC*H*), 7.67 (1H, d, *J* = 2.2 Hz, $C_3N_2H_3$), 7.53-7.49 (4H, m, ArC*H*), 7.50 (1H, s, $C_3N_2H_3$), 6.20 (1H, dd, *J* = 6.6 Hz, 2.5 Hz, C*H*), 3.81 (1H, dd, *J* = 17.6 Hz, 5.7 Hz, C*H*), 3.42 (1H, dd, *J* = 17.6 Hz, 7.4 Hz, C*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 196.1 (*C*=O), 170.2 (*C*=O), 149.8 (Ar*C*), 137.1 (Ar*C*), 136.8 ($C_2N_2CH_3$), 136.1 (Ar*C*), 134.3 (Ar*C*H), 133.9 (Ar*C*), 133.1 (Ar*C*H), 130.7 ($C_2N_2CH_3$), 129.5 (Ar*C*H), 128.8 (Ar*C*H), 128.6 (Ar*C*H), 128.3 (Ar*C*H), 128.2 (Ar*C*H), 126.2 (Ar*C*H), 126.0 (Ar*C*H), 125.6 (Ar*C*), 124.8 (Ar*C*H), 122.9 ($C_2N_2CH_3$), 43.7 (CH_2), 26.8 (*C*H); HRMS (ESI^+) ($M+H^+$) found 394.1556 $C_{25}H_{19}N_3O_2$ requires 394.1552.

9.9.1.7. 2-(3-Methylnaphthalen-1-yl)-3-(2-oxo-2-phenylethyl) isoindolin-1-one 364



Isolated as a yellow solid, following method above using tetrabutylammoniumbromide salt (TBAB) (0.07 g, 0.2 mmol), 2-carboxybenzaldehyde **351** (0.15 g, 1.0 mmol), 3-oxo-3-phenylpropanoic acid **3** (0.49 g, 3.0 mmol) and 3-methylnaphthalen-1-amine **357** (0.95 g, 6.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 5: 5, R_f = 0.5) to yield isoindoline derivative **364** (0.10 g, 67% yield); m.p = 110-112 ºC; υ_{max} (ATR/cm^{-1})= 3059, 2987, 2925, 1766, 1666; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.09 (1H, d, *J* = 8.3 Hz, ArC*H*), 7.99-7.93 (3H, m, ArC*H*), 7.68-7.46 (8H, m, ArC*H*), 7.40 (1H, s, ArC*H*), 7.38 (1H, t, *J* = 4.2 Hz, ArC*H*), 7.37 (1H, s, ArC*H*), 6.21 (1H, dd, *J* = 6.6 Hz, 3.1 Hz, C*H*), 3.81 (1H, dd, *J* = 17.6 Hz, 5.8 Hz,C*H*), 3.41 (1H, dd, *J* = 17.3 Hz, 4.9 Hz, C*H*), 2.58 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 196.0 (*C*=O), 169.2 (*C*=O), 149.7 (Ar*C*), 134.3 (Ar*C*), 133.9 (Ar*C*), 130.6 (Ar*C*H), 130.1 (Ar*C*), 129.8 (Ar*C*H), 129.4 (Ar*C*H), 128.9 (Ar*C*H), 128.2 (Ar*C*H), 128.1 (Ar*C*H), 125.8 (Ar*C*H), 125.2 (Ar*C*H), 125.1 (Ar*C*), 124.8 (Ar*C*H), 123.3 (Ar*C*H), 122.9 (Ar*C*), 121.3 (Ar*C*H), 120.6 (Ar*C*H), 101.7 (Ar*C*), 43.7 (*C*H_2), 29.8 (*C*H), 20.8 (*C*H_3); HRMS (ESI$^+$) (M+H$^+$) found 393.1560 $C_{26}H_{20}N_2O_2$ requires 393.1554.

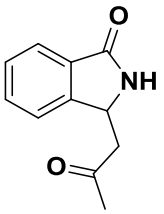
9.9.1.8. 2-(Benzo[d]thiazol-2-yl)-3-(2-oxo-2-phenylethyl) isoindolin-1-one 365



Isolated as a yellow solid, following method above using tetrabutylammoniumbromide salt (TBAB) (0.07 g, 0.2 mmol), 2-carboxybenzaldehyde **351** (0.15 g, 1.0 mmol), 3-oxo-3-phenylpropanoic acid **3** (0.49 g, 3.0 mmol) and 2-aminobenzothiazole **357** (0.91 g, 6.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 6 : 4, R_f = 0.5) to yield isoindoline derivative **365** (0.06 g, 45% yield), m.p = 142-143 ºC; υ_{max} (ATR/cm^{-1})= 3057, 2987, 2926, 1766, 1722, 1686; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.05-7.87 (5H, m, ArC*H*), 7.83-7.31 (8H, m, ArC*H*), 6.22 (1H, dd, *J* = 14.4 Hz, 4.5 Hz, C*H*), 4.70 (1H, dd, *J* = 17.3 Hz, 2.9 Hz, C*H*), 3.81 (1H, dd, *J* = 17.6 Hz, 5.7 Hz, C*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 196.1 (*C*=O), 168.9 (*C*=O), 149.8 (Ar*C*), 136.2 (Ar*C*), 133.9 (Ar*C*), 131.2 (Ar*C*H), 130.6 (Ar*C*), 129.5 (Ar*C*H), 128.9 (Ar*C*), 128.2 (Ar*C*H), 126.5 (Ar*C*H), 125.9 (Ar*C*H), 125.7 (Ar*C*H), 124.6 (Ar*C*H), 124.5 (Ar*C*), 124.2 (Ar*C*H), 122.8

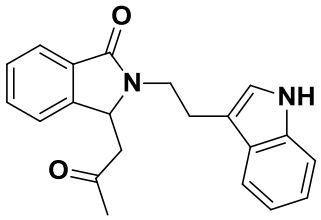
(Ar*C*H), 121.6 (Ar*C*H), 121.3 (Ar*C*H), 43.7 (*C*H_2), 29.7 (*C*H); HRMS (ESI^+) ($M+H^+$) found 385.0960 $C_{23}H_{16}N_2O_2S$ requires 385.0957.

9.9.1.9. 3-(2-Oxopropyl) isoindolin-1-one 366



Isolated as a pale solid, following method above using tetrabutylammoniumbromide salt (TBAB) (0.07 g, 0.2 mmol), 2-carboxybenzaldehyde **351** (0.15 g, 1.0 mmol), 3-oxobutanoicacid **2** (0.30 g, 3.0 mmol) and ammonium hydroxide **352** (32%, 2 mL, 6.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 6: 4, R_f = 0.3) to yield isoindoline derivative **366** (0.09 g, 60% yield); m.p = 130-132 ºC; υ_{max} (ATR/cm^{-1})= 3441, 3053, 2986, 1702, 1619; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.88 (1H, d, *J* = 7.5 Hz, ArC*H*), 7.59 (1H, dd, *J* = 7.4 Hz, 1.9 Hz, ArC*H*), 7.50 (1H, t, *J* = 7.4 Hz, ArC*H*), 7.40 (1H, dd, *J* = 7.5 Hz, 1.8 Hz, ArC*H*), 6.70 (1H, s, N*H*), 4.90 (1H, dd, *J* = 4.5 Hz, 2.2 Hz, C*H*), 3.22 (1H, dd, *J* = 7.3 Hz, 3.4 Hz, C*H*), 2.60 (1H, dd, *J* = 8.4 Hz, 4.3 Hz, C*H*), 2.26 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 206.6 (*C*=O), 169.9 (*C*=O), 145.2 (Ar*C*), 132.0 (Ar*C*H), 131.8 (Ar*C*), 128.5 (Ar*C*H), 124.2 (Ar*C*H), 122.2 (Ar*C*H), 52.9 (*C*H), 48.46 (*C*H_2), 29.7 (*C*H_3); HRMS (ESI^+) ($M+H^+$) found 190.0863 $C_{11}H_{11}NO_2$ requires 190.0863.

9.9.1.10. 2-(2-(1H-Indol-3-yl) ethyl)-3-(2-oxopropyl) isoindolin-1-one 367



Isolated as an orange solid, following method above using tetrabutylammoniumbromide salt (TBAB) (0.07 g, 0.2 mmol), 2-carboxybenzaldehyde **351** (0.15 g, 1.0 mmol), 3-oxobutanoicacid **2** (0.30 g, 3.0 mmol) and 3-(2-aminoethyl) indole **285** (0.96 g, 6.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 7: 3, R_f = 0.5) to yield isoindoline derivative **367** (0.08 g, 55% yield); m.p = 101-102 ºC; υ_{max}(ATR/cm^{-1})= 3468, 3056, 2986, 2927, 1713, 1681; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.06 (1H, s, ArC*H*), 7.86 (1H, d, *J* = 6.6 Hz, ArC*H*), 7.71 (1H, d, *J* = 7.8 Hz, ArC*H*), 7.67-7.07

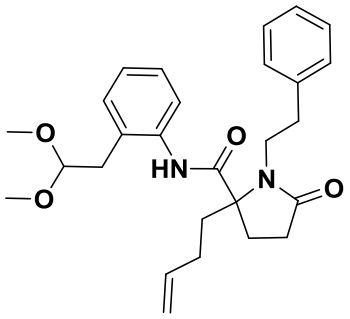
(6H, m, ArC*H*), 5.0 (1H, dd, *J* = 4.1 Hz, 2.1 Hz, C*H*), 4.27 (1H, dd, *J* = 7.3 Hz, 2.8 Hz, C*H*), 3.48 (1H, dd, *J* = 13.9 Hz, 6.8 Hz, C*H*), 3.17 (2H, t, *J* = 7.3 Hz, CH_2), 2.84 (1H, dd, *J* = 7.6 Hz, 3.1 Hz, C*H*), 2.55 (1H, dd, *J* = 8.5 Hz, 2.1 Hz, C*H*), 2.04 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 205.8 (*C*=O), 168.3 (*C*=O), 145.6 (Ar*C*), 136.3 (Ar*C*), 132.0 (Ar*C*), 131.6 (Ar*C*H), 128.4 (Ar*C*H), 127.3 (Ar*C*), 123.5 (Ar*C*H), 122.5 (Ar*C*H), 122.2 (Ar*C*H), 119.6 (Ar*C*H), 118.8 (Ar*C*H), 112.9 (Ar*C*H), 111.2 (Ar*C*H), 55.5 (*C*H_2), 46.3 (*C*H_2), 41.1 (*C*H), 30.4 (*C*H_2), 29.7 (*C*H_3); HRMS (ESI$^+$) (M+H$^+$) found 333.1604 $C_{21}H_{20}N_2O_2$ requires 333.1598.

9.10. Synthesis of pyroglutamic acid analogues from convertible isocyanide 149[354]

9.10.1. General synthesis of Ugi products

A solution of keto acid (1 mmol) in TFE (5 mL) was added to a solution of 2-phenylethanamine **225** (1.25 mmol) in TFE (5 mL) and stirred for 45 minutes. 1-(2,2-Dimethoxyethyl)-2-isocyanobenzene **149** (1.0 mmol) was then added and the reaction mixture was stirred at room temperature for 48 h. The excess solvent was removed under reduced pressure and the residue re-dissolved in ethyl acetate (20 mL). The organic phase was successively washed with hydrochloric acid (2 M, 10 mL), saturated sodium hydrogen carbonate (10 mL), brine (10 mL), dried over magnesium sulphate and concentrated under reduced pressure. The crude cyclic amides were purified by column chromatography to obtain the titled cyclic amide (60-75%).

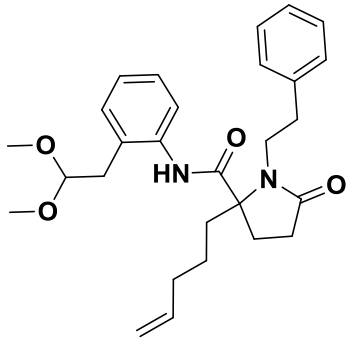
9.10.1.1. 2-(But-3-en-1-yl)-*N*-(2-(2,2-dimethoxyethyl) phenyl)-5-oxo-1-phenethyl pyrrolidine-2-carboxamide 381



Isolated as a brown oil, following method above using 4-oxooct-7-enoic acid **178** (0.15 g, 1 mmol), 2-phenylethanamine **225** (0.12 g, 1.25 mmol) and 1-(2,2-dimethoxyethyl)-2-isocyanobenzene **149** (0.19 g, 1.0 mmol) for 48 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 6: 4, R_f = 0.3) to yield Ugi product **381** (0.11 g, 75% yield); υ_{max} (ATR/cm^{-1})= 3331, 3065, 2937, 2836, 1686, 1585, 1515, 1452; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 9.04 (1H, s, N*H*), 7.75 (1H, d, *J* = 7.4 Hz, ArC*H*), 7.32-

7.11 (8H, m, ArC*H*), 5.92-5.82 (1H, m, C*H*), 5.15-5.04 (2H, m, CH_2), 4.47 (1H, t, *J* = 5.2 Hz, C*H*), 3.61 (1H, ddd, *J* = 18.1 Hz, 6.7 Hz, C*H*), 3.40 (3H, s, OCH_3), 3.35 (3H, s, OCH_3), 3.08 (1H, ddd, *J* = 16.9 Hz, 6.1 Hz, C*H*), 2.92-2.81 (2H, m, CH_2), 2.71-2.51 (4H, m, 2CH_2), 2.46-2.37 (4H, m, 2CH_2), 2.1-1.99 (2H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 175.6 (*C*=O), 172.1 (*C*=O), 138.8 (Ar*C*), 137.1 (Ar*C*H), 136.1 (Ar*C*), 131.2 (Ar*C*H), 128.6 (Ar*C*H), 128.5 (Ar*C*), 127.7 (Ar*C*H), 126.5 (Ar*C*H), 125.5 (Ar*C*H), 124.4 (Ar*C*H), 124.2 (Ar*C*H), 115.5 (*C*H_2), 106.4 (*C*H), 70.3 (*C*), 60.3 (*C*H), 54.4 (O*C*H_3), 53.9 (O*C*H_3), 44.2 (*C*H_2), 36.8 (*C*H_2), 34.5 (*C*H_2), 33.8 (*C*H_2), 29.8 (*C*H_2), 29.20 (*C*H_2), 27.5 (*C*H_2); HRMS (ESI^+) found 473.2414 $C_{27}H_{34}N_2O_4Na$ $[M+Na]^+$ requires 473.2411.

9.10.1.2. *N*-(2-(2, 2-dimethoxyethyl) phenyl)-5-oxo-2-(pent-4-en-1-yl)-1-phenethyl pyrrolidine-2-carboxamide 382

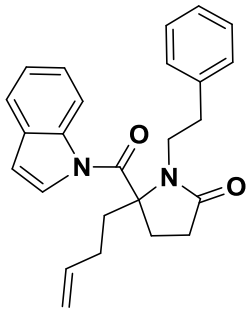


Isolated as a brown dark oil, following method above using 4-oxonon-8-enoic acid **179** (0.17 g, 1 mmol), 2-phenylethanamine **225** (0.12 g, 1.25 mmol) and 1-(2,2-dimethoxyethyl)-2-isocyanobenzene **149** (0.19 g, 1.0 mmol) for 48h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 6: 4, R_f = 0.3) to yield Ugi product **382** (0.1 g, 60% yield); υ_{max} (ATR/cm^{-1})= 3333, 3027, 2937, 2845, 1684, 1587, 1520, 1450; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.99 (1H, m, N*H*), 7.75 (1H, d, *J* = 8 Hz, ArC*H*), 7.33-7.12 (8H, m, ArC*H*), 5.87-5.77 (1H, m, C*H*), 5.1-5.0 (2H, m, CH_2), 4.46 (1H, t, *J* = 5.4 Hz, C*H*), 3.56 (1H, ddd, *J* = 18.9 Hz, 6.8 Hz, C*H*), 3.40 (3H, s, OCH_3), 3.36 (3H, s, OCH_3), 3.06 (1H, ddd, *J* = 17.1 Hz, 6.2 Hz, C*H*), 2.92-2.85 (2H, m, CH_2), 2.65-2.54 (4H, m, 2CH_2), 2.45-2.38 (4H, m, 2CH_2), 2.25-2.12 (2H, m, CH_2), 1.94-1.54 (2H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 175.5 (*C*=O), 172.0 (*C*=O), 138.8 (Ar*C*), 137 (Ar*C*H), 136.2 (Ar*C*), 131.2 (Ar*C*H), 128.7 (Ar*C*H), 128.6 (Ar*C*), 127.6 (Ar*C*H), 126.6 (Ar*C*H), 125.5 (Ar*C*H), 124.5 (Ar*C*H), 115.5 (*C*H_2), 106.5 (*C*H), 70.4 (*C*), 54.5 (O*C*H_3), 53.9 (O*C*H_3), 44.2 (*C*H_2), 36.9 (*C*H_2), 34.5 (*C*H_2), 33.8 (*C*H_2), 29.7 (*C*H_2), 29.2 (*C*H_2), 28.3 (*C*H_2), 27.5 (*C*H_2); HRMS (ESI^+) found 487.2570 $C_{28}H_{36}N_2O_4Na$ $[M+Na]^+$ requires 487.2567.

9.10.2. General synthesis of *N*-acylindole products

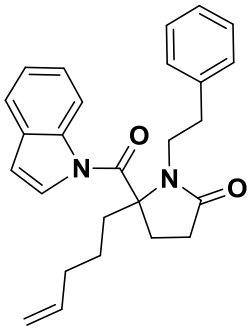
To a solution of Ugi product (**381**, **382**) (1.0 mmol, 1.0 eq) in toluene (5 mL), *dl* camphorsulphonic acid (0.5 mmol, 0.5 eq) was added. The reaction mixture was stirred at 80 °C for 5 h, then allowed to cool, quenched with saturated sodium hydrogen carbonate (10 mL) and extracted with ethyl acetate (3 x 20 mL). The organic combined layers were dried with sodium sulphate, filtered and concentrated under reduced pressure. Purification was achieved by column chromatography to obtain the titled *N*-acylindole products (70-82%).

9.10.2.1. 5-(But-3-en-1-yl)-5-(1H-indole-1-carbonyl)-1-phenethylpyrrolidin-2-one 383



Isolated as a yellow viscous oil, following method above using Ugi product **381** (0.22 g, 0.5 mmol) and *dl* camphorsulphonic acid (0.06 g, 0.25 mmol) at 80 °C for 5 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 5: 5, R_f = 0.4) to yield *N*-acylindole product **383** (0.18 g, 82% yield); υ_{max} (ATR/cm^{-1})= 3065, 3029, 2932, 1691, 1537, 1450, 1397; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.53 (1H, d, *J* = 8.3 Hz, ArC*H*), 7.58 (1H, d, *J* = 7.5 Hz, ArC*H*), 7.41 (1H, t, *J* = 7.4 Hz, ArC*H*), 7.34-7.14 (6H, m, ArC*H*), 6.67 (1H, d, *J* = 3.6 Hz, ArC*H*), 5.92-5.84 (1H, m, C*H*), 3.49-3.30 (2H, m, CH_2), 2.86-2.79 (2H, m, CH_2), 2.73-2.61 (4H, m, 2CH_2), 2.43-2.18 (4H, m, 2CH_2), 2.02-1.99 (2H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 175.1 (*C*=O), 171.6 (*C*=O), 138.5 (Ar*C*), 136.7 (Ar*C*), 136.5 (*C*H), 129.4 (Ar*C*), 128.7 (Ar*C*H), 128.5 (Ar*C*), 126.5 (Ar*C*H), 125.8 (Ar*C*H), 124.4 (Ar*C*H), 123.7 (Ar*C*H), 120.9 (Ar*C*H), 117.1 (Ar*C*H), 115.8 (*C*H_2), 110.5 (Ar*C*H), 71.5 (*C*), 44.5 (*C*H_2), 35.7 (*C*H_2), 33.7 (*C*H_2), 29.6 (*C*H_2), 27.6 (*C*H_2), 27.0 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 387.2067 $C_{25}H_{26}N_2O_2$ requires 387.2067.

9.10.2.2. 5-(1H-Indole-1-carbonyl)-5-(pent-4-en-1-yl)-1-phenethylpyrrolidin-2-one 384

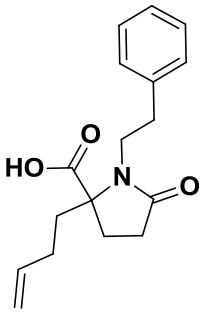


Isolated as a yellow viscous oil, following method above using Ugi product **382** (0.20 g, 0.5 mmol) and *dl* camphorsulphonic acid (0.06 g, 0.25 mmol) at 80 °C for 5 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 5: 5, R_f = 0.4) to yield *N*-acylindole product **384** (0.14 g, 70% yield); υ_{max}(ATR/cm^{-1})= 3060, 3029, 2932, 1696, 1542, 1450, 1387; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.53 (1H, d, *J* = 8.4 Hz, ArC*H*), 7.52 (1H, d, *J* = 7.6 Hz, ArC*H*), 7.4 (1H, t, *J* = 7.2 Hz, ArC*H*), 7.35-7.14 (5H, m, ArC*H*), 6.66 (1H, d, *J* = 3.8 Hz, ArC*H*), 5.88-5.77 (1H, m, C*H*), 5.12-5.04 (2H, m, CH_2), 3.42-3.30 (2H, m, CH_2), 2.86-2.76 (2H, m, CH_2), 2.72-2.60 (2H, m, CH_2), 2.40-2.32 (4H, m, 2CH_2), 1.60-1.27 (4H, m, 2CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 175.2 (*C*=O), 171.8 (*C*=O), 138.6 (Ar*C*), 137.5 (Ar*C*), 136.7 (*C*H), 129.5 (Ar*C*), 128.8 (Ar*C*H), 128.5 (Ar*C*H), 126.5 (Ar*C*H), 125.7 (Ar*C*H), 124.3 (Ar*C*H), 123.8 (Ar*C*H), 120.9 (Ar*C*H), 117.2 (Ar*C*H), 115.9 (*C*H_2), 110.4 (Ar*C*H), 71.7 (*C*), 44.6 (*C*H_2), 36.1 (*C*H_2), 33.7 (*C*H_2), 29.7 (*C*H_2), 27.8 (*C*H_2), 24.3 (*C*H_2), 22.2 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 401.2219 $C_{26}H_{28}N_2O_2$ requires 401.2224.

9.10.3. General synthesis of pyroglutamic acid analogues

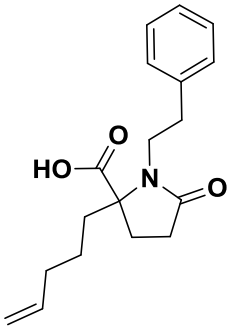
To a solution of *N*-acylindole products (**383**, **384**) (1.0 mmol, 1.0 eq) in DMF (2 mL) and H_2O (2 mL), Cs_2CO_3 (1.0 mmol, 1.0 eq) was added. The reaction mixture was stirred at 22 °C for 6 h, then diluted with H_2O (10 mL), made basic with NaOH (1M) and extracted with ethyl acetate (2 x 20 mL). The aqueous layer was then acidified with HCl (1M) and extracted with ethyl acetate (2 x 20 mL). The organic combined layers were dried with sodium sulphate, filtered and concentrated under reduced pressure. Purification was achieved by column chromatography to obtain the titled pyroglutamic acid derivatives (85-90%).

