Sulfonyl Hydrazides as Potential Candidates for Herbicides and Antibiotics Targeting DHDPS; an Essential Enzyme in the Lysine Biosynthesis Pathway

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Table of Contents

Abstract

Multidrug resistant bacteria are predicted to be the cause of 10 million deaths in 2020.[1] As the majority of pathogenic bacteria species exhibit resistance to the antibiotics currently available, there is a clear need for new and effective antibacterial compounds. Developing agents against novel biological targets also presents the opportunity to avoid current mechanisms of bacterial resistance.

Resistance is also a significant issue in agriculture, with 500 unique cases of herbicide resistant weeds expected by 2020.[2] With food production being critical to a growing world population, the need for the development of innovative herbicidal agents against novel targets should not be underestimated.

Dihydrodipicolinate synthase (DHDPS) is common to both bacteria and plants, and is thus a potential target for the development of inhibitors as antibacterial or herbicides. This enzyme undertakes the first committed enzymatic step in the lysine biosynthesis pathway, where aspartate semi aldehyde (ASA) condenses with pyruvate (PYR). The absence of lysine biosynthesis in humans may be advantageous due to the lack of homologous human enzymes.

This thesis discusses the generation of a library of low molecular weight compounds and their subsequent evaluation against DHDPS for potential application as antibiotics or herbicidal agents.

The lead compound, an aromatic sulfonyl hydrazine, was identified via a high throughput screening. In the first half of the project, 30 compounds were synthesised for testing in order to obtain structure-activity relationship information relating to this class of compounds against DHDPS. Analogues were generated using functionalised aromatic and heterocyclic rings. Variation of the alkyl chain length on the sulphur atom and exploration of the hydrazine and sulfonyl functionality was also carried out.

Elaboration of the compound at the sulfonyl group was undertaken in the second half of the project, where a total of 36 compounds were produced. Establishing the synthetic route as envisaged was found to be more challenging than anticipated. Success was obtained by the synthesis of extended alkyl sulfonyl chlorides which were coupled with β-oxo esters, further reaction with aromatic hydrazides providing the desired compounds. Structural

optimisation was then attempted by changing the aromatic moiety and altering the position of the linking amide bond.

This study has provided a series of sulfonyl hydrazides with significant activity against DHDPS. Compounds which can successfully inhibit DHDPS have potential value as antibiotic and herbicidal agents.

Abbreviations

°C	Celsius
µM	micromolar
AAA	Aminoadipic acid pathway
ADP	Adenosine diphosphate
AGAR	Australian Group on Antimicrobial Resistance
AMP	Adenosine monophosphate
Arg	Arginine
ASA	*L*-Aspartate-semialdehyde
ATP	Adenosine triphosphate
CDC	Centres for Disease Control and Prevention
DAP	Diaminopimelic acid pathway
DEPT	Distortionless Enhancement by Polarization Transfer
DHDP	Dihydrodipicolinate
DHDPR	Dihydrodipicolinase reductase
DHDPS	Dihydrodipicolinase synthase
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
h	hours
HEK293	Human kidney cells
HepG2	Human liver cells
HRAC	Herbicide Resistance Action Committee
HRMS	High-Resolution Mass Spectrometry
HTPA	4-Hydroxy-2,3,4,5-tetrahydro-*L,L*-dipicolinic acid
HTS	High Throughput Screen
IC_{50}	The half maximal inhibitory concentration
K'_i	The inhibitory (dissociation) constant
K_i	The reversible inhibitor constant
K_{ii}	Dissociation constant for uncompetitive inhibition
K_{is}	Dissociation constant for competitive inhibition
LCMS	Liquid chromatography-Mass spectrometry
LiHMDS	Lithium bis(trimethylsilyl)amide
LRMS	Low-Resolution Mass Spectrometry
Lys	Lysine
M	Molar
m/z	mass/charge
meso-DAP	*meso-L,L'*-2,6-Diaminopimalate
mM	millimolar
MS	Mass Spectrometry
MTT	MethylThiazolyldiphenyl-Tetrazolium bromide
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazol-3-ium
$NADP^+$	Nicotinamide adenine dinucleotide phosphate
NADPH	Reduced from of nicotinamide adenine dinucleotide phosphate
NCS	*N*-Chlorosuccinimide

nM	nanomolar
nm	nanometre
NMR	Nuclear Magnetic Resonance Spectroscopy
o/n	over night
o-ABA	*ortho*-aminobenzaldehyde
ppm	part per million
PYR	pyruvate
RNA	Ribonucleic acid
RP-HPLC	Reverse Phase-High Pressure Liquid Chromatography
rt	room temperature
SAR	Structure Activity Relationship
S_N2	Nucleophilic substitution reaction
THDP	*L*- 2,3,4,5,-Tetrahydrodipicolinate
TLC	Thin Layer Chromatography
TPPO	Triphenylphosphine oxide
Tyr	Tyrosine
USA	United States of America
V. Vinifera	*Vitis vinifera*
UV	Ultra Violet
WEHI	Walter and Eliza Hall Institute
WHO	World Health Organisation
WSSA	Weed Science Society of America
α-KPA	α-Ketopimelic acid

Statement of Authorship

Except where reference is made in the text of the thesis, this thesis contains no material published elsewhere or extracted in whole or in part from a thesis submitted for the award of any other degree or diploma.

No other person's work has been used without due acknowledgement in the main text of this thesis.

This thesis has not been submitted for the award of any degree or diploma in any other tertiary institution.

Throughout this study, collaboration and ideas were conversed with fellow project members from the Perugini Laboratory and Soares da Costa Laboratory of the Biochemistry and Genetics Department, La Trobe Institute for Molecular Science. In particular, all biological studies described in this thesis were undertaken by Dr Tatiana Soares da Costa and Cody J Hall of the Soares da Costa and Perugini Laboratory.

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Chapter 1 Introduction

1.1 Antibiotics

1.1.1 The discovery of antibiotics

The historic discovery of penicillin was made by Sir Alexander Fleming in 1928 while studying the bacteria *Staphylococcus aureus* (Figure 1.1).[3-5] Fleming noticed a zone of inhibition on the bacterial culture, where a common mould, *Penicillium notatum*, was growing, which led to the discovery of first ever antibiotic reported scientifically.[3-5]

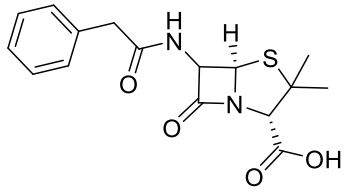


Figure 1.1: Structure of Penicillin.

Despite the challenges in isolation of Penicillin, it was one of the revolutionary natural products which played a crucial role during Second World War. The discovery led to saving countless lives allowing Sir Alexander Fleming to be awarded a joint Nobel Prize in Medicine in 1945.[6, 7] It took more than a decade to generate an X-ray crystal structure of Penicillin, leading the total synthesis of the compound to be completed in 1957 by Sheehan *et al*.[8]

Following the work from Sir Alexander Fleming, a revolutionary period for antibiotic drug discovery started in the 1940s. Isolation of streptomycin was performed using the soil bacteria *Streptomycetes sp.* by Waksman in 1943 (Figure 1.2).[9] This was the first drug used in treating tuberculosis.

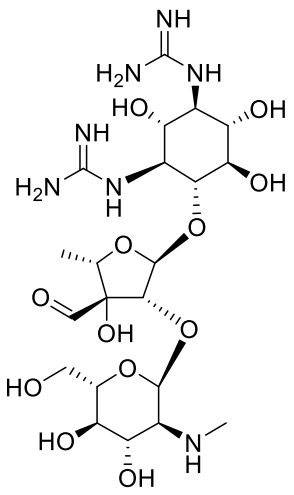


Figure 1.2: Structure of Streptomycin.

Many more compounds derived from natural sources became viable antibacterial drugs, and later synthetic compounds also came in to the combat against bacterial diseases.

1.1.2 Classes of antibiotics

Antibiotics can be classified according to the mode of action, spectrum of activity and method of administration.[10-13] Currently there are 12 classes of antibiotics according to chemical structure as tabulated in Table 1.1.

The modes of action of antibacterial compounds can vary from disruptions to the cell wall either by inhibiting peptidoglycan synthesis or aggregating inside the cell wall, to changes of the cell wall dynamics, disruptions on the ribosomal subunits to inhibit protein synthesis and inhibiting DNA replication/transcription (Figure 1.3 and Table 1.1). All of these modes of actions result in slow bacterial growth and eventually lead to the bacterial death.

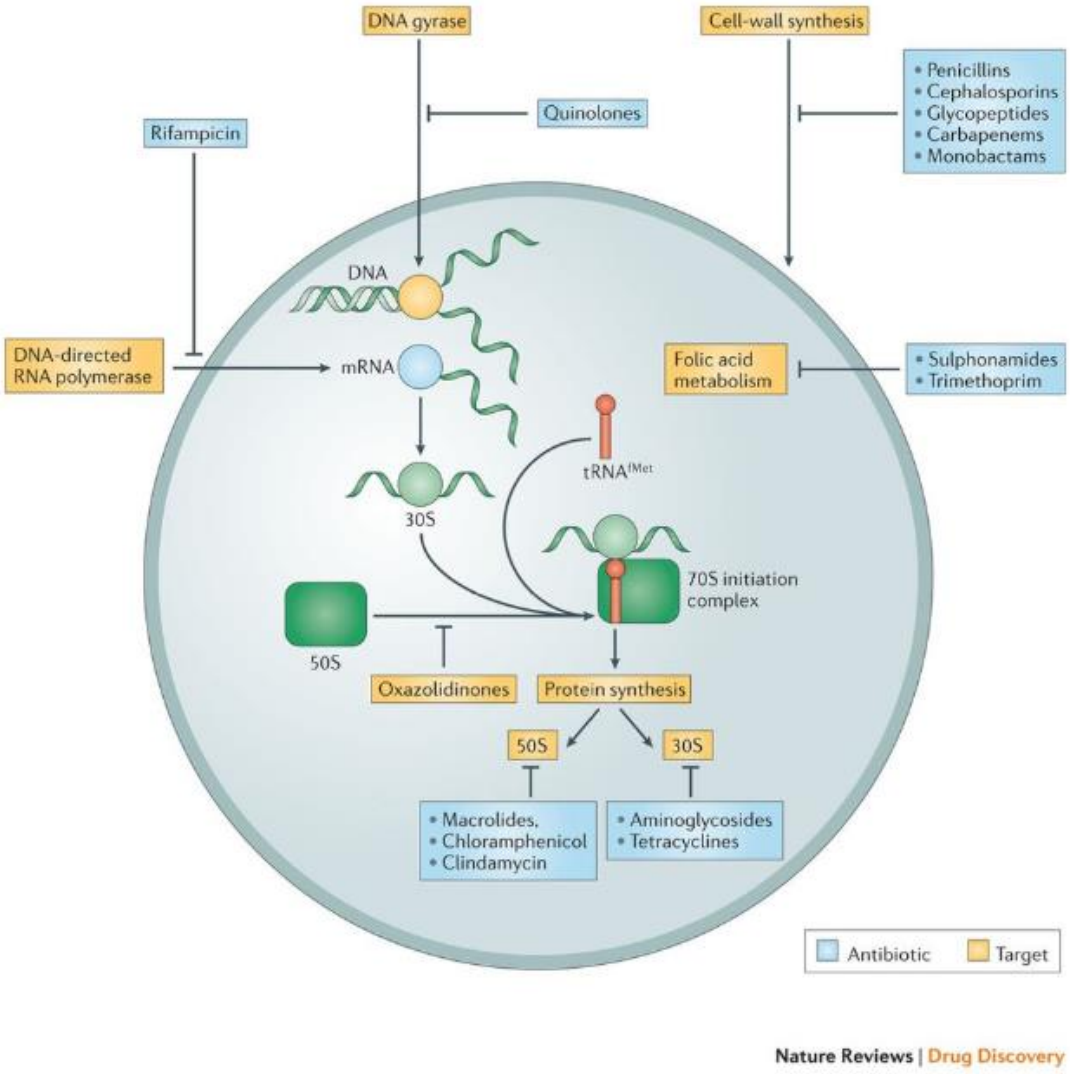


Figure 1.3: Main antibacterial drug targets in bacteria (adapted from Lewis).[14]

Table 1.1: Antibiotic classes according to chemical structure.[10-13]

Class of antibiotic	Common structural feature	Examples of drugs	Mode of antibacterial action	Discovery era
β-lactams	Contains a β-lactam ring	Penicillin Amoxicillin Flucloxacillin Cefalexin	Inhibit cell wall biosynthesis by disrupting the synthesis of peptidoglycan	Late 1920s
Sulfonamides	Contains a sulfonamide group	Prontosil Sulfadiazine Sulfisoxazole	Prevents growth and multiplication	Early 1930s
Aminoglycosides	Contains amino-sugar substructures	Streptomycin Neomycin Kanamycin	Inhibit the synthesis of proteins by binding to the 30S or 50S ribosomal subunits	Early 1940s
Tetracyclines	Contains 4 adjacent cyclic hydrocarbon rings	Tetracycline Doxycycline Limecycline Oxytetracycline	Inhibit the synthesis of proteins by Inhibiting the binding of aminoacyl-tRNA to the mRNA-ribosome complex	Late 1940s
Chloramphenicol	Very distinct structures per compound	Chloramphenicol	Inhibits the protein synthesis by binding to the 50S subunit of the ribosome	Late 1940s

Macrolides	Contains a 14-, 15-, or 16-membered macrolide ring	Erythromycin Clarithromycin Azithromycin	Inhibits protein synthesis by binding to the 50S subunit of the ribosome	1950s
Glycopeptides	Consists of carbohydrates linked to a peptide	Vancomycin Teicoplanin	Inhibits synthesis of peptidoglycan	Late 1950s
Ansamycins	Contains an aromatic ring bridged by an aliphatic chain	Geldanamycin Rifamycin Naphthomycin	Inhibits RNA synthesis	Late 1950s
Quinolones	Contains fused aromatic rings with an attached carboxylic acid group	Ciprofloxacin Levofloxacin Trovafloxacin	Inhibits DNA replication and transcription by binding to the DNA gyrase or the topoisomerase IV enzyme	Early 1960s
Streptogramins	Combination of two drugs from different drug classes which act synergistically	Pristinamycin IIA Pristinamycin IA	Synergism between two modes of actions described above, depending on the combination of drugs	Early 1960s
Oxazolidinones	Contains 2-oxazolidone	Linezolid Posizolid Tedizolid Cycloserine	Inhibits protein synthesis by binding to the ribosomal 50S subunit	Late 1970s
Lipopeptides	Contain a lipid bonded to a cyclic peptide	Daptomycin Surfactin	Disrupts the cell membrane by aggregation and altering the curvature	Late 1980s

1.1.3 Mechanisms of antibacterial resistance

It should be noted that a new class of antibiotic has not been developed since late 1980s.

Despite the initial effectiveness of antibiotics, bacteria have evolved to be resistant to the drugs. Five key mechanisms for resistance in bacteria have been identified as mentioned below.[15, 16]

a) Mutations alter the target and/or target site of the drug, so that the drug no longer binds.
b) Changing the target pathway to by-pass the target.
c) Membrane permeability is reduced to decrease the drug uptake or efflux pumps actively reduce the concentration of the drug inside the cell.
d) Inactivation or modification of the drug through metabolism.
e) Increasing the production of the target enzyme.

1.1.4 Current situation of antibiotic resistance

According to the data obtained from the Centres for Disease Control and Prevention (CDC) in the United States of America (USA), 23 000 people died due to drug resistant bacteria in 2013 and 2 million people were infected.[17, 18] The World Health Organisation (WHO) estimate that 25 000 people in 2011 died in the European Union only due to drug resistant bacteria.[19]

WHO claims that *Escherichia coli*, *Neisseria Gonorrhoeae*, *Staphlylococcus aureus* and *Mycobacterium tuberculosis* currently exhibit extreme resistance to most of the antibiotic drugs on the market.[20] According to the 2014 report from the Australian Group on Antimicrobial Resistance (AGAR), *Staphylococcus aureus* is a serious health threat in Australia due to its continuing resistance to antibiotic treatments.[21] In 2016, an estimation of 490 000 new cases of global multidrug-resistant tuberculosis was made by WHO.[22]

Moreover, a small group of highly resistant bacterial strains has been encountered firstly in hospital environments and still persists in such environments.[23-25] This group of bacteria is named "ESKAPE" and it includes *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanni*, *Pseudomonas aeruginosa* and *Enterobacter* species.

There is a clear need of new antibiotics to combat antibiotic resistant bacteria. Modern drug discovery processes such as genome mapping, chemical libraries and computational

medicinal chemistry can assist traditional structure activity relationship driven chemical approaches in multidisciplinary research to generate novel classes of antibiotics to combat these ESKAPE strains and other resistant bacteria.[26]

1.2 Herbicides

1.2.1 Weeds

According to the Weed Science Society of America (WSSA) there are four types of weeds.[27] A weed is a plant which can cause economical or ecological losses or can create health problems to humans or animals. A noxious weed is a plant that is classified by a government as dangerous to agriculture, wildlife, public health and environment. An invasive weed is very persistent and can spread widely in natural ecosystem and in most cases, same weeds are also classified as noxious weeds by authorities. Finally, a 'superweed" is a weed which has evolved to resist the herbicides commonly in use and are can be very harmful to agricultural practice and/or the natural ecosystem.

Australia has identified 32 weeds to be of national significance which are invasive/noxious and can cause problems to the environment and economy.[28]

1.2.2 Current herbicides and mode of action

There are a large number of herbicides available in the market to treat weeds, which can be classified according to their mode of action. Most of the herbicides have cellular targets, as illustrated in Figure 1.4, which was adapted from a review published by Delye and co-workers in 2013.[29] The letters designate the classification of herbicide according to the Herbicide Resistance Action Committee (HRAC) as used in Australia (Table 1.2).

Herbicides can act on plants' biological pathways such as photosynthesis, fatty acid synthesis, amino acid synthesis, cell wall synthesis, tetrahydroflorate synthesis or ATP synthesis. Furthermore, they can also interrupt processes such as microtubule organisation, hormone transport or hormone-based gene regulation. A few common herbicides are tabulated in Table 1.3.

Table 1.2: HRAC classification codes for herbicide modes of action.

HRAC group	**Herbicide mode of action**
A	Inhibition of acetyl-CoA carboxylase (ACCase)
B	Inhibition of acetohydroxyacid synthase (AHAS, ALS)
C	Inhibition of photosystem II protein D1 (psbA)
D	Diversion of the electrons transferred by the photosystem I ferredoxin (Fd)
E	Inhibition of protoporphyrinogen oxidase (PPO)
F	Inhibition of phytoene desaturase (PDS) or 4-hydroxyphenylpyruvate dioxygenase (4-HPPD) or of an unknown protein
G	Inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase)
H	Inhibition of glutamine synthase
I	Inhibition of dihydropteroate synthase
K1, K2	Enhancement of tubulin depolymerisation
K3	Inhibition of fatty acid synthase (FAS)
L	Inhibition of cellulose-synthase
M	Uncoupling of oxidative phosphorylation
N	Inhibition of fatty acid elongase
O	Stimulation of transport inhibitor response protein 1 (TIR1)
P	Inhibition of auxin transport
Z	Unknown

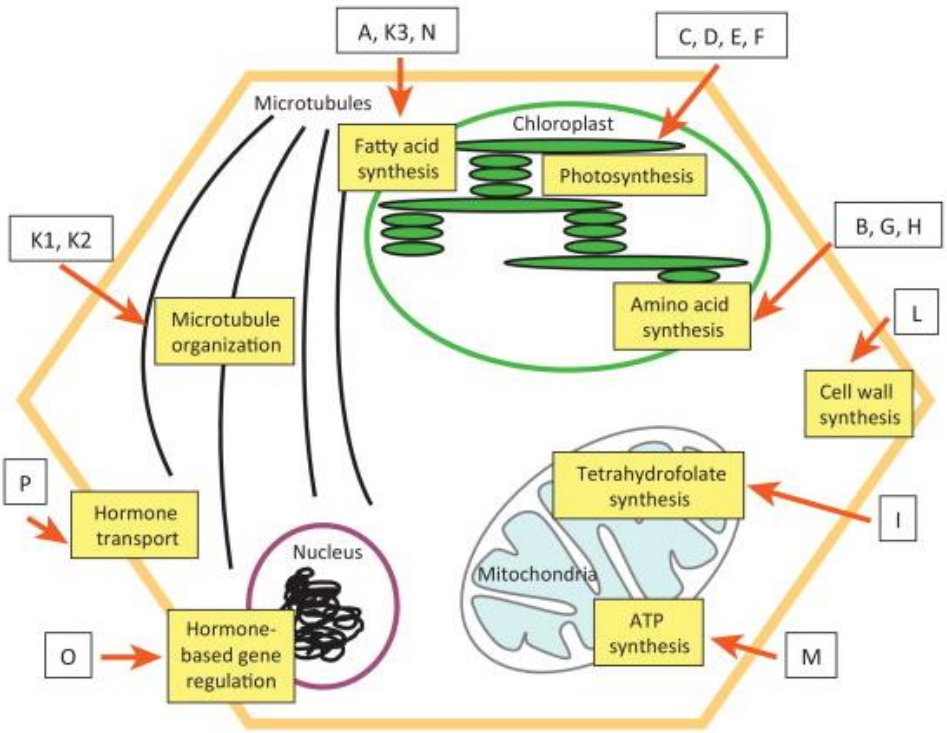
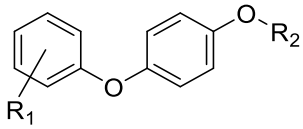
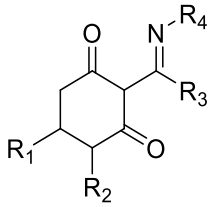
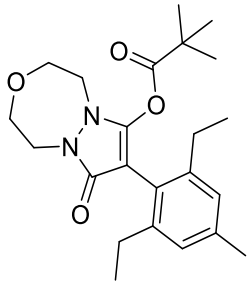
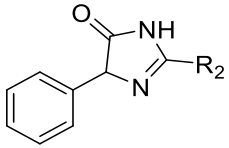
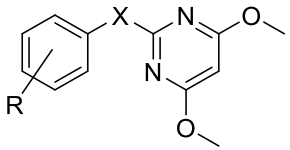
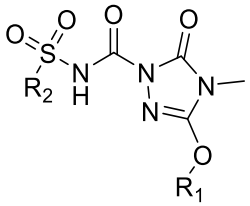
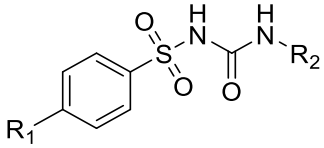
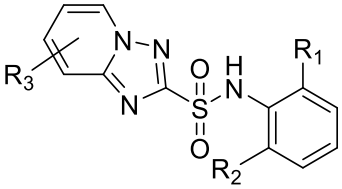
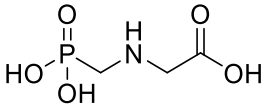


Figure 1.4: Herbicide classification by mode of action adapted from Delye and co-workers.[29]

The most common herbicides act on fatty acid synthesis and amino acid synthesis. Table 1.3 tabulates classification with the mode of action, the targeted pathway or process, corresponding chemical families and some common names of the most widely used herbicides classes; A, B, and G.[2, 29, 30]

Table 1.3: Modes of action of the most common herbicides.[2, 29, 30]

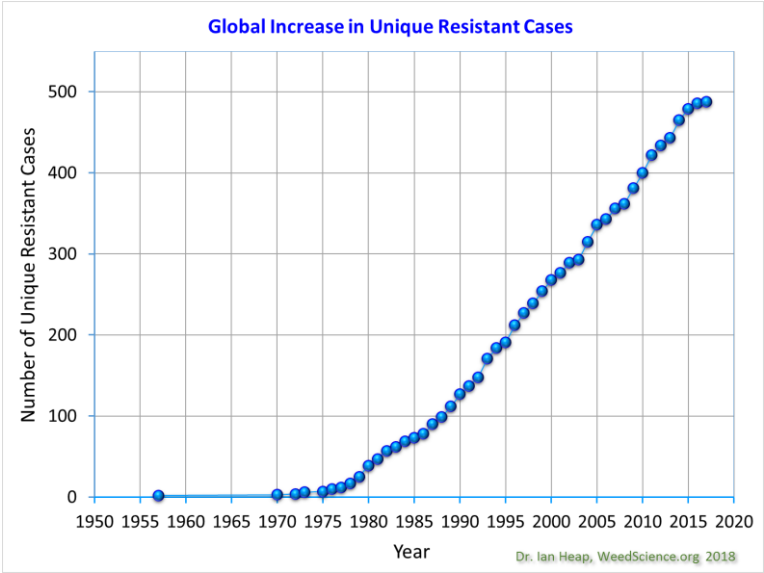
HRAC group	Mode of action	Target pathway or process	Chemical family/ies	Common names of herbicides
A	Inhibition of acetyl-CoA carboxylase (ACCase)	Fatty acid biosynthesis	Aryloxyphenoxypropionate 	Clincher Tecoma Axial XL Fusilade DX
			Cyclohexanedione 	
			Phenylpyrazolin 	
B	Inhibition of acetohydroxyacid synthase (AHAS, ALS)	Amino acid biosynthesis (Leu, Ile, Val)	Imidazolinone 	Raptor Beyond Clearmax

			Pyrimidinylthiobenzoic acid 	Londax FirstRate
			Sulfonylaminocarbonyltriazolinones 	
			Sulfonylurea 	
			Triazolopyrimidine 	
G	Inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase)	Amino acid biosynthesis (Phe, Trp, Tyr)	Organophosphorus 	Roundup Touchdown

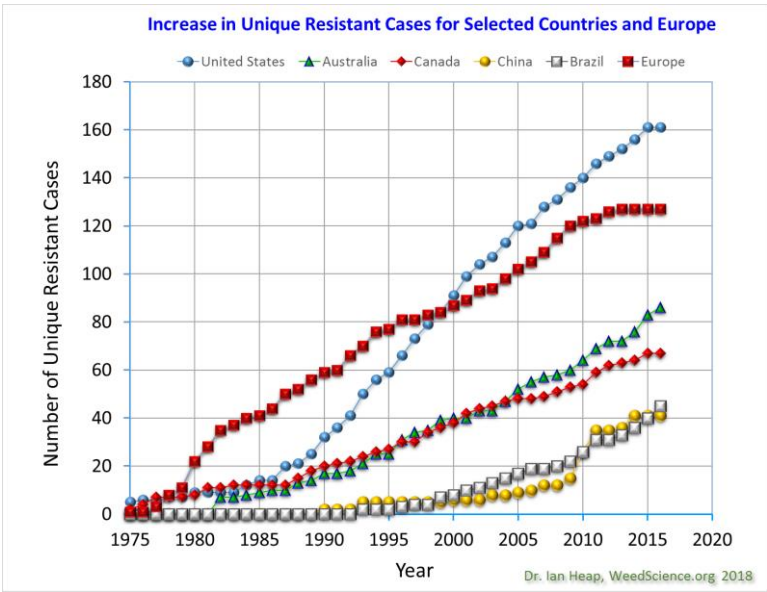
1.2.3 Herbicide resistance

The use of herbicides to control weeds was followed by the rise of herbicide resistance with the first reported case of resistance in the 1950s.[31] Resistance occurs in plants naturally due to genetic or variant selection by tissue culture or mutagenesis.[32] According to WSSA, the term herbicide resistance defines plants which survive and reproduce followed by treatments with herbicides upon which wild type plants will die.[32]

There is an increasing number of unique cases each year, likely to reach 500 cases world-wide by 2020 (Figure 1.5 A). There is also a steady increase in the number of unique herbicide resistant cases in Australia, as evident from Figure 1.5 (B).



(A)



(B)

Figure 1.5: (A) Global increase in unique herbicide resistant cases, (B) Increase in unique cases for selected countries.[2]

The number of cases of herbicide resistant weeds is highest in the United States of America with 162 cases, while Australia is in the second place with 91 cases.[2] The last reported herbicide resistant weed per each Australian state is tabulated in Table 1.4 according to the International Survey of Herbicide-Resistant Weeds.[2] Annual Bluegrass is spreading across the states of Australia as visible from the data in the three adjacent states, NSW, SA and VIC.

Table 1.4: Most recent herbicide resistant weeds in Australian states.[2]

Reported year	Scientific name	Common name	State
2017	*Poa annua*	Annual Bluegrass	New South Wales (NSW)
2018	*Conyza sumatrensis*	Sumatran Fleabane	Queensland (QLD)
2017	*Poa annua*	Annual Bluegrass	South Australia (SA)
2017	*Poa annua*	Annual Bluegrass	Victoria (VIC)
2017	*Lactuca saligna*	Willow-leaved lettuce	Western Australia (WA)

There are six noxious weeds; *Clidemia hirta, Limnocharis flava, Mikania micrantha, Miconia calvescens, Miconia racemosa, Miconia nervosa* which are targeted for eradication nationwide in Australia, showcasing the necessity of efficient weed control methods.[33]

The use of herbicides with the same site of action for prolonged times causes increased herbicide resistance. Therefore, it is important to rotate herbicides with different sites of action to avoid this issue and as the first step of preparation to avoid herbicide resistant weeds.[31, 34]

1.2.4 Modes of herbicide resistance

There are two major mechanisms by which weeds become resistant to herbicides, namely target site resistance and non-target site resistance.[29, 35]

Target site resistance occurs through production of excess target protein through gene amplification and/or genetic changes which results in mutations to the target site.

Any physiological mechanism which aims to reduce the amount of herbicide reaching the target is called non-target-site resistance. Decreased absorption of the compound, increased efflux of the compound out of the cell and enhanced metabolism are the most common methods of non-target-site resistance.

Cross-resistance is also common in plants where one resistance mechanism is able to protect the plant from multiple herbicides.[34]

Herbicide resistant weeds needs to be controlled or preferably eliminated for the ecology and agricultural benefits. Generally weeds have the ability to develop resistance to more than one herbicide active ingredient causing the combat against these problematic.[34-36]

There is a critical need of new herbicides with novel targets which plants have not evolved to be resistant.

1.3 Lysine biosynthesis

The lysine biosynthesis pathway, which can be found in both bacteria and plants and contains multiple enzymes. Those could be potentially explored in searching for new antibiotic and herbicidal agents.

Lysine (Figure 1.6) is an essential amino acid to humans and must be obtained from the diet. Unlike humans, bacteria and plants synthesise lysine.[37, 38] Lysine is essential in protein synthesis.[39, 40]

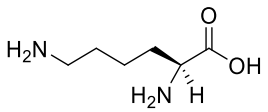


Figure 1.6: Structure of lysine.