9.10.3.1. 2-(But-3-en-1-yl)-5-oxo-1-phenethylpyrrolidine-2-carboxylic acid 385



Isolated as a colourless viscous oil, following method above using *N*-acylindole product **383** (0.19 g, 0.5 mmol) and Cs_2CO_3 (0.16 g, 0.5 mmol) in DMF (1 mL) and H_2O (1 mL). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 5: 5, R_f = 0.2) to yield pyroglutamic acid derivative **385** (0.16 g, 85% yield); υ_{max} (ATR/cm^{-1})= 3456, 3024, 2920, 2848, 1691, 1450, 1319; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.60-7.13 (5H, m, ArC*H*), 5.92-5.82 (1H, m, C*H*), 5.16-5.06 (2H, m, CH_2), 3.40-3.28 (2H, m, CH_2), 2.85 (2H, t, *J* = 8.6 Hz, CH_2), 2.69 (2H, t, *J* = 5.8 Hz, CH_2), 2.43-2.19 (4H, m, 2CH_2), 2.07-1.99 (2H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 180.1 (*C*=O), 168.6 (*C*=O), 139.4 (Ar*C*), 139.1 (*C*H), 128.6 (Ar*C*H), 127.5 (Ar*C*H), 125.8 (Ar*C*H), 115.2 (*C*H_2), 70.1 (*C*), 45.3 (*C*H_2), 34.5 (*C*H_2), 32.9 (*C*H_2), 32.6 (*C*H_2), 30.0 (*C*H_2), 26.8 (*C*H_2); HRMS (ESI^+) ($M+H^+$) found 288.1594 $C_{17}H_{21}NO_3$ requires 288.1594.

9.10.3.2. 5-Oxo-2-(pent-4-en-1-yl)-1-phenethylpyrrolidine-2-carboxylic acid 386



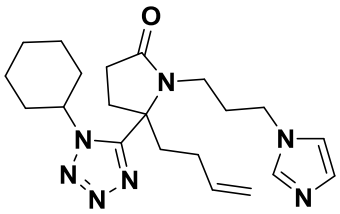
Isolated as a colourless viscous oil, following method above using *N*-acylindole product **384** (0.20 g, 0.5 mmol) and Cs_2CO_3 (0.16 g, 0.5 mmol) in DMF (1 mL) and H_2O (1 mL). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 5: 5, R_f = 0.2) to yield pyroglutamic acid derivative **386** (0.16 g, 80% yield); υ_{max} (ATR/cm^{-1})= 3452, 3026, 2933, 2845, 1689, 1459; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.25-7.16 (5H, m, ArC*H*), 5.90-5.86 (1H, m, C*H*), 5.18-5.09 (2H, m, CH_2), 3.47-3.32 (2H, m, CH_2), 2.88 (2H, t, *J* = 5.9 Hz, CH_2), 2.61 (2H, t, *J* = 5.4 Hz, CH_2), 2.47-2.20 (4H, m, 2CH_2), 2.10-1.98 (4H, m, 2CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C= 181.1 (*C*=O), 167.8 (*C*=O), 138.8 (Ar*C*), 138.2

(*C*H), 129.2 (Ar*C*H), 128.4 (Ar*C*H), 125.9 (Ar*C*H), 115.5 (*C*H_2), 71.0 (*C*), 45.6 (*C*H_2), 34.6 (*C*H_2), 33.0 (*C*H_2), 32.8 (*C*H_2), 30.7 (*C*H_2), 28.2 (*C*H_2), 26.8 (*C*H_2); HRMS (ESI^+) ($M+H^+$) found 302.1774 $C_{18}H_{23}NO_3$ requires 302.1769.

9.11. A general procedure for the synthesis of lactam-tetrazoles

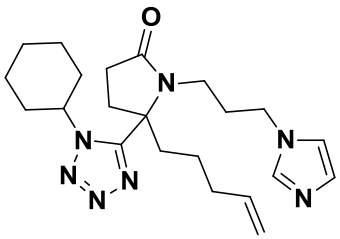
To a solution of Ugi-tetrazoles (**289-298**) (0.25 mmol) in dry tetrahydrofuran (2 mL) at room temperature, 1,1′-carbonyldiimidazole (CDI) (0.37 mmol) was added at once and the reaction mixture was stirred at room temperature for 24 hours. The reduced pressure was used to remove the excess tetrahydrofuran and the purification was achieved by column chromatography to obtain the titled γ-, δ- and ε-lactam tetrazoles.

9.11.1. 1-(3-(1H-Imidazol-1-yl) propyl)-5-(but-3-en-1-yl)-5-(1-cyclohexyl-1H-tetrazol-5-yl) pyrrolidin-2-one 387



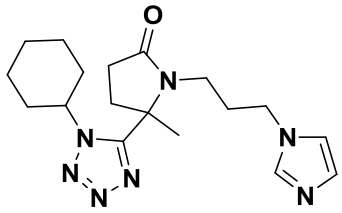
Isolated as a viscous pale oil, following method above using Ugi-tetrazole **289** (0.10 g, 0.25 mmol) and 1,1′-carbonyldiimidazole (CDI) (0.059 g, 0.37 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.5) to yield γ-lactam tetrazole **387** (0.077 g, 77% yield); υ_{max} (ATR/cm^{-1})= 3308, 2932, 2858, 1650, 1509; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.59 (1H, s, $C_3N_2H_3$), 7.10 (1H, d, J = 5.3 Hz, $C_3N_2H_3$), 6.99 (1H, d, J = 5.3 Hz, $C_3N_2H_3$), 5.84-5.75 (1H, m, C*H*), 5.08-5.03 (2H, m, CH_2), 4.75-4.71 (1H, m, C*H*), 4.04 (2H, t, J = 7.4 Hz, CH_2), 3.78-3.70 (4H, m, 2CH_2), 3.18 (2H, t, J = 7.5 Hz, CH_2), 2.20-1.99 (6H, m, 3CH_2), 1.94-1.20 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176.5 (*C*=O), 172.0 (N_4C), 137.0 (*C*H), 136.6 (C_2N_2CH), 129.3 (C_2N_2CH), 118.8 (C_2N_2CH), 115.8 (*C*H_2), 69.7 (*C*), 48.7 (*C*H), 45.2 (*C*H_2), 39 (*C*H_2), 33.4 (*C*H_2), 32.9 (*C*H_2), 29.9 (*C*H_2), 29.7 (*C*H_2), 28.8 (*C*H_2), 27.7 (*C*H_2), 25.4 (*C*H_2), 24.9 (*C*H_2); HRMS (ESI^+) ($M+H^+$) found 398.2665 $C_{21}H_{31}N_7O$ requires 398.2663.

9.11.2. 1-(3-(1H-Imidazol-1-yl) propyl)-5-(1-cyclohexyl-1H-tetrazol-5-yl)-5-(pent-4-en-1-yl)pyrrolidin-2-one 388



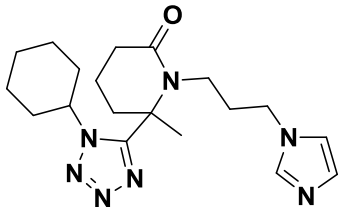
Isolated as a viscous pale oil, following method above using Ugi-tetrazole **290** (0.10 g, 0.25 mmol) and 1,1′-carbonyldiimidazole (CDI) (0.059 g, 0.37 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.4) to yield γ-lactam tetrazole **388** (0.075 g, 75% yield); υ_{max} (ATR/cm^{-1})= 3290, 3116, 2939, 2855, 1667, 1533; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.58 (1H, s, C_3N_2H), 7.29 (1H, d, J = 9.8 Hz, C_3N_2H), 6.97 (1H, d, J = 9.9 Hz, C_3N_2H), 5.81-5.71 (1H, m, C*H*), 5.06-5.00 (2H, m, CH_2), 4.72-4.70 (1H, m, C*H*), 4.06-3.99 (2H, m, CH_2), 3.78-3.69 (4H, m, 2CH_2), 3.22-3.09 (2H, m, CH_2), 2.59-2.35 (4H, m, 2CH_2), 2.21-1.99 (4H, m, 2CH_2), 1.72-1.06 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176.6 (*C*=O), 172.2 (N_4C), 137.5 (*C*H), 137 (C_2N_2CH), 129.4 ($C_{22}N_2C$H), 118.8 (C_2N_2CH), 115.9 (*C*H_2), 69.9 (*C*), 48.7 (*C*H), 45.2 (*C*H_2), 39 (*C*H_2), 33.8 (*C*H_2), 33.7 (*C*H_2), 32.9 (*C*H_2), 29.9 (*C*H_2), 29.8 (*C*H_2), 29.1 (*C*H_2), 25.4 (*C*H_2), 24.8 (*C*H_2), 24.8 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 412.2823 $C_{22}H_{33}N_7O$ requires 412.2819.

9.11.3. 1-(3-(1H-Imidazol-1-yl) propyl)-5-(1-cyclohexyl-1H-tetrazol-5-yl)-5-methyl pyrrolidin-2-one 389



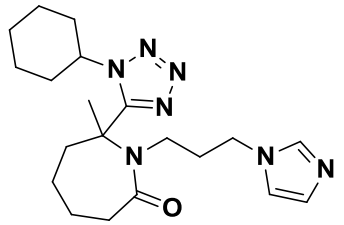
Isolated as a viscous yellow oil, following method above using Ugi-tetrazole **291** (0.09 g, 0.25 mmol) and 1,1′-carbonyldiimidazole (CDI) (0.059 g, 0.37 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.5) to yield γ-lactam tetrazole **389** (0.077 g, 86% yield); υ_{max} (ATR/cm^{-1})= 3103, 2932, 2852, 1791, 1664; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.67 (1H, s, C_3N_2H), 7.10 (1H, d J = 12.2 Hz, C_3N_2H), 7.02 (1H, d, J = 12.3 Hz, C_3N_2H), 5.53-5.35 (1H, m, C*H*), 4.06 (2H, t, J = 6.4 Hz, CH_2), 3.73 (2H, d, J = 8.6 Hz, CH_2), 3.36-3.00 (2H, m, CH_2), 2.51-2.45 (4H, m, 2CH_2), 2.01-1.12 (10H, m, 5CH_2), 1.50 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176.3 (*C*=O), 172.1 (N_4C), 137.1 (C_2N_2CH), 129.2 (C_2N_2CH), 118.8 (C_2N_2CH), 67.1 (*C*), 48.8 (*C*H), 45.0 (*C*H_2), 38.5 (*C*H_2), 33.1 (*C*H_2), 29.5 (*C*H_2), 25.4 (*C*H_2), 25.0 (*C*H_2), 24.8 (*C*H_2), 23.4 (*C*H_2), 22.8 (*C*H_3); HRMS (ESI$^+$) (M+H$^+$) found 358.2349 $C_{18}H_{27}N_7O$ requires 358.2350.

9.11.4. 1-(3-(1H-Imidazol-1-yl) propyl)-6-(1-cyclohexyl-1H-tetrazol-5-yl)-6-methyl piperidin-2-one 390



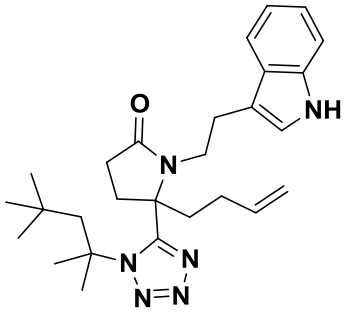
Isolated as a viscous colourless oil, following method above using Ugi-tetrazole **292** (0.09 g, 0.25 mmol) and 1,1′-carbonyldiimidazole (CDI) (0.059 g, 0.37 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.3) to yield δ-lactam tetrazole **390** (0.073 g, 82% yield); υ_{max} (ATR/cm^{-1})= 3140, 2925, 2855, 1700, 1617, 1533; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.5 (1H, s, C_3N_2H), 7.1 (1H, d, J = 9.3 Hz, C_3N_2H), 6.95 (1H, d, J = 9.3 Hz, C_3N_2H), 5.63-5.61 (1H, m, CH_2), 4.81 (4H, t, J = 5.5 Hz, $2CH_2$), 4.04-3.96 (2H, m, CH_2), 3.76-3.23 (2H, m, CH_2), 2.51-2.28 (4H, m, $2CH_2$), 1.86 (3H, s, CH_3), 1.41-1.09 (10H, m, $5CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 172 (*C*=O), 171.6 (N_4C), 137.1 (C_2N_2CH), 129.7 (C_2N_2CH), 118.7 (C_2N_2CH), 66.4 (*C*), 48.9 (*C*H), 45.2 (CH_2), 44.3 (CH_2), 38.2 (CH_2), 36.2 (CH_2), 33.2 (CH_2), 32.9 (CH_2), 30.5 (CH_2), 25.4 (CH_2), 24.9 (CH_2), 17.5 (CH_3); HRMS (ESI$^+$) (M+H$^+$) found 372.2516 $C_{19}H_{29}N_7O$ requires 372.2506.

9.11.5. 1-(3-(1H-Imidazol-1-yl) propyl)-7-(1-cyclohexyl-1H-tetrazol-5-yl)-7-methyl azepan-2-one 391



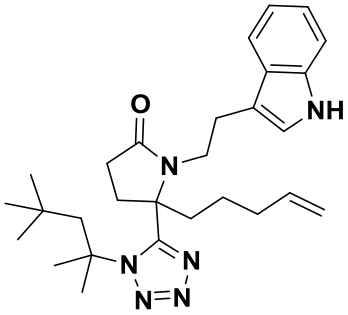
Isolated as a viscous colourless oil, following method above using Ugi-tetrazole **293** (0.10 g, 0.25 mmol) and 1,1′-carbonyldiimidazole (CDI) (0.059 g, 0.37 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.3) to yield ε-lactam tetrazole **391** (0.055 g, 55% yield); υ_{max} (ATR/cm^{-1})= 3049, 2983, 2942, 2859, 1733, 1506; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.84 (1H, s, C_3N_2H), 7.15 (1H, d, J = 6.7 Hz, C_3N_2H), 6.95 (1H, d, J = 6.7 Hz, C_3N_2H), 5.14-5.09 (1H, m, C*H*), 4.07 (2H, t, J = 7.4 Hz, CH_2), 3.67 (2H, t, J = 9.8 Hz, CH_2), 2.31 (2H, t, J = 7.4 Hz, CH_2), 2.00-1.96 (4H, m, $2CH_2$), 1.94-1.91 (4H, m, $2CH_2$), 1.63-119 (10H, m, $5CH_2$), 1.61 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 173.8 (*C*=O), 158.7 (N_4C), 136.9 (C_2N_2CH), 129.2 (C_2N_2CH), 118.8 (C_2N_2CH), 58.9 (*C*), 56.4 (*C*H), 51.6 (CH_2), 45.2 (CH_2), 40.4 (CH_2), 39.8 (CH_2), 33.7 (CH_2), 33.5 (CH_2), 25.7 (CH_2), 24.9 (CH_2), 24.4 (CH_2), 23.3 (CH_3); HRMS (ESI$^+$) (M+H$^+$) found 386.3000 $C_{20}H_{31}N_7O$ requires 386.3001.

9.11.6. 1-(2-(1H-Indol-3-yl) ethyl)-5-(but-3-en-1-yl)-5-(1-(2,4,4-trimethylpentan-2-yl)-1H-tetrazol-5-yl) pyrrolidin-2-one 392



Isolated as a viscous brown oil, following method above using Ugi-tetrazole **294** (0.12 g, 0.25 mmol) and 1,1′-carbonyldiimidazole (CDI) (0.059 g, 0.37 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.3) to yield γ-lactam tetrazole **392** (0.08 g, 68% yield); υ_{max} (ATR/cm^{-1})= 3400, 3048, 2952, 2866, 1664, 1513; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.63 (1H, s, C_8H_5NH), 8.30 (1H, brs, N*H*), 7.72 (1H, d, *J* = 7.6 Hz, ArC*H*), 7.38 (1H, d, *J* = 7.6 Hz, ArC*H*), 7.21 (1H, t, *J* = 7.1 Hz, ArC*H*), 7.14 (1H, t, *J* = 7.0 Hz, ArC*H*), 5.87-5.76 (1H, m, C*H*), 5.09-4.99 (2H, m, CH_2), 3.60-3.42 (2H, m, CH_2), 3.23-3.06 (4H, m, 2CH_2), 2.49 (2H, t, *J* = 8.6 Hz, CH_2), 2.26-2.11 (4H, m, 2CH_2), 1.78 (6H, s, 2CH_3), 1.40 (2H, s, CH_2), 0.97 (9H, s, 3CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176.7 (*C*=O), 172.5 (N_4C), 141.5 (*C*H), 137.1 (ArC), 121.3 (ArC*H*), 119.6 (Ar*C*H), 119.2 (Ar*C*), 115.5 (Ar*C*H), 112.9 (*C*H_2), 111.3 (Ar*C*H), 71.7 (*C*), 63.9 (*C*), 55.8 (*C*H_2), 54.5 (*C*H_2), 52.6 (*C*H_2), 43.9 (*C*), 33.4 (*C*), 31.7 (*C*H_2), 30.7 (3*C*H_3), 29.9 (*C*H_2), 28.9 (*C*H_2), 27.9 (2*C*H_3), 25.6 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 463.3192 $C_{27}H_{38}N_6O$ requires 463.3180.

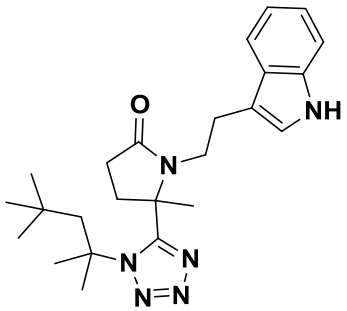
9.11.7. 1-(2-(1H-Indol-3-yl) ethyl)-5-(pent-4-en-1-yl)-5-(1-(2,4,4-trimethylpentan-2-yl)-1H-tetrazol-5-yl) pyrrolidin-2-one 393



Isolated as a viscous brown oil, following method above using Ugi-tetrazole **295** (0.12 g, 0.25 mmol) and 1,1′-carbonyldiimidazole (CDI) (0.059 g, 0.37 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.4) to yield γ-lactam tetrazole **393** (0.079 g, 66% yield); υ_{max} (ATR/cm^{-1})= 3476, 3328, 2959, 2873, 1674,

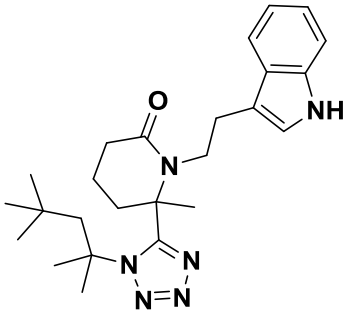
1509; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.62 (1H, s, C_8H_5NH), 8.54 (1H, brs, N*H*), 7.72 (1H, d, *J* = 7.2 Hz, ArC*H*), 7.60 (1H, d, *J* = 7.2 Hz, ArC*H*), 7.39 (1H, t, *J* = 6.2 Hz, ArC*H*), 7.24 (1H, t, *J* = 6.2 Hz, ArC*H*), 5.98-5.72 (1H, m, C*H*), 5.19-4.99 (2H, m, CH_2), 3.49 (2H, t, *J* = 8.4 Hz, CH_2), 3.61 (2H, t, *J* = 7.9 Hz, CH_2), 2.51-2.43 (2H, m, CH_2), 2.23-2.14 (4H, m, $2CH_2$), 2.08 (2H, t, *J* = 10.3 Hz, CH_2), 1.79 (6H, s, $2CH_3$), 1.68 (2H, t, *J* = 4.9 Hz, CH_2), 1.57 (2H, s, CH_2), 0.97 (9H, s, $3CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 177 (*C*=O), 173.5 (N_4C), 140.3 (*C*H), 137.8 (Ar*C*), 122.3 (ArC*H*), 122.2 (Ar*C*), 121.9 (ArC*H*), 119.7 (ArC*H*), 119 (ArC*H*), 115.4 (CH_2), 111.3 (Ar*C*), 112.7 (Ar*C*H), 70.7 (*C*), 62.9 (*C*), 55.7 (CH_2), 54.3 (CH_2), 52.5 (CH_2), 42.8 (*C*), 33.9 (*C*), 31.6 (CH_2), 31.5 ($3CH_3$), 30.9 (CH_2), 29.9 (CH_2), 29.3 (CH_2), 28.9 ($2CH_3$), 27.9 (CH_2); HRMS (ESI$^+$) (M+H$^+$) found 477.3343 $C_{28}H_{40}N_6O$ requires 477.3336.

9.11.8. 1-(2-(1H-Indol-3-yl) ethyl)-5-methyl-5-(1-(2,4,4-trimethylpentan-2-yl)-1H-tetrazol-5-yl) pyrrolidin-2-one 394



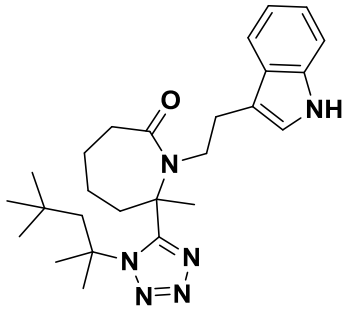
Isolated as a viscous brown oil, following method above using Ugi-tetrazole **296** (0.11 g, 0.25 mmol) and 1,1′-carbonyldiimidazole (CDI) (0.059 g, 0.37 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.5) to yield *γ*-lactam tetrazole **394** (0.09 g, 82% yield); υ_{max} (ATR/cm^{-1})= 3304, 2952, 2921, 2870, 1674, 1454; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.18 (1H, s, C_8H_5NH), 7.68 (1H, d, *J* = 7.5 Hz, ArC*H*), 7.39 (1H, d, *J* = 7.4 Hz, ArC*H*), 7.22 (1H, t, *J* = 4.6 Hz, ArC*H*), 7.15 (1H, t, *J* = 4.9 Hz, ArC*H*), 3.82-3.69 (2H, m, CH_2), 3.39-3.15 (2H, m, CH_2), 2.47 (2H, t, *J* = 5.3 Hz, CH_2), 2.34 (2H, t, *J* = 8.8 Hz, CH_2), 1.54 (6H, s, $2CH_3$), 1.34 (2H, s, CH_2), 1.30 (3H, s, CH_3), 0.95 (9H, s, $3CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176 (*C*=O), 172.5 (N_4C), 136.3 (Ar*C*), 127.4 (ArC*H*), 122.3 (Ar*C*), 121.9 (Ar*C*H), 119.7 (ArC*H*), 118.9 (ArC*H*), 112.9 (ArC*H*), 111.9 (Ar*C*), 111.2 (ArC*H*), 68 (*C*), 55.6 (*C*), 52.2 (CH_2), 43.7 (CH_2), 42.4 (*C*), 33.2 (*C*), 31.9 ($3CH_3$), 31.9 (CH_3), 31.6 (CH_2), 30.9 (CH_2), 29.8 ($2CH_3$), 24.7 (CH_2); HRMS (ESI$^+$) (M+H$^+$) found 423.2879 $C_{24}H_{34}N_6O$ requires 423.2867.

9.11.9. 1-(2-(1H-Indol-3-yl) ethyl)-6-(1-(2,4-dimethylpentan-2-yl)-1H-tetrazol-5-yl)-6-methyl piperidin-2-one 395



Isolated as a viscous brown oil, following method above using Ugi-tetrazoles **297** (0.11 g, 0.25 mmol) and 1,1′-carbonyldiimidazole (CDI) (0.059 g, 0.37 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.4) to yield δ-lactam tetrazole **395** (0.093 g, 85% yield); υ_{max} (ATR/cm^{-1})= 3259, 3056, 2956, 2859, 1650, 1616, 1550; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.36 (1H, s, C_8H_5NH), 7.80 (1H, d, J = 7.7 Hz, ArC*H*), 7.30 (1H, d, J = 7.6 Hz, ArC*H*), 7.20 (1H, t, J = 6.9 Hz, ArC*H*), 7.15 (1H, t, J = 6.8 Hz, ArC*H*), 4.12-3.26 (2H, m, CH_2), 3.04-2.83 (2H, m, CH_2), 2.52 (2H, t, J = 6.2 Hz, CH_2), 2.22 (2H, t, J = 12.3 Hz, CH_2), 1.76 (2H, t, J = 6.7 Hz, CH_2), 1.65 (6H, s, 2CH_3), 1.41 (2H, s, CH_2), 1.36 (3H, s, CH_3), 0.97 (9H, s, 3CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176.2 (*C*=O), 172.5 (N_4C), 136.3 (Ar*C*), 127.3 (Ar*C*H), 122.3 (Ar*C*), 121.9 (Ar*C*H), 119.7 (Ar*C*H), 118.9 (Ar*C*H), 112.9 (Ar*C*H), 111.3 (Ar*C*), 68.1 (*C*), 55.6 (*C*), 52.2 (*C*H_2), 42.32 (*C*), 33.3 (*C*H_2), 31.6 (*C*), 31.5 (3*C*H_3), 30.9 (*C*H_3), 29.8 (*C*H_2), 28.8 (*C*H_2), 28.2 (2*C*H_3), 24.8 (*C*H_2), 23.0 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 437.3029 $C_{25}H_{36}N_7O$ requires 437.3023.