There are two synthetic pathways for the biosynthesis of lysine. The aminoadipic acid pathway (AAA) is used by fungi and *Thermus thermophiles* bacteria to produce lysine from α-ketoglutarate and acetyl coenzyme A.[41] Bacteria except *Thermus thermophiles* and plants synthesise lysine via the diaminopimelic acid (DAP) pathway (Figure 1.7), which is the pathway of interest in this project.[41]

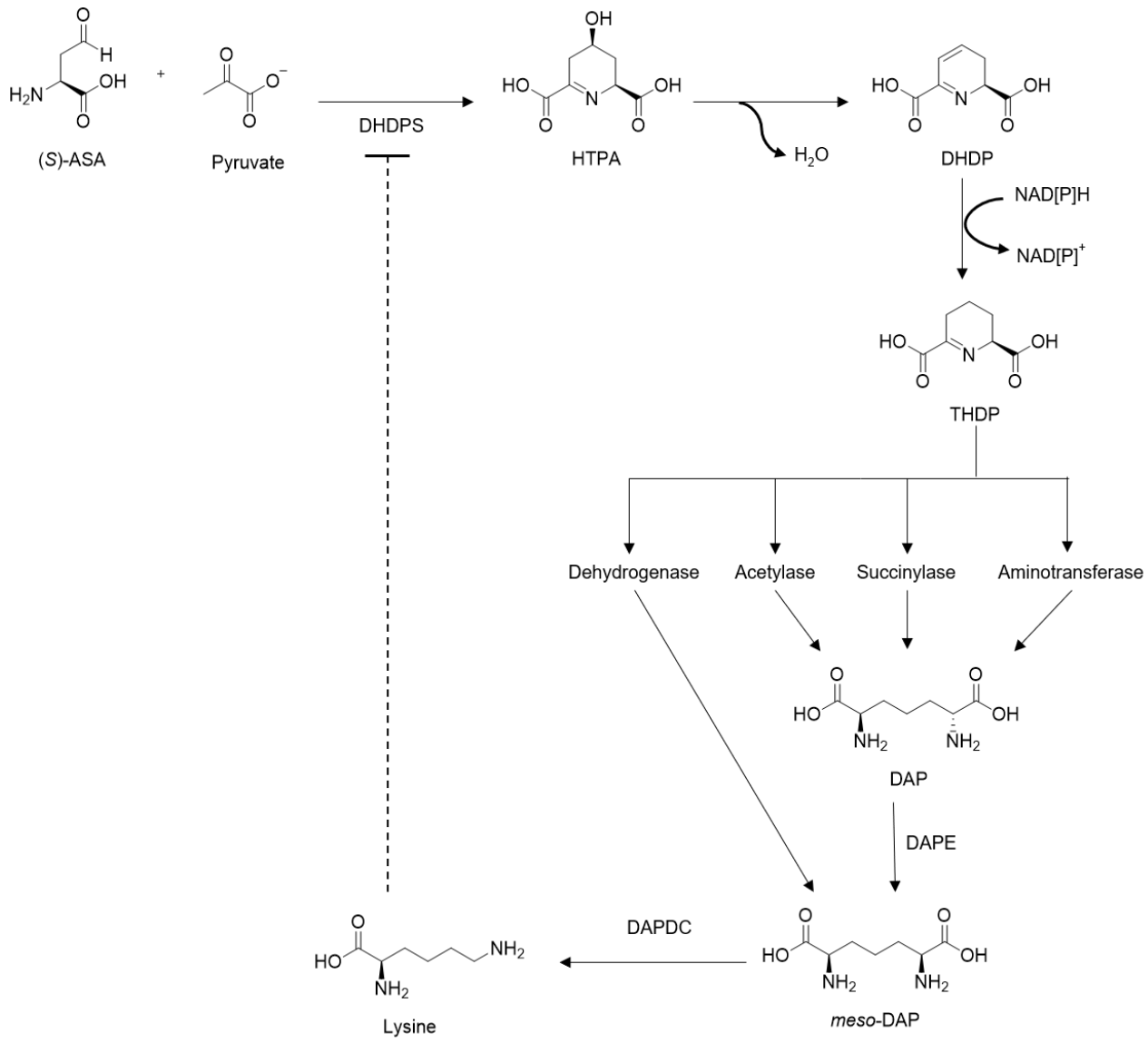


Figure 1.7: Lysine biosynthesis - diaminopimelic acid pathway.

DAP synthesis begins with the condensation of pyruvate (PYR) and *L*-aspartate-semialdehyde (ASA) by the enzyme dihydrodipicolinase synthase (DHDPS) to produce 4-hydroxy-2,3,4,5-tetrahydro-*L*,*L*-dipicolinic acid (HTPA) which is an unstable heterocycle.[42] HTPA undergoes a non-enzymatic dehydration to produce dihydrodipicolinate (DHDP) which is then reduced by a NAD(P)H-dependent enzyme, dihydrodipicolinase reductase (DHDPR), to yield *L*-2,3,4,5,-tetrahydrodipicolinate (THDP). Synthesised THDP can then undergo one of four pathways depending upon the species undertaking the biosynthesis.

The succinylase pathway is typically used by most Gram-negative and Gram-positive bacteria for the synthesis of *meso*-DAP, which consists of four enzymatic steps. Only a few *Bacillus* species utilize the acetylase pathway which is also four enzymatic steps in

length.[39] The aminotransferase pathway of two enzymatic steps, which is catalysed by the enzyme diaminopimelate aminotransferase, is followed by plants.[43] Lastly, *Corynebacterium* and some *Bacillus* species synthesise *meso*-DAP via a dehydrogenase pathway of one enzymatic step where NADPH-dependent enzyme diaminopimelate dehydrogenase is utilized.

Regardless of the pathway taken, *meso-L,L'*-2,6-diaminopimalate (*meso*-DAP) (Figure 1.8) is produced, which then undergoes decarboxylation by diaminopimelate decarboxylase to give lysine.

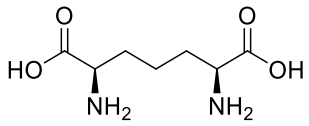


Figure 1.8: Structure of *meso*-DAP.

In Gram-negative bacteria *meso*-DAP plays an important role of a cross-linking agent in the peptidoglycan layer of the cell wall as well as being a precursor to lysine. Lysine also acts as a negative feedback control to inhibit the biosynthesis by allosteric binding to DHDPS, the initial enzyme of DAP pathway.[39, 40]

1.3.1 Enzyme target of interest; DHDPS

Enzyme DHDPS catalyses the first committed step in lysine biosynthesis (Figure 1.9).[44]

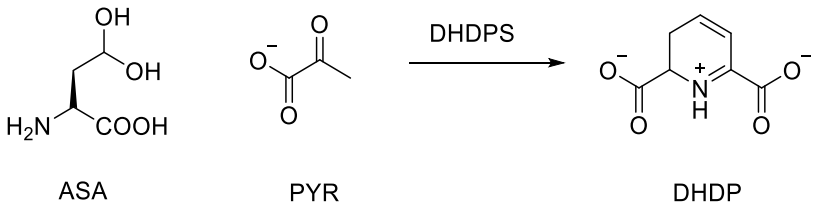


Figure 1.9: First step in lysine biosynthesis pathway catalysed by DHDPS.

PYR first condenses with a Lys residue in the active site forming a Schiff base followed by tautomerisation to generate an enamine (Figure 1.10).[45, 46] This enamine then reacts with ASA in an Aldol-type reaction to generate a cyclic enzyme bound intermediate, followed by transamination to generate a free cyclic alcohol, HTPA. This unstable heterocyclic intermediate dehydrates immediately to generate DHDP.

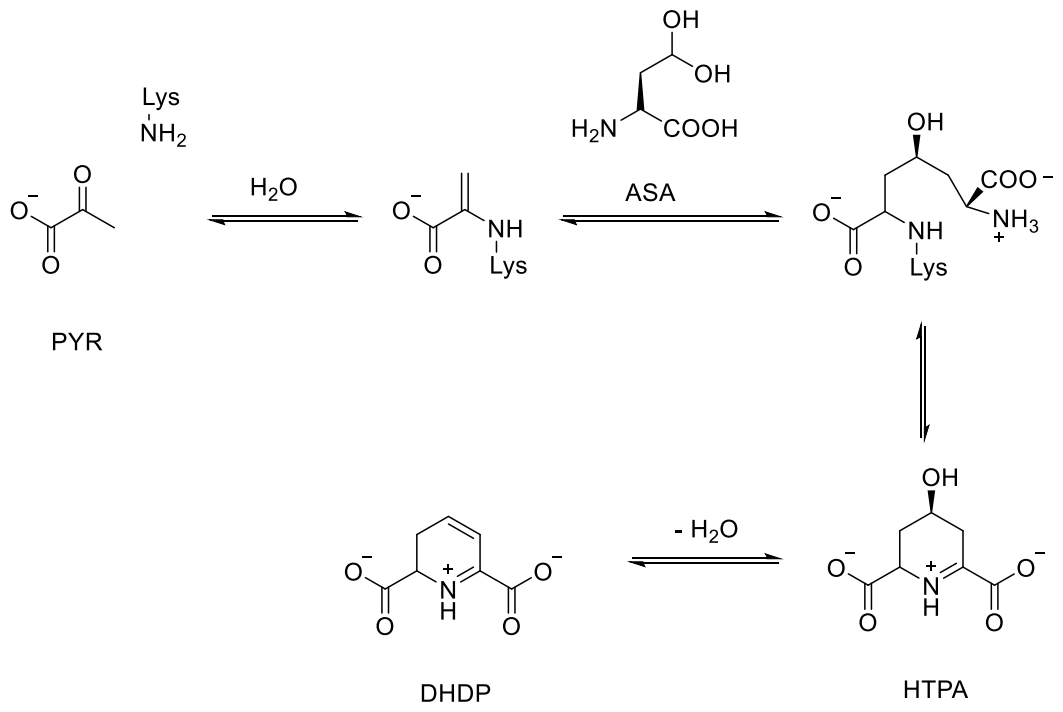


Figure 1.10: Synthesis of DHDP from condensation of ASA and PYR by DHDPS.

Characterisation of DHDPS was first achieved in 1965 and it attracted the scientific community in search for potential antibiotics.[47-49] The crystal structure was deduced in 1995 which described the active sites of *E. coli* DHDPS.[50] In 1997, Blicking and co-workers revealed five crystal structures of chemical compound bound DHDPS complexes where the chemical compounds were pyruvate, pyruvate and succinate *β*-semialdehyde, *R*-ketopimelic acid, dipicolinic acid and *l*-lysine.[51]

The quaternary structure of DHDPS varies from Gram-positive bacteria to Gram-negative bacteria to plants (Figure 1.11).

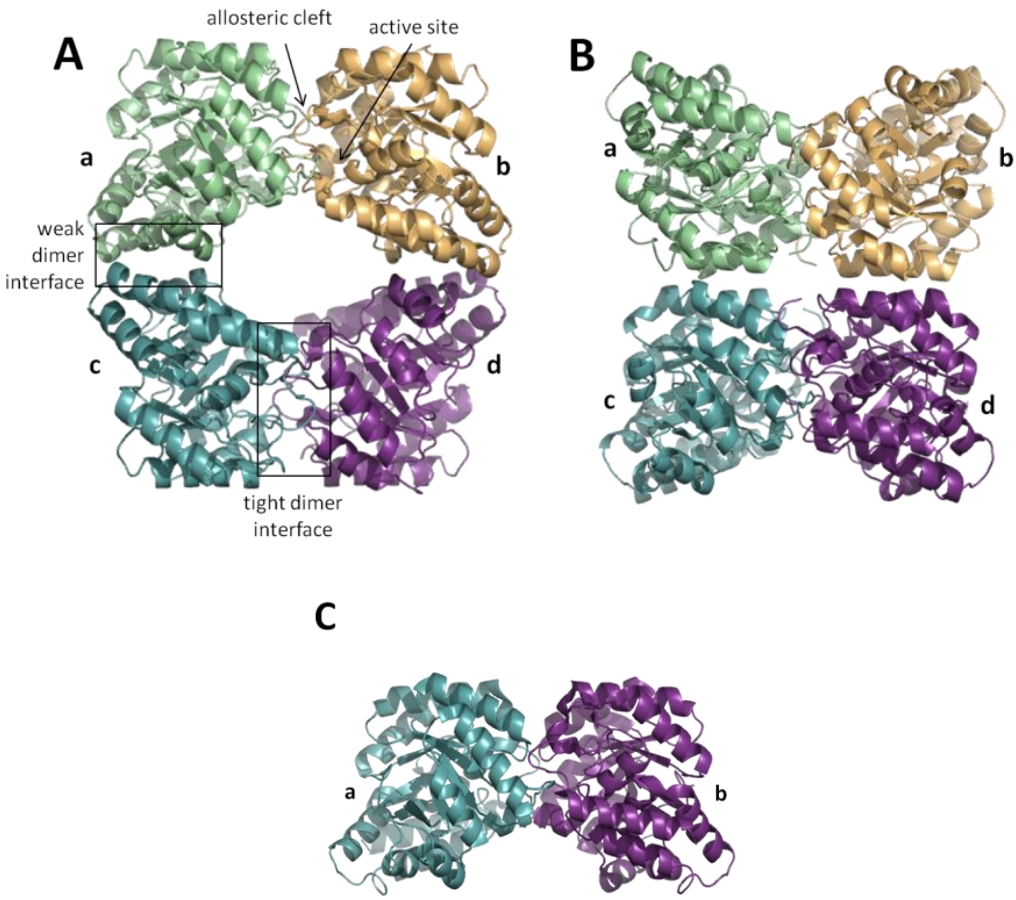


Figure 1.11: DHDPS enzyme structure of Gram-negative bacteria (A), plants (B) and Gram-positive bacteria (C) where a, b, c and d refers to monomeric units of the protein.[37]

In Gram-negative bacteria, such as *E. coli*, the enzyme exhibits as a tetramer with two sub units. Each subunit consists of two tightly bound monomers. The dimers are in "head-to-head" arrangement with the active sites located in the cavities created by the monomers of the dimer (Figure 1.11 A).[37, 44] In the active site of *E. coli*, Tyrosine 44 and Tyrosine 199 are present while Tyrosine 107 interdigitates the two monomers at the tight dimer interface giving rise to two active sites per dimer.[44] The allosteric site of the enzyme is located in the crevice on the interface of the tight dimer (Figure 1.11 A).[37, 44] The active site and the allosteric site are apart but connected via a water channel.[44, 51]

Plant DHDPS shows the quaternary structure as a "back-to-back" arrangement of dimers (Figure 1.11 B).[44] However DHDPS is identified as a dimer in Gram-positive bacteria with similar enzymatic activity to *E.coli* DHDPS (Figure 1.11 C).[44, 52]

1.3.2 Regulation of DHDPS

The regulation of lysine biosynthesis occurs by the allosteric inhibition of DHDPS by lysine. The allosteric site is located in the monomer-monomer interface of dimers.

Surprisingly, the active site and the allosteric sites of both Gram-negative bacteria and plants enzymes are conserved regardless of the arrangement of the tight dimers.[44]

Feedback inhibition by lysine is stronger in plants compared to that in bacteria.[44, 51] The negative feedback is stronger in Gram-negative bacteria compared to that of Gram-positive bacteria, which does not show any inhibition to lysine.[44, 51]

The crystal structure of lysine bound to DHDPS indicates that two lysine molecules are needed for effective allosteric inhibition.[51] Studies undertaken by Blicking and co-workers also show that affinity for the second lysine binding site is higher when the first is already bound.[51] *E. coli* DHDPS is inhibited by lysine uncompetitively with respect to PYR and noncompetitively with respect to aspartate.[51]

1.3.3 Measuring the inhibition of DHDPS

For the development of inhibitors of DHDPS, it is essential to have appropriate assays available. There are three assays available in the literature to measure the inhibition of DHDPS.[47]

The imidazole assay monitors the rise in absorption at 270 nm and measures enzyme kinetics.[47] Autoxidation of DHDPS generates dipicolinic acid and upon the addition of imidazole buffer forms imidazolium dipicolinate, resulting in the increased absorption (Figure 1.12). This assay is commonly used due to the ease of performance. However, due to the longer lag phase and the kinetics of the oxidation step not yet determined, this assay can generate inconsistent results as described by Turner and co-workers in 2005.[45]

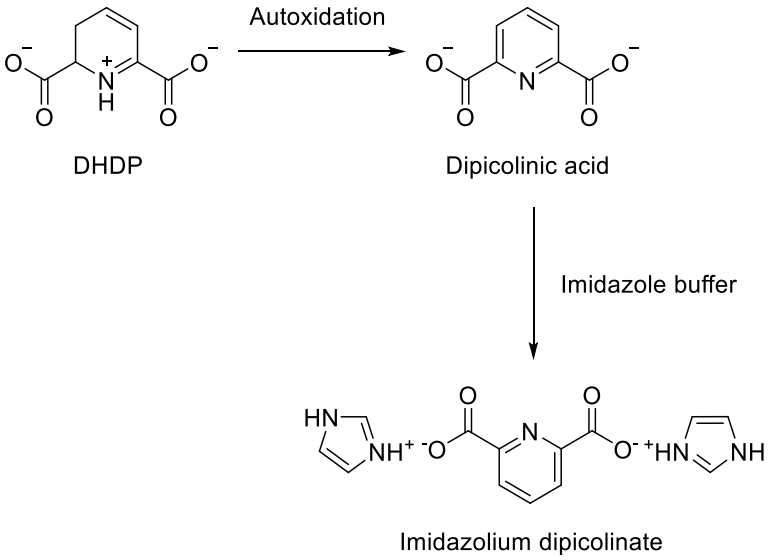


Figure 1.12: Imidazole assay.

The *o*-aminobenzaldehyde (*o*-ABA) assay is also widely used as a qualitative assay, mainly in the purification of the DHDPS enzyme.[45, 47, 53] This assay measures the absorbance at 540 nm of the purple chromophore, (*S*)-6-((2-carboxyphenyl)carbamoyl)-5,6-dihydropyridine-2-carboxylic acid, generated from the reaction of HTPA with *o*-ABA (Figure 1.13).

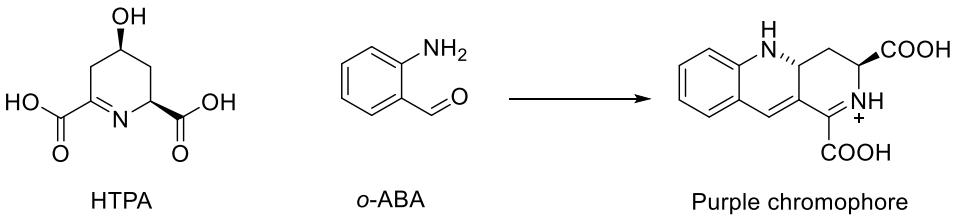


Figure 1.13: *o*-ABA assay.

The DHDPS-DHDPR coupled assay is used to measure the rate of consumption of DHDPS indirectly.[45, 47] Absorption is measured at 340 nm which corresponds to the utilization of DHDP by DHDPR and NADPH giving an indirect measurement of DHDPS; the measured absorption is proportional to the activity of DHDPS (Figure 1.14).

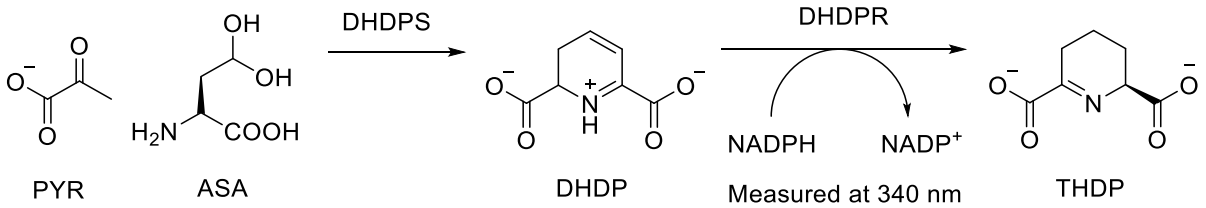
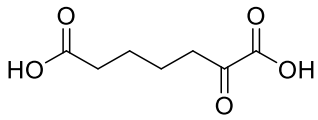


Figure 1.14: DHDPS-DHDPR coupled assay.

1.3.4 Inhibitors of DHDPS

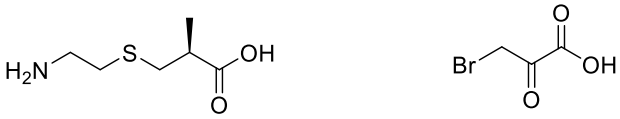
In 1974, Yamakura and Ikeda reported the first inhibitor of DHDPS as ε-diketopimelic acid.[54] DHDPS was competitively inhibited with respect to ASA and PYR with K_i values of 0.22 mM and 0.16 mM respectively (Figure 1.15). They also tested ATP, ADP, AMP, citric acid, *L*-malic acid, α-ketoglutaric acid, fumaric acid, succinic acid, *L*-glutamic acid, and *L*-threonine to discover they do not inhibit DHDPS.



ε-Diketopimelic acid (α-Ketopimelic acid (α-KPA))
K_i 0.22 vs ASA
K_i 0.16 vs PYR

Figure 1.15: First reported inhibitor of DHDPS.

In 1992, Laber and co-workers identified two inhibitors of DHDPS (Figure 1.16).[55] (*S*)-(2-Aminoethyl)-*L*-cysteine and bromopyruvic acid inhibit DHDPS with K_i values of 4.6 mM and 1.6 mM, respectively. Moreover, they identified that DHDPS is 50% inhibited by 1.0 mM *L*-lysine, 1.2 mM sodium dipicolinate and 4.6 mM *S*-2-aminoethyl-*L*-cysteine. However, no inhibition was observed for 3-fluoropyruvate, α-oxobutyrate, oxaloacetate, α -oxoglutarate, phenylpyruvate, 2,6-pipecolinate, aspartate or asparagine.



(*S*)-(2-Aminoethyl)-L-cysteine
K_i 4.6 mM

Bromopyruvic acid
K_i 1.6 mM

Figure 1.16: Inhibitors of DHDPS developed by Laber and co-workers.[55]

Couper and co-workers developed three inhibitors with low micromolar concentration through the synthesis of 30 pyridine and pieridine analogues (Figure 1.17).[56] Compounds A and B both inhibit DHDPS with IC_{50} values of 0.2 mM, which were the best two compounds in this series. Both compounds A and C were found to be non-competitive inhibitors of DHDPS. Compound A inhibits DHDPS with K_i values of 0.29 mM and 0.06 mM with respect to ASA and PYR. Similarly, compound C inhibits DHDPS with K_i values of 1.25 mM and 0.34 mM for ASA and PYR, respectively. All the other inhibitors shown in Figure 1.17 are low micromolar inhibitors.

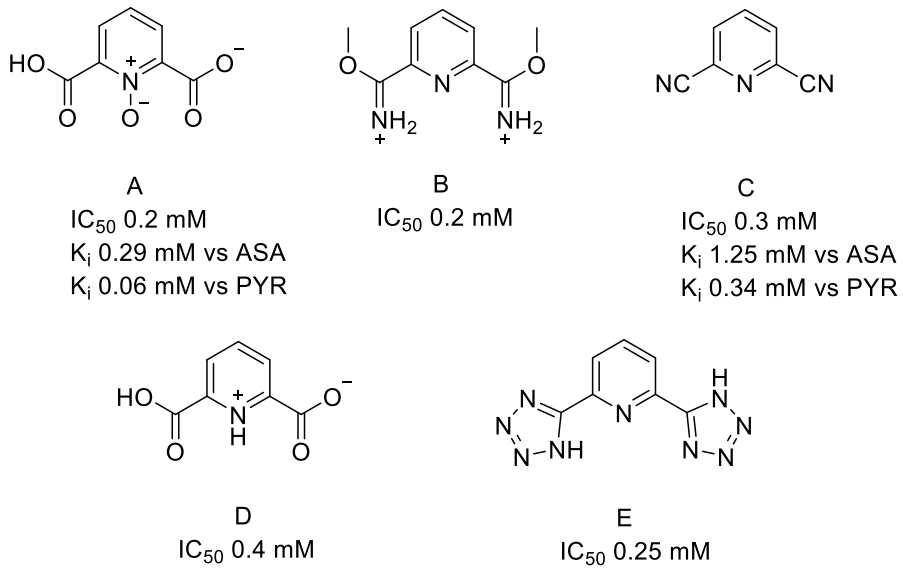


Figure 1.17: Inhibitors of DHDPS developed by Couper and Co-workers.[56]

Karsten discovered that the allosteric inhibitor, *L*-lysine (Figure 1.18), inhibits DHDPS around 90% at saturation conditions in 1997.[57] Additionally, lysine binds to the allosteric site of DHDPS at pH 8 with a K_i of 0.3 mM whereas at low pH, the K_i increases to 5 mM. Substrate analogues were also screened to identify possible inhibitors and the K_{is} or K_{ii} values were reported with respect to ASA and PYR (Figure 1.18). It is important to note that all of the inhibitors were potent at either low millimolar or high micromolar concentrations.

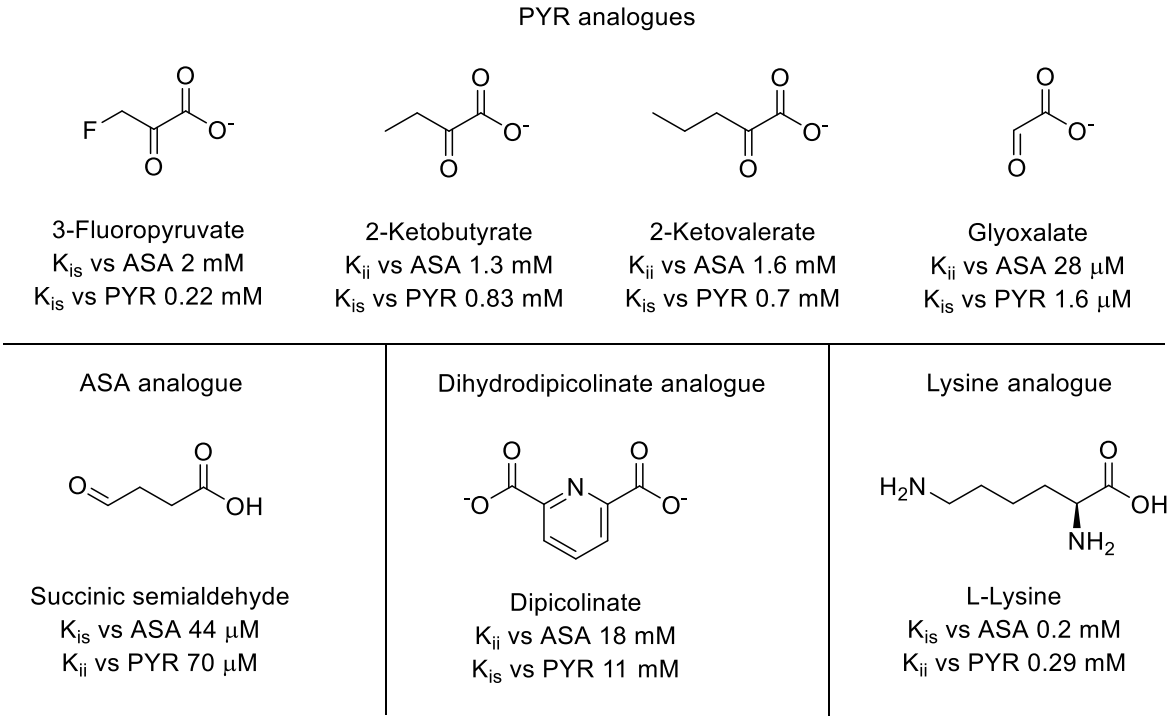


Figure 1.18: Reported inhibitors of DHDPS by Karsten.[57]

Coulter and co-workers explored the development of DHDPS inhibitors in 1999 based on the hypothesised cyclic lactol structure of ASA.[48] Homoserine lactone was discovered as a non-competitive inhibitor of DHDPS with respect to both substrates, ASA and PYR with K_i of 12 mM and 11.5 mM respectively. Reversible, non-competitive inhibition was observed for 2-aminocyclopentanone with a K_i of 1.8 mM with respect to ASA. (*R*)-Cysteine sulfonic acid and (*S*)-glutamic acid were uncompetitive inhibitors of DHDPS with K_i' of 7.35 mM and 1.15 mM with respect to ASA while (*S*)-aspartic acid was found to be a mixed type inhibitor with a K_i' of 3 mM with respect to ASA. Moreover, (*S*)-3-aminopyrrolid-2-one, (*S*)-asparagine, (*S*)-methyl-(*R*)-cysteine and the corresponding sulfoxide and sulfone were not inhibitors of DHDPS.

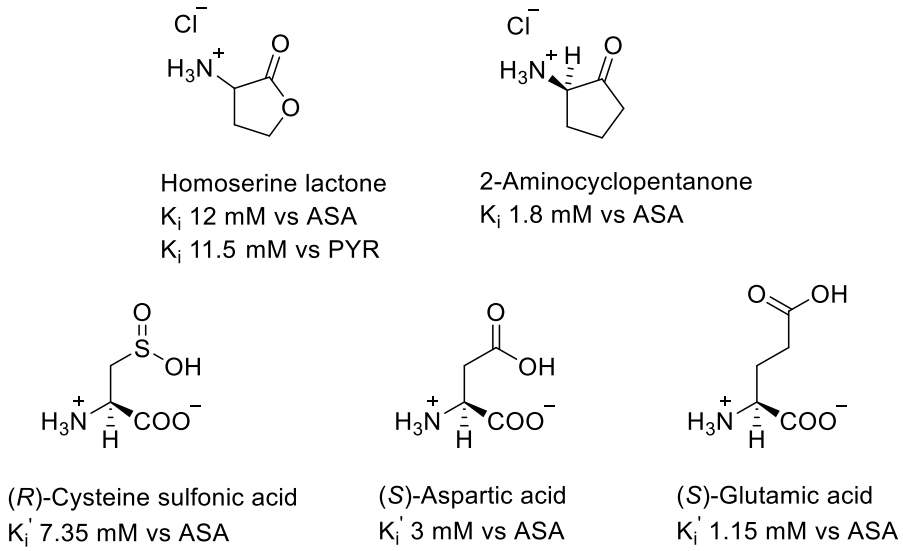


Figure 1.19: DHDPS inhibitors developed by Coulter and co-workers.[48]

In 2005, Turner and co-workers investigated inhibitors for DHDPS inspired by the heterocyclic intermediate, HTPA.[45] Two compounds out of the library of nitrogen containing heterocyclic compounds were active with IC_{50} of 14 mM and 22 mM as shown in Figure 1.20. None of the sulphur containing heterocyclic compounds were active.

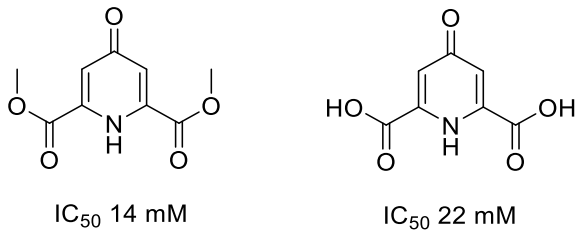
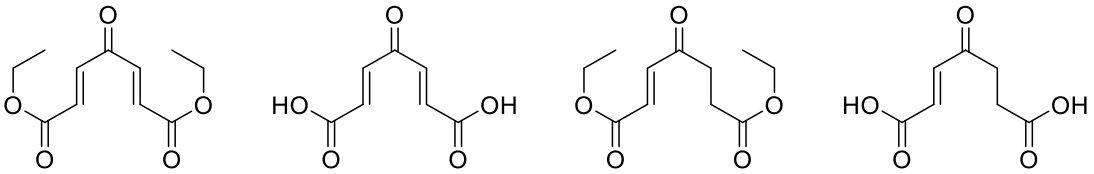


Figure 1.20: Inhibitors developed by Turner and co-workers.[45]

In the same year, Turner and co-workers reported two irreversibly binding inhibitors (Figure 1.21; A and C) for DHDPS and these were inspired by the enzyme bound acyclic condensation product of ASA and PYR.[46] However, the values for either IC_{50} or K_i were not disclosed until 2008 as part of an extended series as illustrated in Figure 1.21.[58] According to their findings, di-enes (Figure 1.21; A and B) are better inhibitors than mono-enes (Figure 1.21; C and D) with the Michael acceptor in all of the active compounds able to irreversibly bind with DHDPS.



A
K_i^{app} 4.95 mM

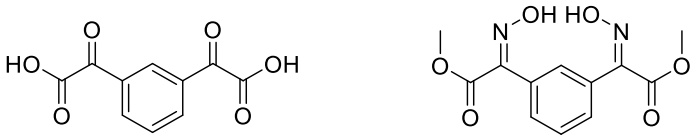
B
K_i^{app} 1.63 mM

C
K_i^{app} 10.9 mM

D
K_i^{app} 32.4 mM

Figure 1.21: Inhibitors developed by Turner and co-workers.[46, 58]

An extension of the potential inhibitors inspired by the acyclic enzyme bound intermediate generated two compounds with low millimolar K_i^{app} values where compounds E and F were best out of the eight synthesised compounds (Figure 1.22).[59]

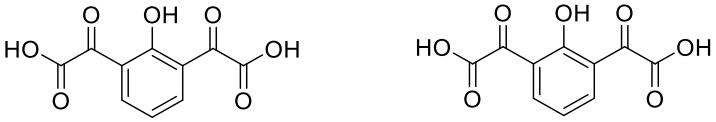


E
K_i^{app} 2.96 mM

F
(*Z*,*Z*):(*E*,*Z*) = 3:2
K_i^{app} 0.33 mM

Figure 1.22: Inhibitors developed by Boughton and co-workers.[59]

The Hutton group continued the development of inhibitors based on their initial findings to discover slow and tight binding of two compounds with low K_i values (Figure 1.23).[60] Binding was confirmed by mass spectrometry.[60] These compounds also relate closely to the enzyme bound acyclic intermediate.