9.11.10. 1-(2-(1H-Indol-3-yl) ethyl)-7-methyl-7-(1-(2,4,4-trimethylpentan-2-yl)-1H-tetrazol-5-yl) azepan-2-one 396



Isolated as a viscous brown oil, following method above using Ugi-tetrazole **298** (0.11 g, 0.25 mmol) and 1,1′-carbonyldiimidazole (CDI) (0.059 g, 0.37 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.5) to yield ε-lactam tetrazole **396** (0.049 g, 45% yield); υ_{max} (ATR/cm^{-1})= 3056, 2987, 2952, 2870, 1733, 1457; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.62 (1H, s, C_8H_5NH), 7.62 (1H, d, J = 7.9 Hz, ArC*H*), 7.50 (1H, d, J = 7.9 Hz, ArC*H*), 7.20 (1H, t, J = 7.4 Hz, ArC*H*), 7.08 (1H, t, J = 7.5

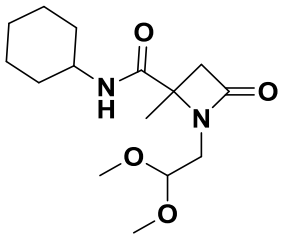
Hz, ArC*H*), 3.68 (2H, t, *J* = 6.5 Hz, CH_2), 3.06-2.98 (2H, m, CH_2), 2.44 (2H, t, *J* = 6.6 Hz, CH_2), 2.29 (2H, t, *J* = 5.9 Hz, CH_2), 2.13 (2H, t, *J* = 7.3 Hz, CH_2), 1.79 (6H, s, 2CH_3), 1.59 (2H, t, *J* = 3.5 Hz, CH_2), 1.49 (2H, s, CH_2), 1.04 (3H, s, CH_3), 0.97 (9H, s, 3CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 177.1 (*C*=O), 176.4 (N_4C), 136.3 (Ar*C*), 127.3 (Ar*C*), 122.3 (Ar*C*H), 121.9 (Ar*C*H), 119.6 (Ar*C*H), 118.9 (Ar*C*H), 112.9 (Ar*C*), 111.2 (Ar*C*H), 68.1 (*C*), 55.7 (*C*), 52.2 (*C*H_2), 42.4 (*C*), 37.8 (*C*H_2), 33.2 (*C*), 31.5 (3*C*H_3), 29.8 (*C*H_3), 29.8 (*C*H_2), 28.8 (*C*H_2), 28.2 (2*C*H_3), 27.6 (*C*H_2), 25.2 (*C*H_2), 22.9 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 451.3000 $C_{26}H_{38}N_6O$ requires 451.3000.

9.12. Synthesis of diones derivatives

9.12.1. General synthesis of Ugi adducts (cyclic amide)

To a solution of ketoacids (1.0 eq) in methanol (5 mL) at room temperature aminoacetaldehyde dimethylacetal **400** (1.2 eq) was added at once and the mixture was stirred for 45 minutes to ensure imine formation. A cyclohexylisocyanide **113** (1.0 eq) was added at once and the reaction mixture was stirred at room temperature for 48-72 hours. The excess methanol was evaporated under reduced pressure and the residue was dissolved in dichloromethane (10 mL). The reaction was then worked up by quenching once with hydrochloric acid (10%, 10 mL) to ensure removal of the most unreacted amine. The mixture was extracted with sodium hydroxide (5 M, 50 mL) to ensure the removal of the most unreacted ketoacid. The organic combined layers were dried with sodium sulphate, filtered and concentrated under reduced pressure. Purification was achieved by column chromatography to obtain the titled Ugi adducts.

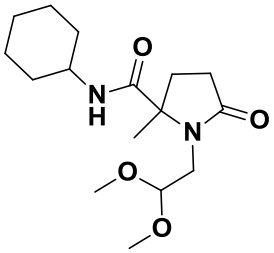
9.12.1.1. *N*-Cyclohexyl-1-(2,2-dimethoxyethyl)-2-methyl-4-oxoazetidine-2-carboxamide 401



Isolated as a colourless oil, following the general method above using 3-oxobutanoic acid **5** (0.20 g, 2.0 mmol), aminoacetaldehyde dimethylacetal **400** (0.26 g, 2.5 mmol) and cyclohexylisocyanide **113** (0.21 g, 2.0 mmol) for 48 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 2: 8, R_f = 0.2) to yield cyclic amide **402** (0.13 g, 65% yield); υ_{max} (ATR/cm^{-1})= 3346, 2986, 2937, 2849, 1702, 1667, 1528, 1444;

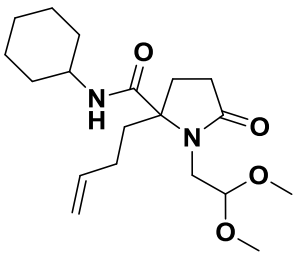
^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 5.66 (1H, d, *J* = 7.9 Hz, N*H*), 4.72 (1H, t, *J* = 5.7 Hz, C*H*), 3.67 (3H, s, OCH_3), 3.47 (3H, s, OCH_3), 3.43-3.40 (1H, m, C*H*), 3.4 (2H, dd, *J* = 8.8 Hz, 4.1 Hz, CH_2), 3.2 (2H, s, CH_2), 1.73-1.21 (10H, m, 5CH_2), 1.44 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 174.7 (*C*=O), 156.6 (*C*=O), 102.9 (*C*H), 62.0 (*C*), 55.2 (O*C*H_3), 54.3 (O*C*H_3), 52.5 (*C*H), 47.9 (*C*H_2), 45.3 (*C*H_2), 33.4 (*C*H_2), 25.8 (*C*H_2), 25.7 (*C*H_2), 25.0 (*C*H_3); HRMS (ESI$^+$) (M+H$^+$) found 299.1970 $C_{15}H_{26}N_2O_4$ requires 299.1965.

9.12.1.2. *N*-Cyclohexyl-1-(2,2-dimethoxyethyl)-2-methyl-5-oxopyrrolidine-2-carbox amide 402



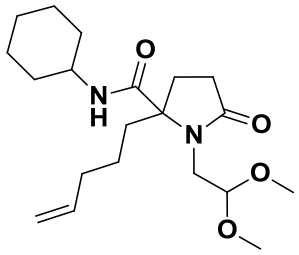
Isolated as a colourless oil, following the general method above using 4-oxopentanoic acid **5** (0.23 g, 2.0 mmol), aminoacetaldehyde dimethylacetal **400** (0.27 g, 2.5 mmol) and cyclohexylisocyanide **113** (0.22 g, 2.0 mmol) for 48 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.4) to yield cyclic amide **402** (0.138 g, 60% yield); υ_{max} (ATR/cm^{-1})= 3328, 2980, 2935, 2853, 1691, 1665, 1535, 1448; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 5.08 (1H, t, *J* = 5.7 Hz, C*H*), 3.76-3.66 (1H, m, C*H*), 3.57 (1H, d, *J* = 6.2Hz, N*H*), 3.50 (3H, s, OCH_3), 3.46 (3H, s, OCH_3), 2.93 (1H, dd, *J* = 6.8 Hz, 3.1 Hz, CH_2), 2.48-2.39 (2H, m, CH_2), 2.37-2.29 (2H, m, CH_2), 1.96-1.41 (10H, m, 5CH_2), 1.48 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 177.7 (*C*=O), 172.5 (*C*=O), 101.6 (*C*H), 67.9 (*C*), 56.2 (O*C*H_3), 54.4 (O*C*H_3), 48.7 (*C*H), 44 (*C*H_2), 34 (*C*H_2), 33.2 (*C*H_2), 32.7 (*C*H_2), 29.5 (*C*H_2), 25.6 (*C*H_2), 25.1 (*C*H_2), 21.8 (*C*H_3); HRMS (ESI$^+$) found 335.1944 $C_{16}H_{28}N_2O_4Na$ [M+Na]$^+$ requires 335.1941.

9.12.1.3. (2-But 3-en-1-yl)-*N*-cyclohexyl-1-(2,2-dimethoxyethyl)-5-oxopyrrolidine-2-carboxamide 403



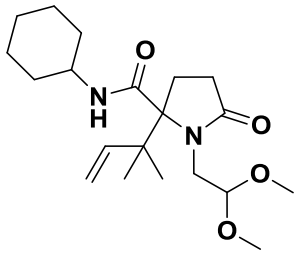
Isolated as a pale oil, following the general method above using 4-oxooct-7-enoic acid **178** (0.10 g, 0.64 mmol), aminoacetaldehyde dimethylacetal **400** (0.18 g, 0.80 mmol) and cyclohexylisocyanide **113** (0.07 g, 0.64 mmol) for 72 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 4: 6, R_f = 0.4) to yield cyclic amide **403** (0.04 g, 40% yield); υ_{max}(ATR/cm^{-1})= 3334, 3074, 2935, 2860, 1691, 1659, 1535, 1451; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.52 (1H, d, *J* = 7.6 Hz, N*H*), 5.85-5.75 (1H, m, C*H*), 5.09-4.99 (2H, m, CH_2), 3.76-3.66 (1H, m, C*H*), 3.53 (3H, s, OCH_3), 3.48 (3H, s, OCH_3), 2.91 (2H, dd, *J* = 7.2 Hz, 3.1 Hz, CH_2), 2.49-2.30 (2H, m, CH_2), 2.22-2.18 (2H, m, CH_2), 1.93-1.82 (4H, m, CH_2), 1.80-1.28 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 177.9 (*C*=O), 172.5 (*C*=O), 137.0 (*C*H), 115.4 (*C*H_2), 101.7 (*C*H), 70.7 (*C*), 56.6 (O*C*H_3), 54.9 (O*C*H_3), 48.7 (*C*H), 45.2 (*C*H_2), 33.9 (*C*H_2), 33.0 (*C*H_2), 32.9 (*C*H_2), 29.9 (*C*H_2), 29.7 (*C*H_2), 27.7 (*C*H_2), 25.7 (*C*H_2); HRMS (ESI$^+$) found 375.2259 $C_{19}H_{32}N_2O_4Na$ $[M+Na]^+$ requires 375.2254.

9.12.1.4. *N*-Cyclohexyl-1-(2,2-dimethoxyethyl)-5-oxo-2-(pent-4-en-1-yl) pyrrolidine-2-carboxamide 404



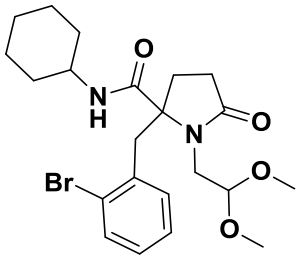
Isolated as a colourless oil, following the general method above using 4-oxonon-8-enoic acid **179** (0.10 g, 0.588 mmol), aminoacetaldehyde dimethylacetal **400** (0.078 g, 0.735 mmol) and cyclohexylisocyanide **113** (0.06 g, 0.588 mmol) for 72 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 4: 6, R_f = 0.4) to yield cyclic amide **404** (0.03 g, 30% yield); υ_{max} (ATR/cm^{-1})= 3334, 3074, 2935, 2857, 1691, 1659, 1535, 1454; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.52 (1H, d, *J* = 7.52 Hz, N*H*), 5.83-5.72 (1H, m, C*H*), 5.05-4.97 (2H, m, CH_2), 3.76-3.66 (1H, m, C*H*), 3.53 (3H, s, OCH_3), 3.49 (3H, s, OCH_3), 2.98 (2H, dd, *J* = 7.2 Hz, 2.9 Hz, CH_2), 2.48-2.29 (2H, m, CH_2), 2.24-2.05 (4H, m, CH_2), 1.94-1.87 (2H, m, CH_2), 1.77-1.59 (4H, m, CH_2), 1.50-1.08 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 178.1 (*C*=O), 172.7 (*C*=O), 137.8 (*C*H), 115.5 (*C*H_2), 101.6 (*C*H), 70.9 (*C*), 56.6 (O*C*H_3), 54.9 (O*C*H_3), 48.7 (*C*H), 45.3 (*C*H_2), 34.3 (*C*H_2), 33.8 (*C*H_2), 33.0 (*C*H_2), 32.9 (*C*H_2), 29.9 (*C*H_2), 29.7 (*C*H_2), 25.7 (*C*H_2), 25.3 (*C*H_2); HRMS (ESI$^+$) found 389.4900 $C_{20}H_{34}N_2O_4Na$ $[M+Na]^+$ requires 389.4910.

9.12.1.5. *N*-Cyclohexyl-1-(2,2-dimethoxyethyl)-2-(2-methylbut-3-en-2-yl)-5-oxo pyro lidine-2-carboxamide 405



Isolated as a yellow oil, following the general method above using 5, 5-dimethyl-4-oxohept-6-enoic acid **181** (0.17 g, 1.0 mmol), aminoacetaldehyde dimethylacetal **400** (0.15 g, 1.5 mmol) and cyclohexylisocyanide **113** (0.10 g, 1.0 mmol) for 72 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 4 : 6, R_f = 0.3) to yield cyclic amide **405** (0.06 g, 35% yield); υ_{max} (ATR/cm^{-1})= 3325, 2929, 2854, 1701, 1658, 1537; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.99 (1H, d, *J* = 7.2 Hz, N*H*), 6.21 (1H, dd, *J* = 17.7 Hz, 10.8 Hz, C*H*), 5.45 (1H, dd, *J* = 8.1 Hz, 3.9 Hz, C*H*), 5.09-4.99 (2H, m, CH_2), 3.79-3.70 (1H, m, C*H*), 3.50 (3H, s, OCH_3), 3.45 (3H, s, OCH_3), 3.40-3.29 (2H, m, CH_2), 2.40-2.05 (2H, m, CH_2), 1.95-1.08 (10H, m, 5CH_2), 1.32 (3H, s, CH_3), 1.18 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 177.9 (*C*=O), 171.9 (*C*=O), 145.2 (*C*H), 113.2 (*C*H_2), 101.4 (*C*H), 73.3 (*C*), 56.6 (O*C*H_3), 54.9 (O*C*H_3), 48.8 (*C*H), 48.1 (*C*H_2), 44.8 (*C*), 33.0 (*C*H_2), 29.6 (*C*H_2), 28.8 (*C*H_2), 25.8 (*C*H_3), 25.7 (*C*H_2), 25.5 (*C*H_2), 25.4 (*C*H_2), 23.5 (*C*H_3); HRMS (ESI^+) found 389.3415 $C_{20}H_{34}N_2O_4Na$ $[M+Na]^+$ requires 389.2411.

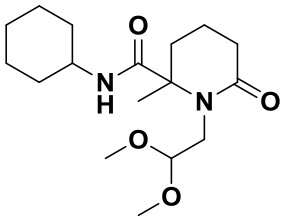
9.12.1.6. 2-(2-Bromobenzyl)-*N*-cyclohexyl-1-(2,2-dimethoxyethyl)-5-oxopyrrolidine-2-carboxamide 406



Isolated as a yellow oil, following the general method above using 5-(2-bromophenyl)-4-oxopentanoic acid **183** (0.269 g, 1.0 mmol), aminoacetaldehyde dimethylacetal **400** (0.15 g, 1.5 mmol) and cyclohexylisocyanide **113** (0.10 g, 1.0 mmol) for 72 h. The crude compound was purified by column chromatography (dichloromethane : methanol 9.5: 0.5, R_f = 0.25) to yield cyclic amide **406** (0.12 g, 48% yield); υ_{max} (ATR/cm^{-1})= ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.85 (1H, d, *J* = 7.9 Hz, N*H*), 7.54 (1H, d, *J* = 3.9 Hz, ArC*H*), 7.30 (1H, d, *J* = 2.1 Hz,

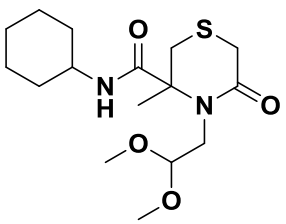
ArC*H*), 7.21 (1H, t, *J* = 7.5 Hz, ArC*H*), 7.09 (1H, t, *J* = 7.7 Hz, ArC*H*), 5.25 (1H, dd, *J* = 8.5 Hz, 3.6 Hz, C*H*), 3.81-3.74 (1H, m, C*H*), 3.71 (2H, s, CH_2), 3.48 (3H, s, OCH_3), 3.34 (3H, s, OCH_3), 3.31 (1H, dd, *J* = 13.9 Hz, 3.7 Hz, C*H*), 2.52 (1H, dd, *J* = 13.9 Hz, 8.6 Hz, C*H*), 2.38-1.99 (4H, m, 2CH_2), 1.97-1.07 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 177.9 (*C*=O), 171.3 (*C*=O), 136.4 (Ar*C*), 133.4 (Ar*C*H), 131.7 (Ar*C*H), 128.9 (Ar*C*H), 127.8 (Ar*C*H), 126.3 (Ar*C*), 101.4 (*C*H), 72.8 (*C*), 56.9 (O*C*H_3), 54.7 (O*C*H_3), 49.0 (*C*H), 46.5 (*C*H_2), 38.7 (*C*H_2), 32.9 (*C*H_2), 32.8 (*C*H_2), 29.3 (*C*H_2), 25.3 (*C*H_2); HRMS (ESI$^+$) found 489.1359 $C_{22}H_{31}BrN_2O_4Na$ $[M+Na]^+$ requires 489.1359.

9.12.1.7. *N*-Cyclohexyl-1-(2,2-dimethoxyethyl)-2-methyl-6-oxopiperidine-2-carbox amide 407



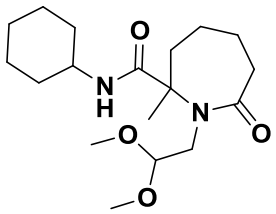
Isolated as a yellow oil, following the general method above using 5-oxohexanoic acid **168** (0.162 g, 1.25 mmol), aminoacetaldehyde dimethylacetal **400** (0.163 g, 1.56 mmol) and cyclohexylisocyanide **113** (0.136 g, 1.25 mmol) for 72 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 4: 6, R_f = 0.3) to yield cyclic amide **407** (0.11 g, 70% yield); υ_{max} (ATR/cm^{-1})= 3321, 2987, 2935, 2857, 1646, 1629, 1629, 1448; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 5.13 (1H, t, *J* = 5.8 Hz, C*H*), 3.78-3.68 (1H, m, C*H*), 3.56-3.3.51 (2H, m, CH_2), 3.49 (3H, s, OCH_3), 3.46 (3H, s, OCH_3), 3.05 (2H, dd, *J* = 5.8 Hz, 2.2 Hz, CH_2), 2.52-2.29 (2H, m, CH_2), 1.99-1.89 (2H, m, CH_2), 1.70-1.08 (10H, m, 5CH_2), 1.51 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 173.2 (*C*=O), 172.6 (*C*=O), 102.9 (*C*H), 66.8 (*C*), 56.6 (O*C*H_3), 55.6 (O*C*H_3), 48.9 (*C*H), 48.2 (*C*H_2), 36.8 (*C*H_2), 33.3 (*C*H_2), 32.9 (*C*H_2), 25.6 (*C*H_2), 25.2 (*C*H_2), 24.4 (*C*H_2), 17.4 (*C*H_3); HRMS (ESI$^+$) found 349.2102 $C_{17}H_{30}N_2O_4Na$ $[M+Na]^+$ requires 349.2098.

9.12.1.8. *N*-Cyclohexyl-4-(2,2-dimethoxyethyl)-3-methyl-5-oxothiomorpholine-3-carbox amide 408



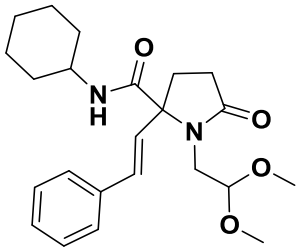
Isolated as a brown oil, following the general method above using 2-((2-oxopropyl) thio) acetic acid **198** (0.14 g, 1.0 mmol), aminoacetaldehyde dimethylacetal **400** (0.15 g, 1.5 mmol) and cyclohexylisocyanide **113** (0.10 g, 1.0 mmol) for 72 h. The crude compound was purified by column chromatography (dichloromethane : methanol 9.5: 0.5, R_f = 0.4) to yield cyclic amide **408** (0.10 g, 68% yield); υ_{max} (ATR/cm^{-1})= 3507, 3331, 2940, 2854, 1650, 1529, 1446; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 4.98 (1H, dd, *J* = 5.6 Hz, 5.2 Hz, C*H*), 3.71-3.63 (1H, m, C*H*), 3.54 (1H, dd, *J* = 13.9 Hz, 4.7 Hz, C*H*), 3.42 (3H, s, OCH_3), 3.40 (3H, s, OCH_3), 3.25 (2H, d, *J* = 5.8 Hz, CH_2), 3.01 (1H, dd, *J* = 13.9 Hz, 6.6 Hz, C*H*), 2.70 (2H, s, CH_2), 1.87-1.05 (10H, m, 5CH_2), 1.54 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 170.6 (*C*=O), 169 (*C*=O), 102.7 (*C*H), 68.6 (*C*), 56.4 (O*C*H_3), 55.9 (O*C*H_3), 49.0 (*C*H), 48.7 (*C*H_2), 38.0 (*C*H_2), 32.6 (*C*H_2), 31.9 (*C*H_2), 25.6 (*C*H_2), 25.0 (*C*H_2), 24.6 (*C*H_3); HRMS (ESI$^+$) found 367.1668 $C_{16}H_{28}N_2O_4SNa$ [M+Na]$^+$ requires 367.1662.

9.12.1.9. *N*-Cyclohexyl-1-(2,2-dimethoxyethyl)-2-methyl-7-oxoazepane-2-carboxamide 409



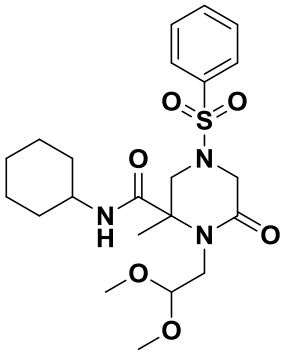
Isolated as a colourless oil, following the general method above using 6-oxoheptenoic acid **32** (0.18 g, 1.25 mmol), aminoacetaldehyde dimethylacetal **400** (0.163 g, 1.56 mmol) and cyclohexylisocyanide **113** (0.136 g, 1.25 mmol) for 72 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.3) to yield cyclic amide **409** (0.072 g, 40% yield); υ_{max} (ATR/cm^{-1})= 3320, 2981, 2930, 2861, 1650, 1645, 1625, 1444; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.04 (1H, d, *J* = 6.6 Hz, N*H*), 4.87 (1H, t, *J* = 5.7 C*H*), 3.84 (2H, dd, *J* = 6.1 Hz, 3.2 Hz, CH_2), 3.78-3.67 (1H, m, C*H*), 3.50 (3H, s, OCH_3), 3.45 (3H, s, OCH_3), 3.23-3.20 (2H, m, CH_2), 2.52-2.32 (4H, m, 2CH_2), 2.23-2.11 (2H, m, CH_2), 1.75-1.02 (10H, m, 5CH_2), 1.54 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 177.9 (*C*=O), 173.9 (*C*=O), 102.7 (*C*H), 64.6 (*C*), 55.8 (O*C*H_3), 55.2 (O*C*H_3), 48.3 (*C*H), 46.2 (*C*H_2), 38.7 (*C*H_2), 37.2 (*C*H_2), 33.2 (*C*H_2), 33.2 (*C*H_2), 30.6 (*C*H_2), 29.6 (*C*H_2), 25.2 (*C*H_2), 22.2 (*C*H_3); HRMS (ESI$^+$) found 363.2263 $C_{18}H_{32}N_2O_4Na$ [M+Na]$^+$ requires 363.2254.

9.12.1.10. (*E*)-*N*-Cyclohexyl-1-(2,2-dimethoxyethyl)-5-oxo-2-styrylpyrrolidine-2-carbox amide 410



Isolated as a yellow solid, following the general method above using (*E*)-4-oxo-6-phenylhex-5-enoic acid **189** (0.204 g, 1mmol), aminoacetaldehyde dimethylacetal **400** (0.15 g, 1.5 mmol) and cyclohexylisocyanide **113** (0.10 g, 1.0 mmol) for 72 h. The crude compound was purified by column chromatography (dichloromethane : methanol 9.5 : 0.5, R_f = 0.3) to yield cyclic amide **410** (0.07 g, 35% yield); m.p= 98-99 ºC; υ_{max} (ATR/cm^{-1})= 3029, 2937, 2835, 2857, 1715, 1671, 1451; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.49 (1H, d, *J* = 7.2 Hz, N*H*), 7.43-7.29 (5H, m, ArC*H*), 6.81 (1H, d, *J* = 7.5 Hz, C*H*), 4.95 (1H, dd, *J* = 11.4 Hz, 4.6 Hz, C*H*), 4.54-4.51 (1H, m, C*H*), 4.39 (1H, d, *J* = 10.8 Hz, C*H*), 3.92 (1H, d, *J* = 8.2 Hz, CH_2), 3.40 (3H, s, OCH_3), 3.09 (3H, s, OCH_3), 2.52-2.28 (4H, m, 2CH_2), 1.81-1.18 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 173.4 (*C*=O), 163.0 (*C*=O), 136.4 (Ar*C*), 129.2 (*C*H), 128.7 (Ar*C*H), 128.6 (Ar*C*H), 127.9 (Ar*C*H), 110.7 (*C*H), 72.7 (*C*), 55.5 (OCH_3), 54.4 (OCH_3), 49.7 (*C*H), 44.2 (*C*H), 33.3 (*C*H_2), 30.9 (*C*H_2), 28.0 (*C*H_2), 25.9 (*C*H_2), 25.3 (*C*H_2), 24.9 (*C*H_2); HRMS (ESI$^+$) found 423.2255 $C_{23}H_{32}N_2O_4Na$ $[M+Na]^+$ requires 423.2254.

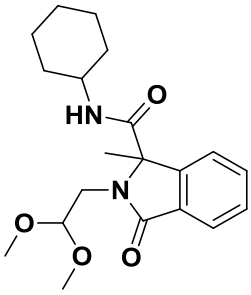
9.12.1.11. ***N*-Cyclohexyl-1-(2,2-dimethoxyethyl)-2-methyl-6-oxo-4-(phenylsulfonyl) piperazine-2-carboxamide 411**



Isolated as a brown oil, following the general method above using 2-(*N*-(2-oxopropyl) phenylsulfonamido) acetic acid **195** (0.27 g, 1.0 mmol), aminoacetaldehyde dimethylacetal **400** (0.15 g, 1.5 mmol) and cyclohexylisocyanide **113** (0.10 g, 1.0 mmol) for 72 h. The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.2) to yield cyclic amide **411** (0.16 g, 60% yield); υ_{max} (ATR/cm^{-1})= 3331, 3066, 2935, 2852, 1652, 1531, 1443, 1355; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.78 (2H, d, *J* = 3.6 Hz, ArC*H*), 7.65 (1H, t, *J* = 8 Hz, ArC*H*), 7.57 (1H, t, *J* = 7.5 Hz, ArC*H*), 7.21 (1H, d, *J* = 7.8 Hz,

ArC*H*), 5.01 (1H, dd, *J* = 5.9 Hz, 5.2 Hz, C*H*), 3.87 (2H, d, *J* = 4.5 Hz, CH_2), 3.79-3.65 (1H, m, C*H*), 3.54 (1H, dd, *J* = 16.7 Hz, 3.8 Hz, C*H*), 3.45 (3H, s, OCH_3), 3.41 (3H, s, OCH_3), 3.04 (1H, dd, *J* = 14.1 Hz, 6.2 Hz, C*H*), 2.81 (1H, d, *J* = 12.2 Hz, C*H*), 1.91-1.09 (10H, m, 5CH_2), 1.50 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 169.3 (*C*=O), 166.8 (*C*=O), 135.2 (Ar*C*), 133.6 (Ar*C*H), 129.4 (Ar*C*H), 127.8 (Ar*C*H), 103.2 (*C*H), 65.9 (*C*), 56.4 (O*C*H_3), 55.5 (O*C*H_3), 53.6 (*C*H_2), 46.7 (*C*H_2), 47.8 (*C*H_2), 32.9 (*C*H_2), 32.6 (*C*H_2), 25.5 (*C*H_2), 21.3 (*C*H_3); HRMS(ESI$^+$) found 490.1989 $C_{22}H_{33}N_3O_6SNa$ $[M+Na]^+$ requires 490.1982.

9.12.1.12. ***N*-Cyclohexyl-2-(2,2-dimethoxyethyl)-1-methyl-3-oxoisoindoline-1-carbox amide 412**

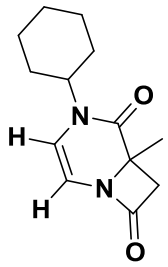


Isolated as a colourless solid, following the general method above using 2-acetylbenzoic acid **170** (0.328 g, 2.0 mmol), aminoacetaldehyde dimethylacetal **400** (0.21 g, 2.0 mmol) and cyclohexylisocyanide **113** (0.218 g, 2.0 mmol) for 48 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 4: 6, R_f = 0.3) to yield cyclic amide **412** (0.18 g, 57% yield); m.p= 123-125 ºC; υ_{max} (ATR/cm^{-1})= 3324, 3100, 3045, 2935, 2853, 1691, 1672, 1529; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.80 (1H, d, *J* = 7.5 Hz, ArC*H*), 7.74 (1H, d, *J* = 7.8 Hz, ArC*H*), 7.58 (1H, t, *J* = 8.3 Hz, ArC*H*), 7.48 (1H, t, *J* = 7.5 Hz, ArC*H*), 5.47 (1H, d, *J* = 4.2 Hz, N*H*), 3.75 (1H, t, *J* = 4.7 Hz, C*H*), 3.66-3.57 (1H, m, C*H*), 3.55 (3H, s, OCH_3), 3.54 (3H, s, OCH_3), 3.21 (2H, dd, *J* 7.2 Hz,3.2 Hz, CH_2), 1.97- 1.03 (10H, m, 5CH_2), 1.73 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 168 (*C*=O), 148.1 (Ar*C*), 132.7 (Ar*C*H), 128.8 (Ar*C*H), 123.4 (Ar*C*H), 122.5 (Ar*C*H), 101.8 (*C*H), 69.9 (*C*), 56.5 (O*C*H_3), 54.7 (O*C*H_3), 49.0 (*C*H), 44.6 (*C*H_2), 32.7 (*C*H_2), 25.5 (*C*H_2), 24.9 (*C*H_2), 22.4 (*C*H_3); HRMS (ESI$^+$) found 383.1939 $C_{20}H_{28}N_2O_4Na$ $[M+Na]^+$ requires 383.1941.

9.12.2. **General synthesis of diones derivatives**[189]

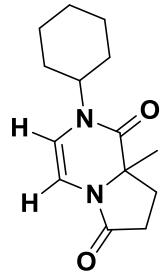
General approach for expansion Ugi products reaction: Ugi product (0.2 mmol) was dissolved in formic acid HCOOH (1.0 mL, 26.5 mmol) and stirred for 5-6 h at room temperature. The organic combined was concentrated under reduced pressure. Purification was achieved by column chromatography to afford the corresponding diones derivatives.

9.12.2.1. 4-Cyclohexyl-6-methyl-1,4-diazabicyclo[4.2.0]oct-2-ene-5,8-dione 413



Isolated as a colourless solid, following method above using *N*-cyclohexyl-1-(2,2-dimethoxyethyl)-2-methyl-4-oxoazetidine-2-carboxamide **401** (0.11 g, 0.4 mmol) and formic acid **124** (2 mL, 53.0 mmol) for 5 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.2) to yield dione **413** (0.08 g, 75% yield); m.p = 69-70 ºC; υ_{max} (ATR/cm^{-1})= 2932, 2858, 1733, 1691, 1650, 1438, 1418; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 6.28 (1H, d, *J* = 6.7 Hz, C*H*), 5.49 (1H, d, *J* = 6.7 Hz, C*H*), 4.45-4.33 (1H, m, C*H*), 3.76 (2H, s, CH_2), 1.90-1.09 (10H, m, 5CH_2), 1.74 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 167.8 (*C*=O), 153.7 (*C*=O), 109.3 (*C*H), 105.4 (*C*H), 62.2 (*C*H), 52.9 (*C)*, 30.7 (*C*H_2), 25.6 (*C*H_2), 25.4 (*C*H_2), 23.4 (*C*H_3); HRMS (ESI$^+$) (M+H$^+$) found 235.1400 $C_{13}H_{18}N_2O_2$ requires 235.1400.

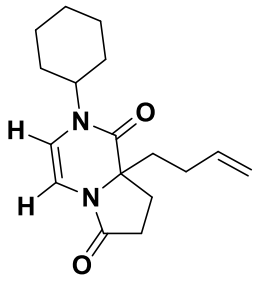
9.12.2.2. 2-Cyclohexyl-8a-methyl-8,8-a-dihydropyrrolo[1,2-a]pyrazine-1,6 (2H, 7H)-dione 414



Isolated as a pink solid, following method above using *N*-cyclohexyl-1-(2,2-dimethoxyethyl)-2-methyl-5-oxopyrrolidine-2-carboxamide **402** (0.24 g, 0.8 mmol) and formic acid **124** (4 mL, 106 mmol) for 5 h. The crude compound was purified by flash column chromatography (ethyl acetate : petroleum ether 4: 6, R_f = 0.4) to yield dione **414** (0.24 g, 85% yield); m.p = 139-141 ºC; υ_{max}(ATR/cm^{-1})= 2926, 2855, 1701, 1663, 1418, 1396; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 6.38 (1H, d, *J* = 5.9 Hz, C*H*), 5.90 (1H, d, *J* = 5.9 Hz, C*H*), 4.45-4.37 (1H, m, C*H*), 2.64-2.40 (2H, m, CH_2), 2.26-2.20 (2H, m, CH_2), 1.89-.10 (10H, m, 5CH_2), 1.29 (3H, m, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C= 171.9 (*C*=O), 167.7 (*C*=O), 112.5 (*C*H), 105

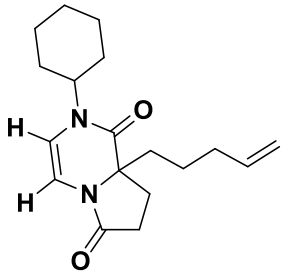
(*C*H), 62.8 (*C*H), 52.4 (*C*), 31.7 (*C*H_2), 30.8 (*C*H_2), 29.6 (*C*H_2), 25.7 (*C*H_2), 25.6 (*C*H_2), 20.7 (*C*H_3); HRMS (ESI^+) ($M+H^+$) found 249.1595 $C_{13}H_{18}N_2O_2$ requires 249.1598.

9.12.2.3. 8a-(But-3-en-1-yl)-2-cyclohexyl-8,8-a-dihydropyrrolo[1,2-a]pyrazine-1,6 (2H, 7H)-dione 415



Isolated as a pale oil, following method above using (2-but-3-en-1-yl)-*N*-cyclohexyl-1-(2,2-dimethoxyethyl)-5-oxopyrrolidine-2-carboxamide **403** (0.23 g, 0.65 mmol) and formic acid **124** (3.2 mL, 84.8 mmol) for 5 h. The crude compound was purified by flash column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.3) to yield dione **415** (0.13 g, 60% yield); υ_{max}(ATR/cm^{-1})= 3120, 3078, 2935, 2860, 1707, 1665; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 6.39 (1H, d, *J* = 5. 4 Hz, C*H*), 5.90 (1H, d, *J* = 5.9 Hz, C*H*), 5.79-5.69 (1H, m, C*H*), 5.06-4.97 (2H, m, CH_2), 4.45-4.39 (1H, m, C*H*), 2.63-2.42 (2H, m, CH_2), 2.32-2.27 (2H, m, CH_2), 2.09-2.03 (2H, m, CH_2), 1.88-1.83 (2H, m, CH_2), 1.78-1.34 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 172.6 (*C*=O), 166.9 (*C*=O), 136.8 (*C*H), 115.5 (*C*H_2), 112.9 (*C*H), 105.4 (*C*H), 64.5 (*C*), 52.5 (CH), 33.7 (*C*H_2), 31.6 (*C*H_2), 30.9 (*C*H_2), 30.0 (*C*H_2), 27.5 (*C*H_2), 26.9 (*C*H_2), 25.7 (*C*H_2); HRMS (ESI^+) ($M+H^+$) found 289.1910 $C_{17}H_{24}N_2O_2$ requires 289.1911.

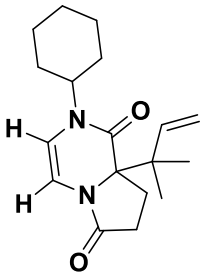
9.12.2.4. 2-Cyclohexyl-8a-(pent-4-en-1-yl)-8,8-a-dihydropyrrolo[1,2-a]pyrazine-1,6 (2H, 7H)-dione 416



Isolated as a pale oil, following method above using *N*-cyclohexyl-1-(2,2-dimethoxyethyl)-5-oxo-2-(pent-4-en-1-yl) pyrrolidine-2-carboxamide **404** (0.30 g, 0.81 mmol) and formic acid **124** (4 mL, 106 mmol) for 5 h. The crude compound was purified by flash column chromatography (ethyl acetate : petroleum ether 3: 7, R_f = 0.5) to yield dione **416** (0.135 g,

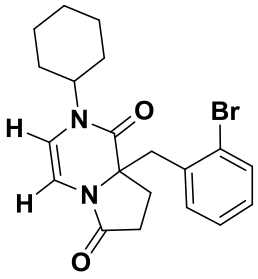
45% yield); υ_{max} (ATR/cm^{-1})= 3123, 3071, 2935, 2853, 1707, 1672, 1451; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 6.38 (1H, d, *J* = 5.8 Hz, C*H*), 5.89 (1H, d, *J* = 5.9 Hz, C*H*), 5.79-5.69 (1H, m, C*H*), 5.03-4.97 (2H, m, CH_2), 4.45-4.38 (1H, m, C*H*), 2.62-2.40 (2H, m, CH_2), 2.31-2.24 (2H, m, CH_2), 2.04 (2H, m, CH_2), 1.88-1.82 (2H, m, CH_2), 1.77-1.33 (12H, m, 6CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 172.6 (*C*=O), 167.2 (*C*=O), 137.7 (*C*H), 115.4 (*C*H_2), 112.8 (*C*H), 105.4 (*C*H), 64.7 (*C*), 52.4 (*C*H), 34.0 (*C*H_2), 33.5 (*C*H_2), 31.7 (*C*H_2), 30.9 (*C*H_2), 30.1 (*C*H_2), 27.0 (*C*H_2), 25.7 (*C*H_2), 25.6 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 303.2067 $C_{18}H_{26}N_2O_2$ requires 303.2067.

9.12.2.5. 2-Cyclohexyl-8a-(2-methylbut-3-en-2-yl)-8,8-a-dihydropyrrolo[1,2-a]pyrazine-1,6 (2H, 7H)-dione 417



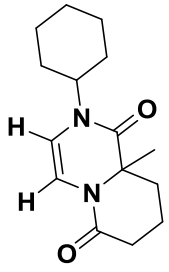
Isolated as a yellow solid, following method above using *N*-cyclohexyl-1-(2,2-dimethoxyethyl)-2-(2-methylbut-3-en-2-yl)-5-oxopyrrolidine-2-carboxamide **405** (0.14 g, 0.40 mmol) and formic acid **124** (2 mL, 53.0 mmol) for 6 h. The crude compound was purified by flash column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.25) to yield dione **417** (0.10 g, 75% yield); m.p = 113-115 ºC; υ_{max} (ATR/cm^{-1})= 2929, 2857, 1709, 1660, 1417, 1350; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 6.53 (1H, d, *J* = 5.9 Hz, C*H*), 5.96 (1H, dd, *J* = 18.1 Hz, 10.7 Hz, C*H*), 5.80 (1H, d, *J* = 5.9 Hz, C*H*), 5.09-5.03 (2H, m, CH_2), 4.54-4.47 (1H, m, C*H*), 2.51-2.32 (4H, m, 2CH_2), 1.85-1.27 (10H, m, 5CH_2), 1.11 (6H, s, 2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 175.8 (*C*=O), 165.7 (*C*=O), 142 (*C*H), 114.1 (*C*H_2), 113.4 (*C*H), 108.1 (*C*H), 52.7 (*C*H), 46.4 (*C*), 31.7 (*C*H_2), 31.2 (*C*H_2), 30.9 (*C*H_2), 27.4 (*C*H_2), 25.6 (*C*H_2), 25.4 (*C*), 22.7 (*C*H_3), 22.3 (*C*H_3); HRMS (ESI$^+$) (M+H$^+$) found 303.2071 $C_{18}H_{26}N_2O_2$ requires 303.2067.

9.12.2.6. 8a-(2-Bromobenzyl)-2-cyclohexyl-8,8-a-dihydropyrrolo[1,2-a]pyrazine-1,6 (2H, 7H)-dione 418



Isolated as a pale solid, following method above using 2-(2-bromobenzyl)-*N*-cyclohexyl-1-(2,2-dimethoxyethyl)-5-oxopyrrolidine-2-carboxamide **406** (0.18 g, 0.4 mmol) and formic acid **124** (2 mL, 53.0 mmol) for 5 h. The crude compound was purified by flash column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.4) to yield dione **418** (0.13 g, 77% yield); m.p = 129-130 ºC; υ_{max} (ATR/cm^{-1})= 3117, 3053, 2933, 2860, 1702, 1668, 1419; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.54 (1H, dd, *J* = 7.9 Hz, 2 Hz, ArC*H*), 7.21 (1H, t, *J* = 7.5 Hz, ArC*H*), 7.12 (1H, t, *J* = 8.5 Hz, ArC*H*), 7.04 (1H, d, *J* = 9.3 Hz, ArC*H*), 6.41 (1H, d, *J* = 5.9 Hz, C*H*), 6.78 (1H, d, *J* = 5.9 Hz, C*H*), 4.48-4.41 (1H, m, C*H*), 3.23 (1H, d, *J* = 13.8 Hz, C*H*), 3.01 (1H, d, *J* = 13.8 Hz, C*H*), 2.54 (1H, dd, *J* = 14.2 Hz, 8.7 Hz, C*H*), 2.37-2.28 (2H, m, CH_2), 2.17 (1H, dd, *J* = 17.9 Hz, 9.2 Hz, C*H*), 1.88-1.06 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 173.0 (*C*=O), 166.7 (*C*=O), 134.1 (Ar*C*), 133.2 (Ar*C*H), 132.1 (Ar*C*H), 129.1 (Ar*C*H), 127.5 (Ar*C*H), 125.6 (Ar*C*), 112.6 (*C*H), 105.6 (*C*H), 65.6 (*C*), 52.6 (*C*H), 38.5 (*C*H_2), 31.7 (*C*H_2), 30.6 (*C*H_2), 29.9 (*C*H_2), 27.8 (*C*H_2), 25.7 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 403.1022 $C_{20}H_{23}BrN_2O_2$ requires 403.1016.

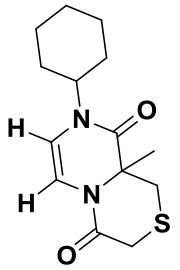
9.12.2.7. 2-Cyclohexyl-9a-methyl-7,8,9,9-a-tetrahydro-1H-pyrido[1,2-a]pyrazine-1,6 (2H)-dione 419



Isolated as a white solid, following method above using *N*-cyclohexyl-1-(2,2-dimethoxyethyl)-2-methyl-6-oxopiperidine-2-carboxamide **407** (0.06 g, 0.2 mmol) and formic acid **124** (1 mL, 26.5 mmol) for 5 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 4: 6, R_f = 0.5) to yield dione **419** (0.046 g, 70% yield); m.p = 153-156 ºC; υ_{max} (ATR/cm^{-1})= 2926, 2858, 1640, 1476, 1447; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 6.66 (1H, d, *J* = 6.1 Hz, C*H*), 5.88 (1H, d, *J* = 6.3 Hz, C*H*), 4.42-

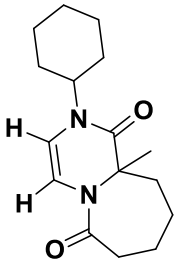
4.33 (1H, m, C*H*), 2.58-2.37 (2H, m, CH_2), 2.30-2.26 (2H, m, CH_2), 2.11-2.02 (2H, m, CH_2), 1.94-1.35 (10H, m, 5CH_2), 1.33 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 168.7 (*C*=O), 167.7 (*C*=O), 111 (*C*H), 107.7 (*C*H), 60.4 (*C*H), 52.5 (*C*), 31.5 (*C*H_2), 31.5 (*C*H_2), 30.6 (*C*H_2), 30.0 (*C*H_2), 25.7 (*C*H_2), 25.6 (*C*H_2), 21.3 (*C*H_3); HRMS (ESI$^+$) (M+H$^+$) found 263.1753 $C_{15}H_{22}N_2O_2$ requires 263.1754.

9.12.2.8. 8-Cyclohexyl-9a-methyl-1,9-a-dihydropyrazino[2,1-c][1,4]thiazine-4,9 (3H, 8H)-dione 420



Isolated as a brown solid, following method above *N*-cyclohexyl-4-(2,2-dimethoxyethyl)-3-methyl-5-oxothiomorpholine-3-carboxamide **408** (0.13 g, 0.4 mmol) and formic acid (2 mL, 53.0 mmol) for 6 h. The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.4) to yield dione **420** (0.092 g, 70% yield); m.p = 144-146 ºC; υ_{max} (ATR/cm^{-1})= 3124, 3052, 2934, 2859, 1644, 1406; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 6.61 (1H, d, *J* = 6.4 Hz, C*H*), 5.88 (1H, d, *J* = 6.4 Hz, C*H*), 4.33-4.27 (1H, m, C*H*), 3.50 (1H, d, *J* = 8.9 Hz, C*H*), 3.25-3.20 (2H, m, CH_2), 3.13 (1H, ddd, *J* = 14.9 Hz, 10.8 Hz, C*H*), 1.83-1.31 (10H, m, 5 CH_2), 1.49 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 166.5 (*C*=O), 164.0 (*C*=O), 111.6 (*C*H), 108.1 (*C*H), 62.6 (*C*), 52.8 (*C*H), 33.6 (*C*H_2), 31.4 (*C*H_2), 30.8 (*C*H_2), 30.5 (*C*H_2), 25.6 (*C*H_2), 20.5 (*C*H_3); HRMS (ESI$^+$) (M+H$^+$) found 281.1320 $C_{14}H_{20}N_2O_2S$ requires 281.1318.