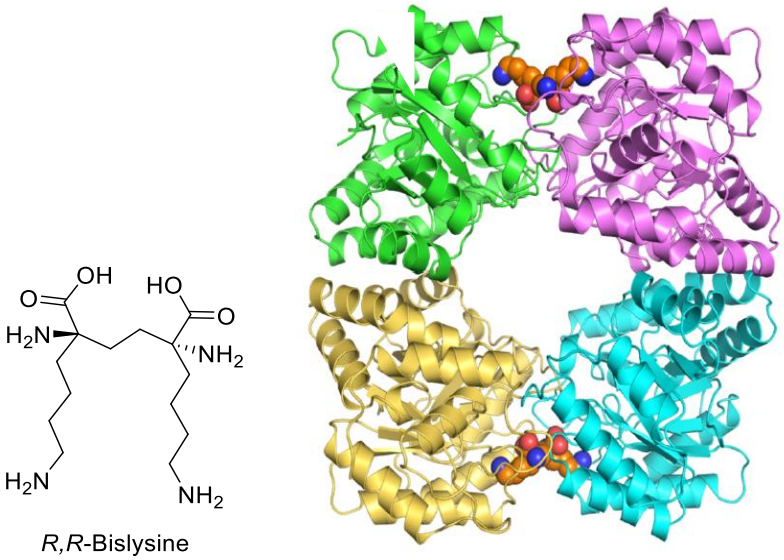
G
K_i^{app} 12 mM
$K^*_i{}^{app}$ 0.04 mM

H
$K^*_i{}^{app}$ 0.29 mM

Figure 1.23: Inhibitors developed by Boughton and co-workers.[60]

In 2016, Skovpen and co-workers developed the first potent inhibitor for the allosteric binding site.[61] This inhibitor consists of two lysine molecules which are connected by an ethylene bridge, hence named bislysine; with a K_i of 200 nM and an IC_{50} of 400 nM against

Campylobacter jejuni. A crystal structure of the bislysine bound *C. jejuni* DHDPS enzyme in a 2.2 Å resolution was also reported.



A B

Figure 1.24: (*R*,*R*)-Bislysine (A) and (*R*,*R*)-Bislysine bound *Campylobacter jejuni* DHDPS (B).[61]

More recently, Shrivastava and co-workers conducted a study in 2016 on α-ketopimelic acid to study the binding and also developed a series of structural analogues of α-KPA.[62] The validity of the previous studies conducted in 1974 by Yamakura and Ikeda were confirmed through co-crystallised structures of α-KPA bound DHDPS. Moreover, their studies measured the IC_{50} values of α-KPA and one analogue was found to exhibit low micromolar inhibition against DHDPS as shown in Figure 1.25.

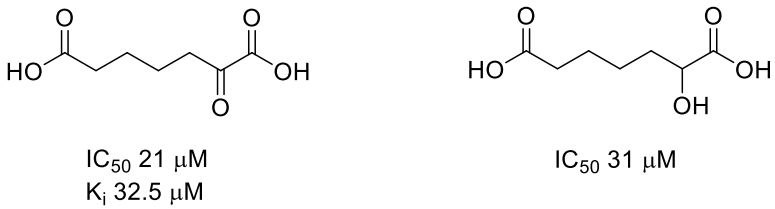


Figure 1.25: Inhibitors of DHDPS reported by Shrivastava and co-workers.[62]

Unfortunately to date most of the active site inhibitors which have been reported are of low millimolar potency. While the allosteric site inhibitor reported exhibit nanomolar potency, antibacterial activity has not been demonstrated.

As most of the inhibitor data was reported, in a variety of measurements such as K_i, K_i^{app}, K_{is}, $K^{*}_{i}{}^{app}$ and IC_{50}, it is therefore difficult to make direct comparisons.

All of the studies to date were conducted on isolated DHDPS enzyme of *Escherichia coli*.[45, 46, 48, 55-60, 62] Three exceptions were when DHDPS was used from *Bacillus subtilis* in 1974,[54] *Campylobacter jejuni* in 2016,[61] and *Mycobacterium tuberculosis* in 2016 where *B. subtilis* was Gram- positive and *C. jejuni* and *E. coli* were Gram-negative.[62] None of the above discussed inhibitors were tested on whole bacteria or plant DHDPS for possible antibacterial or herbicidal activity respectively.

In 2018, our group published a patent on the thiazolidinediones and analogous heterocycles as inhibitors of *E.coli* DHDPS with low micromolar IC_{50} values (Figure 1.26).[63]

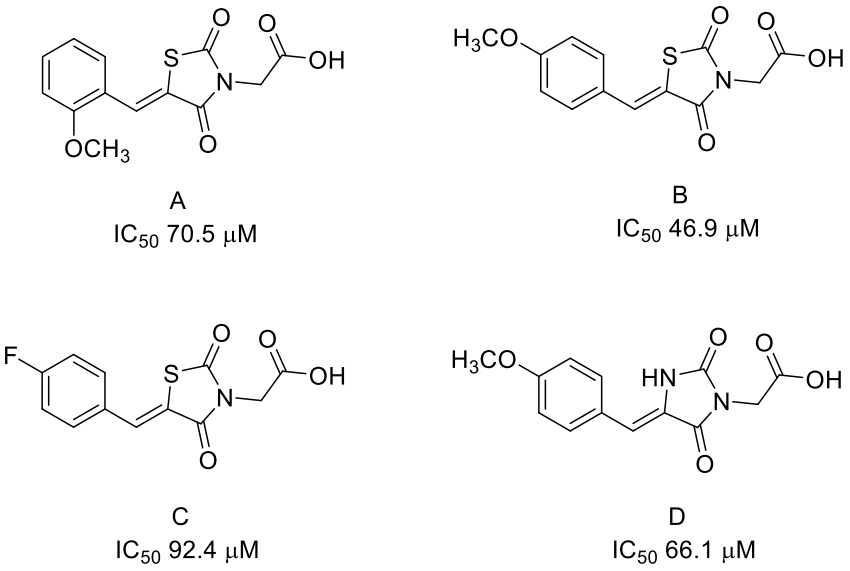


Figure 1.26: Selected inhibitors from WO2018187845A1.[63]

Compounds A and B inhibited isolated DHDPS of *Arabidopsis thaliana*, a small flowering weed, as well as exhibited herbicide-like properties when tested on seedling studies. Most interestingly, compounds A, B, and C were selected for the antibacterial assays, but they did not show any inhibition on both Gram-negative (seven species tested) and Gram-positive (six species tested) bacteria. This lack of antibacterial activity is a desirable property for herbicide agents. No seedling development was observed at 125 μM concentration and above in Agar plates. Moreover, selected compounds A, B, and D are non-toxic to human cells when tested via MTT viability assay using Defensin as the positive control on human liver cells (HepG2) and human kidney cells (HEK293).[64] These

compounds are the first herbicide-like compounds reported in the literature acting on the enzyme DHDPS, with further experimental data to be reported in due course.

1.4 Novel leads for DHDPS inhibition

In 2010, a high throughput screen (HTS) was commissioned by Perugini and co-workers against DHDPS using nearly 88, 000 compounds from a library created by the Walter and Eliza Hall Institute (WEHI).[65] The library was narrowed to 38 compounds using the *o*-ABA assay as described in section 1.3.3. Six compounds were pursued by the Abbott group as potential lead compounds and are illustrated in Figure 1.27.

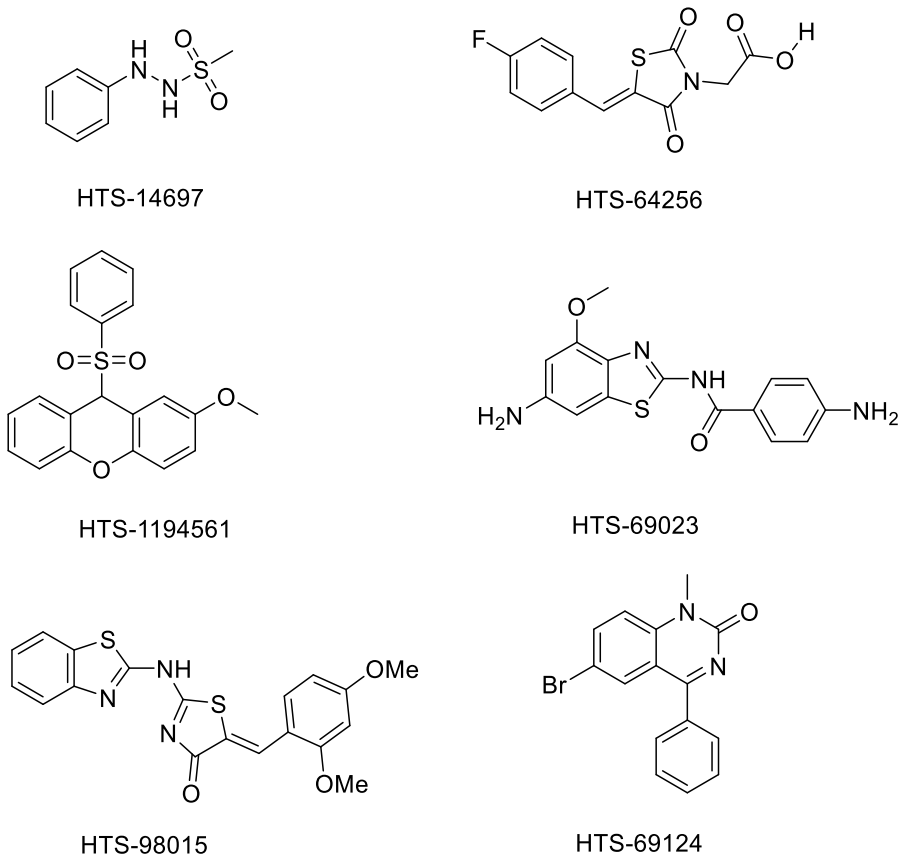


Figure 1.27: Potential DHDPS inhibitors identified from high throughput screening.

Compounds of HTS 1194561, HTS 69023 and HTS 69124 and a number of analogues were synthesised but were found to be poorly soluble or had poor activity against *E. coli* DHDPS with IC_{50} above 125 μM while precursors used in the coupling reaction of HTS 98015 had low IC_{50} values. Due to the toxicity to human cells, both human liver cells (HepG2) and human kidney cells (HEK293), those two compounds were not pursued

further. Development of potential herbicidal agents using HTS-64256 has led to the patent already discussed.[63] This project focuses on development of analogues of HTS-14697.

In 2015, the synthesis of a series of eight analogues of HTS 14697 was undertaken (Figure 1.28).[66]

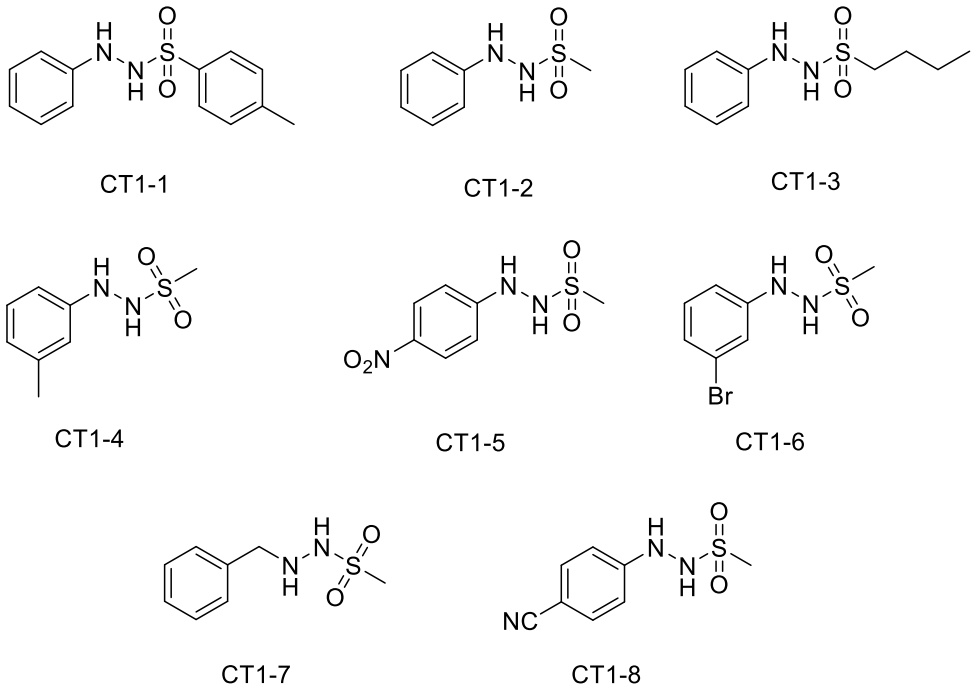
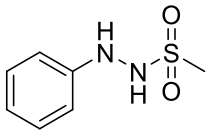
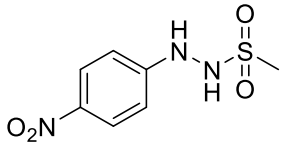
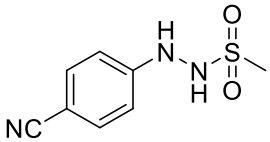


Figure 1.28: Analogues of HTS-14697 synthesised in 2015 with IC_{50} against *E. coli* DHDPS.

The compounds were tested against five species, two Gram-positive bacteria (*Streptococcus pneumonia*, *Staphylococcus aureus*), two Gram-negative bacteria (*Escherichia coli*, *Vibrio cholera*) and plant (*Vitis vinifera*) by Dr. Soares da Costa in collaboration with A/Prof. Perugini.[66] Table 1.5 represents the IC_{50} for two best inhibitors developed by Gardhi and HTS-14697 (CT1-2) as a reference.[66] *N'*-(4-Nitrophenyl)methane sulfonohydrazine (CT1-5) showed the most promising results against all the species tested with an IC_{50} of 72 µM against Gram-negative bacteria and an IC_{50} of 76 µM against plant DHDPS (Table 1.5).

Table 1.5: Preliminary biological results from 2015.[66]

Compound	IC$_{50}$ (µM)				
	S. pneumonia	***S. aureus***	***E. coli***	***V. cholera***	***V. vinifera***
	332	500	129	73	338
	128	105	72	54	75.8
	298	422	152	93	200

1.5 Project aims

This project aimed to synthesise novel antibacterial and herbicidal agents which are potent and effective against DHDPS. In light of the previous work on HTS-14697, this project will explore the synthesis of an expanded library of compounds. This work is significant due to the need of new inhibitors of novel targets in order to combat rising antibacterial and herbicidal resistance.

Chapters 2 and 3 discuss the organic synthetic strategies used in the generation of the library of compounds with variations at the aromatic ring and substitution at the sulfonyl position. A library of analogues was generated by varying the substitutions in the aromatic ring, ring expansion/contraction or by synthesising heterocyclic analogues. The structure activity relationship of the synthesised compounds is also discussed in detail.

Chapters 4 and 5 discusses the extensions made at the sulfonyl sulphur to yield a terminal α-keto acid functionality. Chapter 4 is mainly focused on the troubleshooting undertaken to achieve the target compound using various synthetic methodologies. Utilisation of the chemistry discovered in Chapter 4 to generate a library of compounds is discussed in Chapter 5. The structure activity relationship of the extensive library of compounds is also discussed in both chapters.

The project was guided by the biological data continuously. Chapter 6 concludes the work discussed in this thesis along with possible future work followed by the experimental methods in Chapter 7.

Chapter 2 Synthesis and Biological Evaluation of Sulfonyl Hydrazides; Part I

2.1 Introduction

In 2015, the synthesis of the lead compound HTS-14697/**CT1-2** (Figure 2.1) was undertaken as a potential inhibitor of DHDPS, followed by the generation of seven analogues.[66]

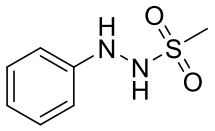
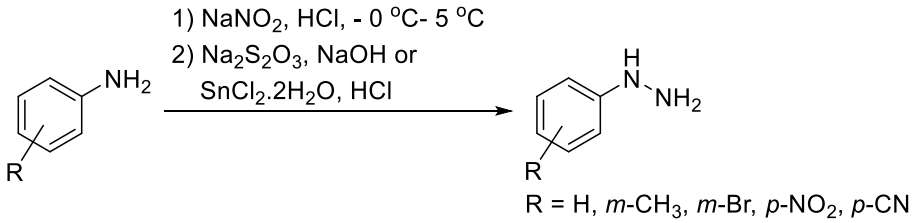


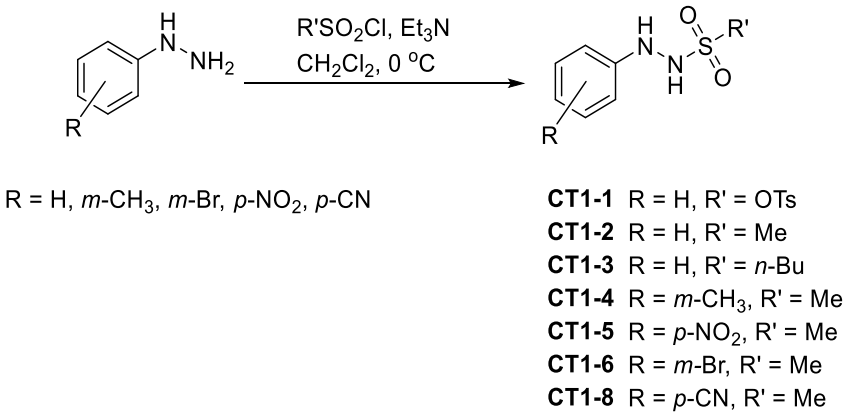
Figure 2.1: HTS 14697 (**CT1-2**).

Firstly, aromatic amines were diazotised followed by the reduction of the diazonium salt to obtain the corresponding hydrazine (Scheme 2.1). Sodium nitrite was used as the nitrosating reagent to generate the diazonium salt at low temperatures (0 – 5 °C) to stop the production of corresponding phenols. Hydrochloric acid created an acidic environment to avoid the formation of diazohydroxides.[67-69] Nitrous acid was then generated *in situ*. It was evident that the diazotization reaction was complete when the solution became clear. Reduction of the diazonium salts were achieved in the second step by the use of sodium thiosulfate or tin (II) chloride.



Scheme 2.1: General reaction scheme for the preparation of aromatic hydrazines.

The synthesised aromatic hydrazines were then reacted with two equivalents of substituted sulfonyl chloride in the presence of triethylamine as the base to obtain HTS-14697 and its analogues via an S_N2 reaction (Scheme 2.2). The reaction was carried out at lower reaction temperatures due to the exothermic reaction associated with hydrochloric acid generation.



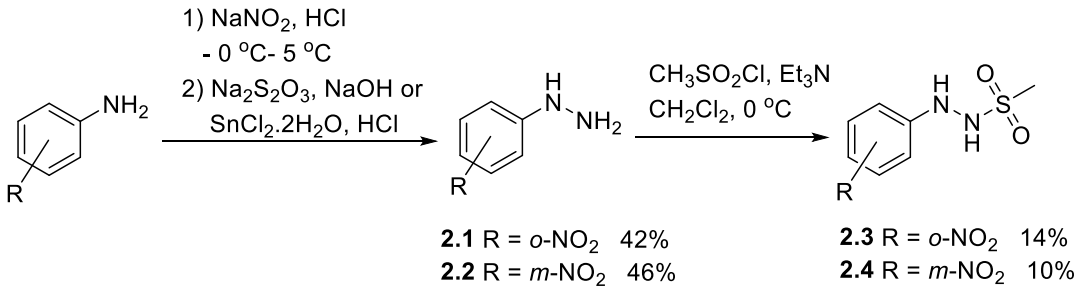
Scheme 2.2: General reaction scheme for the preparation of HTS-14697 analogues.

In vitro testing of the eight compounds was carried out using a DHDPS-DHDPR coupled assay. Preliminary results (Figure 1.28, Table 1.5) indicated that changing the methyl substitution (**CT1-2**) on the sulfonyl group to a *n*-butyl group (**CT1-3**) or a tosyl group (**CT1-1**) was not tolerated, nor was the addition of a methyl (**CT1-4**) or a bromo group (**CT1-6**) at the *meta* position of the aromatic ring. However, *para* substitution appeared more promising with a nitrile group (**CT1-8**) resulting in an IC_{50} of 152 ± 8 µM and the nitro group (**CT1-5**) with an IC_{50} of 72 ± 3 µM (Table 1.5).

This chapter outlines the work undertaken to synthesise HTS-14697 analogues in light of the findings in 2015.[66] Modifications were made to the aromatic ring to explore the best aromatic substituent and the best substitution position on the ring, while extensions to the alkyl chain length at the sulfonyl group were also investigated. Bioisostere replacements of the methyl group at the sulphur were also explored along with alterations made to the hydrazine functionality to explore its importance in the biological activity.

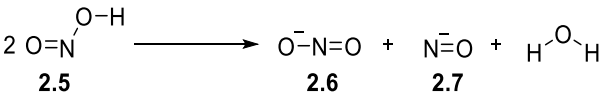
2.2 Optimisation of the position of the nitro group on the aromatic ring

Given the potency of **CT1-5**, it was of interest to find the best position of the nitro group on the aromatic ring. Synthesis of both the *ortho* nitro and *meta* nitro analogues was carried out via diazotisation of the relevant aromatic amines and subsequent reduction, followed by coupling with methanesulfonyl chloride (Scheme 2.3).



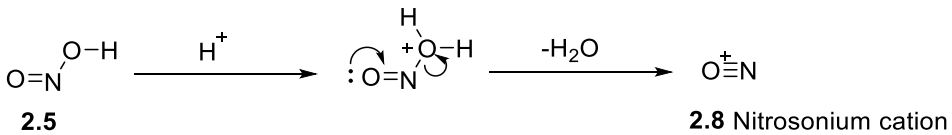
Scheme 2.3: Synthesis of compounds with nitro functionality at the *meta* and *ortho* position of the aromatic ring.

Formation of diazonium salts at lower temperatures is achieved when aromatic amines react with nitrous acid as discussed above. Generation of nitrous acid **2.5** was carried out *in situ* at low reaction temperatures due to its instability, where decomposition can lead to the formation of nitrogen dioxide **2.6**, nitric oxide **2.7** and water (Scheme 2.4).



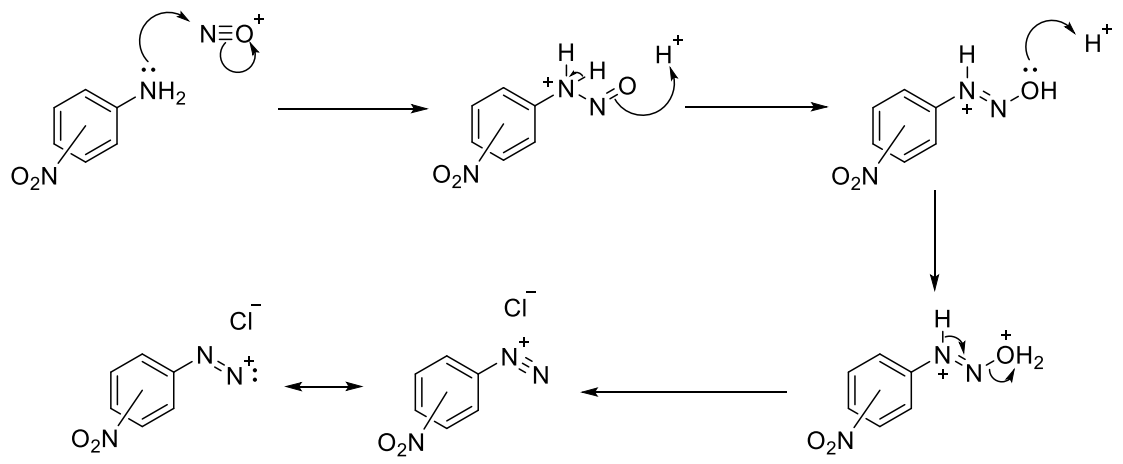
Scheme 2.4: Decomposition of nitrous acid generally at temperatures above 5 °C.

Generated nitrous acid **2.5** undergoes protonation and dehydration to generate the reactive nitrosonium cation **2.8** (Scheme 2.5).



Scheme 2.5: Generation of the nitrosonium cation.

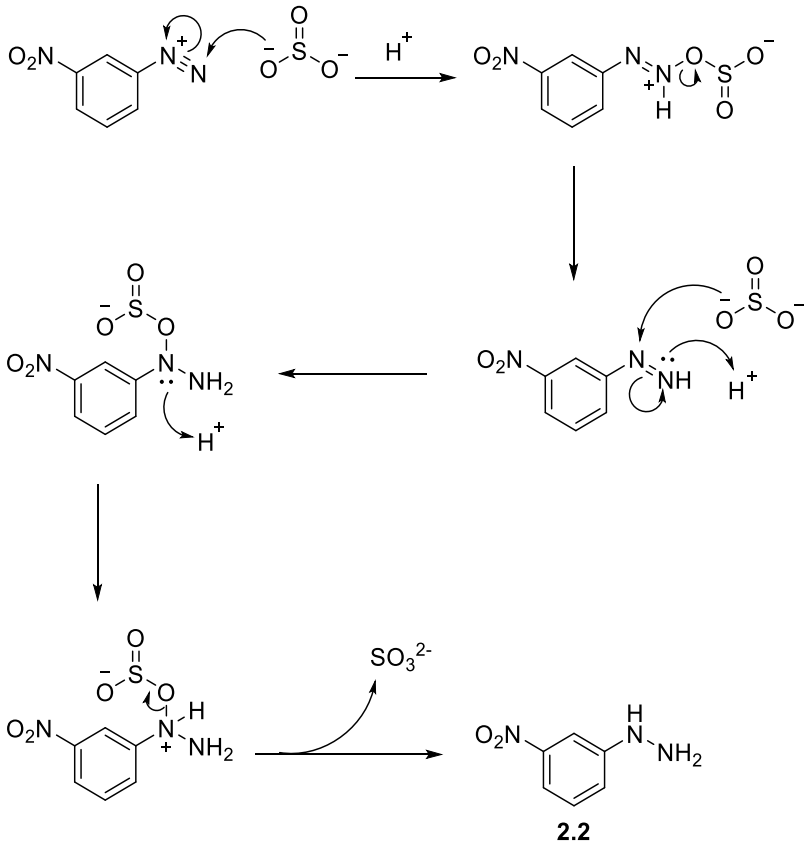
Nitrosonium cation then gets attacked by the nitrogen lone pair of the aromatic aniline. This reaction generates an *N*-nitrosoammonium intermediate and further proton transfers followed by dehydration results in the generation of the corresponding diazonium salts (Scheme 2.6).



Scheme 2.6: Proposed reaction mechanisms for the formation of the nitrosonium cation and the diazonium salt from nitroanilines.

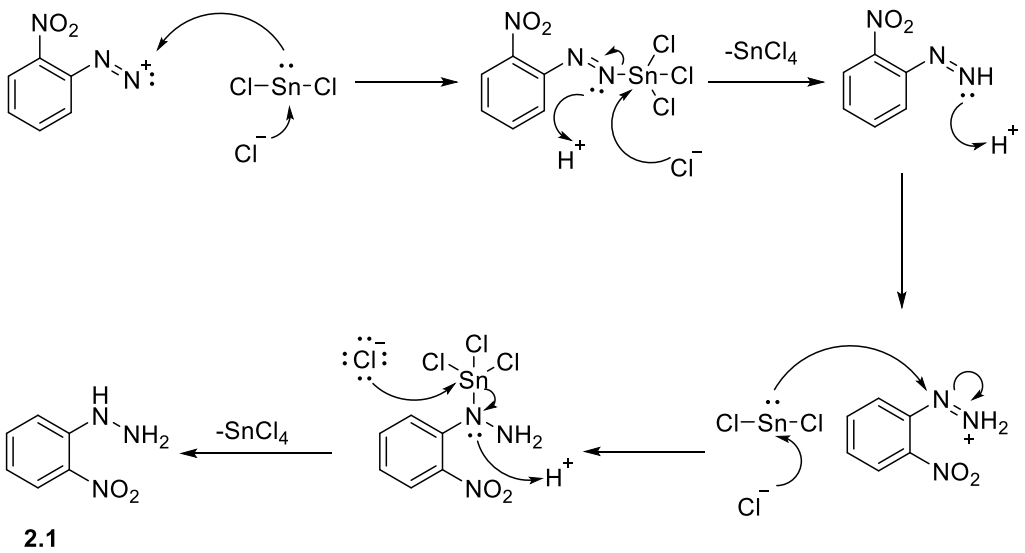
Literature methods use either sodium sulphite or tin (II) chloride for the reduction of the diazonium salts of nitro anilines.[70-72] A clear correlation between the diazonium reducing agents and the aromatic substituents was not observed in the literature. As a result, when lower yields of the hydrazine were obtained from a particular method, the method was changed to the other and usually a better yield was obtained.

For the reduction of *m*-nitroaniline, sodium sulphite was used and a moderate yield of 46% was obtained. Generation of the azo-sulphite salt followed by proton transfers yielded the corresponding hydrazine (Scheme 2.7). ^{1}H NMR spectroscopy analysis showed a downfield shift of all the aromatic protons as expected. This effect is due to the presence of two electron withdrawing NH groups which results in the aromatic protons to be more deshielded.". Compared to the clear pattern of proton signals obtained from *m*-nitroaniline where a doublet at 7.59 ppm, a singlet at 7.49 ppm, a triplet at 7.28 ppm, and another doublet at 6.94 ppm occurred, the ^{1}H NMR signals obtained from (3-nitrophenyl)hydrazine were completely different. Doublets were overlapped and observed in the region of 8.55 ppm – 5.50 ppm with an integration of two protons, whereas a sharp singlet and a clear doublet were observed at 8.40 ppm and 8.23 ppm respectively. One broad signal corresponding to one proton was obtained at 10.20 ppm which corresponds to the NH proton. Proton exchange with water could have been used to confirm this possibility, as the terminal protons would have disappeared in the ^{1}H NMR spectrum.



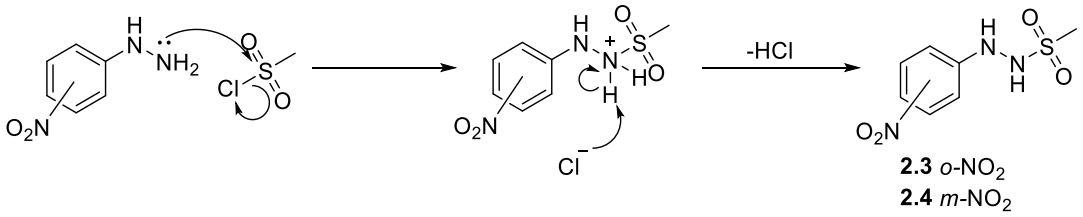
Scheme 2.7: Proposed reaction mechanism for the reduction of 3-nitrobenzenediazonium salt with sodium sulphite.

The reduction of the *o*-nitro diazonium salt was achieved in a moderate yield when tin (II) chloride was used. A lone pair of electrons from stannous chloride attacks the electron-deficient nitrogen. Proton transfers followed by the oxidation of stannous to stannic chloride occurs facilitating the aromatic hydrazine formation (Scheme 2.8). ^{1}H NMR spectroscopy analysis showed a downfield shift of all of the aromatic protons as expected, where two doublets were obtained at 8.14 ppm, and 7.30 ppm and two triplets were obtained at 7.73 ppm and 7.07 ppm corresponding to one proton each. The pattern in the aromatic proton splitting is as expected of a *meta* substituted phenyl ring. Only one peak was obtained for the hydrazine motif of the compound at 9.21 ppm. This might be due to proton exchange of water with the terminal hydrogens.



Scheme 2.8: Proposed reaction mechanism for the reduction of 2-nitrobenzenediazonium salt with stannous (II) chloride.

Generated aromatic nitrohydrazines were then subjected to S_N2 type substitution to generate compounds **2.3** and **2.4** (Scheme 2.9).



Scheme 2.9: Proposed reaction mechanism for the reaction of aromatic nitro hydrazines with methanesulfonyl chloride.

Two equivalents of methanesulfonyl chloride and triethylamine was used to react with **2.2** and **2.4** was obtained in a low yield of 10% after purification by column chromatography. A sharp singlet obtained at 3.37 ppm corresponding to three protons along with the four aromatic protons at 8.23 ppm as a doublet, 7.85 ppm as a triplet, 7.69 ppm as a singlet, and 7.67 ppm as a triplet confirmed the structure of the final product in ^{1}H NMR analysis. The mass peak obtained at 232.0 *m/z* also confirms the identity of the product which corresponds to the $(M^+ + H)^+$.

Similarly, two equivalents of methanesulfonyl chloride were used with **2.1** for the synthesis of **2.3**. Unlike in the previous coupling reactions, a solid was observed after the reaction time. The filtrate and the solid were isolated and analysed.

In the ^{1}H NMR spectra of the concentrated filtrate in deuterated chloroform, a peak at 9.72 ppm was obtained for the NH proton. Aromatic peaks were observed at 8.25 ppm as a doublet, 7.64 ppm as a triplet, 7.42 ppm as a doublet, and 7.09 ppm as another triplet and a singlet at 3.44 ppm, which corresponds to six protons. This was likely to be two methyl substitutions which are chemically equivalent. The ^{13}C NMR spectrum also gave one signal at 43.1 ppm in the methyl region, confirming the chemical equivalency of the two methyl groups. Therefore, it was proposed that the filtrate contains disubstituted compound **2.3** in high purity. This was further confirmed by the peak obtained at 331.8951 from the HRMS analysis which corresponds to the $(M^{+} + Na)^{+}$.

The isolated solid was approximately a 50:50 mixture of two products **2.3** and **2.4**, and was confirmed by ^{1}H NMR spectroscopy in deuterated dimethylsulfoxide (Figure 2.2). Two sets of peaks were observed in the aromatic region, which equates to eight protons where four triplets and four doublets integrate to one proton each (Figure 2.2). Two peaks at 3.53 ppm and 3.05 ppm with integrations of six and three, respectively, were observed. The peaks appearing at 10.82 ppm and 10.24 ppm corresponds to the NH protons.

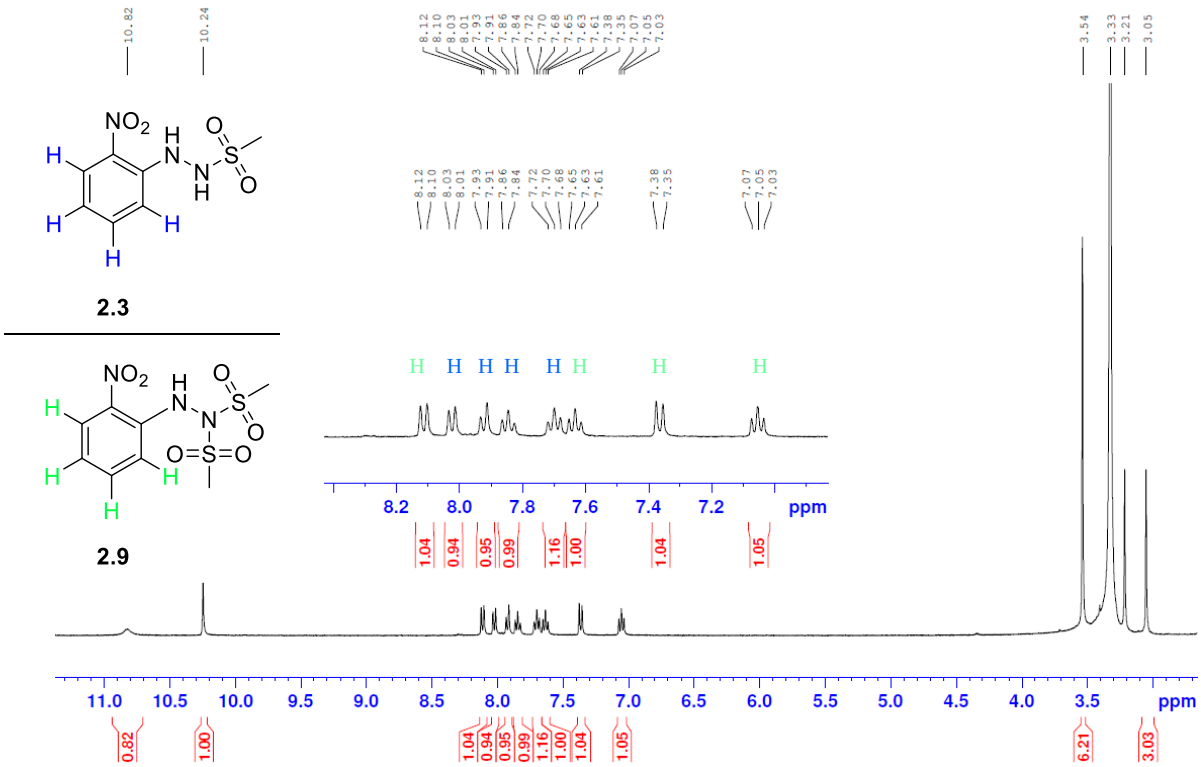
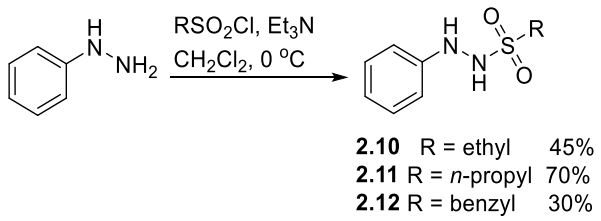


Figure 2.2: ^{1}H NMR spectrum of the 50:50 mixture of **2.3** and **2.4**.

Isolation of **2.3** for further biological testing was performed via column chromatography. The ^{1}H NMR spectrum of the pure product in deuterated dimethylsolfoxide had the methyl peak appearing at 3.05 ppm and two NH protons corresponding to the hydrazine motif at 9.40 ppm and 9.35 ppm with integrations of one each. Four aromatic protons were observed at 8.05 ppm, 7.62 ppm, 7.53 ppm and 6.88 ppm as two doublets and two triplets, respectively.

2.3 Variation of the sulfonyl alkyl chain

The next investigation was to explore the tolerance of alternate aryl and alkyl substitutions at the sulfonyl group with smaller groups than the *n*-butyl (**CT1-3**) and tosyl (**CT1-1**) which had not been tolerated previously. Phenylhydrazine was reacted with either ethylsulfonyl chloride, *n*-propylsulfonyl chloride or phenylmethanesulfonyl chloride to synthesise three analogues. The reactions proceed through S_N2 type substitution where triethylamine scavenges the generated hydrochloric acid (Scheme 2.10).



Scheme 2.10: Synthesis of different substitutions at the sulphur.

The ^{1}H NMR spectra of **2.10** and **2.11** both contain similar aromatic regions where three protons and two protons give a multiplet and a doublet, respectively. The multiplet appears overlaid with the chloroform solvent peak, therefore the integration was set from the methyl peak for both analogues. For **2.10**, a triplet was observed at 1.36 ppm for the methyl protons and a quartet was observed at 3.00 ppm for the CH_2 protons in ^{1}H NMR analysis. For **2.11**, a triplet was observed at 0.99 ppm for methyl protons and a multiplet and a quartet were observed at 1.87 ppm and 2.95 ppm respectively for the CH_2 protons (Figure 2.3). The ^{1}H NMR analysis supports the synthesis of both of the analogues and the ^{13}C NMR spectra strengthens the claims along with the DEPT-135 where ethyl carbons show negative peaks. For **2.10** one negative peak was observed at 46.4 ppm where a positive peak was observed in the ^{13}C NMR spectra. Similarly, two negative peaks were observed

at 53.5 ppm and 16.8 ppm for **2.11** in DEPT-135 whereas they were positive peaks in the ^{13}C NMR spectra.

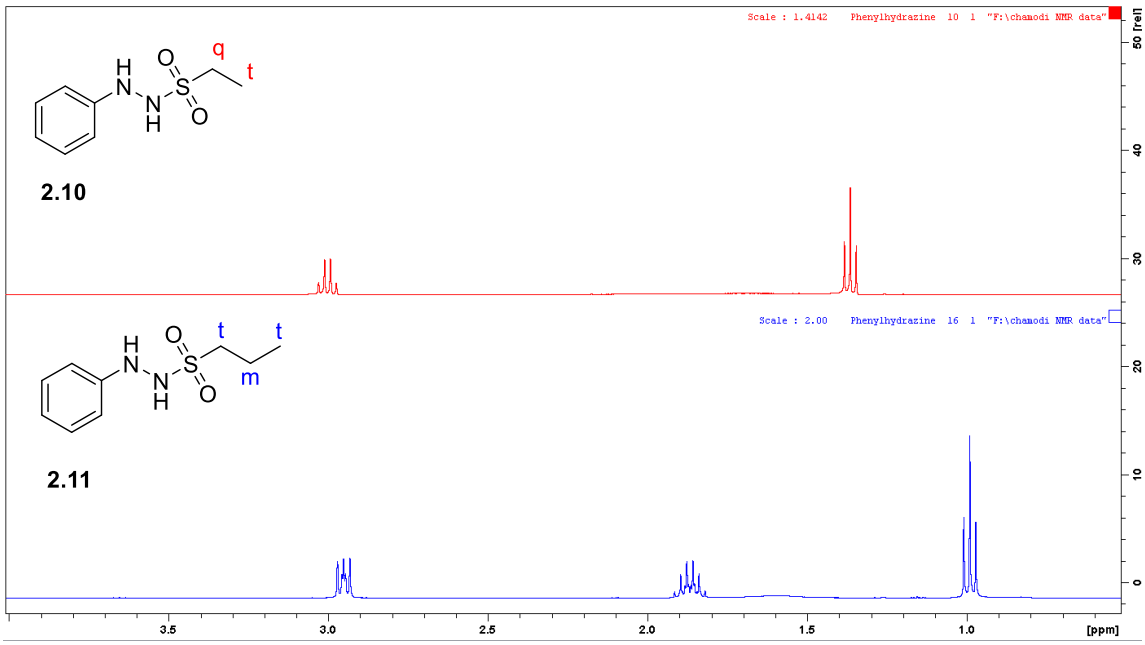
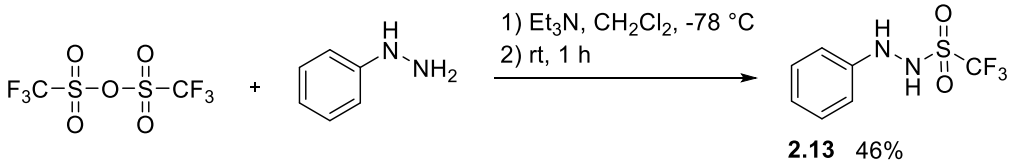


Figure 2.3: ^{1}H NMR spectra of **2.10** and **2.11** in the range of 0 – 4 ppm.

Compound **2.12** was a beige colour solid which had very low solubility in chloroform, therefore the NMR analysis was carried out in deuterated dimethylsulfoxide. The ^{1}H NMR spectra showed the hydrazine NH protons as sharp singlets at 9.10 ppm and 7.95 ppm. Aromatic protons were observed as triplets, doublets and multiplets in the range of 7.41 – 6.71 ppm and the CH_2 peak was observed at 4.38 ppm due its deshielded nature. This is due its positioning where it is sandwiched between the aromatic ring and the sulfonyl functionality. Both aromatic rings of the compound contain symmetric planes therefore, only 8 carbons were observed in the ^{13}C NMR spectra at 149.2 ppm, 131.5 ppm, 130.0 ppm, 129.1 ppm, 128.8 ppm, 128.5 ppm, 119.5 ppm, and 113.2 ppm along with the CH_2 carbon at 55.6 ppm, which appeared upfield compared to the aromatic carbons. The CH_2 carbon appeared as a negative peak as expected in the DEPT-135 spectra, confirming the structure of the synthesised compound.

A common isostere for a methyl group is the trifluoromethyl group. Even though the steric parameters of the fluorine are similar to hydrogen, the electronic effects are different due to the high electron-withdrawing nature of the fluorine. This strong electronic effect might have interesting interactions with the DHDPS enzyme.

Synthesis of **2.13** was achieved via a literature cited method using triflic anhydride, triethylamine in dichloromethane to obtain the product in a 46% yield (Scheme 2.11).[73] The NMR studies showed matching peaks with the literature reference where the ^{1}H NMR spectra cited peaks as a multiplet in the region of 7 ppm. The fluorine NMR spectrum showed a single peak at -77.9 ppm, further confirming the identity of the product.



Scheme 2.11: Synthesis of 1,1,1-trifluoro-N'-phenylmethanesulfonohydrazide.

2.4 Investigation of *para* substitution on the aromatic ring

Preliminary work by Gardhi[66] as addressed in section 2.1, suggested that *para*-substitution may be preferred. The nitro group is a strongly *meta*-directing deactivating group on an aromatic ring. The reactivity of a nitro substituted aromatic ring is poor due to the withdrawal of electrons inductively via the σ bond between the nitrogen and the aromatic ring along with the resonance electron withdrawal via π electron flow from the ring to the nitrogen (Figure 2.4). The nitrile group is also a *meta*-directing deactivating group.

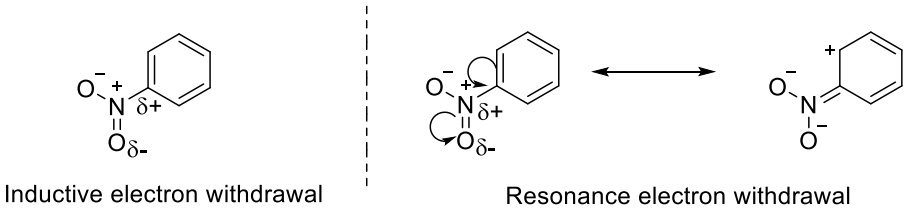
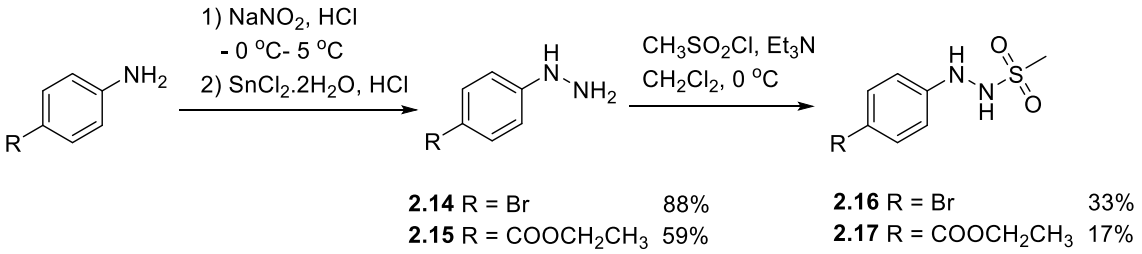


Figure 2.4: Two types of electron withdrawals from the nitro group.

Investigations to find the best substituent to have at the *para* position of the aromatic ring were carried out by the synthesis of analogues with other ring deactivators (Scheme 2.12).



Scheme 2.12: *para* substituted analogue generation.

The synthesis of **2.14** was carried out by following literature methods.[74, 75] The work up of the reaction required basification with 5M sodium hydroxide followed by an extraction. ^{1}H NMR signals corresponded to the literature results where two doublets were obtained for the aromatic protons at 7.18 ppm and 6.69 ppm along with two broad peaks at 6.68 ppm (for the NH) and 3.98 ppm (for the terminal NH_2). The hydrazine was then carried forward for the coupling reaction using methanesulfonyl chloride, triethylamine in dichloromethane, however the desired product was not obtained. Different reaction conditions were attempted where reaction temperatures, solvents and bases were altered without success.

Compound **2.14** was re-synthesised, but the work up of the reaction was carried out without basifying the reaction mixture. As a result, the obtained hydrazine was not the free hydrazine but (4-bromophenyl)hydrazine hydrochloride. The aromatic protons appeared downfield compared to the free hydrazine, with ^{1}H NMR signals observed at 7.44 ppm and 6.89 ppm together with the NH peak at 8.30 ppm and the terminal NH_2 peak at 9.94 ppm. The hydrazine salt was then carried forward for coupling with methanesulfonyl chloride under general conditions to obtain the desired product in a moderate yield after purification by column chromatography. The ^{1}H NMR spectrum showed that the aromatic protons are slightly upfield compared to that of **2.14**, which was as expected due to the substitution of an electron-rich group at the terminal NH_2. A clear peak for the methyl protons was observed at 2.95 ppm as well as the two NH peaks appearing at 9.04 ppm and 8.13 ppm. Moreover, the *m/z* of 187.1 and 189.1 corresponds to the ^{79}Br and ^{81}Br isotopes further confirming the identity of the product.

The synthesis of **2.15** was achieved using ethyl 4-aminobenzoate, sodium nitrite and tin (II) chloride. The work up was performed without base workup due to the difficulties encountered in the previous synthesis of **2.16**. The hydrochloride salt of 4-hydrazinylbenzoic acid was obtained in 59% yield. The ^{1}H NMR spectrum shows a

downfield shift of the aromatic peaks for the **2.14** compared to the aromatic protons of the ethyl 4-aminobenzoate starting material. The aromatic proton peaks of the hydrazine appear as doublets at 7.87-7.84 ppm and 6.96-6.93 ppm compared to the doublets at 7.62-7.60 ppm and 6.54-6.52 ppm for ethyl 4-aminobenzoate. Also, the clear broad peaks of the hydrazine functionality appearing at 8.78 ppm for one proton and 10.06 ppm for two protons in the ^{1}H NMR spectra confirms that the desired product was obtained. There was only a slight shift of the peaks appearing for the ethyl functionality in the ^{1}H NMR spectra.

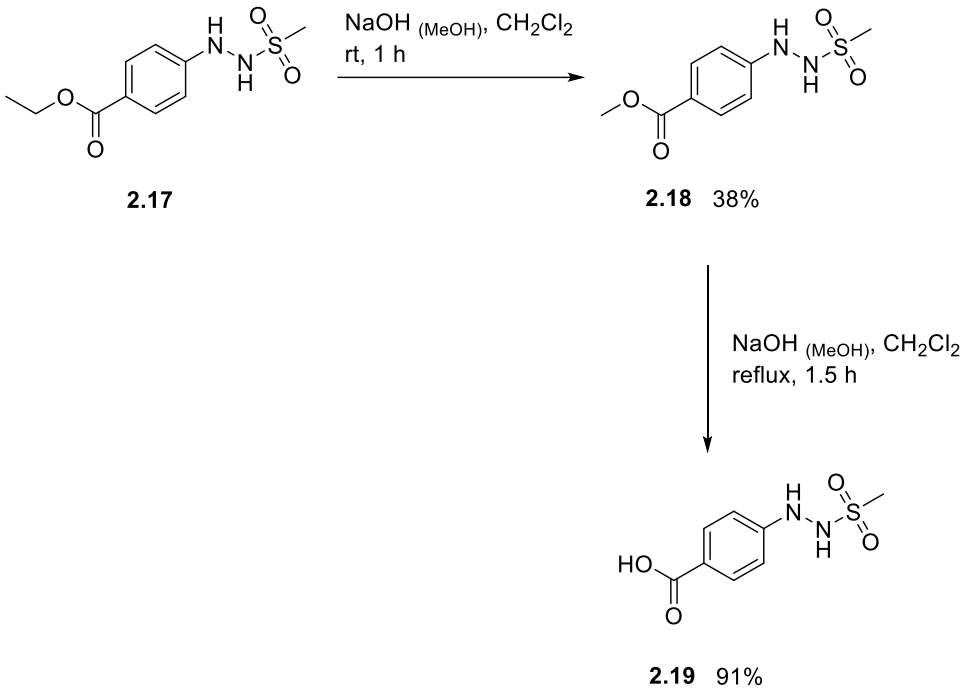
The coupling reaction of **2.15** with methanesulfonyl chloride was performed in dichloromethane with triethylamine as the base. The desired product was obtained in 17% yield. ^{1}H NMR spectra of the final compound after column chromatography confirmed the synthesis of **2.17**, where a new methyl functionality appeared at 3.47 ppm with an integration of three protons. Also, the aromatic protons shifted downfield compared to **2.15**, further confirming the identity of **2.17**. The aromatic protons appear as doublets at 8.22-8.20 ppm and 8.07-8.05 ppm with an integration of two protons each. The NH peaks were not observed, most likely due to the proton exchange with water as the sample NMR spectrum contained considerable water contamination. The region for the CH_2 functionality in the ^{1}H NMR spectra was shifted downfield in comparison to the ethyl 4-aminobenzoate and **2.15**. The CH_3 peak was observed at 1.34 ppm as a triplet and the CH_2 peak was observed at 4.36 ppm as a quartet in **2.17** whereas in ethyl 4-aminobenzoate and **2.15**, the CH_3 peak was observed at 1.24 ppm as a triplet and the CH_2 peak was observed at 4.17 ppm as a quartet.

For the synthesis of 4-aminobenzoic acid hydrazine, the general route was attempted using 4-aminobenzoic acid as the starting material, with sodium nitrite and sodium sulphite as the reducing agents. Formation of the hydrazine was achieved successfully in a low yield of 10%. Two doublets were observed at 7.61-7.59 ppm and 6.85-6.83 ppm in the ^{1}H NMR spectra which were downfield shifted compared to that of *p*-aminobenzoic acid. After the mass spectrometry analysis in the negative mode, the major peak obtained at 150.9000 *m/z* confirms the identity of the product which corresponds to the $(M - H)^-$ ion.

Difficulties were encountered during the coupling reaction of 4-aminobenzoic acid hydrazine with methanesulfonyl chloride. Unfortunately, only starting material was recovered. Different reaction temperatures, reaction times, bases and solvents were attempted but without success.

As an alternative route, ester hydrolysis was attempted on the previously synthesised **2.17** using methanolic sodium hydroxide solution in dichloromethane (Scheme 2.13). However, only the trans-esterified methyl ester product (**2.18**) was isolated in a moderate yield of 38%.

In the ^{1}H NMR spectra, the aromatic protons were not much shifted compared to **2.17**, but two singlets were obtained at 3.98 ppm and 3.25 ppm with integrations of three protons each. The peak obtained at 3.98 ppm corresponds to the methyl ester as these protons are more deshielded than the sulfonyl methyl at 3.25 ppm.



Scheme 2.13: *para* substituted ester and acid analogue generation.

Further hydrolysis of **2.18** (Scheme 2.13) was attempted at room temperature but no further reaction was noticeable by TLC. Therefore, the reaction mixture was refluxed and monitored via TLC until complete consumption of **2.18** was observed after 1.5 hours. Once the reaction mixture was cooled to room temperature, a solid precipitated. The target compound, **2.19** was isolated by filtration, and washing with cold methanol resulted in an excellent yield of 91%. Analysis by ^{1}H NMR spectroscopy showed only one peak integrated to three protons at 3.39 ppm corresponding to the methyl at the sulfonyl functionality. The aromatic protons were shifted upfield compared to **2.18**, as doublets at 8.04-8.02 ppm and 7.23-7.21 ppm with integration of two protons were observed. This shift is due to the poor electron-withdrawing properties of the carboxylic acid group

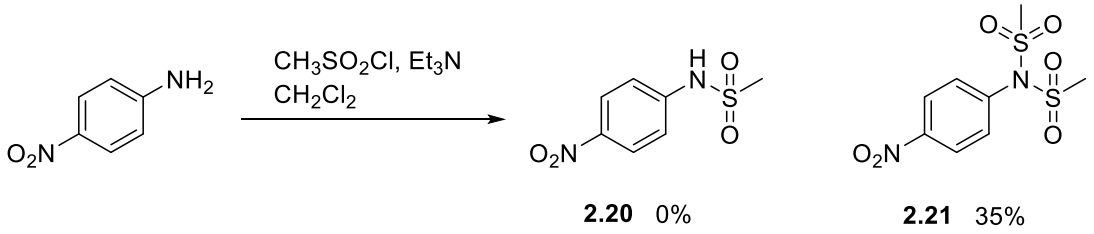
compared to an ester group, making the aromatic ring more shielded compared to when it was bound to a highly electron-withdrawing group.

2.5 Investigation of the hydrazine functionality

The importance of the hydrazine group in the inhibition of DHDPS enzyme was next explored. Three different alterations were made by synthesising five analogues.

Firstly, removal of one nitrogen from the hydrazine moitif was carried out. This change retains the nitrogen to sulphur/phenyl bond while reducing the number of nitrogens from two to one. The analogues generated would enable examination if there is any role played by both nitrogens in the hydrazine motif as well as any size effect.

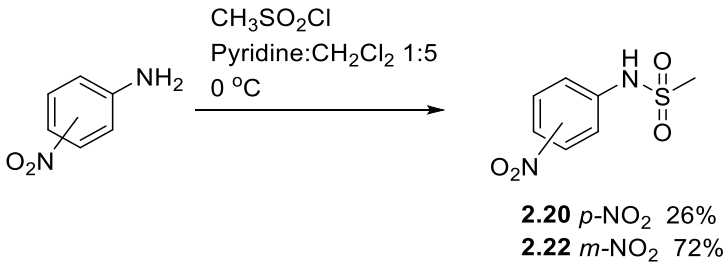
Synthesis of **2.20** was first attempted as shown in Scheme 2.14 by the use of two equivalents of methanesulfonyl chloride, and triethylamine in dichloromethane. The ^{1}H NMR spectrum after the workup of the crude material obtained had a peak appearing at 3.5 ppm, which corresponds to six protons and the aromatic region had an integration of four protons. It was evident from the NMR analysis, that the reaction conditions did not produce the desired mono-substituted product, but produced the di-substituted product as shown in Scheme 2.14. Furthermore, the HRMS gave a mass corresponding to $(M + Na)^{+}$ of 316.9869 confirming the identity of the product as di-substituted **2.21**.



Scheme 2.14: Attempted synthesis of **2.20**.

An alternative synthetic method for a similar analogue to **2.20** was found in the literature.[76] This method uses low reaction temperatures and pyridine as the base as well dichloromethane as a solvent respectively in 1:5 ratio (Scheme 2.15). This method resulted in the successful synthesis of CT1-7 in a 26% yield. The ^{1}H NMR spectra of CT2-7 contained a doublet of doublets for the aromatic protons at 8.22-8.20 ppm and 7.37-7.33 ppm, along with the methyl protons at 3.17 ppm. The HRMS gave the mass peak

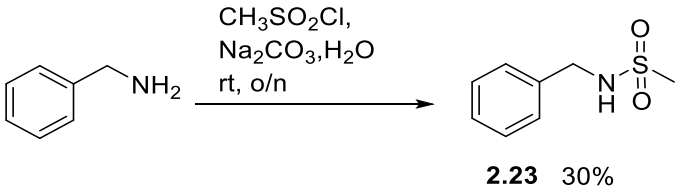
corresponding to the $(M + Na)^+$ ion at 239.0096, further confirming the desired product had been isolated.



Scheme 2.15: Mono-substituted sulfonamide synthesis.

Using to the method described above, synthesis of the *m*-nitro analogue **2.22** was achieved in a 72% yield. The aromatic region of the ^1H NMR spectra contained a singlet at 8.04 ppm, a doublet at 7.92 ppm, and a multiplet at 7.61 ppm, which collectively corresponds to four aromatic protons. The methyl protons were observed at 3.09 ppm and the NH proton appeared at 10.39 ppm. In the HRMS two peaks were observed at 217.1042 and 239.0096, which corresponds to $(M + H)^+$ and $(M + Na)^+$, respectively, as further evidence of the product.

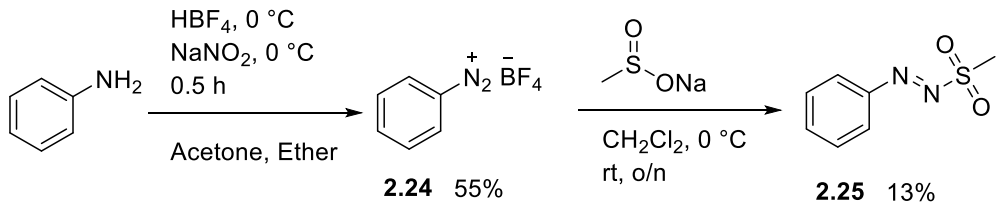
The second variation retained the hydrazine chain length but replaced the nitrogen with a carbon next to the phenyl ring. The biological data obtained from this analogue will clarify whether positioning of the nitrogen next to the phenyl ring is important for the binding to the enzyme, or whether it is just the chain length which is required for the observed activity of HTS-14697. Benzylamine was used to synthesise compound **2.23** (Scheme 2.16). The reaction is S_N2 type and was performed in water. Sodium carbonate was dissolved in water to generate a basic environment to scavenge the hydrochloric acid generated when the reaction is progressing. One equivalent of methanesulfonyl chloride was used to obtain the product.



Scheme 2.16: Synthesis of **2.23**.

In the ^{1}H NMR spectra, all the aromatic protons were observed together in the range of 7.38-7.26 ppm as a multiplet with an integration of five protons. The CH_2 protons gave a doublet at 4.35 ppm with an integration of two protons. The NH was observed at 7.72 ppm as a broad singlet and the methyl protons appeared at 2.87 ppm with an integration of three protons. In the DEPT-135 spectra of **2.23**, one negative peak was obtained at 47.2 ppm which corresponds to the CH_2 carbon. The rest of the peaks in the DEPT-135 spectra were positive peaks and appeared at 128.9 ppm, 128.7 ppm, 128.2 ppm, 127.9 ppm, 127.5 ppm, 127.4 ppm, and 41.2 ppm, which corresponds to the rest of the carbons in the compound.

Thirdly, an azo compound **2.25**, where a rigid double bond will be imposed instead of the flexible NH-NH functionality, was synthesised as the final exploration into the importance of the hydrazine functionality. Synthesis was performed following literature methods (Scheme 2.17).[77, 78]

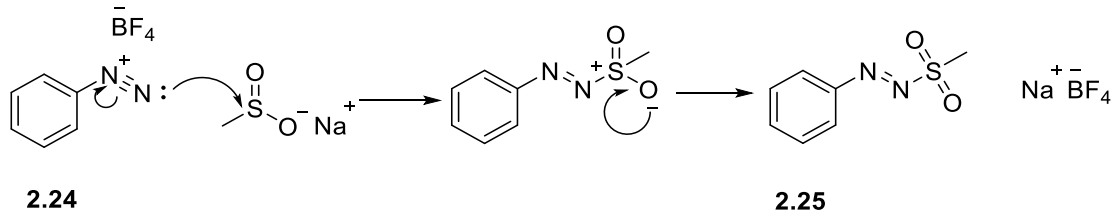


Scheme 2.17: Synthesis of **2.25**.

The first step of the reaction generates a diazonium salt containing tetrafluoroborate as the counter ion. This diazo tetrafluoroborate salt **2.24** is a stable solid at room temperature. The reaction was monitored with LCMS in both positive and negative modes. In the positive mode, 105.06 *m/z* was observed with the expected mass for the benzenediazonium salt. The ^{1}H NMR spectra of the isolated salt matched with the literature data where the aromatic protons appeared as a multiplet at 7.40 ppm with an integration of four protons and at 7.17 ppm a triplet was observed with an integration of one proton.