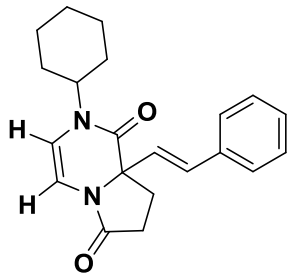
9.12.2.9. 2-Cyclohexyl-10a-methyl-8,9,10,10-a-tetrahydropyrazino[1,2-a]azepine-1,6 (2H, 7H)-dione 421



Isolated as a colourless solid, following method above using *N*-cyclohexyl-1-(2,2-dimethoxyethyl)-2-methyl-7-oxoazepane-2-carboxamide **409** (0.068 g, 0.2 mmol) and formic

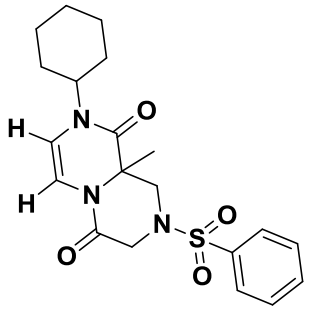
acid **124** (1 mL, 53.0 mmol) for 6 h. The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3: 7, R_f = 0.3) to yield dione **421** (0.040 g, 60% yield); m.p = 123-124 ºC; υ_{max} (ATR/cm^{-1})= 2945, 2869, 1644, 1476, 1448; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 6.62 (1H, d, *J* = 6.2 Hz, C*H*), 5.82 (1H, d, *J* = 6.3 Hz, C*H*), 3.58-3.42 (1H, m, C*H*), 2.24-2.13 (2H, m, CH_2), 1.87-1.81 (2H, m, CH_2), 1.56-1.49 (4H, m, 2CH_2), 1.44-1.12 (10H, m, 5CH_2), 1.34 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 174.3 (*C*=O), 169.5 (*C*=O), 110.2 (*C*H), 105.6 (*C*H), 69.6 (*C*), 67.9 (*C*H), 38.5 (*C*H_2), 34.7 (*C*H_2), 31.5 (*C*H_2), 31.1 (*C*H_2), 25.3 (*C*H_2), 25.3 (*C*H_2), 23.8 (*C*H_2), 21.7 (*C*H_3); HRMS (ESI$^+$) (M+H$^+$) found 277.2753 $C_{16}H_{24}N_2O_2$ requires 277.2749.

9.12.2.10. (*E*)-2-Cyclohexyl-8a-styryl-8,8-a-dihydropyrrolo[1,2-a]pyrazine-1,6 (2H, 7H)-dione 422



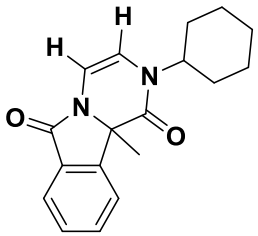
Isolated as a yellow solid, following method above using (*E*)-*N*-cyclohexyl-1-(2,2-dimethoxyethyl)-5-oxo-2-styrylpyrrolidine-2-carboxamide **410** (0.08 g, 0.2 mmol) and formic acid **124** (1 mL, 26.5 mmol) for 6 h. The crude compound was purified by flash column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.4) to yield dione **422** (0.044 g, 55% yield); m.p = 132-134 ºC; υ_{max} (ATR/cm^{-1})= 3028, 2938, 2857, 1715, 1671, 1451; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.44-7.26 (5H, m, ArC*H*), 6.19 (1H, d, *J* = 5.1 Hz, C*H*), 6.07 (1H, d, *J* = 5.1 Hz, C*H*), 5.31 (1H, d, *J* = 11.2 Hz, C*H*), 4.52-4.45 (1H, m, C*H*), 4.26 (1H, dd, *J* = 12.2 Hz, 3.6 Hz, C*H*), 2.63-2.52 (2H, m, CH_2), 2.21-2.26 (2H, t *J* = 15.3 Hz, CH_2), 1.96-1.27 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176.7 (*C*=O), 165.5 (*C*=O), 143.9 (Ar*C*), 128.7 (Ar*C*H), 127.7 (Ar*C*H), 127.4 (Ar*C*H), 117.4 (*C*H), 108.4 (*C*H), 101.9 (*C*H), 61.4 (*C*H), 50.5 (*C*), 43.2 (*C*H_2), 33.2 (*C*H_2), 31.9 (*C*H_2), 31.3 (*C*H_2), 25.5 (*C*H_2); HRMS (ESI$^+$)(M+H$^+$) found 337.1853 $C_{21}H_{24}N_2O_2$ requires 337.1849.

9.12.2.11. 2-Cyclohexyl-9a-methyl-8-(phenylsulfonyl)-7,8,9,9-a-tetrahydro-1H-pyrazino [1,2-a]pyrazine-1,6 (2H)-dione 423



Isolated as a colourless solid, following method above using *N*-cyclohexyl-1-(2,2-dimethoxyethyl)-2-methyl-6-oxo-4-(phenylsulfonyl) piperazine-2-carboxamide **411** (0.18 g, 0.4 mmol) and formic acid **124** (2 mL, 53.0 mmol) for 6 h. The crude compound was purified by flash column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.25) to yield dione **423** (0.14 g, 82% yield); m.p = 153-156 ºC; υ_{max} (ATR/cm^{-1})= 3125, 3061, 2930, 2860, 1665, 1448, 1408; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 7.84 (2H, d, *J* = 4.1 Hz, ArC*H*), 7.67 (1H, t, *J* = 4.9 Hz, ArC*H*), 7.60 (2H, t, *J* = 7.4 Hz, ArC*H*), 6.54 (1H, d, *J* = 6.3 Hz, C*H*), 5.92 (1H, d, *J* = 6.3 Hz, C*H*), 4.31 (2H, dd, *J* = 24 Hz, 2 Hz, C*H*), 4.31-4.30 (1H, m, C*H*), 3.24 (1H, d, *J* = 17 Hz, C*H*), 2.77 (1H, d, *J* = 12.9 Hz, C*H*), 1.89-1.14 (10H, m, 5CH_2), 1.54 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 164.8 (*C*=O), 162.1 (*C*=O), 134.7 (Ar*C*), 133.7 (Ar*C*H), 129.5 (Ar*C*H), 127.8 (Ar*C*H), 111.9 (*C*H), 106.3 (*C*H), 59.9 (*C*), 52.8 (*C*H), 49.4 (*C*H_2), 48.5 (*C*H_2), 31.4 (*C*H_2), 30.5 (*C*H_2), 25.5 (*C*H_2), 20.2 (*C*H_3); HRMS (ESI$^+$) (M+H$^+$) found 404.1648 $C_{20}H_{25}N_3O_4S$ requires 404.1639.

9.12.2.12. 2-Cyclohexyl-10b-methyl pyrazino[2,1-a]isoindole-1-6(2H, 10bH)-dione 424



Isolated as a pale solid, following method above using *N*-cyclohexyl-2-(2,2-dimethoxyethyl)-1-methyl-3-oxoisoindoline-1-carboxamide **412** (0.11 g, 0.30 mmol) and formic acid **124** (1.5 mL, 39.5 mmol) for 5 h. The crude compound was purified by flash column chromatography (ethyl acetate: petroleum ether 6: 4, R_f = 0.4) to yield dione **424** (0.08 g, 80% yield); m.p = 159-161 ºC; υ_{max} (ATR/cm^{-1})= 3116, 3052, 2931, 2857, 1707, 1672, 1464; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.00 (1H, d, *J* = 8 Hz, ArC*H*), 7.87 (1H, d, *J* = 7.6 Hz, ArC*H*), 7.87 (1H, t, *J* = 7.6 Hz, ArC*H*), 7.54 (1H, t, *J* = 7.6 Hz, ArC*H*), 6.68 (1H, d, *J* = 5.8 Hz, C*H*), 6.06 (1H, d, *J* = 5.8 Hz, C*H*), 4.44-4.36 (1H, m, C*H*), 1.92-10 (10H, m, 5CH_2), 1.52 (3H, s, CH_3); ^{13}C

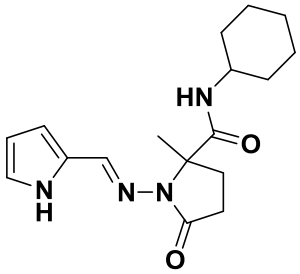
NMR (100 MHz, $CDCl_3$) δ_C = 166.3 (*C*=O), 165.0 (*C*=O), 145.5 (Ar*C*), 132.5 (Ar*C*), 130.1 (Ar*C*H), 128.9 (Ar*C*H), 124.9 (Ar*C*H), 123.7 (Ar*C*H), 113.5 (*C*H), 105.8 (*C*H), 63.5 (*C*), 52.9 (*C*H), 31.6 (CH_2), 30.9 (CH_2), 25.7 (CH_2), 23.9 (CH_3); HRMS (ESI^+) ($M+H^+$) found 297.1594 $C_{18}H_{20}N_2O_2$ requires 297.1598.

9.13. Synthesis of pyrrole-oxopyrrolidine derivatives

9.13.1. General method

To a solution of ketoacids (2.0 mmol) and *tert*-butyl carbazate **428** (2.0 mmol) in methanol (10 mL), solid ammonium chloride NH_4Cl **63** (1.0 mmol) was added and followed by water (2 mL). When a clear solution forms the cyclohexylisocyanide **113** (2.0 mmol) was added and the reaction mixture was stirred at r.t for 48 h. The mixture was then partitioned between ethyl acetate (50 mL) and water (50 mL). The organic phase was separated, dried over sodium sulphate, filtered and concentrated under reduced pressure. The residual product was deprotected by stirring in a solution of HCl (12-14%) in methanol (40 mL) for 3h at r.t (until the Boc group was removed according to TLC check). The resulted was concentrated to form gooey residue which was dissolved in ethanol (10 mL), followed by pyrrole-2-carboxaldehyde **431** (2.0 mmol) and trimethylamine (2.4 mmol). The reaction mixture was then heated at 40 ºC for 4 h, cooled to r.t, silica gel (20 g) added (Bright-dark yellow crude product was absorbed by silica gel). The residual product was concentrated under reduced pressure and purified by column chromatography to obtain the corresponding pyrrole-oxopyrrolidine derivatives.

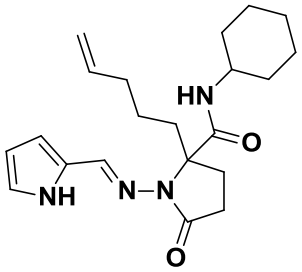
9.13.1.1. (*E*)-1-(((1H-Pyrrol-2-yl) methylene)amino)-*N*-cyclohexyl-2-methyl-5-oxopyro lidine-2-carboxamide 432



Isolated as a dark green solid, following method above using 4-oxopentanoic acid **5** (0.23 g, 2.0 mmol), *tert*-butyl carbazate **428** (0.26 g, 2.0 mmol), ammonium chloride NH_4Cl **63** (0.05 g, 1.0 mmol), cyclohexylisocyanide **113** (0.21 g, 2.0 mmol), pyrrole-2-carboxaldehyde **431** (0.19 g, 2.0 mmol) and trimethylamine (0.25 mL, 2.4 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9.0 : 0.5, R_f = 0.3) to yield pyrrole-oxopyrrolidine **432** (0.17g, 76% yield); m.p = 182-183 ºC; υ_{max} (ATR/cm^{-1})= 3318,

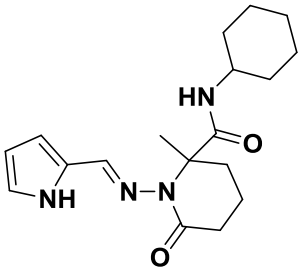
2930, 2855, 1657, 1595; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 9.20 (1H, s, N*H*), 9.12 (1H, s, C*H*), 6.97 (1H, d, *J* = 2.1 Hz, C_4NH_4), 6.62 (1H, dd, *J* = 3.7 Hz, 1.9 Hz, C_4NH_4), 6.41 (1H, d, *J* = 7.6 Hz, N*H*), 6.30 (1H, dd, *J* = 6.1 Hz, 2.6 Hz, C_4NH_4), 3.82-3.73 (1H, m, C*H*), 2.61-2.55 (2H, m, CH_2), 2.48 (2H, t, *J* = 7.6 Hz, CH_2), 1.97-1.10 (10H, m, $5CH_2$), 1.59 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 172.3 (*C*=O), 171.6 (*C*=O), 148.1 (C_3NCH_4), 127.4 (C_3NCH_4), 122.7 (C_3NCH_4), 115.9 (C_3NCH_4), 110.6 (*C*H), 68.4 (*C*), 48.3 (*C*H), 32.9 (CH_2), 32.7 (CH_2), 29.9 (CH_2), 25.4 (CH_2), 24.6 (CH_2), 23.5 (CH_3); HRMS (ESI^+) ($M+H^+$) found 317.1978 $C_{17}H_{24}N_4O_2$ requires 317.1972.

9.13.1.2. (*E*)-1-(((1H-Pyrrol-2-yl) methylene)amino)-*N*-cyclohexyl-5-oxo-2-(pent-4-en-1-yl)pyrrolidine-2-carboxamide 433



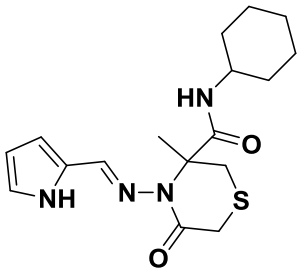
Isolated as a dark green viscous oil, following method above using 4-oxonon-8-enoic acid **179** (0.34 g, 1.0 mmol), *tert*-butyl carbazate **428** (0.26g, 2.0 mmol), ammonium chloride NH_4Cl **63** (0.05 g, 1.0 mmol), cyclohexylisocyanide **113** (0.21g, 2.0 mmol), pyrrole-2-carboxaldehyde **431** (0.19 g, 2.0 mmol) and trimethylamine (0.25 mL, 2.4 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9.0: 0.5, R_f = 0.4) to yield pyrrole-oxopyrrolidine **433** (0.23 g, 70% yield); υ_{max} (ATR/cm^{-1})= 3310, 2930, 2860, 1657, 1603; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 9.22 (1H, s, N*H*), 8.96 (1H, s, C*H*), 7.00 (1H, d, *J* = 3.1 Hz, C_4NH_4), 6.64 (1H, dd, *J* = 5.1 Hz, 1.9 Hz, C_4NH_4), 6.33 (1H, dd, *J* = 5.2 Hz, 1.9 Hz, C_4NH_4), 5.80-5.7 (1H, m, C*H*), 5.0-4.9 (2H, m, CH_2), 3.83-3.76 (1H, m, C*H*), 2.64-2.55 (2H, m, CH_2), 2.48-1.92 (4H, m, $2CH_2$), 1.82-1.97 (4H, m, $2CH_2$), 1.76-1.21 (10H, m, $5CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 171.9 (*C*=O), 171.1 (*C*=O), 148.3 (C_3NCH_4), 138.1 (C_3NCH_4), 127.5 (C_3NCH_4), 122.7 (C_3NCH_4), 115.8 (*C*H), 115.3 (CH_2), 110.7 (*C*H), 71.1 (*C*), 48.1 (*C*H), 35.9 (CH_2), 33.6 (CH_2), 32.8 (CH_2), 29.5 (CH_2), 26.0 (CH_2), 25.7 (CH_2), 24.5 (CH_2), 24.1 (CH_2); HRMS (ESI^+) ($M+H^+$) found 371.2446 $C_{21}H_{30}N_4O_2$ requires 371.2442.

9.13.1.3. (*E*)-1-(((1H-Pyrrol-2-yl) methylene)amino)-*N*-cyclohexyl-2-methyl-6-oxo piperidine-2-carboxamide 434



Isolated as a green solid, following method above using 5-oxohexanoic acid **168** (0.26 g, 2.0 mmol), *tert*-butyl carbazate **428** (0.26 g, 2.0 mmol), ammonium chloride NH_4Cl **63** (0.05 g, 1.0 mmol), cyclohexylisocyanide **113** (0.21 g, 2.0 mmol), pyrrole-2-carboxaldehyde **431** (0.19 g, 2 mmol) and trimethylamine (0.25mL, 2.4 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9.0: 0.5, R_f = 0.4) to yield pyrrole-oxopyrrolidine **434** (0.17 g, 67% yield); m.p = 145-146 ºC; υ_{max} (ATR/cm^{-1})= 3272, 2930, 2856, 1724, 1654; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 9.15 (1H, s, C*H*), 6.97 (1H, d, *J* = 2.1 Hz, C_4NH_4), 6.61 (1H, dd, *J* = 3.9 Hz, 2.4 Hz, C_4NH_4), 6.31 (1H, dd, *J* = 6.3 Hz, 2.5 Hz, C_4NH_4), 3.82-3.73 (1H, m, C*H*), 2.62-2.56 (2H, m, CH_2), 2.48 (2H, t, *J* = 7.6 Hz, CH_2), 1.96-1.83 (2H, m, CH_2), 1.69-1.11 (10H, m, $5CH_2$), 1.58 (3H, s, CH_3); ^{13}CNMR (100 MHz, $CDCl_3$) δ_C = 172.4 (*C*=O), 171.5 (*C*=O), 148.0 (C_3NCH_4), 127.3 (C_3NCH_4), 122.7 (C_3NCH_4), 115.8 (C_3NCH_4), 110.6 (*C*H), 68.3 (*C*), 48.2 (*C*H), 32.9 (CH_2), 32.8 (CH_2), 29.9 (CH_2), 29.2 (CH_2), 25.4 (CH_2), 24.7 (CH_2), 24.5 (CH_2), 23.4 (CH_3); HRMS (ESI^+) ($M+H^+$) found 331.2141 $C_{18}H_{26}N_4O_2$ requires 331. 2129.

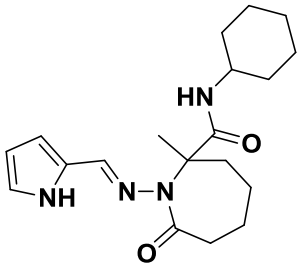
9.13.1.4. (*E*)-4-(((1H-Pyrrol-2-yl)methylene)amino)-*N*-cyclohexyl-3-methyl-5-oxo thiomorpholine-3-carboxamide 435



Isolated as a dark green solid, following method above using 2-((2-oxopropyl)thio) acetic acid **198** (0.29 g, 2.0 mmol), *tert*-butyl carbazate **428** (0.26 g, 2.0 mmol), ammonium chloride NH_4Cl **63** (0.05 g, 1.0 mmol), cyclohexylisocyanide **113** (0.21 g, 2.0 mmol), pyrrole-2-carboxaldehyde **431** (0.19 g, 2.0 mmol) and trimethylamine (0.25 mL, 2.4 mmol). The crude compound was purified bycolumn chromatography (dichloromethane: methanol 9.0: 0.5, R_f = 0.3) to yield pyrrole-oxopyrrolidine **435** (0.18 g, 65% yield), m.p = 156-157 ºC; υ_{max}

(ATR/cm^{-1})= 3315, 2927, 2852, 1732, 1628; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 9.10 (1H, s, C*H*), 6.90 (1H, d, *J* = 2.2 Hz, C_4NH_4), 6.60 (1H, dd, *J* = 4.7 Hz, 1.9 Hz, C_4NH_4), 6.28 (1H, dd, *J* = 5.6 Hz, 1.9 Hz, C_4NH_4), 3.83-3.74 (1H, m, C*H*), 2.58 (2H, dd, *J* = 12.2 Hz, 6.7 Hz, CH_2), 2.48 (2H, dd, *J* = 9.3 Hz, 6.1 Hz, CH_2), 1.69-1.12 (10H, m, 5CH_2), 1.59 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 172.3 (*C*=O), 171.6 (*C*=O), 148.1 (C_3NCH_4), 127.4 (C_3NCH_4), 122.6 (C_3NCH_4), 115.9 (C_3NCH_4), 110.5 (*C*H), 68.4 (*C*), 48.3 (*C*H), 32.8 (*C*H_2), 32.7 (*C*H_2), 29.8 (*C*H_2), 25.3 (*C*H_2), 24.6 (*C*H_2), 23.5 (*C*H_3); HRMS (ESI$^+$) (M+H$^+$) found 349.1698 $C_{17}H_{24}N_4O_2S$ requires 349.1693.

9.13.1.5. (*E*)-1-(((1H-Pyrrol-2-yl) methylene)amino)-*N*-cyclohexyl-2-methyl-7-oxo azepane-2-carboxamide 436



Isolated as a green solid, following method above using 6-oxo-heptanoic acid **32** (0.28 g, 2.0 mmol), *tert*-butyl carbazate **428** (0.26 g, 2.0 mmol), ammonium chloride NH_4Cl **63** (0.05 g, 1.0 mmol), cyclohexylisocyanide **113** (0.21g, 2.0 mmol), pyrrole-2-carboxaldehyde **431** (0.19 g, 2.0 mmol) and trimethylamine (0.25 mL, 2.4 mmol). The crude compound was purified by column chromatography (dichloromethane: methanol 9.0: 0.5, R_f = 0.3) to yield pyrrole-oxopyrrolidine **436** (0.12 g, 45% yield); m.p = 92-93 ºC; υ_{max} (ATR/cm^{-1})= 3273, 2935, 2858, 1713, 1636; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 8.37 (1H, s, C*H*), 7.02 (1H, d, *J* = 2.2 Hz, C_4NH_4), 6.66 (1H, dd, *J* = 3.5 Hz, 1.9 Hz, C_4NH_4), 6.31 (1H, t, *J* = 3.0 Hz, C_4NH_4), 4.18-4.08 (1H, m, C*H*), 2.50-2.45 (2H, m, CH_2), 2.41-2.37 (4H, m, 4CH_2), 2.16 (3H, s, CH_3), 1.83-1.16 (12H, m, 6CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 178.1 (*C*=O), 172.5 (*C*=O), 150.7 (C_3NCH_4), 127.0 (C_3NCH_4), 123.7 (C_3NCH_4), 117.5 (C_3NCH_4), 110.5 (*C*H), 60.9 (*C*), 48.2 (*C*H), 43.2 (*C*H_2), 33.8 (*C*H_2), 33.1 (*C*H_2), 29.9 (*C*H_3), 29.3 (*C*H_2), 25.5 (*C*H_2), 24.7 (*C*H_2), 24.2 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 345.2293 $C_{19}H_{28}N_4O_2$ requires 345.2285.

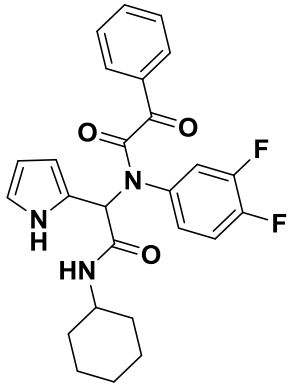
9.14. Synthesis of pyrrolo-pyridine derivatives

9.14.1. General synthesis of pyrrol-2-yl-oxo-acetamides (Ugi adducts)[355]

To a solution pyrrole-2-carbaldehyde **431** (3.0 mmol), substituted anilines (3.0 mmol) and keto acids (3.0 mmol) in methanol (6 mL), cyclohexylisocyaine **113** (3.0 mmol) was added at

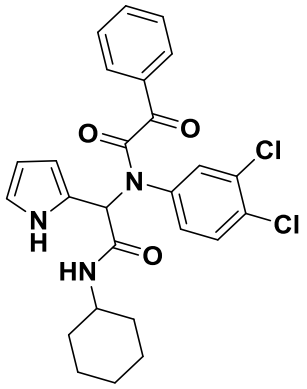
once at room temperature. The reaction mixture was then stirred at room temperature for 24 hours. The reaction mixture was concentrated under reduced pressure and the residual mixture was purified by column chromatography to obtain the titled pyrrol-2-yl-oxo-acetamides (Ugi adducts).