The coupling reaction was then carried out with sodium methanesulfinate where the slightly electron-positive sulphur of the sulfinate gets attacked by the lone pair of electrons from the nitrogen in the diazo salt (Scheme 2.18). Electron transfer then occurs to facilitate the synthesis of stable **2.25**. In the NMR spectra of the purified compound, the methyl peak was observed at 3.22 ppm, which was the expected region for a sulfonyl bound methyl group with an integration of three protons, while the aromatic protons were observed at 7.95 ppm as a doublet, 7.67 ppm as a triplet and 7.58 ppm as a triplet with a combined

integration of five protons. The HRMS of the final compound contained the mass peak of 185. 0285 which corresponds to $(M + H)^+$.

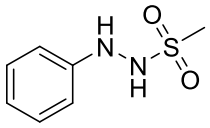
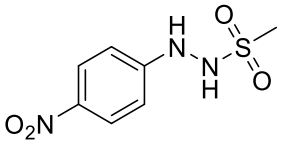
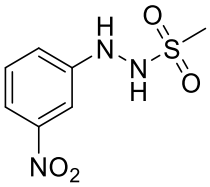
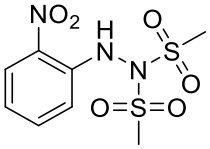


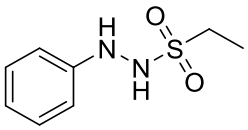
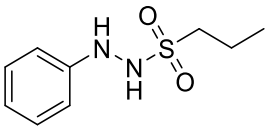
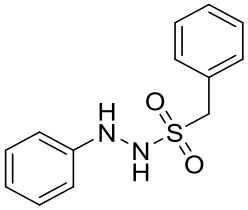
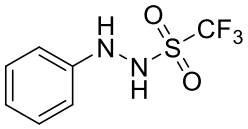
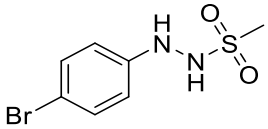
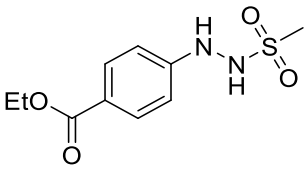
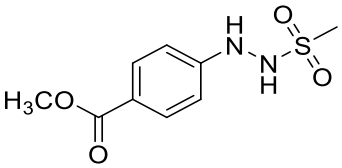
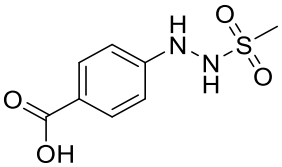
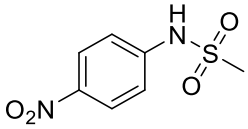
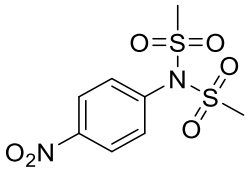
Scheme 2.18: Proposed reaction mechanism for the formation of **2.25**.

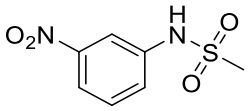
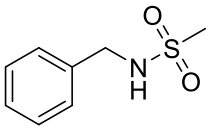
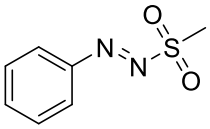
2.6 Biological evaluation of the synthesised analogues

The DHDPS-DHDPR coupled assay, as discussed in Chapter 1, was used by Dr. Tatiana Soares da Costa to measure the IC_{50} values for the synthesised 16 compounds to evaluate the structure-activity relationship (SAR). Obtained IC_{50} values are tabulated in Table 2.1.

Table 2.1: Bioassay results against *E. coli* DHDPS.

Compound Number	Compound	IC_{50} µM
CT1-2		128 ± 5.2
CT1-5		72 ± 3
2.3		>250
2.4		>250
2.9		>250

2.10		>250
2.11		>250
2.12		>250
2.13		>500
2.16		>250
2.17		Unusual behaviour in the assay.
2.18		160 ± 14
2.19		207 ± 18
2.20		300 ± 21
2.21		>250

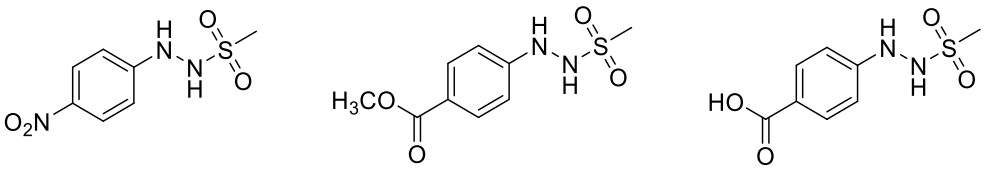
2.22		>250
2.23		>250
2.25		>500

Analysing the results obtained from altering the position of the nitro group, it is evident that the *para* position is the best for inhibiting DHDPS, as *ortho* (**2.3**) and *meta* (**2.4**) nitro analogues did not show any inhibition. It was hypothesised that **CT1-5** binds in the active site with favourable interactions at the nitrogen of the *para* nitro with Ser 250 and Thr 47, and oxygen with the Thr 47. These favourable interactions might not occur when the positioning of the nitro group is different, as a result the synthesised 2.3 and 2.4 were not as potent as CT1-5. Also, the di-substituted sulfonyl analogue **2.9** did not show any inhibition, possibly due to not having enough space in the binding site to fit two bulky sulfonyl groups. This implies that, **2.9** has steric hindrance making it harder to interact with the site of interest for favourable interactions.

The extensions made at the sulfonyl functionality with the ethyl (**2.10)** and *n*-butyl (**2.17)** groups were also not potent against the DHDPS enzyme. Furthermore, the benzyl functionality at the sulphur (**2.12**) was not also tolerated giving an IC_{50} above 250 µM. All of the data strengthened the hypothesis that more hydrophobicity at the sulfonyl functionality leads to the loss of activity against DHDPS. This could possibly be due to the steric hindrance of the bulky groups. Replacement of the methyl with the trifluoromethyl also did not result in any inhibition against DHDPS. This is an indication of how specific the active site is and even a close isosteric replacement can lead to an inactive compound.

The biological assay results of the *para* substituted electron-withdrawing groups (**2.17** – **2.20**) on the aromatic ring showed mild activity against the DHDPS enzyme assay. More specifically the *para* substituted methyl ester **2.18** and the *para* substituted carboxylic acid **2.19** showed inhibition of 160 µM and 207 µM, respectively. The *para* substituted ethyl ester **2.17** showed an unusual behaviour in the coupled assay where a colour change was

observed even without reaction initiation by the addition of ASA. This is possibly due to an interference caused by **2.17** at 340 nm, which is the wavelength used to measure the absorbance of consumed NAPDH. Due to this unusual behaviour of the compound, the IC_{50} value was not obtained. Substitution of a bromo group at the *para* position (**2.16**) was not tolerated by *E. coli* DHDPS where the IC_{50} was above 250 µM. The observed activity of different *para* substituents correlates with their deactivating strengths of the aromatic ring. The nitro group is the most deactivating group, having the lowest IC_{50} against *E. coli* DHDPS with an activity of 71 µM. The methyl ester comes second with an activity of 160 µM; the carboxylic acid is third with an activity of 207 µM and finally bromo with activity above 250 µM.



CT1-5
IC_{50} 72 ± 3 µM

2.18
IC_{50} 160 ± 14 µM

2.19
IC_{50} 207 ± 18 µM

Figure 2.5: Best inhibitors of the series.

The synthesised sulfonamides with nitro functionality at the *para* position (**2.20**) and the *meta* position (**2.22**) were not active against DHDPS having IC_{50} above 250 µM. The di-substituted sulfonamide **2.21** did not show any activity against the isolated enzyme assay. Compound **2.23** was synthesised while keeping the bridge length similar to that of HTS 14697, where a carbon was used to replace the nitrogen atom close to the aromatic ring. The activity of the synthesised compound was above 250 µM. The synthesised azo compound **2.25**, where a rigid double bond was replaced instead of the free rotating single bond between the two nitrogens in the hydrazine group, showed no inhibition against *E. coli* DHDPS. These results strengthens our hypothesis about the specificity of the active site along with the importance of having the hydrazine functionality to bridge the aromatic ring to the sulfonyl sulphur.

2.7 Summary

The SAR of the synthesised 16 compounds summaries in Figure 2.6, highlights the importance of having a highly electron-withdrawing, deactivating group at the *para* position of the aromatic ring with the nitro group being the best substituent. Introducing increased hydrophobicity at the sulfonyl functionality other than a methyl group results in the loss of activity. The importance of having the hydrazine functionality was also observed due to the loss of activity of compounds if the hydrazine functionality was replaced or altered.

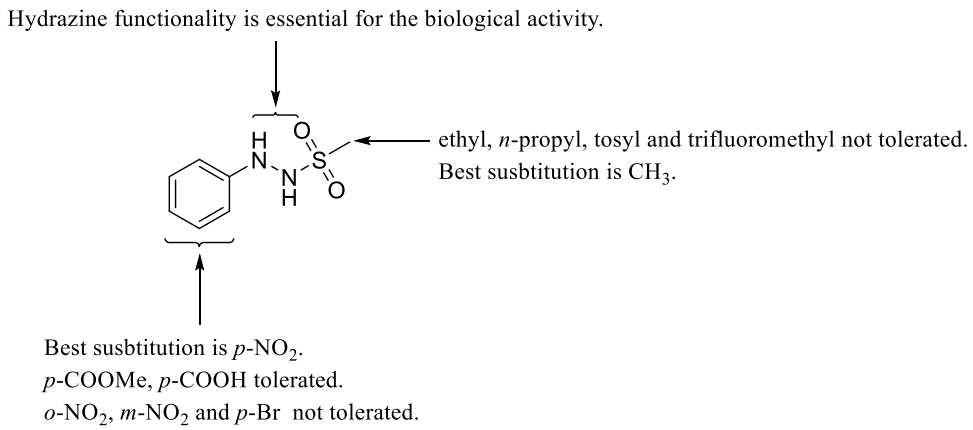


Figure 2.6: SAR summary of sulfonyl hydrazides considered.

Chapter 3 Synthesis and Biological Evaluation of Sulfonyl Hydrazides; Part II

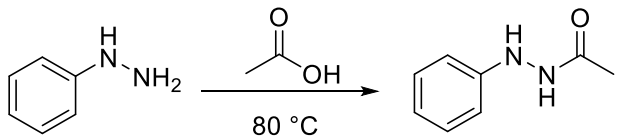
3.1 Introduction

Further work to understand the structure activity relationships of HTS-14697 against DHDPS was undertaken and is detailed in this chapter. Exploration of isosteres of the sulfonyl group is first discussed. Variations made to the aromatic ring with ring expansions, ring contraction and heterocyclic analogues is subsequently discussed.

3.2 Isosteres of the sulfonyl group

3.2.1 Carbonyl group

The first explored isostere for the sulfonyl group was the carbonyl group (Scheme 3.1). Synthesis was achieved from following a literature method.[79] The reaction generated a mixture of two rotamers which was confirmed by ^{1}H NMR spectra to be in a ratio of 2.2:1. The methyl peak occurred at 2.05 ppm and 2.11 ppm for the major and minor isomers, respectively. Similarly, two sets of peaks were obtained for each proton except for the NH protons. The major aromatic proton peaks appeared at 7.23 ppm as a triplet, 6.90 ppm as a triplet, and at 6.83 ppm as a doublet whereas the minor peaks were observed at 7.26 ppm as a triplet, 6.93 ppm as a triplet, and 6.79 ppm as a doublet. Furthermore, the *m/z* peak at 151.0861 observed in the HRMS corresponding to the $(M + H)^{+}$ also confirmed the identity of the product.

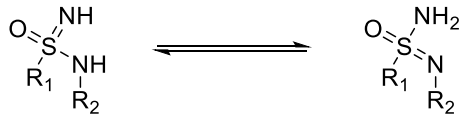


3.1 37%

Scheme 3.1: Synthesis of *N'*-Phenylacetohydrazide **3.1**.

3.2.2 Sulfonimidamide group

Sulfonimidamide is the aza isostere of the sulfonyl group. Tautomerisation is observed in primary and secondary sulfonimidamide where a proton migrates between the nitrogens (Scheme 3.2).



Scheme 3.2: Tautomerisation of sulfonimidamides.

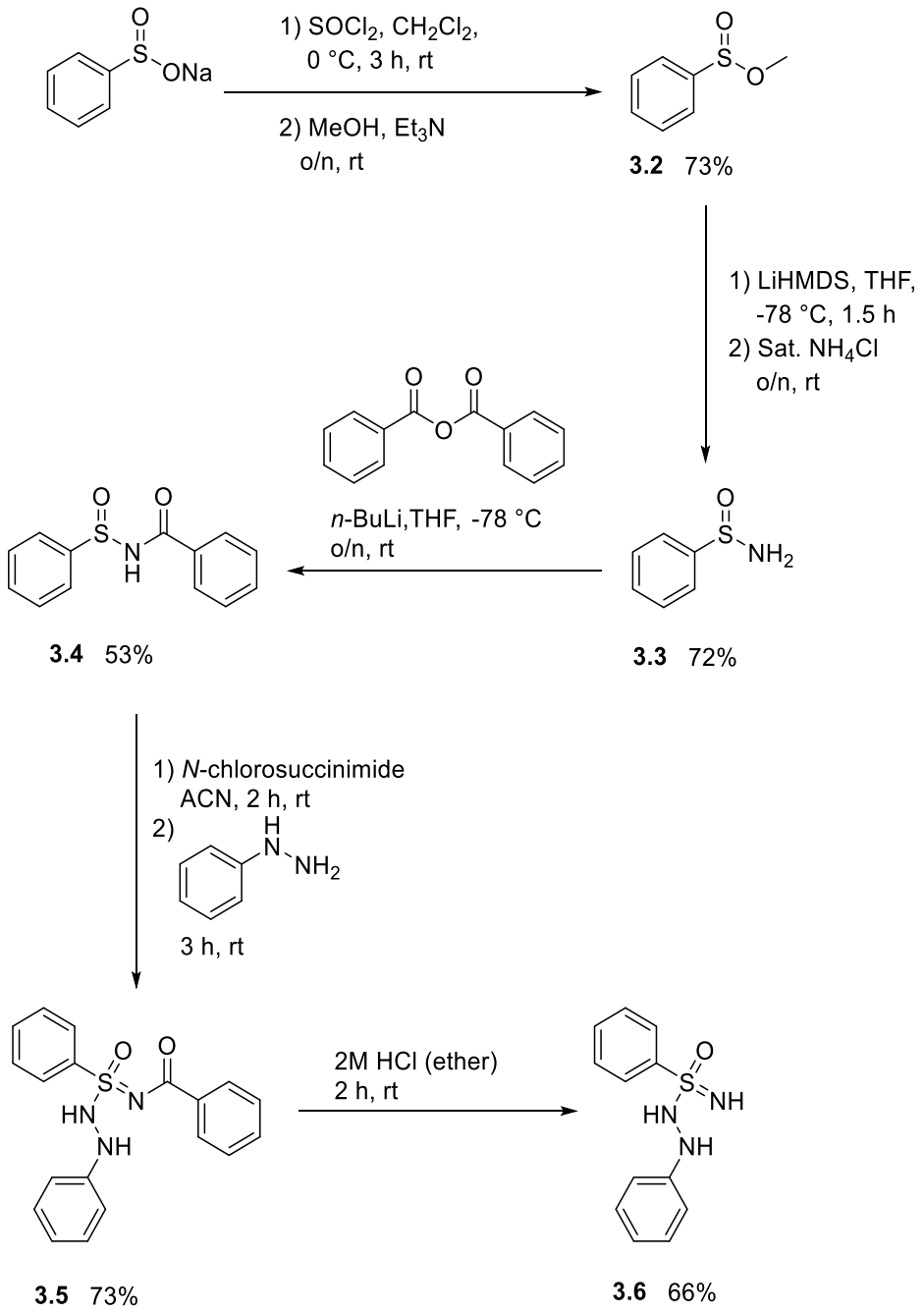
The first sulfinimidamide analogue synthesised was *N'*-phenylmethanesulfonimidhydrazide **3.6** following a literature method (Scheme 3.3) as a model reaction.[80]

Synthesis started from chlorination using thionyl chloride followed by the esterification of sodium benzenesulfinate with methanol to obtain methyl benzenesulfinate **3.2** in a 73% yield (Scheme 3.3). The ^{1}H NMR spectra of the product after purification had a sharp singlet at 3.42 ppm corresponding to the methyl group and two multiplets at 7.70 ppm and 7.64 ppm with integrations of two and three protons respectively, as expected. In the ^{13}C NMR spectra, a new peak was observed at 50.6 ppm corresponding to the newly added methyl group while the aromatic carbons were observed at 144.2 ppm, 133.0 ppm, 129.8 ppm, and 125.6 ppm.

This compound was then hydrolysed using lithium bis(trimethylsilyl)amide (LiHMDS) for the generation of benzenesulfinamide **3.3**. Recrystallization yielded pure product in a good yield of 72%. In the ^{1}H NMR spectrum of **3.3**, a doublet was obtained at 7.75 ppm and a triplet at 7.50 ppm corresponding to the aromatic protons with integration of two and three protons respectively. A broad singlet at 4.35 ppm was also observed with an integration of two protons for the NH_2 group. As expected, the ^{13}C NMR spectrum contained four peaks corresponding to the aromatic carbons at 146.5, 131.1, 128.9, and 125.4 ppm.

N-(Phenylsulfinyl)benzamide **3.4** was the key intermediate for the transformation of the compound to a sulfinimidamide from a sulfinate, where a benzoyl group was introduced as the protecting group. Introduction of greater aromaticity to the compound resulted in an increased solubility in chloroform; recrystallization in 2:1 hexane:ethyl acetate yielded pure compound. The ^{1}H NMR spectrum of the compound showed an NH proton at 8.20

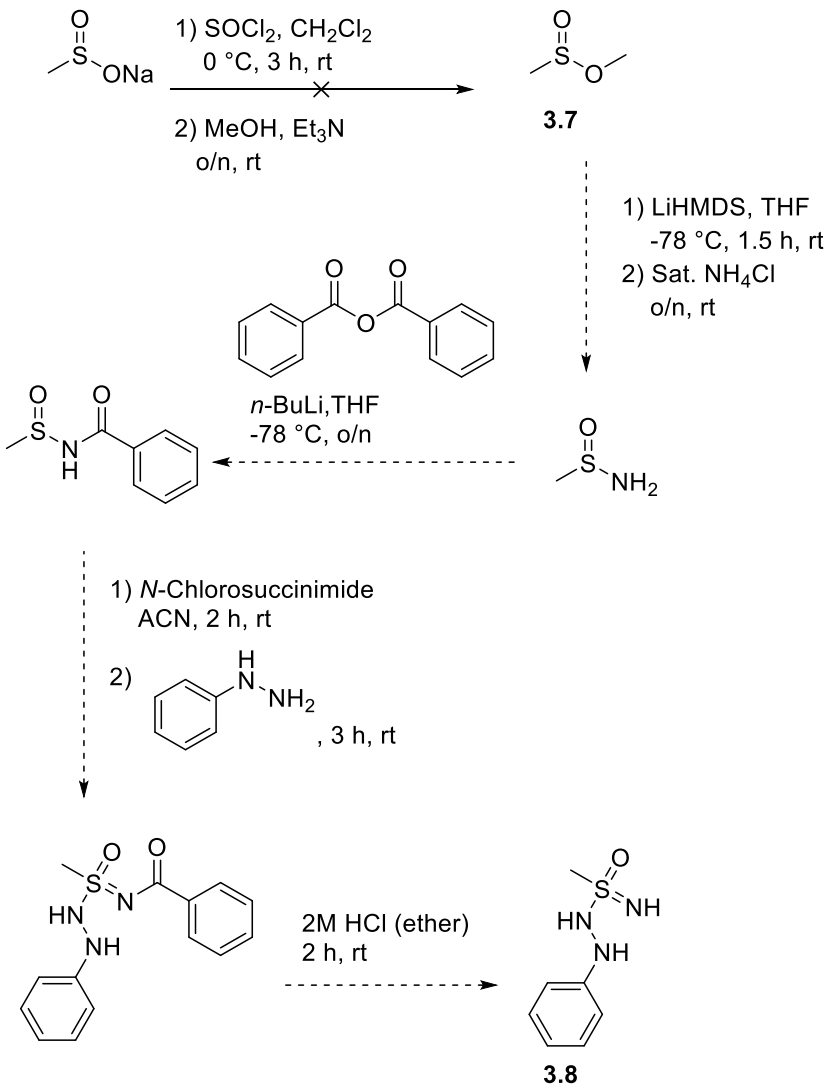
ppm and the aromatic protons in the range of 7.82 – 7.44 ppm with a collective integration of ten protons. The ^{13}C NMR spectrum had a total of nine carbon peaks, where the peak at 167.3 ppm corresponds to the carbonyl carbon and the other peaks at 134.2, 133.6, 132.1, 131.7, 129.6, 128.9, 127.9, and 124.8 ppm correspond to the aromatic carbons.



Scheme 3.3: Synthesis of *N'*-Phenylmethanesulfonimidhydrazide **3.6**.

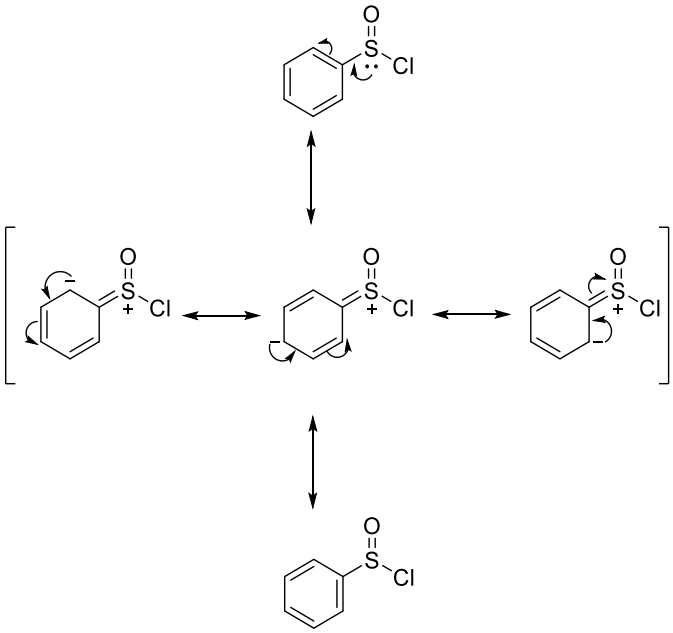
Oxidative chlorination at the sulphur using *N*-chlorosuccinimide (NCS) was performed, which then facilitated the nucleophilic substitution of phenylhydrazine to obtain *N*-(oxo(phenyl)(2-phenylhydrazinyl)-λ^6-sulfanylidene)benzamide **3.5**. After an extraction of the reaction mixture followed by column chromatography, the desired product was confirmed by matching the ^{1}H NMR spectrum with a known literature NMR spectrum,[80] where aromatic protons appeared at 7.82 ppm, 7.55 ppm, 7.45 ppm, and 7.33 ppm as multiplets with a collective integration of 15 protons and the two NH protons appeared at 7.93 ppm and 7.65 ppm. In the carbon NMR spectrum, the carbonyl carbon appeared at 177.8 ppm and the aromatic carbons appeared at 136.7, 132.3, 131.2, 129.6, 129.5, 129.3, 128.9, 128.8, 127.7, 127.5, 127.2, and 125.0 ppm. To further confirm the structure of **3.5**, HRMS was used to give a *m/z* peak at 352.1111 which corresponded to the $(M + H)^+$ ion.

Cleavage of the benzoyl group was then achieved using hydrochloric acid in diethyl ether to obtain the desired compound, **3.6**. The product was purified using column chromatography. The ^{1}H NMR spectra contained multiplets in the region of 7.59 – 7.31 ppm corresponding to ten protons as expected. In addition, HRMS resulted in a peak at 248.0850 *m/z*, which corresponds to the $(M + H)^+$ ion of **3.6**. It was evident that the reaction sequence outlined in Scheme 3.3 was very successful in the synthesis of *N'*-phenylmethanesulfonimidhydrazide **3.6**. Following the successful synthesis of Scheme 3.3, synthesis of *N'*-phenylmethanesulfonimidhydrazide **3.8** was attempted (Scheme 3.4).



Scheme 3.4: Proposed synthesis of **3.8**.

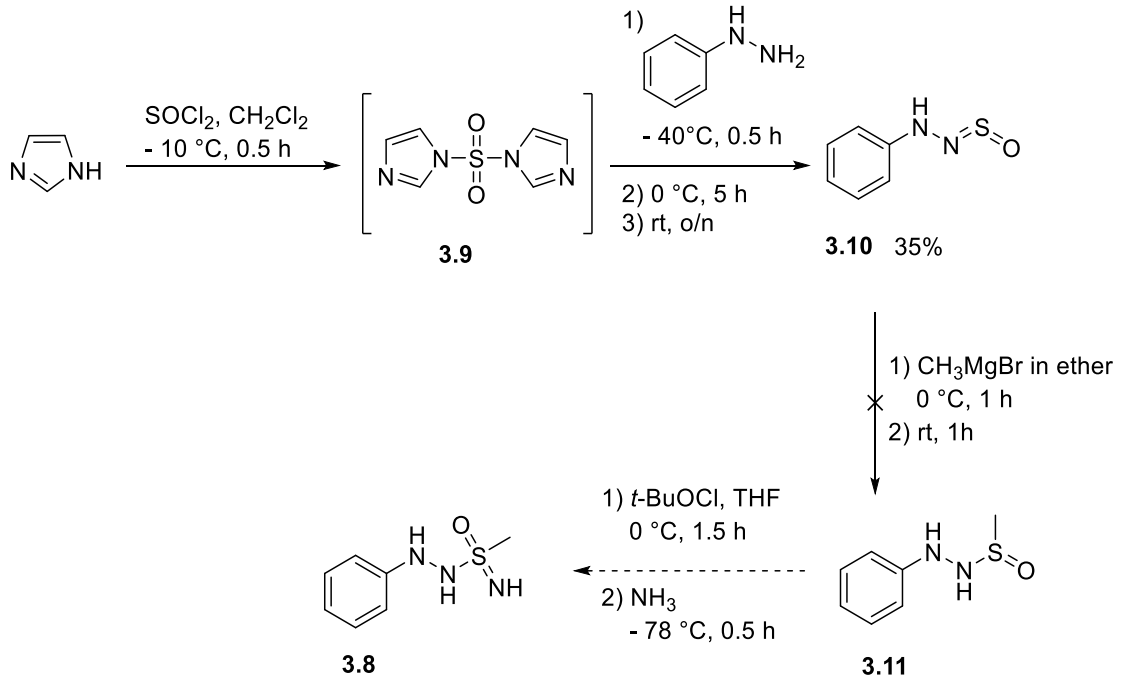
Generation of methyl methanesulfinate **3.7** was unsuccessful. Different temperatures (- 78 °C to -5 °C) were trialled along with different reaction times (5 h – 5 days), but the product was not observed in the ^{1}H NMR spectrum or MS analysis nor the starting material, sodium methanesulfinate. This could be due to the electron-donating nature of the methyl group and the inability to generate resonance structures resulting in an unstable sulfinic chloride. In contrast, in the previous synthesis (Scheme 3.3), the starting material had an electron-rich phenyl ring which had the ability to generate resonance structures, thus making the intermediate sulfinic chloride stable (Scheme 3.5).



Scheme 3.5: Resonance structures of benzenesulfinic chloride.

Literature methods to synthesise methanesulfinic chloride predominantly use dimethyl disulphide which is a flammable liquid with a strong unpleasant odour[81-84] and sulphuryl chloride. As a result, it was decided not to pursue the synthesis of *N'*-phenylmethanesulfonimidhydrazide via these known routes, alongside the explosion risks associated in the isolation due to the possibility of generation of hazardous gases from traces of sulphuryl chloride used[85] and difficulties encountered in purification of the desired product due to the contamination with many sulphur containing by-products.[86]

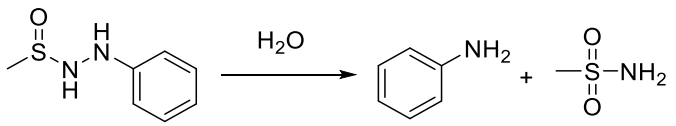
A new approach was attempted for the synthesis of **3.8** (Scheme 3.6).[87] The synthesis started with imidazole, from which 1,1'-sulfonylbis(*1H*-imidazole) **3.9** was generated *in situ* and coupling was then performed with phenylhydrazine to obtain (2-phenylhydrazono)-λ^4-sulfanone **3.10** in a moderate yield. The ^{1}H NMR spectrum of the product contained aromatic protons at 7.23, 6.93, and 6.86 ppm consistent with the literature.[87] The *m/z* peak of 155.0268 obtained in HRMS further confirmed the identity of the product.



Scheme 3.6: Attempted synthesis of **3.8**.

The synthesis of the *N′*-phenylmethanesulfinohydrazide **3.11** was then attempted using 1.5 equivalents of methylmagnesium bromide under anhydrous conditions at 0 °C.[88] The crude ^{1}H NMR spectrum indicated that the reaction had not gone to completion, therefore the number of equivalents of methyl magnesium bromide was doubled. From the repeated reaction a much cleaner crude ^{1}H NMR spectra was obtained where the methyl group appeared at 1.91 ppm and the aromatic protons appeared at 7.23, 7.04, and 6.83 ppm. The NH protons were observed at 7.74 ppm and 7.49 ppm. Column chromatography was performed on the crude sample using 20% ethyl acetate: hexane as the solvent system. Unfortunately, none of the fractions collected had the expected methyl peak although the aromatic protons were present in the second fraction collected. The sample might have degraded on silica due to the sensitiveness of **3.11**.

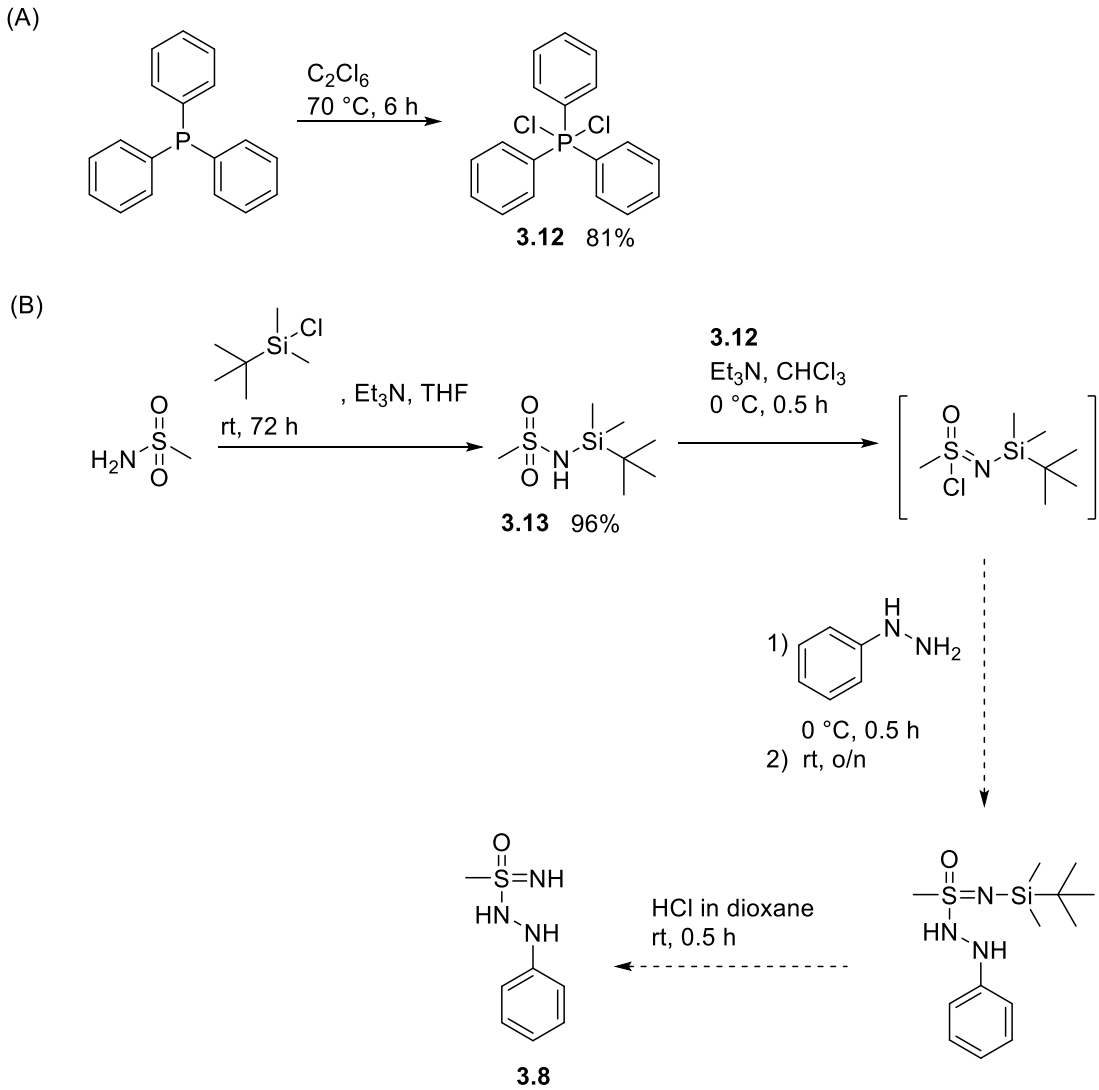
There is no spectral data available for **3.11**. The product, upon contact with water, may easily decompose into aniline and methanesulfonamide, which are more stable than the sulfinohydrazide (Scheme 3.7). Efforts towards this method were not continued.