9.14.1.1. *N*-(2-(Cyclohexylamino)-2-oxo-1-(1H-pyrrol-2-yl) ethyl)-*N*-(3,4-difluoro phenyl)-2-oxo-2-phenylacetamide 451



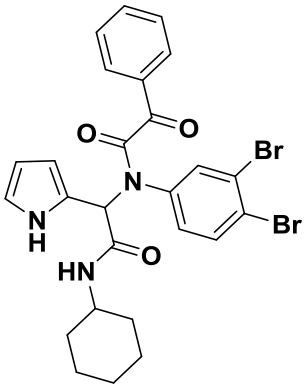
Isolated as a dark brown solid, following method above using pyrrole-2-carbaldehyde **431** (0.28 g, 3 mmol), 3, 4-diflouroaniline **445** (0.38 g, 3 mmol), benzoylformic acid **169** (0.54 g, 3 mmol) and cyclohexylisocyanide **113** (0.32 g, 3.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3:7, R_f = 0.3) to yield Ugi product **451** (0.16 g, 59% yield); m.p = 191-192 ºC; υ_{max} (ATR/cm^{-1})= 3344, 3069, 2930, 2859, 1675, 1602; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 9.56 (1H, s, N*H*), 8.02 (2H, d, *J* = 7.1 Hz, ArC*H*), 7.61 (1H, t, *J* = 7.4 Hz, ArC*H*), 7.51 (1H, t, *J* = 7.9 Hz, ArC*H*), 7.15-7.10 (1H, m, ArC*H)*, 7.01-6.98 (2H, m, ArC*H*), 6.75 (1H, s, ArC*H*), 6.16-6.10 (1H, m, C_4NH_4), 6.12 (1H, dd, *J* = 5.9 Hz, 2.8 Hz, C_4NH_4), 5.87 (1H, d, *J* = 8.1 Hz, C_4NH_4), 5.71 (1H, s, C*H*), 3.93-3.84 (1H, m, C*H*), 1.97-1.12 (10H, m, $5CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 190.1 (*C*=O), 168.2 (*C*=O), 167.0 (*C*=O), 151.5, 150.1 (dd, J_{C-F} = 242, 13.5 Hz, Ar*C*F), 148.5 (dd, J_{C-F} = 240, 13.0 Hz, Ar*C*F), 135.0 (dd, J_{C-F} = 17.0 Hz, Ar*C*H), 132.8 (Ar*C*), 129.4 (Ar*C*H), 128.6 (Ar*C*H), 126.4 (Ar*C*H), 124.2 (Ar*C*H), 122.7 (C_3NCH_4), 119.0 (C_3NCH_4), 117.0 (Ar*C*H), 111.7 (C_3NCH_4), 109.0 (C_3NCH_4), 61.5 (*C*H), 48.9 (*C*H), 32.7 (CH_2), 25.1 (CH_2), 24.3 (CH_2); HRMS (ESI$^+$) (M+H$^+$) found 466.1943 $C_{26}H_{25}F_2N_3O_3$ requires 466.1937.

9.14.1.2. *N*-(2-(Cyclohexylamino)-2-oxo-1-(1H-pyrrol-2-yl) ethyl)-*N*-(3,4-dichloro phenyl)-2-oxo-2-phenylacetamide 452



Isolated as a pink solid, following method above using pyrrole-2-carbaldehyde **431** (0.28 g, 3.0 mmol), 3,4-dichloroaniline **446** (0.486 g, 3.0 mmol), benzoylformic acid **169** (0.54 g, 3.0 mmol) and cyclohexylisocyanide **113** (0.32 g, 3.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3:7, R_f = 0.4) to yield Ugi product **452** (0.23 g, 81% yield); m.p = 248-249 ºC; υ_{max} (ATR/cm^{-1})= 3350, 3062, 2928, 2856, 1675, 1595; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 9.48 (1H, s, N*H*), 8.03 (2H, d, *J* = 7.7 Hz, ArC*H*), 7.63 (1H, t, *J* = 4.1 Hz, ArC*H*), 7.09 (1H, dd, *J* = 8.5 Hz, 2.4 Hz, ArC*H*), 7.01-6.99 (1H, m, ArC*H*), 6.83 (1H, s, ArC*H*), 6.24-6.22 (1H, m, C_4NH_4), 6.18 (1H, dd, *J* = 5.9 Hz, 2.8 Hz, C_4NH_4), 5.72 (1H, d, *J* = 5.3 Hz, C_4NH_4), 5.50 (1H, s, C*H*), 3.93-3.84 (1H, m, C*H*), 1.95-1.11 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 189.6 (*C*=O), 167.7 (*C*=O), 166.7 (*C*=O), 139.9 (Ar*C*), 134.8 (Ar*C*H), 133.3 (Ar*C*), 133.3 (Ar*C*), 131.3 (Ar*C*H), 129.4 (Ar*C*H), 129.0 (Ar*C*H), 128.6 (Ar*C*H), 124.3 (C_3NCH_4), 123.0 (Ar*C*), 120.3 (C_3NCH_4), 117.0 (Ar*C*H), 111.7 (C_3NCH_4), 108.5 (C_3NCH_4), 62.2 (*C*H), 48.9 (*C*H), 33.0 (*C*H_2), 25.3 (*C*H_2), 24.3 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 498.1000 $C_{26}H_{25}Cl_2N_3O_3$ requires 498.1000.

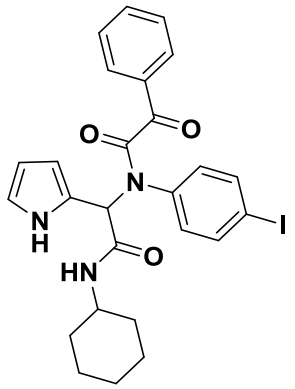
9.14.1.3. ***N*-(2-(Cyclohexylamino)-2-oxo-1-(1H-pyrrol-2-yl) ethyl)-*N*-(3,4-dibromo phenyl)-2-oxo-2-phenylacetamide 453**



Isolated as a pink solid, following method above using pyrrole-2-carbaldehyde **431** (0.28 g, 3.0 mmol), 3,4-dibromoaniline **447** (0.75 g, 3.0 mmol), benzoylformic acid **169** (0.54 g, 3.0 mmol) and cyclohexylisocyanide **113** (0.32 g, 3.0 mmol). The crude compound was purified

by column chromatography (ethyl acetate: petroleum ether 3:7, R_f = 0.4) to yield Ugi product **453** (0.24 g, 85% yield); m.p = 237-238 ºC; υ_{max} (ATR/cm^{-1})= 3316, 3061, 2935, 2852, 1680, 1582; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 9.49 (1H, s, N*H*), 8.03 (2H, d, *J* = 7.1 Hz, ArC*H*), 7.62 (1H, t, *J* = 7.4 Hz, ArC*H*), 7.53-7.49 (2H, m, ArC*H*), 7.05 (1H, dd, *J* = 8.5 Hz, 2.3 Hz, ArC*H*), 7.02-6.99 (1H, m, ArC*H*), 6.82 (1H, s, ArC*H*), 6.23-6.21 (1H, m, C_4NH_4), 6.18 (1H, dd, *J* = 5.8 Hz, 2.8 Hz, C_4NH_4), 5.73 (1H, d, *J* = 7.9 Hz, C_4NH_4), 5.50 (1H, s, C*H*), 3.92-3.83 (1H, m, C*H*), 1.95-1.11 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 189.6 (*C*=O), 168.1 (*C*=O), 166.2 (*C*=O), 139.4 (Ar*C*), 135.0 (Ar*C*H), 134.8 (Ar*C*H), 133.1 (Ar*C*), 129.9 (Ar*C*H), 129.8 (Ar*C*H), 129.2 (Ar*C*H), 128.6 (Ar*C*H), 125.7 (Ar*C*), 125.0 (Ar*C*), 123.2 (C_3NCH_4), 120.6 (C_3NCH_4), 111.9 (C_3NCH_4), 108.4 (C_3NCH_4), 62.2 (*C*H), 48.9 (*C*H), 32.5 (*C*H_2), 25.1 (*C*H_2), 24.3 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 608.0134 $C_{26}H_{25}Br_2N_3O_3Na$ requires 608.0160.

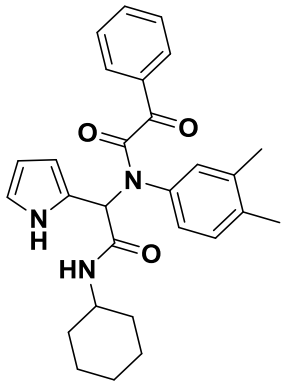
9.14.1.4. ***N*-(2-(Cyclohexylamino)-2-oxo-1-(1H-pyrrol-2-yl) ethyl)-*N*-(4-iodophenyl)-2-oxo-2-phenylacetamide 454**



Isolated as a white solid, following method above using pyrrole-2-carbaldehyde **431** (0.28 g, 3.0 mmol), 4-iodoaniline **448** (0.65 g, 3.0 mmol), benzoylformic acid **169** (0.54 g, 3.0 mmol) and cyclohexylisocyanide **113** (0.32 g, 3.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3:7, R_f = 0.4) to yield Ugi product **454** (0.18 g, 66% yield); m.p = 213-214 ºC; υ_{max} (ATR/cm^{-1})= 3352, 3064, 2930, 2852, 1642, 1517; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 9.55 (1H, s, N*H*), 8.02 (1H, d, *J* = 7.5 Hz, ArC*H*), 7.60 (1H, t, *J* = 7.3 Hz, ArC*H*), 7.48 (4H, t, *J* = 7.8 Hz, ArC*H*), 6.95 (2H, d, *J* = 8.4 Hz, ArC*H*), 6.77 (1H, s, ArC*H*), 6.18-6.15 (1H, m, C_4NH_4), 6.13 (1H, dd, *J* = 5.6Hz, 2.8 Hz, C_4NH_4), 5.81 (1H, d, *J* = 8.0 Hz, C_4NH_4), 5.61 (1H, s, C*H*), 3.91-3.84 (1H, m, C*H*), 1.95-1.11 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 189.9 (*C*=O), 168.1 (*C*=O), 166.8 (*C*=O), 138.9 (Ar*C*), 138.3 (Ar*C*H), 134.8 (Ar*C*H), 133.0 (Ar*C*), 131.1 (Ar*C*H), 129.9 (Ar*C*H), 128.9 (Ar*C*H), 123.5 (C_3NCH_4), 120.4 (C_3NCH_4), 111.8 (C_3NCH_4), 108.3 (C_3NCH_4), 94.9 (Ar*C*),

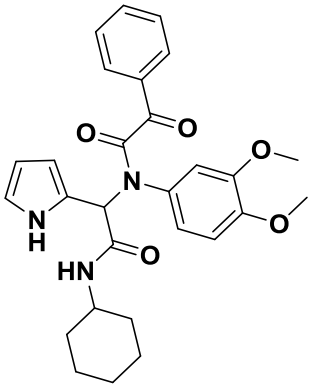
61.9 (*C*H), 49.2 (*C*H), 32.7 (*C*H_2), 25.4 (*C*H_2), 24.7 (*C*H_2); HRMS (ESI^+) ($M+H^+$) found 578.0919 $C_{26}H_{26}IN_3O_3Na$ requires 578.0911.

9.14.1.5. ***N*-(2-(Cyclohexylamino)-2-oxo-1-(1H-pyrrol-2-yl) ethyl)-*N*-(3,4-dimethyl phenyl)-2-oxo-2-phenylacetamide 455**



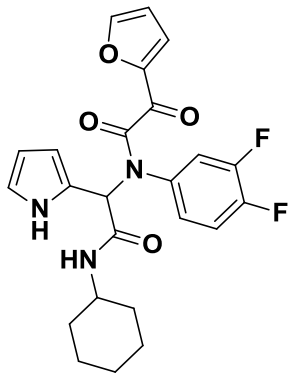
Isolated as a black solid, following method above using pyrrole-2-carbaldehyde **431** (0.28 g, 3.0 mmol), 3,4-dimethylaniline **449** (0.36 g, 3.0 mmol), benzoylformic acid **169** (0.54 g, 3.0 mmol) and cyclohexylisocyanide **113** (0.32 g, 3.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3:7, R_f = 0.3) to yield Ugi product **455** (0.18 g, 72% yield); m.p = 135-136 ºC; υ_{max} (ATR/cm^{-1})= 3316, 3064, 2932, 2850, 1675, 1637; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 9.63 (1H, s, N*H*), 8.06 (2H, d, *J* = 7.5 Hz, ArC*H*), 7.58 (1H, t, *J* = 7.4 Hz, ArC*H*), 7.48 (2H, t, *J* = 7.6 Hz, ArC*H*), 7.00 (1H, s, ArC*H*), 6.95-6.82 (2H, m, ArC*H*), 6.23-6.21 (1H, m, C_4NH_4), 6.17 (1H, dd, *J* = 5.9 Hz, 2.8 Hz, C_4NH_4), 5.67 (1H, d, *J* = 8.4 Hz, C_4NH_4), 5.40 (1H, s, C*H*), 3.91-3.87 (1H, m, C*H*), 2.13 (3H, s, CH_3), 2.06 (3H, s, CH_3), 1.97-1.12 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 190.3 (*C*=O), 168.5 (*C*=O), 166.8 (*C*=O), 137.8 (Ar*C*), 137.4 (Ar*C*), 134.4 (Ar*C*H), 133.4 (Ar*C*), 130.2 (Ar*C*H), 129.9 (Ar*C*H), 129.5 (Ar*C*H), 128.8 (Ar*C*H), 125.9 (Ar*C*H), 124.5 (C_3NCH_4), 120.2 (C_3NCH_4), 111.5 (C_3NCH_4), 108.1 (C_3NCH_4), 63.5 (*C*H), 48.9 (*C*H), 32.8 (*C*H_2), 25.4 (*C*H_2), 24.7 (*C*H_2) 19.6 (*C*H_3), 19.4 (*C*H_3); HRMS (ESI^+) ($M+H^+$) found 458.2448 $C_{28}H_{31}N_3O_3$ requires 458.2438.

9.14.1.6. ***N*-(2-(Cyclohexylamino)-2-oxo-1-(1H-pyrrol-2-yl) ethyl)-*N*-(3,4-dimethoxy phenyl)-2-oxo-2-phenylacetamide 456**



Isolated as a dark red solid following method above using pyrrole-2-carbaldehyde **431** (0.28 g, 3.0 mmol), 3,4-dimethoxyaniline **450** (0.45 g, 3.0 mmol), benzoylformic acid **169** (0.54 g, 3.0 mmol) and cyclohexylisocyanide **113** (0.32 g, 3.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3:7, R_f = 0.2) to yield Ugi product **456** (0.15 g, 55% yield); m.p = 120-122 ºC; υ_{max} (ATR/cm^{-1})= 3350, 3062, 2928, 2854, 1684, 1595; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 9.63 (1H, s, N*H*), 8.00 (2H, d, *J* = 7.2 Hz, ArC*H*), 7.51 (1H, t, *J* = 7.4 Hz, ArC*H*), 7.41 (2H, t, *J* = 7.6 Hz, ArC*H*), 6.71-6.65 (2H, m, ArC*H*), 6.60 (1H, s, ArC*H*), 6.50 (1H, d, *J* = 8.6 Hz, C_4NH_4), 6.09-6.08 (1H, m, C_4NH_4), 6.04 (1H, dd, *J* = 5.8 Hz, 2.7 Hz, C_4NH_4), 5.80 (1H, s, C*H*), 3.85-3.80 (1H, m, C*H*), 3.67 (3H, s, OCH_3), 3.49 (3H, s, OCH_3), 1.88-1.08 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 190.4 (*C*=O), 168.4 (*C*=O), 167.2 (*C*=O), 148.9 (Ar*C*), 148.5 (Ar*C*), 134.4 (Ar*C*H), 133.3 (Ar*C*), 130.9 (Ar*C*), 129.8 (Ar*C*H), 128.8 (Ar*C*H), 123.7 (*C*), 122.3 (Ar*C*H), 119.9 (Ar*C*H), 112.6 (Ar*C*H), 111.3 (C_3NCH_4), 110.3 (C_3NCH_4), 108.2 (C_3NCH_4), 61.0 (*C*H), 55.7 (O*C*H$_3$), 55.7 (O*C*H$_3$), 48.9 (*C*H), 32.7 (*C*H$_2$), 25.4 (*C*H$_2$), 24.7 (*C*H$_2$); HRMS (ESI$^+$) (M+H$^+$) found 490.234 $C_{28}H_{31}N_3O_5$ requires 490.2336.

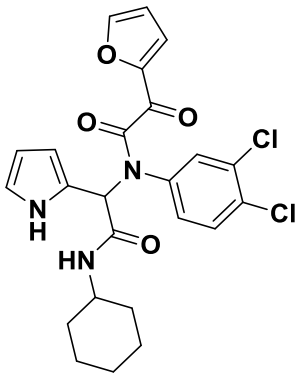
9.14.1.7. ***N*-(2-(Cyclohexylamino)-2-oxo-1-(1H-pyrrol-2-yl) ethyl)-*N*-(3,4-difluoro phenyl)-2-(furan-2-yl)-2-oxoacetamide 457**



Isolated as a brown solid, following method above using pyrrole-2-carbaldehyde **431** (0.28 g, 3.0 mmol), 3,4-diflouroaniline **445** (0.38 g, 3.0 mmol), (2-furyl) glyoxylic acid **201** (0.42 g,

3.0 mmol) and cyclohexylisocyanide **113** (0.32 g, 3.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3:7, R_f = 0.3) to yield Ugi product **457** (0.15 g, 55% yield); m.p = 182-183 ºC; υ_{max} (ATR/cm^{-1})= 3316, 3062, 2932, 2859, 1660, 1512; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 9.54 (1H, s, N*H*), 7.65 (2H, d, *J* = 4.4 Hz, ArC*H*), 7.12 (1H, t, *J* = 8.8 Hz, C_4OH_3), 6.97-6.89 (1H, s, ArC*H)*, 6.72 (1H, dd, *J* = 3.9 Hz, 2.51 Hz, C_4OH_3), 6.58 (1H, dd, *J* = 3.6 Hz, 1.6 Hz, C_4OH_3), 6.11-6.10 (1H, m, C_4NH_4), 6.08 (1H, dd, *J* = 5.8 Hz, 2.8 Hz, C_4NH_4), 5.93 (1H, d, *J* = 7.9 Hz, C_4NH_4), 5.74 (1H, s, C*H*), 3.87-3.79 (1H, m, C*H*), 1.93-1.10 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176.8 (*C*=O), 166.8 (*C*=O), 166.5 (*C*=O), 151.7, 150.0 (dd, $J_{C\text{-}F}$ = 241, 12.0 Hz, Ar*C*F), 149.6 (C_3OCH_3), 149.3 (C_3OCH_3), 149.2 (dd, $J_{C\text{-}F}$ = 240, 13.0 Hz, Ar*C*F), 148.5 (C_3OCH_3), 126.2 (dd, $J_{C\text{-}F}$ = 11.5 Hz, Ar*C*H), 124.2 (C_3NCH_4), 122.8 (C_3OCH_3), 120.3 (Ar*C*H), 126.4 (Ar*C*H), 118.8 (Ar*C*H), 117.4 (C_3NCH_4), 111.8 (C_3NCH_4), 108.5 (C_3NCH_4), 61.6 (*C*H), 49.2 (*C*H), 32.7 (*C*H_2), 25.4 (*C*H_2), 24.7 (*C*H_2); HRMS (ESI$^+$) (M+H$^+$) found 456.1741 $C_{24}H_{23}F_2N_3O_4$ requires 456.1729.

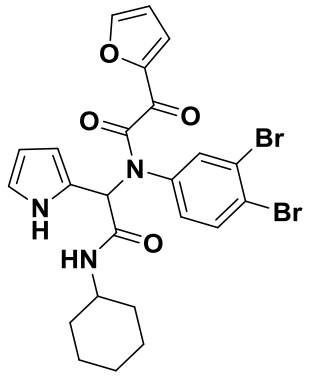
9.14.1.8. ***N*-(2-(Cyclohexylamino)-2-oxo-1-(1H-pyrrol-2-yl) ethyl)-*N*-(3,4-dichloro phenyl)-2-(furan-2-yl)-2-oxoacetamide 458**



Isolated as a white solid, following method above using pyrrole-2-carbaldehyde **431** (0.28 g, 3.0 mmol), 3,4-dichloroaniline **446** (0.486 g, 3.0 mmol), (2-furyl) glyoxylic acid **201** (0.42 g, 3.0 mmol) and cyclohexylisocyanide **113** (0.32 g, 3.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3:7, R_f = 0.4) to yield Ugi product **458** (0.16 g, 59% yield), m.p = 197-199 ºC; υ_{max} (ATR/cm^{-1})= 3341, 3127, 2930, 2852, 1655, 1519; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 9.45 (1H, s, N*H*), 7.67 (2H, d, *J* = 1.7 Hz, ArC*H*), 7.39 (1H, d, *J* = 2.3 Hz, C_4OH_3), 7.08 (1H, dd, *J* = 8.5 Hz, 2.3 Hz, C_4OH_3), 6.81 (1H, s, ArC*H*), 6.61 (1H, dd, *J* = 3.5 Hz, 1.4 Hz, C_4OH_3), 6.61-6.21 (1H, m, C_4NH_4), 6.17 (1H, dd, *J* = 5.9 Hz, 2.80 Hz, C_4NH_4), 5.73 (1H, d, *J* = 7.8 Hz, C_4NH_4), 5.48 (1H, s, C*H*), 3.92-3.80 (1H, m, C*H*), 1.95-1.11 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 187.7

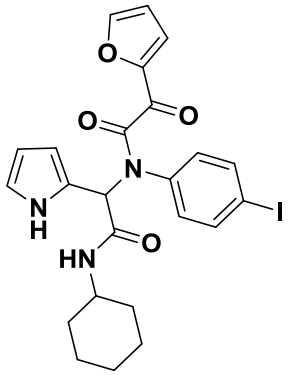
(*C*=O), 176.5 (*C*=O), 166.4 (*C*=O), 149.2 (C_3OCH_3), 138.7 (C_3OCH_3), 133.4 (Ar*C*), 133.1 (Ar*C*), 130.8 (Ar*C*H), 130.8 (Ar*C*H), 128.6 (C_3OCH_3), 124.3 (C_3NCH_4), 120.5 (C_3NCH_4), 113.2 (Ar*C*H), 111.9 (C_3NCH_4), 108.8 (C_3NCH_4), 62.5 (*C*H), 49.1 (*C*H), 32.7 (CH_2), 25.4 (CH_2), 24.6 (CH_2); HRMS (ESI^+) ($M+H^+$) found 488.1121 $C_{24}H_{23}Cl_2N_3O_4$ requires 488.1138.

9.14.1.9. ***N*-(2-(Cyclohexylamino)-2-oxo-1-(1H-pyrrol-2-yl) ethyl)-*N*-(3,4-dibromo phenyl)-2-(furan-2-yl)-2-oxoacetamide 459**



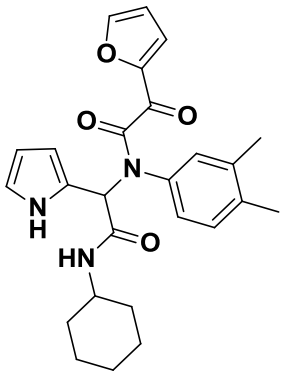
Isolated as a grey solid, following method above using pyrrole-2-carbaldehyde **431** (0.28 g, 3.0 mmol), 3,4-dibromoaniline **447** (0.75 g, 3.0 mmol), (2-furyl) glyoxylic acid **201** (0.42 g, 3.0 mmol) and cyclohexylisocyanide **113** (0.32 g, 3.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3:7, R_f = 0.4) to yield Ugi product **459** (0.22 g, 80% yield); m.p = 183-184 ºC; υ_{max} (ATR/cm^{-1})= 3321, 3131, 2928, 2850, 1662, 1519; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 9.47 (1H, s, N*H*), 7.67 (2H, d, *J* = 2.0 Hz, ArC*H*), 7.52 (1H, d, *J* = 2.6 Hz, C_4OH_3), 7.47 (1H, d, *J* = 9.7 Hz, C_4OH_3), 7.04 (1H, dd, *J* = 8.6 Hz, 2.4 Hz, C_4OH_3), 6.81 (1H, s, ArC*H*), 6.21-6.19 (1H, m, C_4NH_4), 6.16 (1H, dd, *J* = 5.8 Hz, 2.8 Hz, C_4NH_4), 5.77 (1H, d, *J* = 7.8 Hz, C_4NH_4), 5.31 (1H, s, C*H*), 3.87-3.80 (1H, m, C*H*), 1.94-1.10 (10H, m, $5CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176.5 (*C*=O), 166.5 (*C*=O), 166.4 (*C*=O), 149.6 (C_3OCH_3), 149.2 (C_3OCH_3), 139.3 (Ar*C*), 133.7 (Ar*C*), 129.2 (Ar*C*H), 125.8 (C_3NCH_4), 125.2 (Ar*C*), 124.3 (Ar*C*H), 122.9 (C_3NCH_4), 120.5 (C_3OCH_3), 113.2 (C_3OCH_3), 112.1 (C_3NCH_4), 108.5 (C_3NCH_4), 62.4 (*C*H), 49.3 (*C*H), 32.7 (CH_2), 25.5 (CH_2), 24.7 (CH_2); HRMS (ESI^+) ($M+H^+$) found 599.9939 $C_{24}H_{23}Br_2N_3O_4Na$ requires 599.9929.

9.14.1.10. ***N*-(2-(Cyclohexylamino)-2-oxo-1-(1H-pyrrol-2-yl) ethyl)-2-(furan-2-yl)-*N*-(4-iodophenyl)-2-oxoacetamide 460**



Isolated as a grey solid, following method above using pyrrole-2-carbaldehyde **431** (0.28 g, 3.0 mmol), 4-iodoaniline **448** (0.65 g, 3.0 mmol), (2-furyl) glyoxylic acid **201** (0.42 g, 3.0 mmol) and cyclohexylisocyanide **113** (0.32 g, 3.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3:7, R_f = 0.4) to yield Ugi product **460** (0.15 g, 56% yield), m.p = 212-213 ºC; υ_{max} (ATR/cm^{-1})= 3348, 3133, 2937, 2852, 1646, 1519; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 9.49 (1H, s, N*H*), 7.67 (2H, d, *J* = 3.4 Hz, ArC*H*), 7.53 (1H, d, *J* = 8.9 Hz, C_4OH_3), 6.96 (1H, d, *J* = 8.4 Hz, C_4OH_3), 6.79 (1H, s, ArC*H*), 6.58 (1H, dd, *J* = 3.5 Hz, 1.5 Hz, C_4OH_3), 6.19-6.17 (1H, m, C_4NH_4), 6.14 (1H, dd, *J* = 5.7 Hz, 2.7 Hz, C_4NH_4), 5.77 (1H, d, *J* = 7.9 Hz, C_4NH_4), 5.52 (1H, s, C*H*), 3.88-3.79 (1H, m, C*H*), 1.94-1.10 (10H, m, $5CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 176.81 (*C*=O), 166.71 (*C*=O), 166.5 (*C*=O), 149.6 (C_3OCH_3), 148.9 (C_3OCH_3), 139.1 (Ar*C*), 138.5 (Ar*C*H), 130.8 (Ar*C*H), 124.2 (C_3NCH_4), 123.3 (C_3NCH_4), 120.5 (C_3OCH_3), 113.3 (C_3OCH_3), 111.9 (C_3NCH_4), 108.4 (C_3NCH_4), 94.9 (Ar*C*), 62.3 (*C*H), 49.1 (*C*H), 32.7 (CH_2), 25.4 (CH_2), 24.6 (CH_2); HRMS (ESI$^+$) ($M+H^+$) found 568.0706 $C_{24}H_{24}IN_3O_4Na$ requires 568.0704.

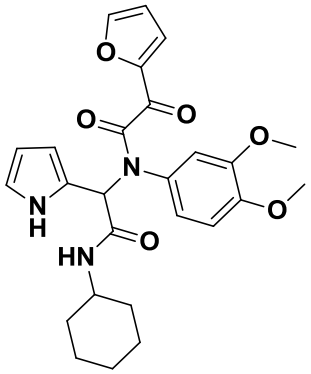
9.14.1.11. ***N*-(2-(Cyclohexylamino)-2-oxo-1-(1H-pyrrol-2-yl) ethyl)-*N*-(3,4-dimethyl phenyl)-2-(furan-2-yl)-2-oxoacetamide 461**



Isolated as a brown solid, following method above using pyrrole-2-carbaldehyde **431** (0.28 g, 3.0 mmol), 3,4-dimethylaniline **449** (0.36 g, 3.0 mmol), (2-furyl) glyoxylic acid **201** (0.42 g, 3.0 mmol) and cyclohexylisocyanide **113** (0.32 g, 3.0 mmol). The crude compound was

purified by column chromatography (ethyl acetate: petroleum ether 3:7, R_f = 0.3) to yield Ugi product **461** (0.18 g, 65% yield); m.p = 177-178 ºC; υ_{max} (ATR/cm^{-1})= 3350, 3124, 2928, 2856, 1666, 1644; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 9.61 (1H, s, N*H*), 7.74 (1H, d, *J* = 3.9 Hz, C_4OH_3), 7.66 (1H, d, *J* = 2.0 Hz, C_4OH_3), 6.99 (1H, m, ArC*H*), 6.94 (1H, d, *J* = 1.1 Hz, C_4OH_3), 6.79 (1H, s, ArC*H*), 6.55 (1H, dd, *J* = 3.6 Hz, 1.7 Hz, ArC*H*), 6.18-6.12 (1H, m, C_4NH_4), 6.13 (1H, dd, *J* = 5.8 Hz, 2.8 Hz, C_4NH_4), 5.71 (1H, d, *J* = 8.1 Hz, C_4NH_4), 5.41 (1H, s, C*H*), 3.89-3.80 (1H, m, C*H*), 2.14 (3H, s, CH_3), 2.09 (3H, s, CH_3), 1.95-1.11 (10H, m, $5CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C= 177.3 (*C*=O), 166.9 (*C*=O), 166.8 (*C*=O), 149.8 (C_3OCH_3), 148.5 (C_3OCH_3), 137.9 (Ar*C*), 137.5 (Ar*C*H), 137.3 (Ar*C*), 130.3 (Ar*C*H), 129.4 (Ar*C*), 125.8 (Ar*C*H), 124.1 (C_3NCH_4), 123.9 (C_3OCH_3), 120.2 (C_3OCH_3), 112.9 (C_3NCH_4), 111.6 (C_3NCH_4), 108.1 (C_3NCH_4), 63.1 (*C*H), 49.2 (*C*H), 32.6 (CH_2), 24.6 (CH_2), 24.6 (CH_2), 19.6 (CH_3), 19.42 (CH_3); HRMS (ESI$^+$) (M+H$^+$) found 448.2249 $C_{26}H_{29}N_3O_4$ requires 448.2231.

9.14.1.12. ***N*-(2-(Cyclohexylamino)-2-oxo-1-(1H-pyrrol-2-yl) ethyl)-*N*-(3,4-dimethoxy phenyl)-2-(furan-2-yl)-2-oxoacetamide 462**



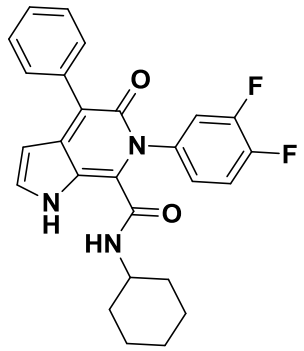
Isolated as a brown solid following method above using pyrrole-2-carbaldehyde **431** (0.28 g, 3.0 mmol), 3,4-dimethoxyaniline **450** (0.45 g, 3.0 mmol), (2-furyl) glyoxylic acid **201** (0.42 g, 3.0 mmol) and cyclohexylisocyanide **113** (0.32 g, 3.0 mmol). The crude compound was purified by column chromatography (ethyl acetate: petroleum ether 3:7, R_f = 0.2) to yield Ugi product **462** (0.12 g, 45% yield), m.p = 235-234 ºC; υ_{max} (ATR/cm^{-1})= 3350, 3127, 2935, 2856, 1673, 1651; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H = 9.54 (1H, s, N*H*), 7.64 (1H, d, *J* = 3.4 Hz, C_4OH_3), 6.74-6.72 (2H, m, ArC*H*), 6.64 (1H, s, ArC*H*), 6.59 (1H, d, *J* = 8.6 Hz, C_4OH_3), 6.53 (1H, dd, *J* = 3.6 Hz, 1.7 Hz, C_4OH_3), 6.18-6.14 (1H, m, C_4NH_4), 6.09 (1H, dd, *J* = 5.9 Hz, 2.8 Hz, C_4NH_4), 5.90 (1H, d, *J* = 8.0 Hz, C_4NH_4), 5.64 (1H, s, C*H*), 3.87-3.80 (1H, m, C*H*), 3.76 (3H, s, OCH_3), 3.62 (3H, s, OCH_3), 1.92-1.13 (10H, m, $5CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C = 177.4 (*C*=O), 167.0 (*C*=O), 166.8 (*C*=O), 149.7 (C_3OCH_3), 149.8 (Ar*C*),

149.1 (Ar*C*), 148.5 (C_3OCH_3), 131.4 (C_3NCH_4), 121.8 (C_3OCH_3), 120.1 (Ar*C*H), 112.9 (C_3NCH_4), 112.1 (Ar*C*H), 111.5 (C_3NCH_4), 110.4 (Ar*C*H), 108.2 (C_3NCH_4), 63.6 (*C*H), 55.8 (OCH_3), 55.7 (OCH_3), 48.9 (*C*H), 32.7 (CH_2), 25.4 (CH_2), 24.6 (CH_2); HRMS (ESI^+) ($M+H^+$) found 480.2146 $C_{26}H_{29}N_3O_6$ requires 480.2129.

9.14.2. Synthesis of pyrrolo-pyridine derivatives

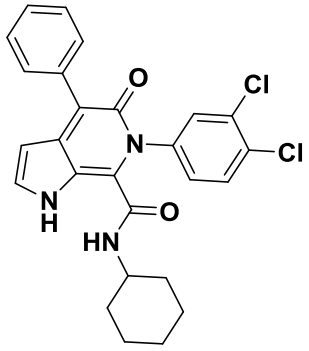
To a solution of pyrrol-2-yl-oxo-acetamides (2.0 mmol) in methanol (10 mL), *para*-toluene sulfonic acid (PTSA) (40 mol%) was added and the reaction mixture was heated at 60 ºC for 2 h. The solvent was removed under reduced pressure and water (20 mL) was added to the mixture. The yellow solid was precipitated, filtered and washed with water to remove all PTSA. The yellow-green solid was then purified by column chromatography to obtain the titled pyrrolo-pyridine derivatives.

9.14.2.1. *N*-Cyclohexyl-6-(3,4-difluorophenyl)-5-oxo-4-phenyl-5,6-dihydro-1H-pyrrolo [2, 3-c] pyridine-7-carboxamide 463



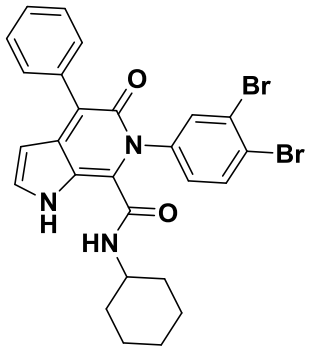
Isolated as a dark green solid, following method above using Ugi adduct **451** (0.447 g, 1.0 mmol) and PTSA (0.08 g, 40 mol%). The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.3) to yield pyrrolo-pyridine **463** (0.28 g, 64% yield); m.p = 196-197 ºC; υ_{max} (ATR/cm^{-1})= 3312, 3071, 2926, 2852, 1635, 1528; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H = 10.97 (1H, s, N*H*), 8.69 (1H, d, *J* = 16.7 Hz, ArC*H*), 8.31 (1H, s, N*H*), 7.65-7.19 (7H, m, ArC*H*), 6.82 (1H, d, *J* = 4.1 Hz, $C_7ON_2H_3$), 6.16 (1H, dd, *J* = 3.0 Hz, 1.7 Hz, $C_7ON_2H_3$), 3.56-3.48 (1H, m, C*H*), 1.59-0.98 (10H, m, $5CH_2$); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C = 159.5 (*C*=O), 157.7 (*C*=O), 150.5, 150.0 (dd, $J_{C\text{-}F}$ = 241, 12.5 Hz, Ar*C*F), 148.1 (dd, $J_{C\text{-}F}$ = 240, 11.8 Hz, Ar*C*F), 142.1 (dd, $J_{C\text{-}F}$ = 13.0 Hz, Ar*C*), 139.9 (Ar*C*H), 137.2 (Ar*C*), 136.4 (Ar*C*), 130.1 (Ar*C*H), 128.2 (Ar*C*H), 127.8 (Ar*C*H), 126.7 ($C_6ON_2CH_3$), 126.5 ($C_6ON_2CH_3$), 121.9 (Ar*C*), 119.4 (Ar*C*H), 117.3 (Ar*C*H), 113.5 ($C_6ON_2CH_3$), 98.6 ($C_6ON_2CH_3$), 48.6 (*C*H), 31.8 (CH_2), 25.6 (CH_2), 24.4 (CH_2); HRMS (ESI^+) ($M+H^+$) found 448.1834 $C_{26}H_{23}F_2N_3O_2$ requires 448.1831.

9.14.2.2. *N*-Cyclohexyl-6-(3,4-dichlorophenyl)-5-oxo-4-phenyl-5,6-dihydro-1H-pyrrolo [2, 3-c] pyridine-7-carboxamide 464



Isolated as a green solid, following method above using Ugi adduct **452** (0.479 g, 1.0 mmol) and PTSA (0.08 g, 40 mol%). The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.4) to yield pyrrolo-pyridine **464** (0.37 g, 78% yield), m.p = 257-258 ºC; υ_{max} (ATR/cm^{-1})= 3307, 3082, 2935, 2852, 1637, 1524; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H = 10.98 (1H, s, N*H*), 8.69 (1H, d, *J* = 8.2 Hz, ArC*H*), 8.31 (1H, s, N*H*), 7.76-7.26 (7H, m, ArC*H*), 7.80 (1H, d, *J* = 4.8 Hz, $C_7ON_2H_3$), 6.16 (1H, dd, *J* = 4.8 Hz, 1.7 Hz, $C_7ON_2H_3$), 3.55-3.47 (1H, m, C*H*), 1.60-0.96 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ_C = 159.5 (*C*=O), 157.7 (*C*=O), 142.1 (Ar*C*), 140.1 (Ar*C*H), 139.7 (Ar*C*), 137.1 (Ar*C*H), 137.2 (Ar*C*), 131.5 (Ar*C*H), 131.4 (Ar*C*), 131.2 (Ar*C*), 130.7 (Ar*C*H), 130.0 (Ar*C*H), 129.9 (Ar*C*H), 128.2 ($C_6ON_2CH_3$), 127.6 ($C_6ON_2CH_3$) 126.9 (Ar*C*), 122.2 (Ar*C*), 113.7 ($C_6ON_2CH_3$), 98.8 ($C_6ON_2CH_3$), 48.3 (*C*H), 31.8 (*C*H$_2$), 25.5 (*C*H$_2$), 24.5 (*C*H$_2$); HRMS (ESI$^+$) (M+H$^+$) found 480.1260 $C_{26}H_{23}Cl_2N_3O_2$ requires 480.1240.

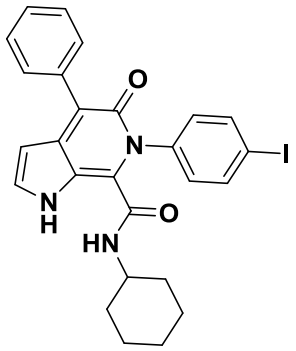
9.14.2.3. *N*-Cyclohexyl-6-(3,4-dibromophenyl)-5-oxo-4-phenyl-5,6-dihydro-1H-pyrrolo [2, 3-c] pyridine-7-carboxamide 465



Isolated as a yellow solid, following method above using Ugi adduct **453** (0.569 g, 1.0 mmol) and PTSA (0.08 g, 40 mol%). The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.2) to yield pyrrolo-pyridine **465** (0.45 g, 80%

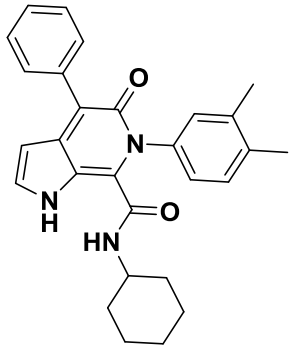
yield); m.p = 253-254 ºC; υ_{max} (ATR/cm^{-1})= 3305, 3057, 2937, 2850, 1633, 1528; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H = 10.96 (1H, s, N*H*), 8.68 (1H, d, *J* = 8.3 Hz, ArC*H*), 8.30 (1H, s, N*H*), 7.86-7.28 (7H, m, ArC*H*), 6.79 (1H, d, *J* = 4.2 Hz, $C_7ON_2H_3$), 6.16 (1H, d, *J* = 4.8 Hz, $C_7ON_2H_3$), 3.55-3.48 (1H, m, C*H*), 1.62-0.98 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ_C = 159.5 (*C*=O), 157.6 (*C*=O), 150.5 (Ar*C*), 142.1 (Ar*C*), 140.1 (Ar*CH*), 140.1 (Ar*C*), 134.4 (Ar*CH*), 136.8 (Ar*CH*), 130.5 (Ar*C*H), 130.1 (Ar*C*H), 128.2 (Ar*CH*), 127.5 ($C_6ON_2CH_3$), 126.9 ($C_6ON_2CH_3$) 122.2 (Ar*C*), 113.7 ($C_6ON_2CH_3$), 98.8 ($C_6ON_2CH_3$), 48.4 (*C*H), 31.8 (*C*H$_2$), 25.5 (*C*H$_2$), 24.6 (*C*H$_2$); HRMS (ESI$^+$) (M+H$^+$) found 570.0220 $C_{26}H_{23}Br_2N_3O_2$ requires 570.0211.

9.14.2.4. *N*-Cyclohexyl-6-(4-iodophenyl)-5-oxo-4-phenyl-5,6-dihydro-1H-pyrrolo[2, 3-c] pyridine-7-carboxamide 466



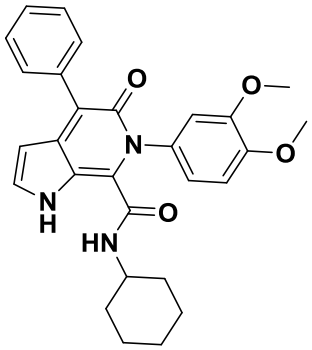
Isolated as a dark green solid, following method above using Ugi adduct **454** (0.537 g, 1.0 mmol) and PTSA (0.08 g, 40 mol%). The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.4) to yield pyrrolo-pyridine **466** (0.413 g, 77% yield); m.p = 221-222 ºC; υ_{max} (ATR/cm^{-1})= 3294, 3051, 2928, 2852, 1642, 1524; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H = 10.91 (1H, s, N*H*), 8.60 (1H, d, *J* = 8.2 Hz, ArC*H*), 8.61-7.13 (8H, m, ArC*H*), 6.96 (1H, d, *J* = 2.1 Hz, $C_7ON_2H_3$), 6.16 (1H, d, *J* = 2.0 Hz, $C_7ON_2H_3$), 3.51-3.46 (1H, m, C*H*), 1.55-0.94 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ_C = 159.6 (*C*=O), 157.7 (*C*=O), 140.8 (Ar*C*), 139.7 (Ar*C*H), 137.6 (Ar*C*H), 137.3 (Ar*C*), 137.1 (Ar*CH*), 131.6 (Ar*CH*), 128.2 (Ar*C*H), 127.9 ($C_6ON_2CH_3$), 126.8 ($C_6ON_2CH_3$), 121.9 (Ar*C*), 113.4 ($C_6ON_2CH_3$), 94.6 ($C_6ON_2CH_3$), 48.3 (*C*H), 31.8 (*C*H$_2$), 25.5 (*C*H$_2$), 24.6 (*C*H$_2$); HRMS (ESI$^+$) (M+H$^+$) found 538.0992 $C_{26}H_{24}IN_3O_2$ requires 538.0992.

9.14.2.5. *N*-Cyclohexyl-6-(3,4-dimethylphenyl)-5-oxo-4-phenyl-5,6-dihydro-1H-pyrrolo [2, 3-c] pyridine-7-carboxamide 467



Isolated as a green solid, following method above using Ugi adduct **455** (0.439 g, 1.0 mmol) and PTSA (0.08 g, 40 mol%). The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.6) to yield pyrrolo-pyridine **467** (0.28 g, 65% yield); m.p = 218-219 ºC; υ_{max} (ATR/cm^{-1})= 3290, 3093, 2928, 2856, 1642, 1528; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H = 10.84 (1H, s, N*H*), 8.56 (1H, d, *J* = 8.7 Hz, ArC*H*), 7.67-7.05 (7H, m, ArC*H*), 6.72 (1H, d, *J* = 5.0 Hz, $C_7ON_2H_3$), 6.15 (1H, d, *J* = 4.7 Hz, 1.7 Hz, $C_7ON_2H_3$), 3.52-3.45 (1H, m, C*H*), 2.26 (3H, s, CH_3), 2.23 (3H, s, CH_3), 1.59-0.98 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ_C = 159.8 (*C*=O), 157.9 (*C*=O), 141.5 (Ar*C*), 139.3 (Ar*C*H), 137.6 (Ar*C*), 137.5 (Ar*C*), 130.1 (Ar*C*H), 129.5 (Ar*C*H), 128.5 (Ar*C*), 128.0 (Ar*C*H), 126.6 ($C_6ON_2CH_3$), 126.5 ($C_6ON_2CH_3$), 121.7 ($C_6ON_2CH_3$), 113.3 (Ar*C*H), 98.5 ($C_6ON_2CH_3$), 48.2 (*C*H), 31.8 (*C*H$_2$), 25.6 (*C*H$_2$), 24.6 (*C*H$_2$), 19.8 (*C*H$_3$), 19.5 (*C*H$_3$); HRMS (ESI$^+$) (M+H$^+$) found 440.2335 $C_{28}H_{29}N_3O_2$ requires 440.2333.

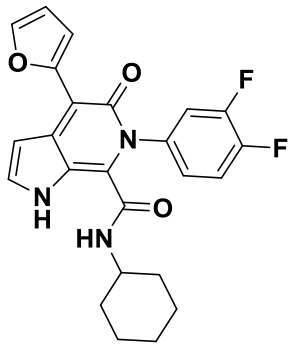
9.14.2.6. *N*-Cyclohexyl-6-(3, 4-dimethoxyphenyl)-5-oxo-4-phenyl-5,6-dihydro-1H-pyrrolo [2, 3-c] pyridine-7-carboxamide 468



Isolated as a black solid, following method above using Ugi adduct **456** (0.471 g, 1.0 mmol) and PTSA (0.08 g, 40 mol%). The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.4) to yield pyrrolo-pyridine **468** (0.27 g, 59% yield); m.p = 165-157 ºC; υ_{max} (ATR/cm^{-1})= 3301, 3055, 2932, 2854, 1640, 1530; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H = 10.86 (1H, s, N*H*), 8.50 (1H, d, *J* = 8.5 Hz, ArC*H*), 8.32 (1H, s,

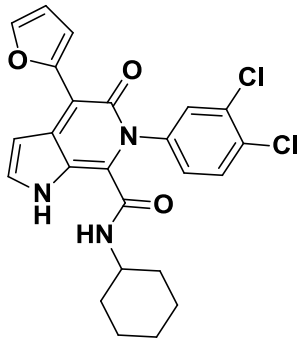
N*H*), 7.67-6.81 (7H, m, ArC*H*), 6.76 (1H, d, *J* = 5.1 Hz, $C_7ON_2H_3$), 6.15 (1H, d, *J* = 4.8 Hz, $C_7ON_2H_3$), 3.78 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 3.52-3.44 (1H, m, C*H*), 1.61-0.91 (10H, m, $5CH_2$); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C = 159.7 (*C*=O), 158.0 (*C*=O), 148.9 (Ar*C*), 148.7 (Ar*C*H), 141.6 (Ar*C*), 139.3 (Ar*C*H), 137.5 (Ar*C*), 132.7 (Ar*C*H), 130.1 (Ar*C*H), 128.1 (Ar*C*H), 126.8 (Ar*C*H), 121.7 ($C_6ON_2CH_3$), 121.3 (Ar*C*), 113.7 ($C_6ON_2CH_3$), 111.5 ($C_6ON_2CH_3$), 98.6 ($C_6ON_2CH_3$), 56.2 (OCH_3), 55.9 (OCH_3), 48.3 (*C*H), 31.9 (CH_2), 25.5 (CH_2), 24.6 (CH_2); HRMS (ESI$^+$) (M+H$^+$) found 472.2238 $C_{28}H_{29}N_3O_4$ requires 472.2231.