Scheme 3.7: Proposed products of **3.11** upon contact with water.

For the synthesis of **3.11**, a one-step method could have been used except the yields reported for the aliphatic sulfonyl chlorides were poor.[89] Therefore, this method was not trialled.

The next approach to synthesising **3.8** was inspired by a research article from Chen and Gibson describing the development of a one-pot conversion of sulfonamides to sulfonimidamides via *in situ* generation of sulfonimidoyl chlorides and coupling them with corresponding amines, followed by hydrolysis (Scheme 3.8).[90]



Scheme 3.8: New approach for the synthesis of **3.8**.

Firstly, dichlorotriphenyl-λ^5-phosphane **3.12** was freshly prepared. Triphenylphosphine and hexachloroethane were stirred in chloroform at 70 °C for six hours to generate the desired compound. The ^{1}H NMR spectra contained peaks which are shifted downfield compared to the triphenylphosphine starting material and matched with the literature ^{1}H

NMR spectra. The *m/z* peak of 335.1249 obtained by HRMS further confirmed the identity of the product.

Next, methanesulfonamide was amino-protected using *tert*-butylchlorodimethylsilane because of the instability of the compound under basic conditions.[90] The synthesised compound **3.13** had ^{1}H NMR peaks appearing at 7.03 ppm for the NH proton, 2.89 ppm for the methyl group at the sulphur, 0.87 ppm for the two methyl groups at silicon and at 0.15 ppm for the three methyl groups on the *tert*-butyl group. The ^{13}C NMR spectra of the compound was as expected, with peaks appearing at 44.5, 26.2, 17.7, and -3.9 ppm (Figure 3.1). The negative valued peak obtained at -3.9 ppm is for the carbons at the silicon and this is due to the carbon being more shielded than the carbon in tetramethylsilane which is used as the zero calibration for the carbon NMR spectra. The *m/z* peak obtained at 210.0975 from HRMS confirms the identity of the product.

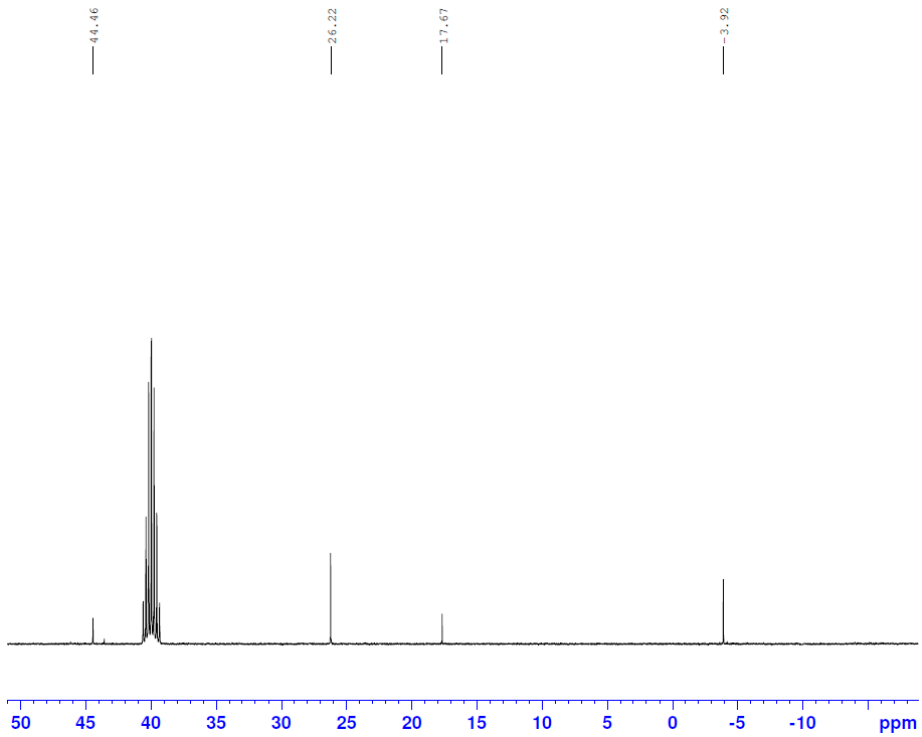


Figure 3.1: Expanded region of the^{13}C NMR spectra of **3.13**.

Freshly prepared **3.12** was then used for the oxidative chlorination at the sulphur. After 0.5 hours, phenylhydrazine was added to the reaction mixture for the nucleophilic substitution. The reaction was then monitored by RP-HPLC, where the consumption of phenylhydrazine was monitored due to the UV inactive nature of **3.13**. The reaction

mixture showed complete consumption of phenylhydrazine after 24 hours. The acidic workup was also used to cleave the *tert*-butyl dimethylsilyl group.

The obtained crude product mostly appeared to be triphenylphosphine oxide (TPPO). Column chromatography was performed a number of times to reduce the amount of TPPO. Trituration with diethyl ether and hexane was then attempted to eliminate the impurity, followed by recrystallization attempts using various solvent systems without any success. The difficulty in removing TPPO has been encountered previously.[91-93]

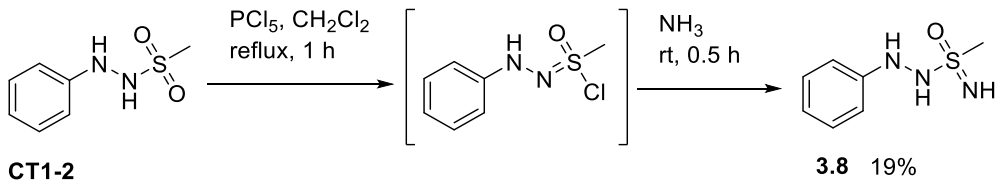
The reaction was repeated again using the same method (Scheme 3.8), but this time addition of oxalyl chloride was performed immediately after the acidic deprotection.[93] In theory, TPPO will react with oxalyl chloride to generate chlorophosphonium chloride which is easy to remove. While a significant reduction of TPPO was observed, complete purification by chromatography was not achieved.

Metal halides can also be used to generate salts with TPPO. Therefore, the next approach to obtain a TPPO free crude product from the reaction mixture was inspired by a method described by Gordon and co-workers.[92] Magnesium chloride was found to be the metal halide of choice, where the use of non-polar solvents like toluene resulted in isolation of high amounts of TPPO-$MgCl_2$. Polar solvents such as dichloromethane were found to generate low amounts of TPPO-$MgCl_2$. Taking all of these factors into consideration, the reaction was repeated again. After acid work up the reaction mixture was concentrated to remove the solvent of the reaction. The residue was then dissolved in toluene followed by the addition of two equivalents of magnesium chloride. The mixture was then heated at 60 °C for 2 hours, filtered and concentrated. For the removal of any residual triethylamine, washing the toluene mixture with water was performed. The crude material was observed to have TPPO comparable to the results from the previous oxalyl chloride method. According to Gordon and co-workers, repeating the procedure resulted in better purification and so two more repetitions were performed. A reduced amount of TPPO was observed, but complete purification was still not achieved after chromatography.

Due to the ongoing difficulties in the purification of the product from the TPPO by-product, no further attempts were made.

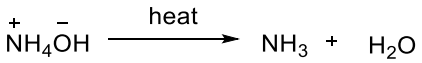
Direct chlorination on a sulfonamide was the next and final approach mentioned in the literature to obtain sulfinimidamides, though only two references to this class of

compounds, published in 1960 by the same authors.[94, 95] Phosphorus pentachloride was used as the direct chlorinating agent (Scheme 3.9).



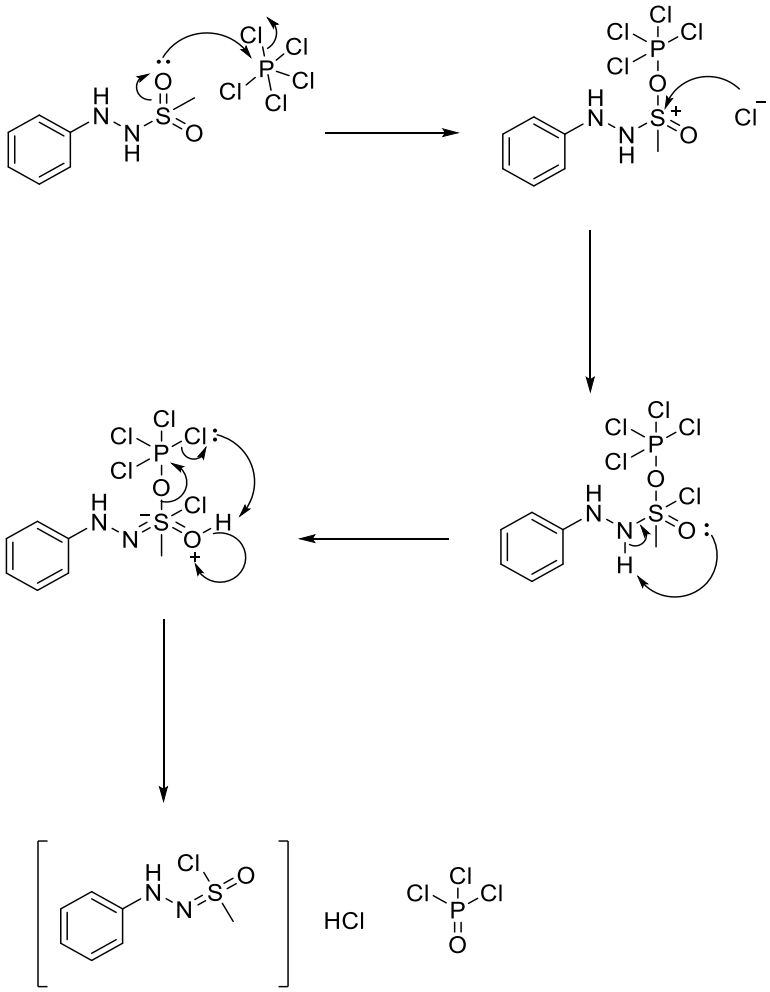
Scheme 3.9: Direct chlorination method to obtain **3.8**.

The phosphorus pentachloride reaction proceeded quickly under reflux temperatures, while monitoring was performed via TLC. Ammonia gas was generated *in situ* by heating an aqueous solution of ammonium hydroxide and dried through a calcium chloride filled drying tube prior to being bubbled through the reaction mixture. (Scheme 3.10).



Scheme 3.10: Decomposition of ammonium hydroxide upon heating.

The proposed reaction mechanism for the sulfonimidoyl chloride generation is shown in Scheme 3.11. The generated sulfonimidoyl chloride was then reacted with ammonia gas to generate the target compound **3.8**.



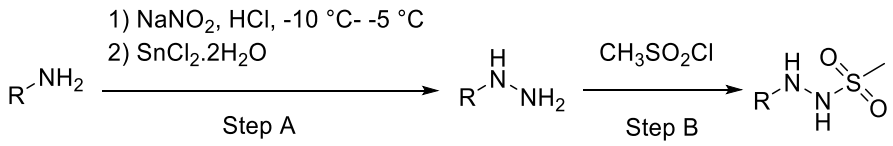
Scheme 3.11: Proposed mechanism for the reaction of **CT1-2** with phosphorus pentachloride.

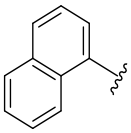
The method was successful in obtaining the product **3.8** in a 19% yield as a purple solid after column chromatography. The ^{1}H NMR spectra contained peaks at 7.89 ppm as a doublet for NH proton, 7.83 ppm as a doublet for two aromatic protons, 7.59 ppm as a doublet for NH proton, 7.49 ppm as a multiplet for three aromatic protons, and 3.15 ppm as a singlet for the methyl protons. The ^{13}C NMR spectrum contained peaks at 141.8, 130.2, 125.8 and 34.9 ppm while the DEPT-135 only showed three peaks, where the quaternary carbon peak in the phenyl ring were not present as expected. The identity of the product was further confirmed by the HRMS analysis where the *m/z* peak was observed at 186.0692 corresponding to the $(M + H)^{+}$ ion.

3.3 Exploration of alternate aromatic and heteroaromatic ring systems

Thiazole and napthyl ring analogues were synthesised using the corresponding amine starting material, where a diazonium salt generation was followed by a reduction using stannous (II) chloride to yield the hydrazine (Table 3.1). The hydrazine was then reacted with methanesulfonyl chloride via S_N2 substitution to generate the final compounds of interest, **3.15** and **3.17**.

Table 3.1: Ring analogues of HTS-14697.



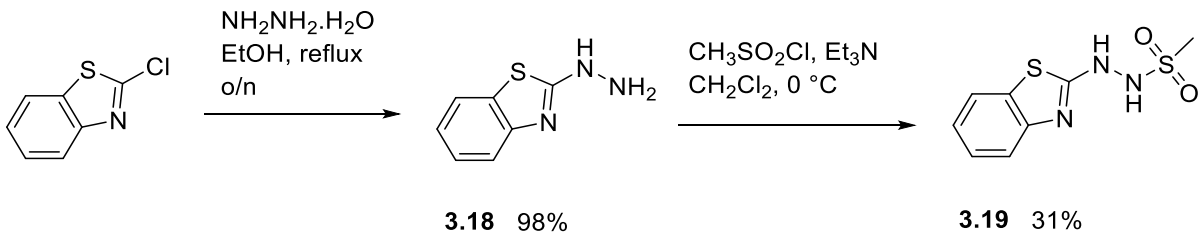
-R	Step A		Step B	
	Compound number	**% Yield**	**Compound number**	**% Yield**
	**3.14**	36	**3.15**	31
	**3.16**	43	**3.17**	31

For the ^{1}H NMR spectra of **3.14**, aromatic protons appeared at 7.95 ppm as a multiplet, 7.55 ppm as a multiplet, 7.45 ppm as a triplet and 6.93 ppm as a doublet with a total integration of seven protons, while NH_2 protons appeared at 5.67 ppm as a broad signal. The HRMS showed a *m/z* peak at 158.0838 confirming the identity of the product. This product was obtained in 30% yield when coupled with methanesulfonyl chloride in dichloromethane with triethylamine as the base. The resulting **3.16** had the methyl peak appearing at 2.99 ppm and the aromatic region from 8.13 ppm – 7.02 ppm in the ^{1}H NMR spectrum. Similarly, the *m/z* peak obtained at 237.0599 from HRMS analysis corresponds to the $(M + H)^+$ ion.

Compound **3.15** showed aromatic protons as doublets at 6.90 ppm and 6.52 ppm along with the terminal NH_2 at 6.83 ppm in the ^{1}H NMR spectrum. The obtained *m/z* peak at 116.0275 from HRMS confirms the identity of the product. For the coupling of **3.15** with methanesulfonyl chloride, pyridine was used as the base and the solvent. The product **3.17**,

was purified by silica gel column chromatography in a 53% yield. The ^{1}H NMR spectrum of the compound contained aromatic peaks at 7.24 ppm and 6.80 ppm along with the CH_3 peak at 2.88 ppm. The HRMS analysis of the compound contained the *m/z* peak appearing at 194.0048 as expected.

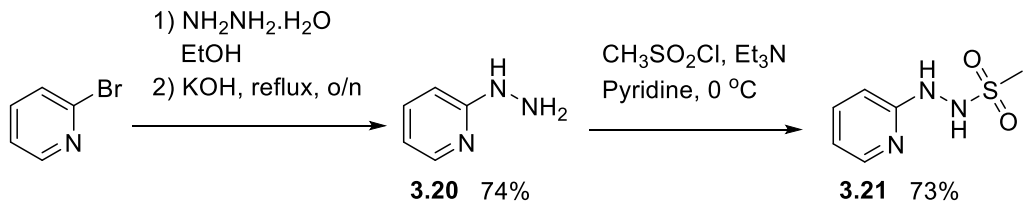
Another biaryl ring analogue was synthesised as shown in Scheme 3.12 providing the benzothiazole ring instead of the phenyl ring. Chlorobenzothiazole undergoes nucleophilic substitution with hydrazine hydrate to generate **3.18** as described in the literature,[96] which then undergoes another nucleophilic substitution with methanesulfonyl chloride to generate **3.19**. The synthesised hydrazine gave a ^{1}H NMR spectrum consistent with the literature, where aromatic peaks were obtained as triplets and doublets at 6.95 ppm, 7.17 ppm, 7.30 ppm, and 7.66 ppm with an integration of one hydrogen each. The NH peak was observed at 8.97 ppm, with NH_2 at 4.99 ppm. Furthermore, the *m/z* peak obtained at 244.0201 from HRMS corresponds to the $(M + H)^+$ ion.



Scheme 3.12: Synthesis of **3.19**.

3.4 Pyridine analogues of HTS-14697

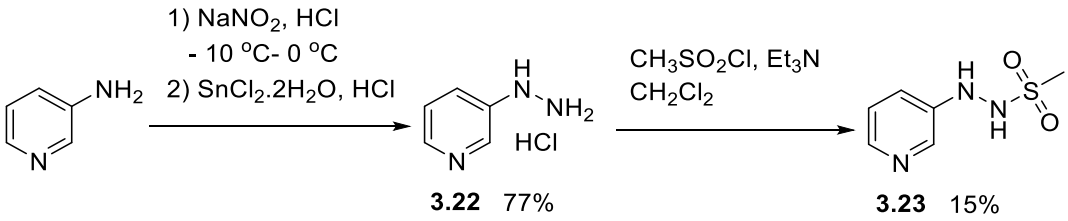
Firstly, the synthesis of **3.21** was performed as outlined in Scheme 3.13 in order to obtain the nitrogen at the *ortho* position of the aromatic ring. Compound **3.20** was synthesised using a method cited from literature where a bromo substituted pyridine ring underwent S_N2 substitution using hydrazine hydrate.[97] The ^{1}H NMR spectra of **3.20** contained a doublet at 8.10 ppm, a triplet at 7.46 ppm, a doublet at 6.68 ppm, and a triplet at 6.65 ppm corresponding to the pyridine ring protons. The NH and NH_2 protons appeared at 6.04 ppm and 3.72 ppm, respectively.



Scheme 3.13: Synthesis of **3.21**.

Coupling of **3.20** with methanesulfonyl chloride was followed to obtain compound **3.21** in a good yield of 73%. The ^{1}H NMR spectra contained peaks corresponding to NH protons at 9.10 ppm and 8.62 ppm. The aromatic pyridine protons appeared at 8.04 ppm as a doublet, 7.55 ppm as a triplet, 6.81 ppm as a doublet, and 6.71 ppm as a triplet. The *m/z* peak obtained at 188.0484 further confirms the identity of the product to be **3.21**.

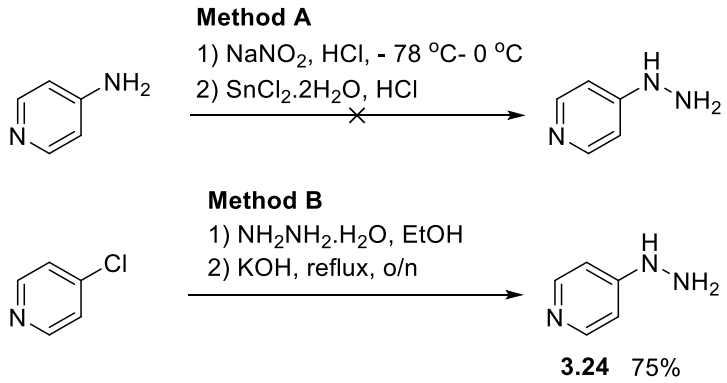
To obtain the nitrogen at the *meta* position, pyridin-3-amine was used as the starting material as cited from a patent for the synthesis of **3.22**,[98] followed by coupling of methanesulfonyl chloride to obtain **3.23** (Scheme 3.14). The identity of the synthesised **3.22** was confirmed by ^{1}H NMR spectroscopy and HRMS. The ^{1}H NMR spectra contained peaks as per the literature and the *m/z* peak obtained at 110.0710 corresponded to the $(M + H)^{+}$ ion. The ^{1}H NMR spectra of **CT2-5** had the methyl peak at 3.43 ppm, aromatic peaks at 8.71 ppm as a doublet, 8.63 ppm as a doublet, 7.71 ppm as a doublet, and 7.44 ppm as a doublet, with integration of one proton each. The HRMS contained a *m/z* peak at 188.0485 corresponding to $(M + H)^{+}$ ion.



Scheme 3.14: Synthesis of **3.23**.

The synthesis of a compound with the nitrogen at the *para* position was attempted via the synthesis of the 4-hydrazinylpyridine as shown in Scheme 3.15. The generation of the 4-hydrazinyl pyridine **3.24** by method A was not achieved, possibly due to the instability of the diazonium salt. This could be due to the electron-rich nature of the nitrogen at the *para* position which can make the diazonium salt very unstable. Lower temperatures such as -

78 °C was also achieved in order to synthesise the **3.24**, along with sodium nitrite under acidic conditions. Unfortunately, all attempts were unsuccessful.

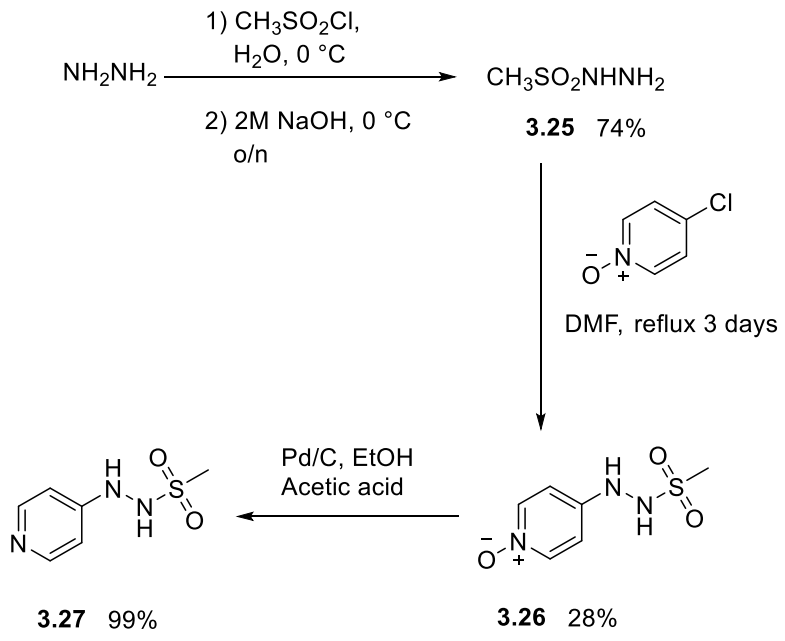


Scheme 3.15: Attempted synthesis for 4-hydrazinylpyridine **2.24**.

Synthesis of **3.24** was then attempted using method B in Scheme 3.15; 4-chloropyridine and hydrazine hydrate was stirred in ethanol and refluxed for 20 hours with a slight alteration to the literature, using ethanol as the solvent instead of 1-propanol.[99, 100] The target compound was obtained in a high yield of 75%. The HRMS gave a *m/z* peak corresponding to the $(M + H)^+$ ion at 110.0710 along with ^{1}H NMR peaks at 8.01 ppm as a doublet, 6.79 ppm as a doublet for aromatic protons, and 7.08 ppm for NH_2 protons.

However, the S_N2 coupling of **3.24** with methanesulfonyl chloride was not a success. Different reaction temperatures and times along with different solvents and bases were used to optimise the reaction, but the coupling was unsuccessful under all of the conditions used.

A different approach was then attempted for the synthesis of the target compound **3.27** (Scheme 3.16). Synthesis of the methanesulfonohydrazide **3.25** was achieved via a nucleophilic substitution reaction as mentioned in the literature with the use of hydrazine hydrate and methenesulfonyl chloride.[101, 102] In the ^{1}H NMR spectra, the methyl peak is observed at 3.00 ppm, the NH peak is observed at 5.55 ppm, and the NH_2 is observed at 3.85 ppm. The HRMS gave a *m/z* peak at 111.0220 corresponding to the molecular ion.



Scheme 3.16: Synthesis of **3.26**.

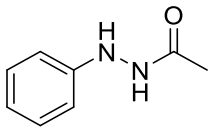
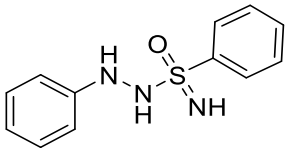
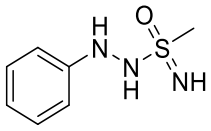
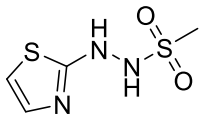
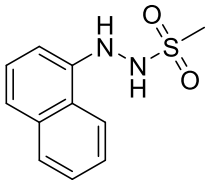
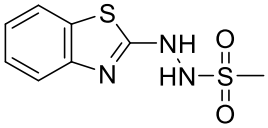
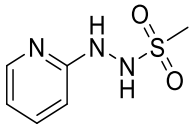
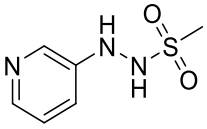
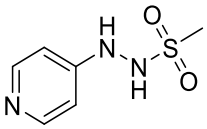
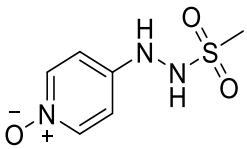
Methanesulfonohydrazide **3.25** was then reacted with 4-chloropyridine-*N*-oxide in dimethylformamide to obtain **3.26** in a moderate yield of 28%. The ^{1}H NMR spectrum showed peaks corresponding to the aromatic protons at 8.68 ppm and 7.92 ppm. The methyl peak was observed at 2.48 ppm. The *m/z* peak obtained at 204.0430 corresponds to the $(M + H)^{+}$ ion.

A catalytic reduction using palladium on carbon in ethanol was performed for **3.26** to obtain **3.27** in an excellent yield of 99%. The ^{1}H NMR spectrum had peaks appearing at 8.94 ppm and 8.24 ppm for the aromatic protons, while methyl protons appeared at 2.50 ppm. Noticeably, the aromatic protons had shifted downfield in **3.27** compared to **3.26** due to the loss of the electron-withdrawing oxygen from the nitrogen. This further ensures the identity of the product to be **3.27**. The white solid had a *m/z* peak at 188.0488 on HRMS analysis, which was as expected.

3.5 Biological evaluation of the synthesised sulfonylhydrazide analogues

Using the DHDPS-DHDPR coupled assay described in 1.3.3, the IC_{50} values of the synthesised compounds against DHDPS were measured and the data collected in Table 3.2.

Table 3.2: IC_{50} values for the synthesised compounds.

Number	Compound	IC_{50}/ µM
3.1		>250
3.6		>250
3.8		352 ± 22
3.17		>250
3.15		143 ± 10
3.19		>250
3.21		109 ± 3.7
3.23		~ 250
3.27		>500
3.26		>500

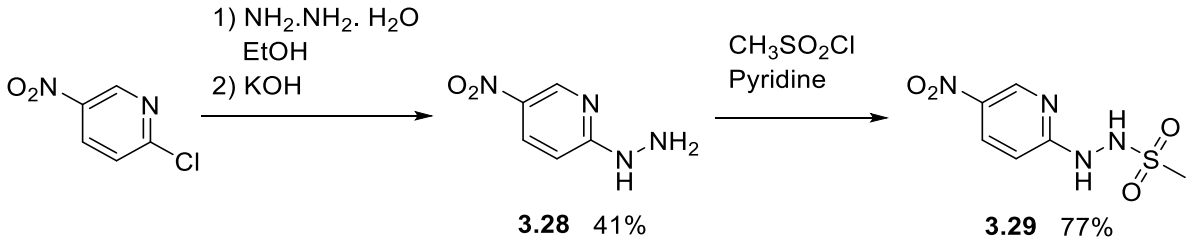
The isostere replacement of the sulfonyl functional group with carbonyl functionality resulted in a complete loss of activity. Disappointingly, despite the challenging syntheses, **3.8** was not active either. As expected, **3.6** was not tolerated, as it appears that the binding pocket cannot tolerate large hydrophobic groups and steric effects as discussed in section 2.6.

Next, exploration of alternate aromatic and heteroaromatic ring systems was undertaken. Unfortunately, the thiazole (**3.17**) and benzothiazole (**3.19**) analogues did not inhibit DHDPS *in vitro*, with IC_{50} values above 250 µM. Interestingly, the α-napthyl analogue (**3.15**) had activity of 143 µM. When considering the ring sizes, both napthyl and benzothiazole rings are larger than phenyl ring. Therefore, it cannot possibley be a size effect which gives rise to the observed activity of compound **3.15**. It is possible that the observed activity is due the high hydrophobicity of the naphthyl ring system with respect to the benzothiazole ring. For a better understanding of the binding of **3.15**, a co-crystallization of the enzyme DHDPS with the compound **3.15** would be useful. However, this experiment was not pursued at this time due to the moderate potency of the compound.

Out of the four synthesised pyridine analogues, only one compound had moderate activity against the DHDPS enzyme. Compound **3.23** which contains the nitrogen at the *meta* position and **3.27** which contains the nitrogen at the *para* position were not tolerated with IC_{50} above 250 µM. When the *para* position contained *N*-oxide functionality the IC_{50} of **3.25** was above 500 µM. However, when the nitrogen was at the *ortho* position, a moderate activity for **3.21** was observed and this was comparable to the IC_{50} of the **CT1-2** which was 128 µM. Furthermore, the compound has an improved log P of -0.13 compared to that of **CT1-2** which was 0.79.

3.6 A small library of analogues based on the SAR of HTS-14697

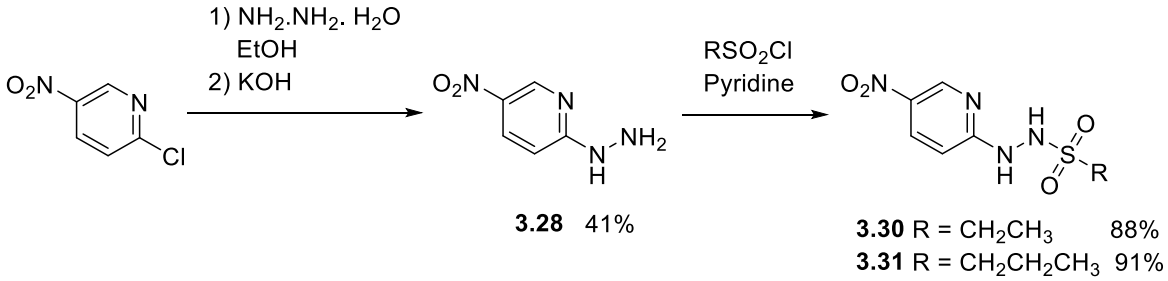
From the biological data obtained in 2.6 and 3.5, it is clear that having an electron-withdrawing group, preferably a nitro group, at the *para* position of the aromatic ring is favourable for DHDPS inhibition. Furthermore, having a pyridine ring and positioning the nitrogen at the *ortho* position with respect to the hydrazine functionality resulted in an increased activity against DHDPS. Combining both of these features should in theory, increase the activity against DHDPS *in vitro*. With this hypothesis, synthesis of **3.29** was attempted as shown in Scheme 3.17.



Scheme 3.17: Synthesis of **3.29**.

2-Chloro-5-nitropyridine was used to produce **3.28**, following the published literature.[103] The synthesised product had its aromatic protons appearing at 8.96, 8.29, and 7.10 ppm in ^{1}H NMR spectra, in accordance with the literature. The coupling reaction of the hydrazine and methanesulfonyl chloride used pyridine as the base and the solvent.[104] the ^{1}H NMR spectrum of compound **2.29** had aromatic protons at 8.34 ppm as a doublet, 8.31 ppm as a doublet, and 6.62 ppm as a doublet along with methyl peaks appearing at 3.03 ppm and NH protons at 10.03 ppm and 9.63 ppm. Two *m/z* peaks were obtained in HRMS analysis at 233.0338 and 255.0157, which correspond to $(M + H)^+$ and $(M + Na)^+$, further confirming the identity of the product to be **3.29**.

Analysis of the preliminary crystal structure of DHDPS bound by **CT2-1** (not yet available for publication) shows more space which can possibly be filled with functionalisation at the sulfonyl end of the molecule. Therefore, two analogues were synthesised extending the carbon chain at the sulfonyl group containing the aromatic core as of **3.29** (Scheme 3.18).

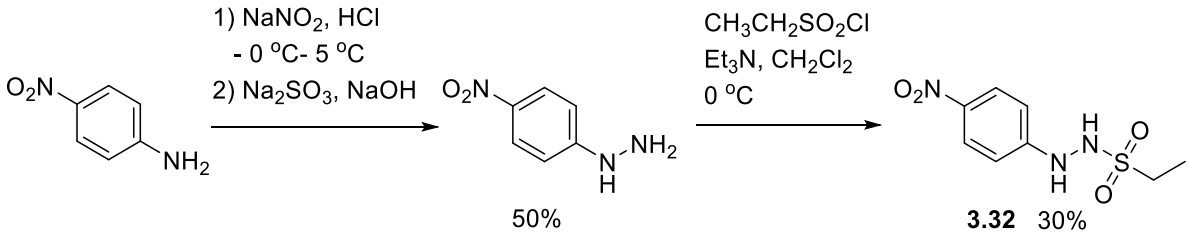


Scheme 3.18: Synthesis of **3.30** and **3.31**.

Synthesised **3.30** and **3.31** contain similar peaks in the ^{1}H NMR spectra for the aromatic region. The distinguishable peaks appear for the CH_2 groups and the terminal CH_3 group. In **3.30**, a quartet was observed at 3.15 ppm and a triplet at 1.24 ppm corresponding to the CH_2 and CH_3 protons, respectively. For **3.31**, the aliphatic protons appeared at 3.13 ppm as a triplet, 1.73 ppm as a multiplet, and 0.97 ppm as a triplet corresponding to two CH_2

groups and the terminal CH_3, respectively. For further structure analysis purposes, HRMS was performed for both **3.30** and **3.31** where *m/z* ions were found at 247.0496 and 261.0650 which correspond to the respective $(M + H)^+$ ions.

As another expansion of the SAR, one more analogue was synthesised to have the para-nitro functionality on the phenyl ring and an ethyl functionality at the sulfonyl group as shown in Scheme 3.19. This compound will also elaborate whether the chemistry at the sulphur end of the compound can be expanded to obtain better activity against DHDPS.



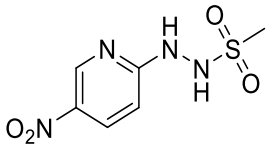
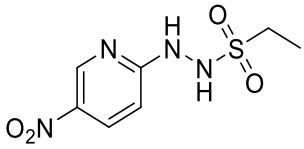
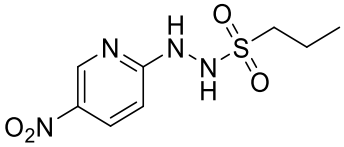
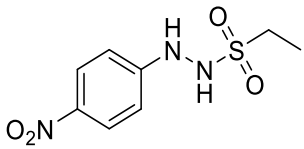
Scheme 3.19: Synthesis of **3.32**.

The ^1H NMR spectrum of compound **3.32** exhibited aromatic protons at 8.45 ppm and 8.10 ppm as doublet of doublets, CH_2 protons at 3.54 ppm as a quartet, and methyl protons at 1.54 ppm as a triplet. The *m/z* peak found at 268.0363 corresponds to the $(M + H)^+$ ion confirming the identity of the product to be **3.32**.

3.6.1 Biological evaluation of the small library of compounds discussed in 3.6

The four compounds were subjected to DHDPS-DHDPR coupled assay with the results tabulated in Table 3.3.

Table 3.3: IC_{50} for the synthesised compounds combining the optimal features.

Number	Compound	IC_{50}/ µM
3.29		66.5 ± 6.0
3.30		163 ± 4.9
3.31		159 ± 6.0
3.32		152 ± 9.1

As hypothesised from the previous SAR data, all of the synthesised compounds had activity against DHDPS.

Compounds **3.30** and **3.31** had moderate activity with IC_{50} of 163 µM and 160 µM, respectively, supporting the crystal structure interpretation for more room to functionalise at the sulphur. Furthermore, compound **3.32** had moderate activity. When compared with compound **2.10** from section 2.3 (where the phenyl ring was a unsubstituted phenyl ring without the *para* nitro functionality, but the functionality at the sulphur was an ethyl group), which did not inhibit DHDPS, it is evident that the interaction made by the nitro functional group inside the active site allows more hydrophobicity at the sulphur.

Compound **3.29** contained a methyl functionality at the sulphur along with a pyridine ring where the nitrogen is at the *ortho* position and a nitro group at the *para* position, with respect to the hydrazine functionality. This combination of structural features identified

from compounds **CT1-5** and **3.21** gave the most potent inhibitor of DHDPS to date (**3.29**) with an IC_{50} of 67 µM.

Toxicity studies were conducted by Dr. Tatiana Soares da Costa on **3.29** using an MTT viability assay using Defensin as the positive control on human liver cells (HepG2) and human kidney cells (HEK293) (Figure 3.2). Compound **3.29** was found to be non-toxic to both cell lines with EC_{50} above 500 µM.

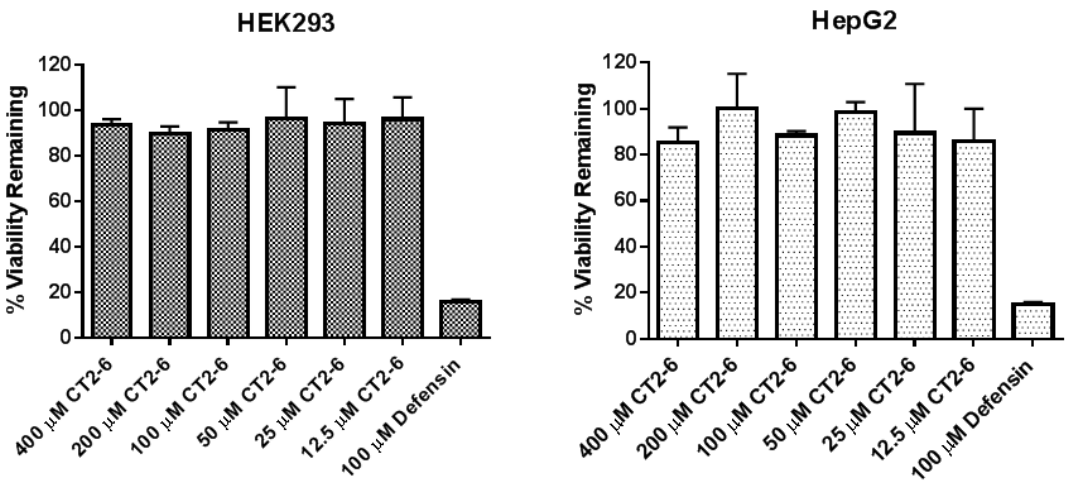


Figure 3.2: CT2-6 in MTT viability assay.

3.7 Summary

A summary of SAR results is illustrated in Figure 3.3. The sulfonyl functional group was found to be essential for the inhibition of DHDPS and alteration or removal of this moiety results in the complete loss of activity against the enzyme.

Alternate aromatic substitutions were not tolerated with the exception of an α-napthyl substitution. Further studies are needed to explore the mode and site of binding of this compound.

A pyridine ring with an *ortho*-nitrogen with respect to the hydrazine functionality, gave an increase of activity against DHDPS compared to HTS-14697. The *meta* and *para* nitrogen containing pyridines did not inhibit DHDPS.

When a pyridine ring with an *ortho*-nitrogen along with a nitro group at the *para* position, previously found to be a group which improved activity against DHDPS, resulted in the

best inhibitor to date with an IC_{50} of 67 µM. This compound is non-toxic to human liver and kidney cells.

There appears to be some scope for extension of the methyl group at the sulphur, but only when the aromatic core is either a phenyl ring with a *para* nitro substitution or a pyridine ring with the nitrogen at *ortho* position and a nitro group at *para* position with respect to the hydrazine functionality.

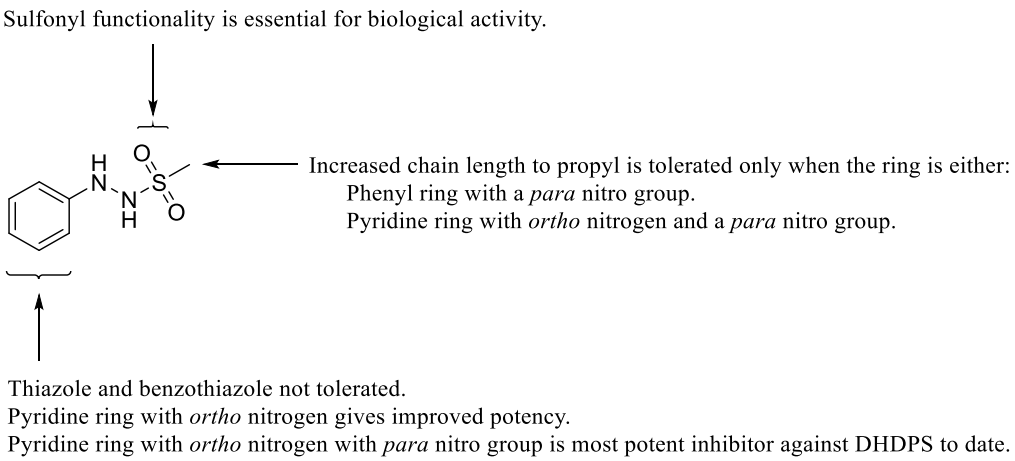
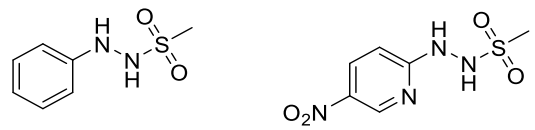


Figure 3.3: A summary of the structure activity relationship of sulfonyl hydrazides discussed in the chapter.

Chapter 4 Extending the alkyl functionality at the sulfonyl sulphur and synthesis of preliminary analogues

4.1 Introduction

Studies on identifying the best inhibitor for the DHDPS active site as outlined in Chapter 2 and 3, led to **3.29** which has two-fold increased activity compared to the HTS-14697 (Figure 4.1).



CT1-2
IC_{50} 128 ± 5.2 μM

3.29
IC_{50} 66.5 ± 6.0 μM

Figure 4.1: CT1-2 and the best inhibitor (**3.29**) identified from previous studies.

DHDPS contains four active sites per enzyme in its tetramer arrangement in Gram-negative bacteria and plants. Each active site contains two binding pockets, where aspartate semialdehyde (ASA) and pyruvate (PYR) bind to form HTPA.

The crystal structure of PYR bound DHDPS of common grape vine, *V. Vinifera*, is shown in Figure 4.2. The binding sites of bacteria and plants are similar except the differences in the amino acid residues. An overlay of active site residues of both *V. Vinifera* and *E. coli* is shown in cyan in Figure 4.2 (B). Lysine, which forms a Schiff base with PYR is coloured yellow. In *V. Vinifera*, PYR interacts with Lys 184 and Tyr 156 whereas, ASA interacts with Arg 161, Tyr 132 and Tyr 131.

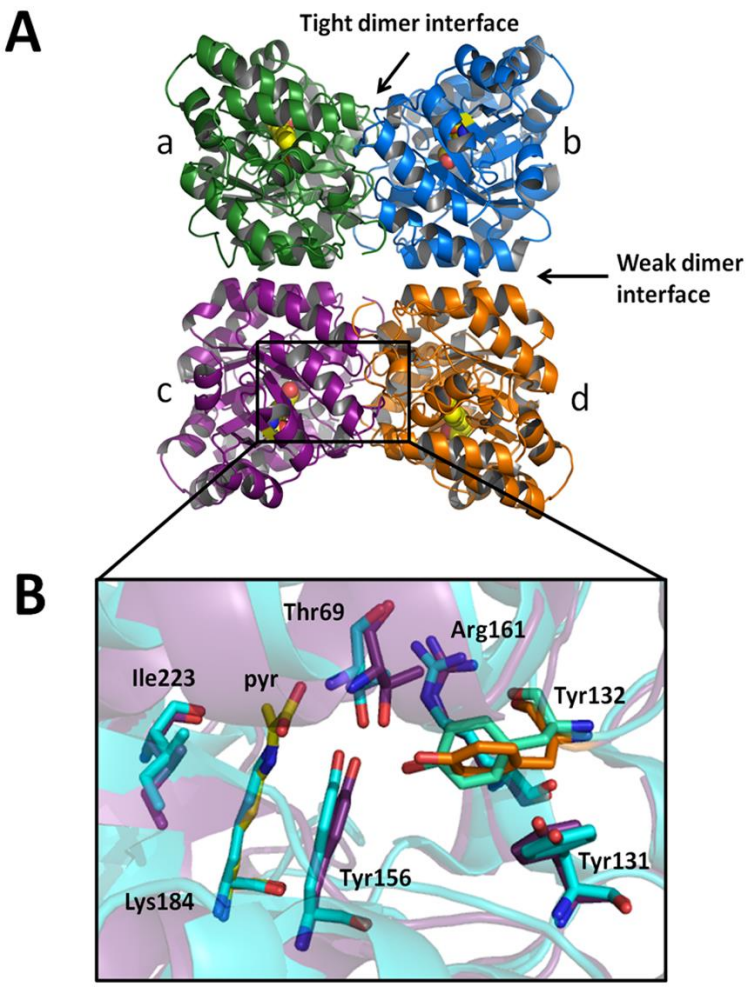


Figure 4.2: Crystal structure of the active site bound pyruvate in plant DHDPS.[105] (A) DHPDS of *V. Vinifera*, (B) PYR bound expanded active site.

Co-crystallisation studies performed by Shrivastava and co-workers in 2016 demonstrated that the well-known DHDPS inhibitor,[51] *α*-ketopimelic acid (*α*-KPA) (Figure 4.3) binds in the PYR binding pocket.[62] The key role of the α-keto group in forming the Schiff base as well as an optimal chain length of seven carbons was established. It was proposed that inhibition of the DHDPS enzyme was achieved by disrupting the tetrameric structure of DHDPS.

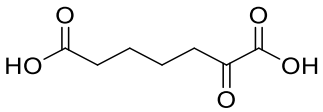


Figure 4.3: α-Ketopimelic acid.

It was observed in our previous work that longer alkyl chains may be tolerated at the sulfonyl group (Chapter 3.6). This chapter outlines the efforts to introduce a pyruvate/α-KPA analogue extending the chain length at the sulphonyl group.

4.2 Design and synthesis of the target compound

Chain extension at the sulfonyl group of HTS-14697 was investigated, with the introduction of an α-keto acid at the terminal functionality of the target compound (Figure 4.4). It was hypothesised that such a chain extension might lead to the molecule reaching out to the pyruvate binding pocket, while the α-keto acid may mimic pyruvate to form a Schiff base. It was intended that the aromatic ring would remain in the adjacent pocket where ASA binds. Such alterations may result in increased interactions of the target compound with the active site of the enzyme, resulting in better inhibitory properties.

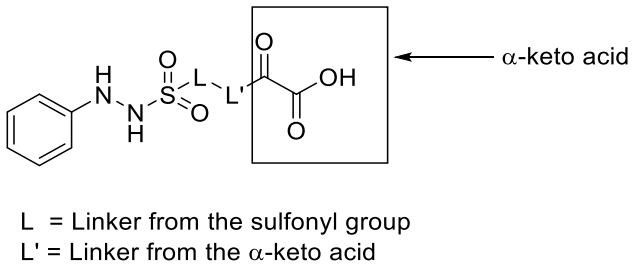


Figure 4.4: Preliminary design of the target compound.

It was decided to initially use α-KPA as the terminal linker, which has a carbon chain of seven carbons with one end containing α-keto acid functionality and the other end with a carboxylic acid (Figure 4.3). It was thought that ester protection might be required in the early stages of the synthesis. With one part of the acid chain being a carboxylic acid, amide bond generation was considered to link the sulfonyl end of the compound.

As a consequence, there needs to be a connecting terminal amine. After examining the preliminary crystal structure data, it was anticipated that L needs to be 2-3 carbons in length in order to reach out to the cavity of pyruvate binding pocket,

After consideration of the above components, the initial target compound was determined as shown in Figure 4.5.

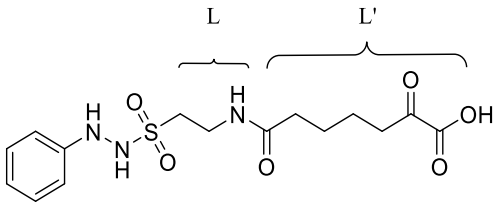
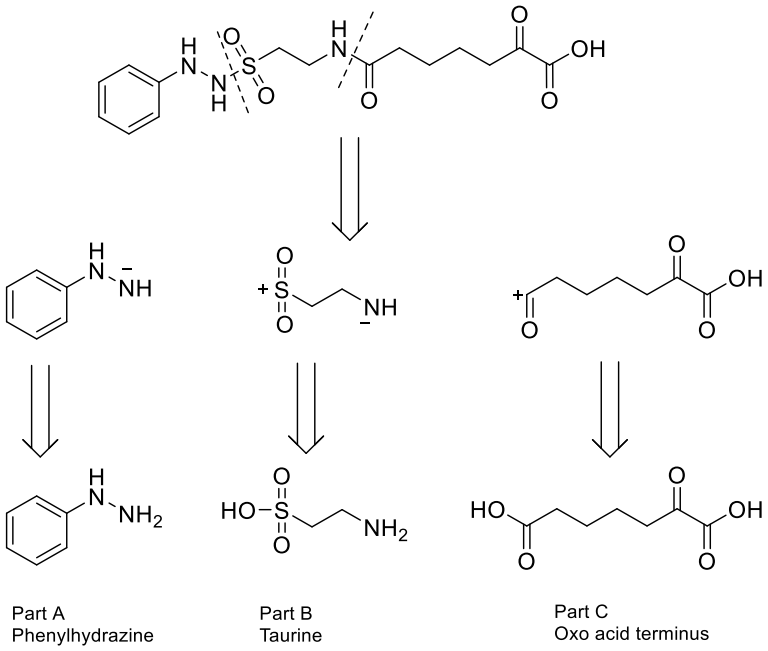


Figure 4.5: Target compound.

For the synthesis of the initial target compound, a retro-synthetic approach was taken as shown in Scheme 4.1.

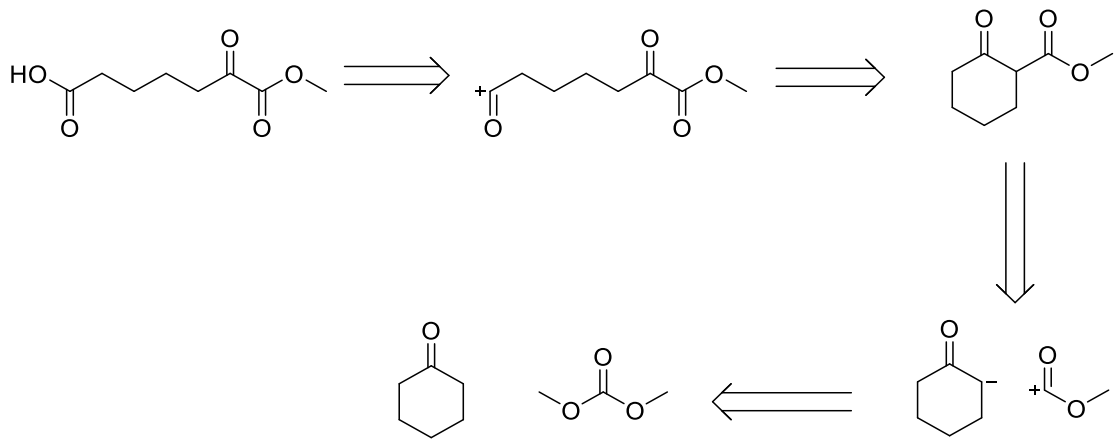


Scheme 4.1: Retrosynthetic analysis of the target compound.

Part A of the molecule, phenylhydrazine, and part B, taurine, are commercially available and were purchased. The α-keto acid terminus requires synthesis as part C.

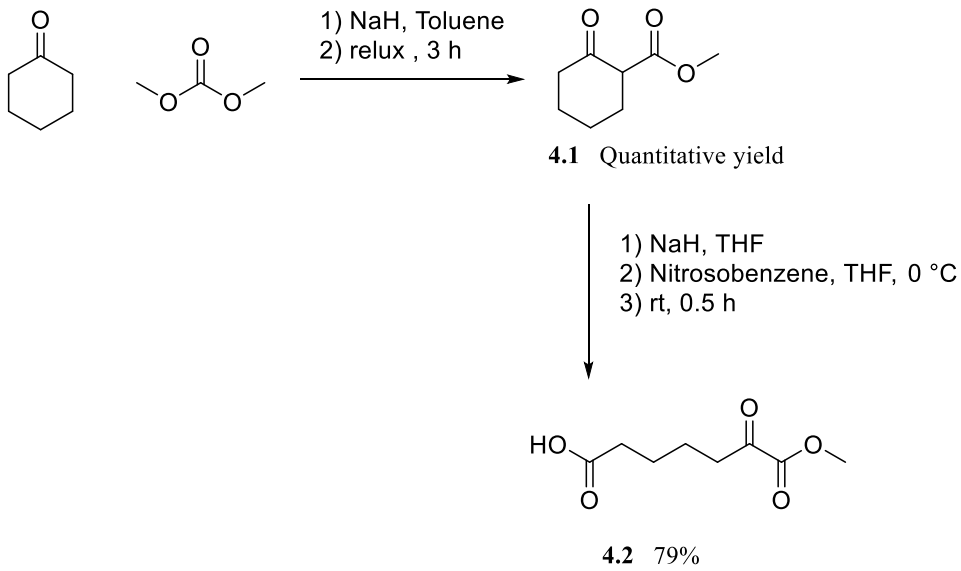
4.3 Synthesis of the α-keto acid terminus, 2-oxoheptanedioic acid

A retro-synthetic approach was undertaken to break the synthetic equivalent of part C to obtain commercially available starting materials from which the synthesis could be undertaken (Scheme 4.2).



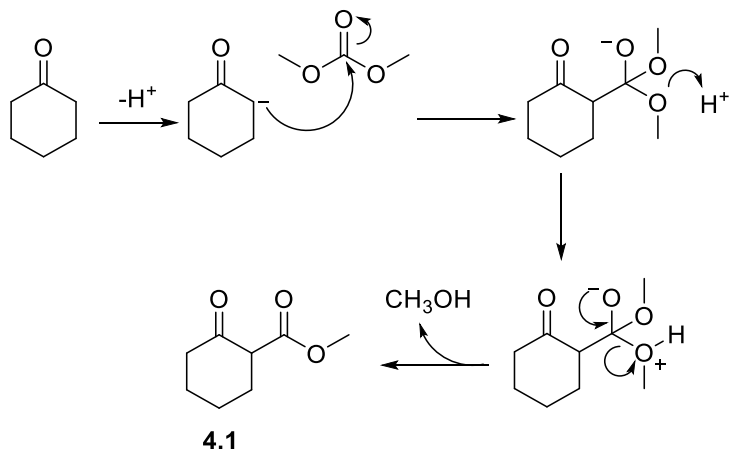
Scheme 4.2: Retro-synthetic approach to synthesise Part C of the target compound.

The synthesis of the target fragment, 7-methoxy-6,7-dioxoheptanoic acid, started from coupling of cyclohexanone with dimethyl carbonate under basic conditions (Scheme 4.3). While the reactions outlined in the literature used cycloheptanone as the cyclic ketone, [106, 107] this reactant was successfully changed to cyclohexanone to obtain the target compound in a quantitative yield. Toluene was used as the solvent due to the known hazards of benzene.



Scheme 4.3: Synthetic route to obtain the terminal α-keto acid functionality.

Sodium hydride is a strong base which can abstract a proton from the slightly acidic α-carbon of cyclohexanone, resulting in the formation of enolate ion (Scheme 4.4). This enolate can then attack dimethyl carbonate followed by proton transfers to generate methyl 2-oxocyclohexane-1-carboxylate **4.1** and methanol as the by-product.

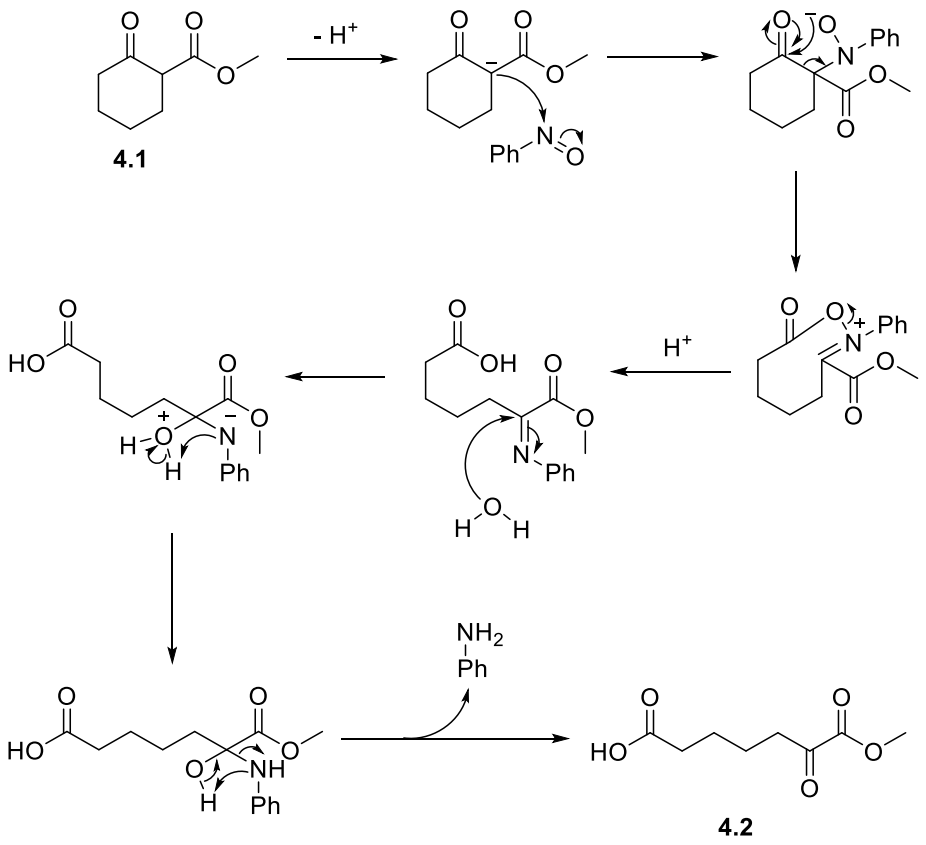


Scheme 4.4: Proposed mechanism for the synthesis of **4.1**.

In the ^{1}H NMR spectra of the synthesised **4.1**, a singlet was observed corresponding to the methyl protons at 3.75 ppm. The two triplets at 2.27 ppm and 2.22 ppm with an integration of two protons, and a multiplet at 1.65 ppm integrated to five protons, were also noted for the cyclohexane ring protons. The *m/z* ion at 179.0675 corresponds to the $(M + Na)^{+}$ ion confirming the identity of the **4.1**.

The obtained β-ketoester **4.1** was then subjected to one-step oxidative carbon-carbon ring opening (Scheme 4.3). Nitrosobenzene (a commercially available precursor) was used as the oxidant in the reaction as outlined Payette and Yamamoto.[108] The method produced **4.2** in a good yield of 79% after purification by column chromatography.

The proposed reaction mechanism for the cleavage of the cyclic ketone is illustrated in Scheme 4.5. The reaction initiation occurs when an enolate ion is generated due to the basic environment, which can then attack the nitrosobenzene. Electron transfers and the ring opening of the cyclohexane generated an eight-membered ring intermediate. Ring opening of the eight-membered ring gives a stable non-cyclic α-keto acid and aniline.



Scheme 4.5: Postulated reaction mechanism of the cyclic ketone cleavage.

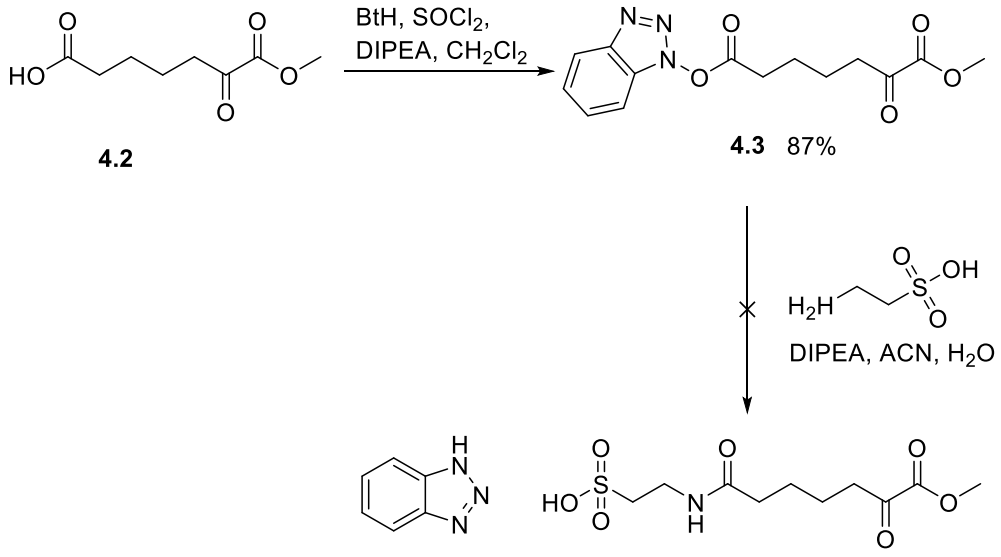
The ^{1}H NMR spectrum of the product contained the methyl group at 3.87 ppm as a singlet without much deviation from the methyl peak of the starting materials. Remaining CH_2 groups appeared as doublets at 2.88 ppm and 2.39 ppm with integrations of two protons each, and a multiplet at 1.69 ppm with an integration of four protons.[109] The carbon NMR spectra contained peaks at 193.6, 178.3, and 165.1 ppm corresponding to carbonyl carbons and peaks at 23.8, 38.8, 33.5, and 29.7 ppm corresponding to CH_2 carbons, and 53.0 ppm corresponding to the terminal methyl carbon. The carbon assignment was confirmed by running a DEPT-135 NMR spectrum where the carbonyl carbons did not appear, CH_2 carbons appeared as negative peaks and the terminal CH_3 carbon appeared as a positive peak.