9.14.2.7. *N*-Cyclohexyl-6-(3,4-difluorophenyl)-4-(furan-2-yl)-5-oxo-5,6-dihydro-1H-pyrrolo [2, 3-c] pyridine-7-carboxamide 469



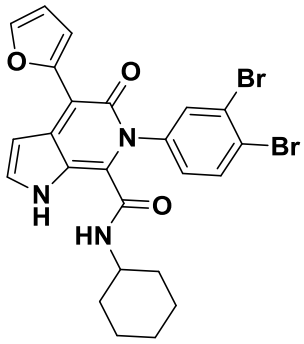
Isolated as a grey solid, following method above using Ugi adduct **457** (0.437 g, 1.0 mmol) and PTSA (0.08 g, 40 mol%). The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f= 0.3) to yield pyrrolo-pyridine **469** (0.240 g, 55% yield); m.p = 161-162 ºC; υ_{max} (ATR/cm^{-1})= 3426, 3051, 2941, 2854, 1631, 1515; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H = 11.11 (1H, s, N*H*), 8.66 (1H, d, *J* = 8.1 Hz, ArC*H*), 7.78 (1H, dd, *J* = 2.8 Hz, 1.8 Hz, C_4OH_3), 7.74 (1H, t, *J* = 2.9 Hz, C_4OH_3), 7.61-7.51 (2H, m, ArC*H*), 7.26-7.18 (1H, m, C_4OH_3), 6.88 (1H, dd, *J* = 3.1 Hz, 1.8 Hz, $C_7ON_2H_3$), 6.59 (1H, dd, *J* = 3.4 Hz, 1.8 Hz, $C_7ON_2H_3$), 3.54-3.47 (1H, m, C*H*), 1.58-0.94 (10H, m, $5CH_2$); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C = 159.5 (*C*=O), 155.7 (*C*=O), 152.4 (C_3OCH_3), 150.9, 150.2 (dd, $J_{C\text{-}F}$ = 240, 14.0 Hz, Ar*C*F), 148.4 (dd, $J_{C\text{-}F}$ = 240, 13.5 Hz, Ar*C*F), 141.8 (C_3OCH_3), 139.7 (dd, $J_{C\text{-}F}$ = 17.0 Hz, Ar*C*H), 137.5 (C_3OCH_3), 136.0 (Ar*C*), 126.7 ($C_6ON_2CH_3$), 122.1 (Ar*C*), 119.5 (Ar*C*H), 119.3 (C_3OCH_3), 117.6 (Ar*C*H), 117.4 ($C_6ON_2CH_3$), 109.6 (Ar*C*H), 100.9 ($C_6ON_2CH_3$), 48.4 (*C*H), 31.8 (CH_2), 25.5 (CH_2), 24.5 (CH_2); HRMS (ESI$^+$) (M+H$^+$) found 438.1628 $C_{24}H_{21}F_2N_3O_3$ requires 438.1624.

9.14.2.8. *N*-Cyclohexyl-6-(3,4-dichlorophenyl)-4-(furan-2-yl)-5-oxo-5,6-dihydro-1H-pyrrolo [2, 3-c] pyridine-7-carboxamide 470



Isolated as a black solid, following method above using Ugi adduct **458** (0.469 g, 1.0 mmol) and PTSA (0.08 g, 40 mol%). The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.4) to yield pyrrolo-pyridine **470** (0.28 g, 60% yield); m.p = 225-226 ºC; υ_{max} (ATR/cm^{-1})= 3312, 3060, 2932, 2859, 1646, 1555; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H = 11.12 (1H, s, N*H*), 8.69 (2H, d, *J* = 8.1 Hz, ArC*H*), 8.18 (1H, d, *J* = 2.4 Hz, C_4OH_3), 7.72 (1H, d, *J* = 3.1Hz, C_4OH_3), 7.24 (1H, s, ArC*H*), 7.15 (1H, dd, *J* = 4.3 Hz, 1.9 Hz, C_4OH_3), 6.88 (1H, dd, *J* = 4.1 Hz, 2.1 Hz, $C_7ON_2H_3$), 6.59 (1H, dd, *J* = 3.1 Hz, 1.9 Hz, $C_7ON_2H_3$), 3.54-3.46 (1H, m, C*H*), 1.60-0.95 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ_C = 159.4 (*C*=O), 155.6 (*C*=O), 152.3 (Ar*C*), 141.9 (C_3OCH_3), 139.8 (Ar*C*H), 139.4 (C_3OCH_3), 137.6 (Ar*C*), 131.5 (Ar*C*H), 130.7 (Ar*C*H), 126.7 (Ar*C*), 122.2 ($C_6ON_2CH_3$), 112.1 (C_3OCH_3), 109.7 (C_3OCH_3), 104.4 ($C_6ON_2CH_3$), 101.1 ($C_6ON_2CH_3$), 100.9 ($C_6ON_2CH_3$), 48.4 (*C*H), 31.82 (*C*H$_2$), 25.54 (*C*H$_2$), 24.58 (*C*H$_2$); HRMS (ESI$^+$) (M+H$^+$) found 470.1042 $C_{24}H_{21}Cl_2N_3O_3$ requires 470.1033.

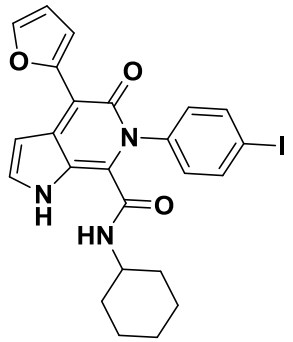
9.14.2.9. ***N*-Cyclohexyl-6-(3, 4-dibromophenyl)-4-(furan-2-yl)-5-oxo-5,6-dihydro-1H-pyrrolo [2, 3-c] pyridine-7-carboxamide 471**



Isolated as a dark green solid, following method above using Ugi adduct **459** (0.559, 1.0 mmol) and PTSA (0.08 g, 40 mol%). The crude compound was purified by column chromatography (dichloromethane: methanol 9.5 : 0.5, R_f = 0.4) to yield pyrrolo-pyridine **471** (0.36 g, 65% yield); m.p = 198-199 ºC; υ_{max} (ATR/cm^{-1})= 3290, 2935, 2941, 2861, 1642,

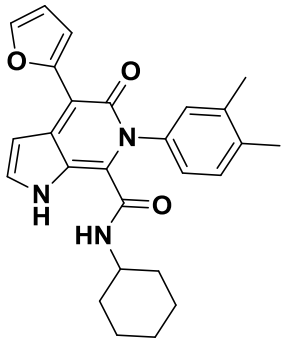
1526; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H = 11.08 (1H, s, N*H*), 8.71 (1H, d, *J* = 8.3 Hz, C_4OH_3), 7.84 (1H, d, *J* = 8.5 Hz, C_4OH_3), 7.75-7.22 (2H, m, ArC*H*), 7.15 (1H, s, ArC*H*), 6.89 (1H, dd, *J* = 4.2 Hz, 1.8 Hz, $C_7ON_2H_3$), 6.57 (1H, dd, *J* = 3.8 Hz, 1.9 Hz, $C_7ON_2H_3$), 3.53-3.45 (1H, m, C*H*), 1.59-0.94 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ_C = 159.6 (*C*=O), 155.8 (*C*=O), 152.2 (Ar*C*), 141.9 (C_3OCH_3), 139.8 (Ar*C*H), 139.8 (Ar*C*), 137.5 (C_3OCH_3), 134.3 (Ar*C*H), 133.9 ($C_6ON_2CH_3$), 126.4 (Ar*C*), 124.4 ($C_6ON_2CH_3$), 112.1 (C_3OCH_3), 109.9 (C_3OCH_3), 101.2 ($C_6ON_2CH_3$), 48.5 (*C*H), 31.8 (*C*H$_2$), 25.5 (*C*H$_2$), 24.5 (*C*H$_2$); HRMS (ESI$^+$) (M+H$^+$) found 560.0009 $C_{24}H_{21}Br_2N_3O_3$ requires 560.0004.

9.14.2.10. ***N*-Cyclohexyl-4-(furan-2-yl)-6-(4-iodophenyl)-5-oxo-5,6-dihydro-1H-pyrrolo [2, 3-c] pyridine-7-carboxamide 472**



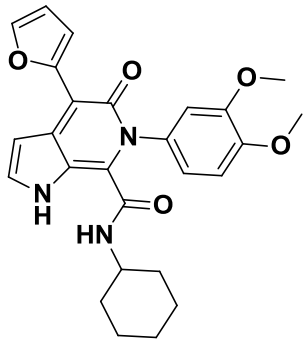
Isolated as a red solid, following method above using Ugi adduct **460** (0.527, 1.0 mmol) and PTSA (0.08 g, 40 mol%). The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.4) to yield pyrrolo-pyridine **472** (0.28 g, 55% yield); m.p = 191-192 ºC; υ_{max} (ATR/cm^{-1})= 3249, 3053, 2930, 2854, 1640, 1535; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H = 10.89 (1H, s, N*H*), 8.61 (1H, d, *J* = 8.1 Hz, C_4OH_3), 8.32 (1H, dd, *J* = 7.9 Hz, 3.4 Hz, C_4OH_3), 8.05-7.39 (4H, m, ArC*H*), 6.57 (1H, d, *J* = 2.2 Hz, $C_7ON_2H_3$), 5.70 (1H, d, *J* = 18.7 Hz, $C_7ON_2H_3$), 3.74-3.65 (1H, m, C*H*), 1.83-0.93 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ_C = 159.5 (*C*=O), 155.7 (*C*=O), 152.6 (Ar*C*), 150.9 (Ar*C*), 141.7 (C_3OCH_3), 139.4 ($C_6ON_2CH_3$), 137.7 (Ar*C*H), 137.4 (C_3OCH_3), 131.6 (Ar*C*H), 127.2 ($C_6ON_2CH_3$), 122.1 (Ar*C*), 112.0 (C_3OCH_3), 109.6 (C_3OCH_3), 104.3 (Ar*C*), 100.9 ($C_6ON_2CH_3$), 94.7 ($C_6ON_2CH_3$), 48.8 (*C*H), 31.7 (*C*H$_2$), 25.5 (*C*H$_2$), 24.6 (*C*H$_2$); HRMS (ESI$^+$) (M+H$^+$) found 528.078 $C_{24}H_{22}IN_3O_3$ requires 528.0779.

9.14.2.11. ***N*-Cyclohexyl-6-(3,4-dimethylphenyl)-4-(furan-2-yl)-5-oxo-5,6-dihydro-1H-pyrrolo [2, 3-c] pyridine-7-carboxamide 473**



Isolated as a black solid, following method above using Ugi adduct **461** (0.429, 1.0 mmol) and PTSA (0.08 g, 40 mol%). The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.4) to yield pyrrolo-pyridine **473** (0.28 g, 67% yield); m.p = 162-164 ºC; υ_{max} (ATR/cm^{-1})= 3410, 3057, 2935, 2854, 1644, 1564; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H = 10.98 (1H, s, N*H*), 8.56 (1H, d, *J* = 8.2 Hz, C$_4$O*H*$_3$), 8.32 (1H, dd, *J* = 3.4 Hz, 1.9 Hz, C$_4$O*H*$_3$), 7.82 (1H, d, *J* = 6.2 Hz, C$_4$O*H*$_3$), 7.76-7.01 (2H, m, ArC*H*), 7.09 (1H, s, ArC*H*), 6.86 (1H, dd, *J* = 3.1 Hz, 1.9 Hz, C$_7$ON$_2$*H*$_3$), 6.57 (1H, dd, *J* = 3.3 Hz, 1.8 Hz, C$_7$ON$_2$*H*$_3$), 3.52-3.44 (1H, m, C*H*), 2.31 (3H, s, C*H*$_3$), 2.24 (3H, s, C*H*$_3$), 1.56-0.93 (10H, m, 5C*H*$_2$); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ_C = 159.7 (*C*=O), 155.9 (*C*=O), 152.8 (Ar*C*), 141.5 (C$_3$O*C*H$_3$), 138.9 (C$_6$ON$_2$*C*H$_3$), 137.2 (Ar*C*), 136.5 (Ar*C*), 130.1 (Ar*C*H), 129.5 (Ar*C*H), 127.8 (C$_6$ON$_2$*C*H$_3$), 126.5 (Ar*C*H), 121.7 (C$_3$O*C*H$_3$), 111.9 (C$_3$O*C*H$_3$), 109.3 (C$_6$ON$_2$*C*H$_3$), 104.3 (C$_3$O*C*H$_3$), 100.8 (C$_6$ON$_2$*C*H$_3$), 48.2 (*C*H), 31.8 (*C*H$_2$), 25.5 (*C*H$_2$), 24.6 (*C*H$_2$), 19.8 (*C*H$_3$), 19.5 (*C*H$_3$); HRMS (ESI$^+$) (M+H$^+$) found 430.2132 $C_{26}H_{27}N_3O_3$ requires 430.2125.

9.14.2.12. *N*-Cyclohexyl-6-(3,4-dimethoxyphenyl)-4-(furan-2-yl)-5-oxo-5,6-dihydro-1H-pyrrolo [2, 3-c] pyridine-7-carboxamide 474



Isolated as a black solid, following method above using Ugi adduct **462** (0.461, 1.0 mmol) and PTSA (0.08 g, 40 mol%). The crude compound was purified by column chromatography (dichloromethane: methanol 9.5: 0.5, R_f = 0.4) to yield pyrrolo-pyridine **474** (0.23 g, 51% yield); m.p = 144-145 ºC; υ_{max} (ATR/cm^{-1})= 3560, 3053, 2930, 2852, 1642, 1559; ^{1}H NMR

(400 MHz, DMSO-d_6) δ_H = 11.01 (1H, s, N*H*), 8.51 (1H, d, *J* = 8.2 Hz, C_4OH_3), 8.32 (1H, dd, *J* = 8.7 Hz, 3.9 Hz, C_4OH_3), 7.76-6.88 (2H, m, ArC*H*), 6.84 (1H, s, ArC*H*), 6.82 (1H, dd, *J* = 8.5 Hz, 2.4 Hz, $C_7ON_2H_3$), 6.58 (1H, dd, *J* = 3.3 Hz, 1.8 Hz, $C_7ON_2H_3$), 3.80 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 3.50-3.47 (1H, m, C*H*), 1.61-0.88 (10H, m, 5CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C = 158.7 (*C*=O), 156.1 (*C*=O), 152.8 (Ar*C*), 149.4 (C_3OCH_3), 149.0 (Ar*C*), 141.6 (C_3OCH_3), 138.9 ($C_6ON_2CH_3$), 137.2 (Ar*C*), 128.0 ($C_6ON_2CH_3$), 121.7 ($C_6ON_2CH_3$), 121.4 (Ar*C*H), 113.7 (Ar*C*H), 111.9 (C_3OCH_3), 111.6 (C_3OCH_3), 109.3 (Ar*C*H), 100.8 ($C_6ON_2CH_3$), 56.3 (O*C*H$_3$), 55.9 (O*C*H$_3$), 48.3 (*C*H), 32.1 (*C*H$_2$), 25.6 (*C*H$_2$), 24.7 (*C*H$_2$); HRMS (ESI$^+$) (M+H$^+$) found 462.2028 $C_{26}H_{27}N_3O_5$ requires 462.2023.

9.15. Screening methodology (SMB Cells)

9.15.1. Preparation of required solutions

1- **RIPA buffer**: A solution was prepared from Tris (50 mM), NaCl (150 mM), Triton-X (1%), sodium deoxycholate (1%) and SDS (0.1%) in 500 ml deionized water and adjusted to pH 8.

2- **10 x TBS**: A solution was prepared from Tris (200 mM) and NaCl (9%) in water (1 L). the solution was adjusted to pH 7.4 and filtered using a 0.22 Mm membrane filter.

3- **10% Tween 20**: Tween 20 (5 mL) was measured out into a 50 mL centrifuge tube and then made up to 50 ml with water and gently mixed until dissolved.

4- **1 x TBS**: A solution was prepared by addition of 10 x TBS (100 mL) to water (900 mL). The total volume was freshly prepared to the amount required.

5- **1 x TBS-T**: A solution was prepared by addition of tween 20 (1 mL, 10%) to TBS (100 mL) (final concentration 0.1%).

9.15.2. Routine culture of scrape mouse brain (SMB cells)

1- SMB cells were grown in T75 flasks at 37℃ under CO_2 (5%) in air and at 95% humidity.

2- Growth media was made up of medium 199 (500 mL) supplemented with new born calf serum (50 mL, 10 %, NBCS), fetal bovine serum (25 mL, 5% FBS) and penicillin-streptomycin (5.75 mL, 1%). This media was sterile filtered before use.

3- Cultured cells were passaged once a week as follows:

- Media was warmed for at least half an hour before use.
- The cells were checked under the microscope to ensure they are healthy.

- The medium was removed and the cells were washed with HBSS (10 mL). The HBSS was then removed, Tryp-LE (5 mL) was added and incubated at 37 ℃ for 5 minutes.
- Media (7 mL) was added to each flask and aspirated. All cells were washed off the flask surface transferred to a centrifuge tube (50 mL) and spun down at 1,000 rpm for 5 minutes.
- The supernatant was removed and the pellet was resuspended in fresh media. Media (10 mL) was added per flask and the cells were counted using a haemcytometer.
- Flasks were seeded at 8 x 10^5 cells/flask in 25 ml media and calculated as follows:
    - Haemocytometer count = 100 cells.

      100 x 10 = 1000 cells/mm^2

      x 1000 = 1,000,000 cells/mL
    - Large plates:

$$1\text{mL} + \text{medium (24mL)} = \frac{800{,}000(\text{cells needed per flask})}{1{,}000{,}000(\frac{\text{cells}}{\text{mL}})}$$

- 96 well plates should be seeded at 10,000 cells/well in media (100 µL). One plate requires approximately 1 x 10^6 cells in media (10 mL). This can be counted using the formula above. In addition, plates were incubated overnight before dosing.
- Waste liquids of these cells must be decontaminated with NaOH (1M) before disposal. NaOH pellets (100 g) were added to waste (2.5 L), and left for at least one hour to ensure full decontamination.

9.15.3. Dosing the cells

1- Cells were dosed with target compounds and incubated with the compounds for 5 days. Compound stocks were normally dissolved in DMSO and diluted to ensure a final DMSO concentration of 0.5%. Initial screens were achieved at concentrations of 1, 10 and 20 µM, while EC_{50} screening was measured over a range of 6 concentrations.

2- Each plate was normally dosed in duplicate (two plates prepared), one was used for dot blot analysis and the one was used for MTT analysis.

3- DMSO stock (10 mM) was diluted in DMSO 200 times to required working concentration. This was then diluted 1:10 into HBSS (stock (5 µL) into HBSS (45 µl)) before was diluted 1:20 into the media (5 µL into media (100 mL)).

4- Each concentration of each compound was dosed in triplicate.

5- Curcumin at 10 μM was used as a positive control, while DMSO (0.5%) was used as a negative control (Figure **56**).

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		A 1 uM	A 1 uM	A 1 uM	C 1 uM	C 1 uM	C 1 uM	E 1 uM	E 1 uM	E 1 uM	DMSO	
C		A 10 uM	A 10 uM	A 10 uM	C 10 uM	C 10 uM	C 10 uM	E 10 uM	E 10 uM	E 10 uM	DMSO	
D		A 20 uM	A 20 uM	A 20 uM	C 20 uM	C 20 uM	C 20 uM	E 20 uM	E 20 uM	E 20 uM	DMSO	
E		B 1 uM	B 1 uM	B 1 uM	D 1 uM	D 1 uM	D 1 uM	F 1 uM	F 1 uM	F 1 uM	DMSO	
F		B 10 uM	B 10 uM	B 10 uM	D 10 uM	D 10 uM	D 10 uM	F 10 uM	F 10 uM	F 10 uM	10 uM C	
G		B 20 uM	B 20 uM	B 20 uM	D 20 uM	D 20 uM	D 20 uM	F 20 uM	F 20 uM	F 20 uM	10 uM C	
H												

Figure 56: The negative control of DMSO (0.5%) includes multiples on each plate.

9.15.4. The assessment of cell viability (MTT assay)

1- The compounds were assessed for toxicity. This test is very important as if all the cells are dead this may lead to false positives in the dot blot assay.

2- The media was made up with a 1:10 dilution of MTT stock solution (5 $mg.mL^{-1}$ in PBS). Media (100 μL) was added to each well and then original media was removed and replaced with media containing the MTT and incubated for 2 hours.

3- The MTT media was removed and cells were resuspended in acidified isopropanol (60 mL) (0.1 M HCl).

4- Absorbance at 570 nm was used to determine cells viability, using a background subtraction of 690 nm, cell viability was calculated as a percentage of the untreated control.

9.15.5. Cell lysis

1- The cells were lysed after 5 days incubation to release the prion protein.

2- The media was removed and RIPA buffer (50 μL) was added and samples were shaken at 200 rpm for 30 minutes.

3- Benzonase (25 μL) (0.2 μl/mL in lysis buffer) was added and samples were shaken for an additional 15 minutes.

9.15.6. Protein concentration determination

1- Protein levels in the samples were measured using a Bradford assay. This assay was used to determine how much of the lysate can be loaded onto the nitrocellulose membrane.

2- Each sample (5 mL) was transferred to a clean 96 well plate and Bradford reagent (250 μL) was added. BSA at different concentrations (0–1 $mg.mL^{-1}$) was used to allow determination of a standard curve.

3- The plates were measured at 570 nm after they were incubated at room temperature for 5 minutes.

4- Protein concentration was determined for the unknown samples using the standard curve.

9.15.7. Dot blot analysis

1- The lysate samples were transferred onto a nitrocellulose membrane using a vacuum manifold.

2- Pieces of filter paper and membrane were prepared using the template and soaked in water before use.

3- The top two portions of the manifold was rinsed with ethanol (10%), followed by water. Two pieces of filter paper were put at the bottom and the membrane was placed on top.

4- The lysate was loaded in the same arrangement (as the plate) and pulled through using a vacuum of 350 mmHg. The membrane was removed and left to dry for an hour.

Once the membrane was dried and it should be processed as follows:

- The membrane was incubated with PK for 1 hour. Stock solution (1.5 mL) was added at 3 $mg.mL^{-1}$ to TBS (28.5 mL) to give a final concentration of 150 $mg.mL^{-1}$.
- The membrane was incubated with PMSF for 10 minutes. Stock (200 mM) was made up in ethanol then 150 mL of stock was added to 30 mL water to give a working concentration.
- The membrane was washed with **TBS-T** 3 x 5 minutes.
- The membrane was washed with aqueous guanadine (1.8 M) for 10 minutes. (Guanadine thiocyanate (5.32 g) in TBS (25 mL)).
- The membrane was washed with **TBS-T** 3 x 5 minutes.
- The membrane was thoroughly mixed with milk solution ((5%), 50 $mg.mL^{-1}$) for 1 hour. (Milk powder (1.25 g) in PBS (25 mL)).
- The membrane was washed with **TBS-T** 3 x 5 minutes.
- The membrane was delt with **8H4** (0.2 $mg.mL^{-1}$) for 1 hour (approx. 10 μL in TBS-T (25 mL).
- The membrane was washed with **TBS-T** 3 x 5 minutes.
- The membrane was treated with **HRP-tagged anti-mouse IgG**, 1:4,000 dilution for 1 hour. This solution (6.25 μL) was made up in TBS-T (25 mL) and made up fresh each time.
- The membrane was washed **TBS-T** 3 x 5 minutes.

- The membrane was washed with TBS 2 x 5 minute. Tween can interfere with the detection solutions so to ensure there is none left 2 washes with TBS were undertaken.
- The membrane was incubated with detection solutions (5 mL) for 3 minutes. EZ-ECL solutions were prepared the same as the GE solutions by mixing equal amounts together. This was done at least 5 minutes before use.
- The membrane was sealed between two pieces of thin plastic and visualised using a ChemiDoc model.

Chapter 10. References

10.1. References

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