4.4 Synthesis of the taurine linker

For synthesis of the taurine motif, a method described from Abo-Dya and Vertesaljai *et al*, was initially trialled.[110, 111] The synthesis used benzotriazole mediated coupling of peptides with taurine. Similarly, coupling of the α-keto acid with taurine was attempted. The α-keto

acid was reacted with benzotriazole to first generate the precursor required (Scheme 4.6).[110, 111]

Upon reacting **4.2** with thionyl chloride in the presence of *N,N*-diisopropylethylamine (DIPEA), generation of an acid chloride occurs followed by the coupling reaction with benzotriazole. This reaction generates hydrochloric acid for DIPEA to scavenge. The reaction proceeded as expected. ^{1}H NMR analysis followed by HRMS confirmed the identity of the product as **4.3**. In the ^{1}H NMR spectra, peaks at 8.30 ppm, 8.13 ppm, 7.66 ppm, and 7.25 ppm correspond to the aromatic protons. The CH_2 peaks appeared at 3.74 ppm, 2.97 ppm, 1.97 ppm, and 1.85 ppm as two triplets and two multiplets, respectively. The methyl peak appeared at 3.88 ppm as a singlet. The *m/z* peak obtained at 306.1083 corresponds to the $(M + H)^+$ ion of **4.3**.



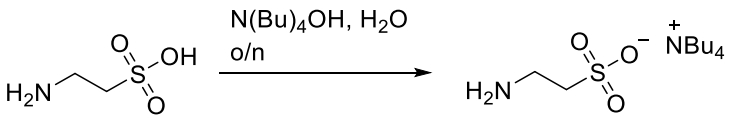
Scheme 4.6: Linking taurine with α-keto acid.

Coupling of taurine with **4.3** was then attempted following the method described by Katrizky.[111] Due to the high polarity of the taurine and the final product, a few drops of water were used to improve the solubility of the compound in acetonitrile. However as the solubility of taurine in water is 62.5 mg/mL at 20 °C, complete solubilisation of taurine was not obtained. The reaction was heated to temperatures higher than room temperature (30 – 70 °C) but no product **4.5** was visible either by NMR spectroscopy and LRMS performed at different reaction times (0.5 hours – 3 days).

Insoluble taurine was clearly visible in the reaction mixture, but the use of more water to solubilise was not advised by Katrizky, as “presence of even trace amounts of water can cause lower reaction yields”.[111]

Reacting taurine with carboxylic acids using 1-ethyl-3-(3-dimethylaminopropyl)carbo diimide (EDCI) and hydroxybenzotriazole (HOBt) coupling in dimethyl formamide (DMF) for 21 hours was then trialled.[112] Utilisation of *iso*-butyl chloroformate and triethylamine in tetrahydrofuran was also attempted as outlined by Sun and co-workers, to couple taurine with a carboxylic acid containing bulky pentacyclic triterpene.[113] Neither of these methods was successful in obtaining the target molecule, where a variety of reaction conditions such as reaction temperatures and times were attempted. Dioxane was also trialled but no reaction was observed even after a week.

The main problem with the coupling appeared to be the insolubility of taurine in organic solvents. This issue needed to be addressed before any further synthetic attempts. The t*etra*-butylammonium salt of taurine was used in the literature as a soluble taurine species for reactions in organic solvents.[114-116] Therefore the synthesis of tetrabutylammonium 2-aminoethane-1-sulfonate **4.6** was performed as shown in Scheme 4.7 to obtain the product in a quantitative yield.



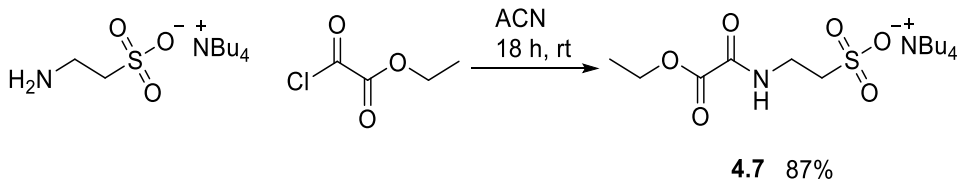
4.6 Quantitative yeild

Scheme 4.7: Synthesis of a soluble taurine species.

The ^{1}H NMR spectra of **4.6** contains peaks corresponding to the *n*-tetrabutylammonium at 3.17 ppm as a triplet, 1.56 ppm as a multiplet, 1.30 ppm as a multiplet, and 0.93 ppm as a triplet, with integrations of eight, eight, eight and twelve protons, respectively. The peaks corresponding to the taurine part appeared at 2.76 ppm as a triplet and 2.48 ppm as a triplet, with integrations of two protons each. The LRMS of the product gave *m/z* peaks at 242.19 corresponding to $(C_{16}H3_{6}N)^{+}$ ion and at 123.83 corresponding to $(C_2H_6NO_3S)^{-}$ in positive and negative modes, respectively.

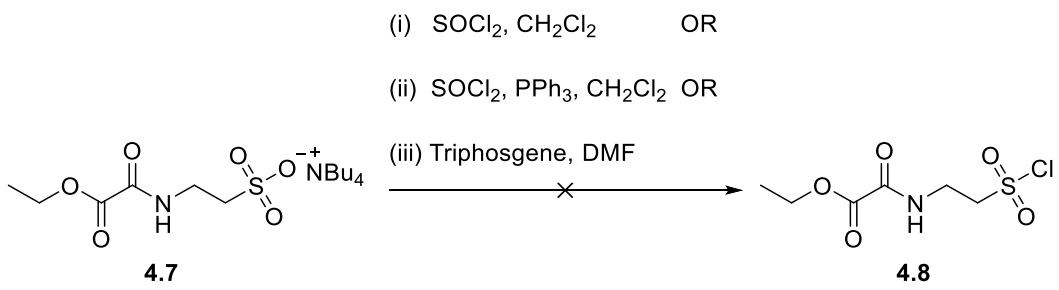
With a soluble taurine salt in hand, the coupling of the simplest β-keto ester, ethyl chloroacetoacetate, was performed as shown in Scheme 4.8 as a trial reaction. The reaction was successful with a good yield of 87%. The LRMS of **4.7** was conducted in both positive

and negative modes to obtain *m/z* ions at 242.28 and 123.83, respectively, corresponding to $(C_{16}H_{36}N)^+$ and $(C_6H_{10}NO_6S)^-$. The ^{1}H NMR spectra contained peaks at 3.16, 1.57, 1.30, and 0.93 ppm, corresponding to the *n*-tetrabutylammonium group and peaks at 4.22, 3.40, 2.59, and 1.25 ppm for the remaining protons. DEPT-135 was performed to allocate the obtained carbon NMR peaks. The ^{13}C NMR spectra of **4.7** contained peaks corresponding to the carbonyl carbons at 159.5 ppm and 158.7 ppm, the CH_2 carbons appeared at 62.9, 62.8, 59.1, 49.8, 36.1, and 24.2 ppm, whereas CH_3 carbons appeared at 20.0 and 13.8 ppm.



Scheme 4.8: Synthesis of **4.7**.

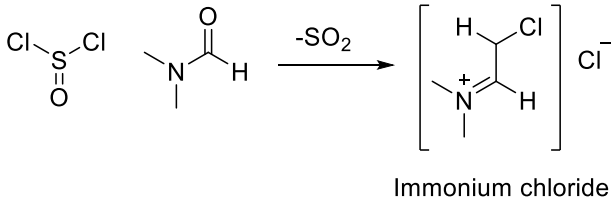
This was followed by attempts at converting the **4.7** to its corresponding sulfonyl chloride **4.8** using the methods outlined in Scheme 4.9. The use of sulfonyl chloride was first attempted as the use of thionyl chloride in synthesising acid chlorides from carboxylic acids are widely known (Scheme 4.9 (i)). However, due to the difference in electronic and steric properties of the sulfonate salt to a carboxylic acid, the method did not yield any product when monitoring the reaction by LCMS and NMR spectroscopy during the reaction progression.



Scheme 4.9: Attempts to convert the tetrabutylammonium sulfonate salt to a sulfonyl chloride.

Using thionyl chloride together with triphenylphosphine has been described in two patents, so a trial of this reaction was then undertaken (Scheme 4.9 (ii)).[117, 118] The reaction was monitored continuously using LCMS but no progression of the reaction was achieved even after two days. The use of dimethylformamide (DMF) in conjunction with thionyl chloride has been shown to generate an immonium chloride as a highly reactive chloride species (Scheme 4.10).[119] One equivalent of DMF to **4.7** was added to the reaction mixture after

two days of no reaction. Monitoring the reaction for three further days indicated there was no reaction progression (Scheme 4.9(iii)).



Scheme 4.10: Generating the reactive chlorine species from thionyl chloride and DMF.

Reynolds and co-workers also encountered difficulties when trying to synthesise sulfonyl chlorides from sulfonic salts, but found success using triphosgene as the chlorinating reagent.[120] Unfortunately, applying the same protocol did not lead to success in our synthesis.

The sensitivity and reactivity of the sulfonic salts might possibly be the reason for the vastly different chlorination methods, hence different sulfonic salts require different conditions to yield the respective sulfonyl chloride.

In 2004, Judd and co-workers published a one-pot method for direct conversion of sulfonic salts to sulfonyl chlorides followed by coupling with an amine using triphenylphosphine ditriflate as the activator (Figure 4.6).[121] The use of tetrabutylammonium salts was recommended for sulfonic acids with low solubility as the choice of counter ion was found to be nontrivial.

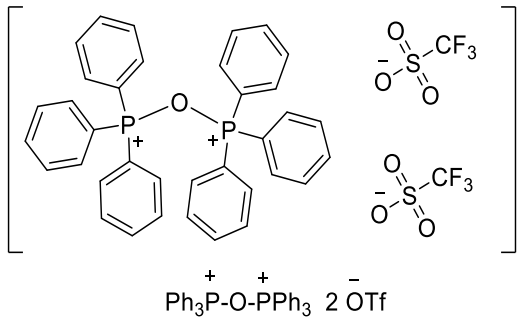
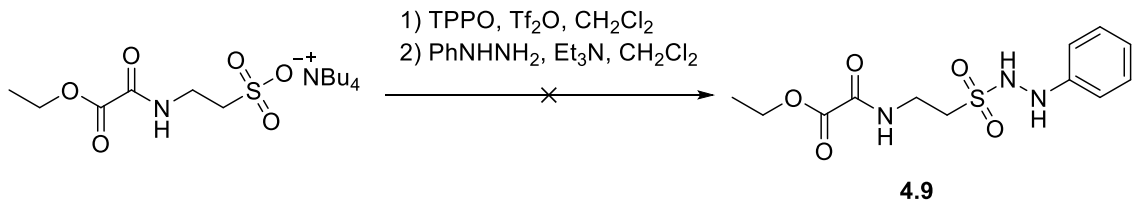


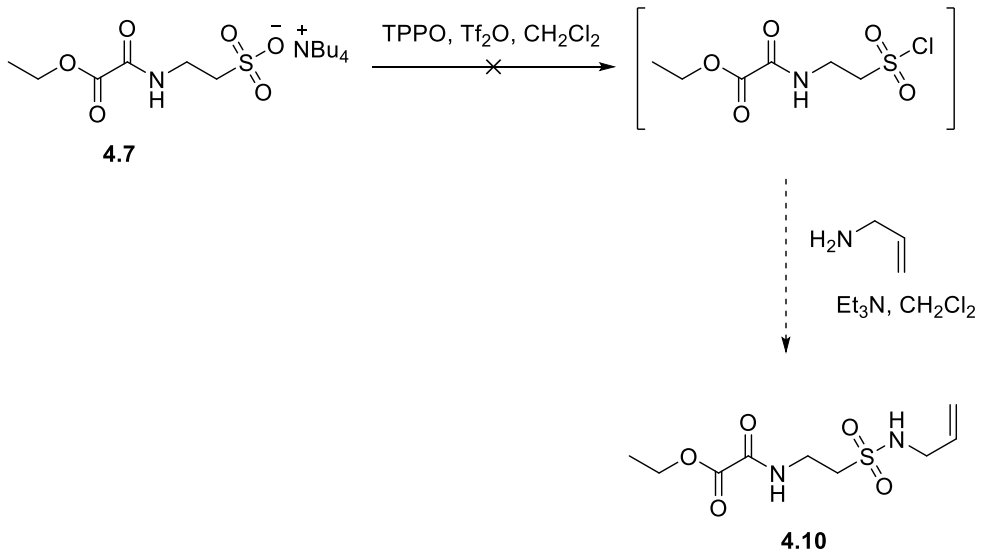
Figure 4.6: Structure of ($[Ph_3P\text{-}O\text{-}PPh_3]^{2+}$ $2[OTf]^-$).

As shown in Scheme 4.11, the synthesis of ethyl 2-oxo-2-((2-((2-phenylhydrazinyl) sulfonyl)ethyl)amino)acetate **4.9** was attempted. However, after 2 days of the reaction monitoring by the LCMS, the chlorinated species was not observed and the starting material was recovered.



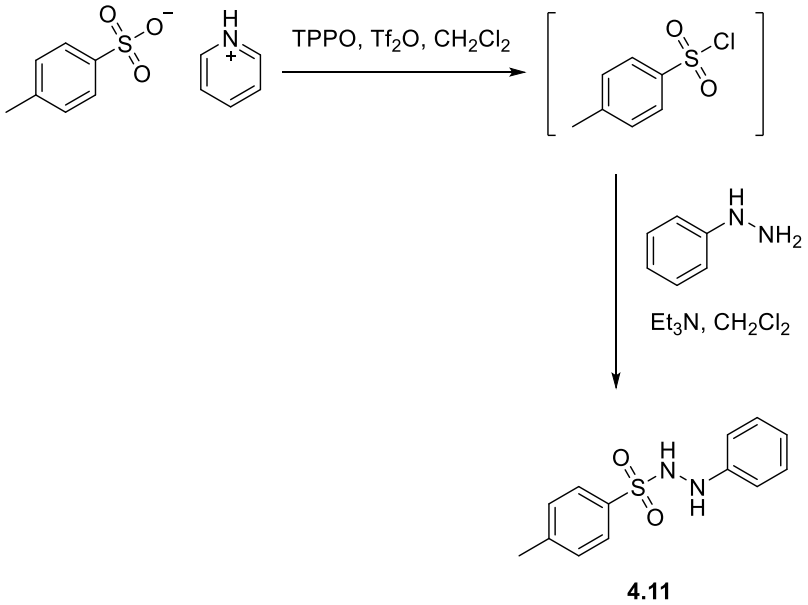
Scheme 4.11: Attempted synthesis of ethyl 2-oxo-2-((2-((2-phenylhydrazinyl)sulfonyl)ethyl) amino)acetate.

To better understand the triphenylphosphine ditriflate methodology two trial reactions were carried out (Scheme 4.12 and Scheme 4.13). Firstly, the amine of phenylhydrazine was replaced by allylamine as used by Judd and co-workers (Scheme 4.12).[121] The reaction was monitored via LCMS for two days with the starting material, **4.7** recovered on the third day.



Scheme 4.12: Trial reaction of **4.7** with allylamine.

However, when the sulfonate salt was swapped to the pyridine salt of *p*-toluenesulfonic acid (Scheme 4.13), reaction progression was observed by LCMS with the observed *m/z* at 190.95 and at 190.93 corresponding to the ^{35}Cl and ^{37}Cl isomers.



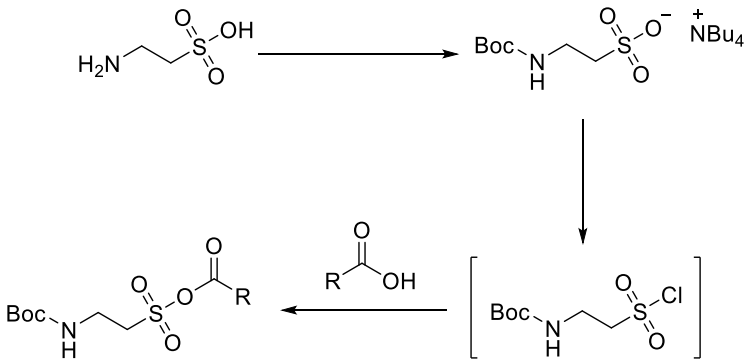
Scheme 4.13: Trial reaction of pyridine salt of *p*-toluenesulfonic acid with phenylhydrazine.

After the complete consumption of the pyridine salt of *p*-toluenesulfonic acid as observed by LCMS, the reaction was terminated and the product **4.11** was purified using column chromatography with 20% ether in hexane. In the ^{1}H NMR spectra peaks for the aromatic protons were observed at 7.68 ppm as a doublet, 7.25 as a doublet, 7.20 ppm as a doublet, 6.81 ppm as a triplet, and 6.71 as a triplet with integrations of two, two, two, one, and two respectively. At 2.39 ppm, a singlet with an integration of three was also seen corresponding to the methyl protons. The carbon NMR spectra of the product contained peaks at 150.8, 137.3, 133.2, 128.5, 128.1, 122.6, and 113.0 ppm corresponding to the aromatic carbons, and 21.3 ppm corresponding to the methyl carbon. A *m/z* peak of 285.0667 was observed in the HRMS analysis corresponding to the $(M + Na)^{+}$ ion, further confirming the identity of the product.

The above two experiments (Scheme 4.12 and Scheme 4.13) imply that the reaction method outlined by Judd and co-workers was not compatible with the starting material of CT4-11A. This strengthens the hypothesis that each sulfonic acid salt is highly specific and responds to different chlorination methods.

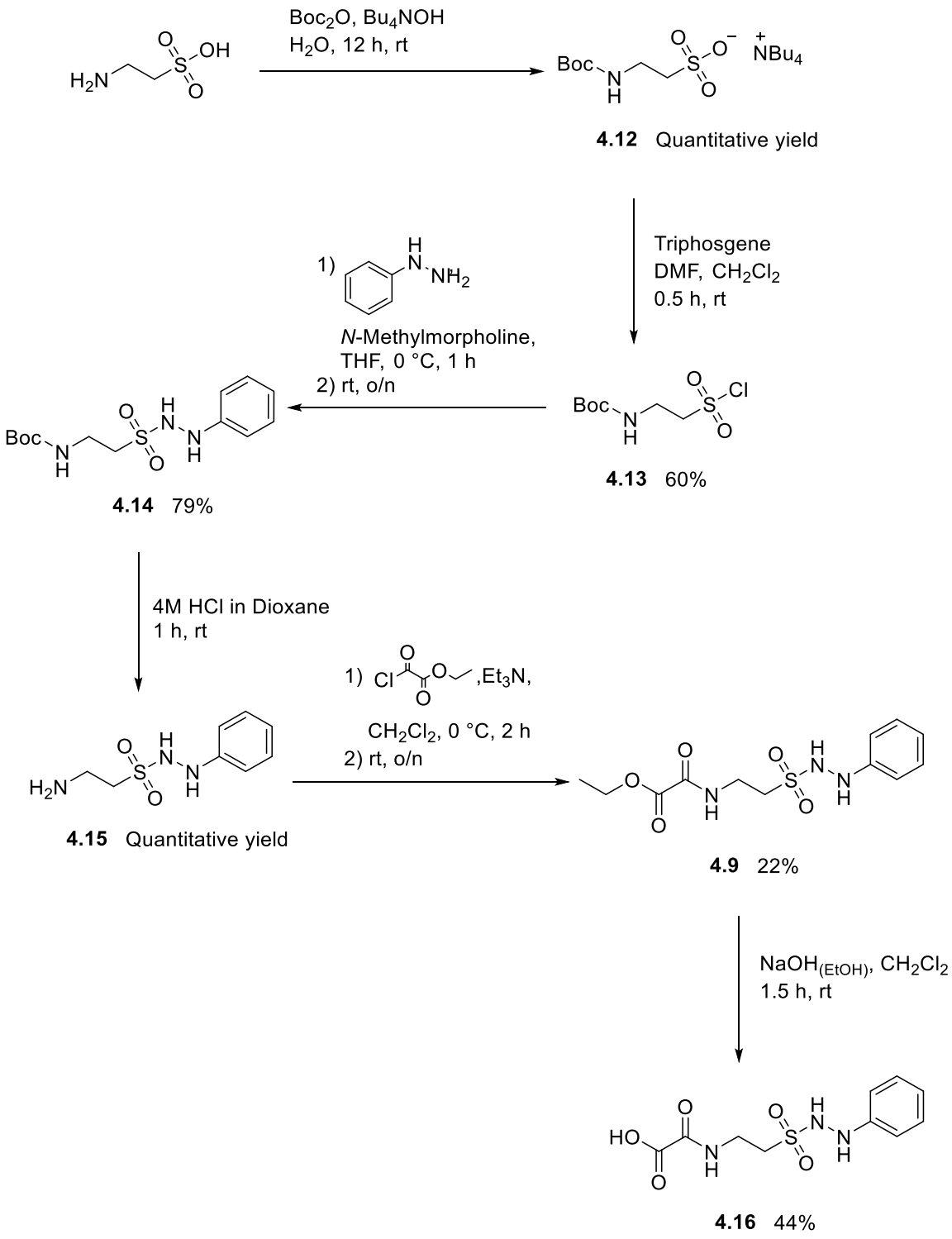
Further investigation to the related literature was conducted. Two research articles published by Leszczynska *et al.*[122] and Xie *et al.*[123] were identified, where taurine was converted to the *N*-Boc taurine tetrabutylammonium salt although the rationale for doing so was not discussed. It was hypothesised that this conversion results in further

improvement in the solubility of the taurine salt in organic solvents. The sulfonyl chloride was produced using triphosgene followed by coupling with the corresponding acid and *N*-Boc deprotection to obtain the free amine (Scheme 4.14).



Scheme 4.14: Reactions with *N*-Boc taurine tetrabutylammnium salt.[122, 123]

Following on from Scheme 4.14, a new synthetic scheme was undertaken as outlined in Scheme 4.15, using phenylhydrazine which has previously been found to be suitable.

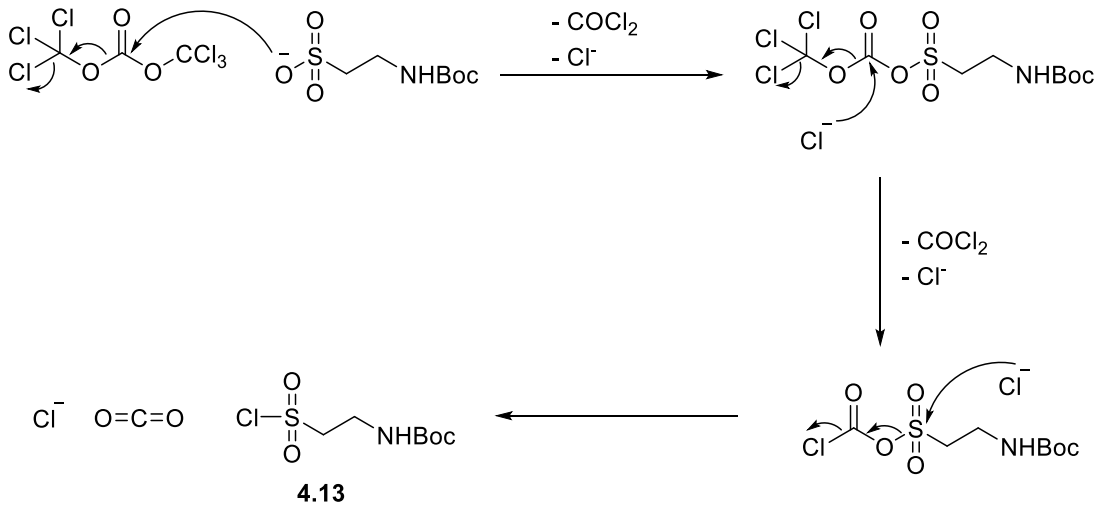


Scheme 4.15: Synthesis of ethyl 2-oxo-2-((2-((2-phenylhydrazinyl)sulfonyl)ethyl)amino)acetate **4.16** via BOC protection.

Amine protection of taurine was performed via *N*-Boc protection and the acid group on the sulfonyl was converted to the quaternary ammonium salt using tetrabutylammonium hydroxide. The reaction generated the salt **4.12** in a quantitative yield. The peaks of the ^{1}H NMR spectra appeared at 3.53 ppm as a multiplet and 2.91 ppm as a multiplet, with

integrations of two protons each corresponding to the CH_2 moieties in the taurine. The peaks corresponding to the quaternary ammonium salt appeared at 3.28 ppm as a multiplet, 1.64 ppm as a multiplet, 1.44 ppm as a multiplet with integrations of eight protons each, and at 1.00 ppm a singlet with an integration of twelve protons. The ^{1}H NMR spectra also contained a peak at 1.39 ppm corresponding to the *N*-Boc protons as a singlet with an integration of nine protons. In the carbon NMR spectra of **4.12**, peaks were observed at 153.1 ppm for the carbonyl carbon, at 53.4 ppm and 30.9 ppm for the taurine carbons, at 58.8 and 13.7 ppm for the Boc carbons, and at 53.3, 28.5, 24.0, and19.7 ppm for the butyl carbons. Peaks for *m/*z ions at 242.19 and 224.06 were found in positive and negative modes, respectively, in the LRMS analysis of **4.12**.

Reacting **4.12** with triphosgene resulted in the generation of the corresponding sulfonyl chloride **4.13**. Triphosgene is a white crystalline solid which is equivalent to three molecules of phosgene.[124] The use of phosgene was crucial as a chlorinating agent but due to its harmful effects and being difficult to handle, triphosgene is reluctantly employed in both industry and research laboratories.[124-126] The proposed reaction mechanism for the reaction between triphosgene and **4.12** is illustrated in Scheme 4.16.



Scheme 4.16: Proposed reaction mechanism of sulfonyl chloride generation with triphosgene.

For the ^{1}H NMR spectra of **4.13**, the absence of the peaks corresponding to the quaternary ammonium salt was noticeable. There were signals at 3.90 ppm and 3.77 ppm corresponding to the CH_2 groups in the taurine skeleton, a peak at 1.44 ppm corresponding to the *tert*-butyloxycarbonyl group, and a broad peak at 5.10 ppm corresponding to the NH_2 group. In the carbon NMR spectrum, the carbonyl carbon appeared at 155.7 ppm, the

CH_2 groups appeared at 65.0 ppm and 38.0 ppm, the quaternary carbon was found at 80.6 ppm, and the CH_3 carbon appeared at 28.3 ppm. Furthermore, a *m/z* ion of 244.7093 was found corresponding to the $(M + H)^+$ of **4.13**.

The coupling of **4.13** with phenylhydrazine was undertaken in tetrahydrofuran with *N*-methylmorphiline (NMM) as the base scavenging the generated hydrochloric acid. The reaction was adopted from the research article published by Dai and co-workers.[123] The reaction was successful in a 79% yield generating **4.14**. Aromatic protons were observed at 7.26, 6.99, and 6.93 ppm with a collective integration of five protons in the ^{1}H NMR spectra. The NH protons appeared at 9.13, 6.30, and 6.04 ppm, the CH_2 protons at 4.05 and 3.29 ppm, and the three CH_3 protons at 1.54 ppm. The HRMS analysis of **4.14** contained a mass peak appearing at 316.3955, corresponding to the molecular ion.

Deprotection of **4.14** was undertaken using trifluoroacetic acid in dichloromethane. As expected, the reaction yielded product quantitatively. The ^{1}H NMR spectra of **4.15** did not contain the protons belonging to the *tert*-butyloxycarbonyl group while all of the other signals were a slightly shifted from what was observed for the **4.14** ^{1}H NMR spectra. In the carbon NMR spectrum of **4.15**, compared to that of **4.14**, peaks corresponding to the protection group were not observed as expected. The only peaks observed were for the aromatic carbons at 150.8, 128.7, 121.7, and 112.8 ppm, and the CH_2 peaks at 62.5 and 30.8 ppm. Moreover, the desired molecular ion was observed using HRMS of **4.15** at *m/z* 216.0803.

The amide bond between ethyl chlorooxoacetate and **4.15** was generated in the presence of triethylamine in dichloromethane. After washing the reaction mixture with base then water, trituration with ether yielded the product **4.9** in a moderate yield of 22%. Comparing the ^{1}H NMR spectra of **4.9** with that of **4.15**, two extra peaks were observed corresponding to the ethyl group of the ester functionality at 3.01 and 1.35 ppm. The other ^{1}H NMR peaks observed were at 7.96, 7.66, and 7.60 ppm, corresponding to the aromatic protons, while the CH_2 peaks were observed at 4.31 and 3.79 ppm. The identity of **4.9** was further confirmed by the molecular ion obtained at 316.0960, which corresponds to the $(M + H)^+$ ion.

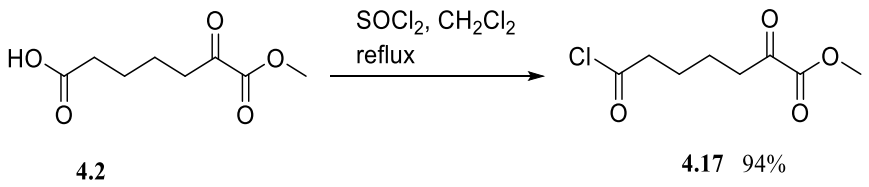
Finally, base hydrolysis was used for the deprotection of the ester to obtain the product **4.16** in a 44% yield after purification. In the ^{1}H NMR spectra, the ethyl protons were not observed, as expected, while the aromatic protons were observed at 7.88, 7.56, and 7.50

ppm. The CH_2 protons were observed at 4.25 and 3.98 ppm. In the HRMS analysis, the molecular ion peak was observed at 286.0501, which corresponds with the $(M - H)^-$ due to the presence of the carboxylic acid group.

4.5 Combination of the synthetic equivalents to synthesise the initial target compound

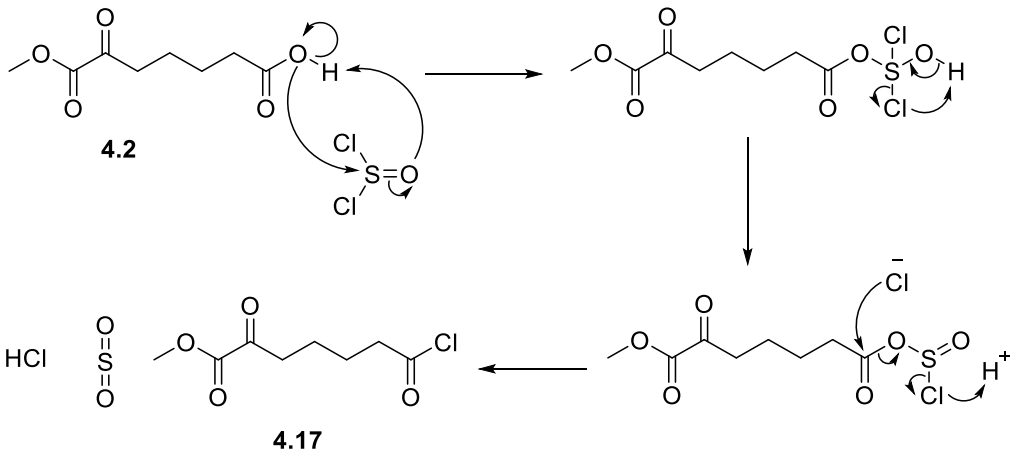
With a synthetic pathway successfully established (Scheme 4.15), further analogue generation was conducted.

Chlorination of **4.2** with thionyl chloride in dichloromethane under reflux condition was used to obtain the acid chloride in a 94% yield after distillation of excess thionyl chloride (Scheme 4.17).



Scheme 4.17: Chlorination of **4.2**.

The reaction of the acid chloride generation occurs via S_N2 substitution as shown in Scheme 4.18 followed by proton transfers.

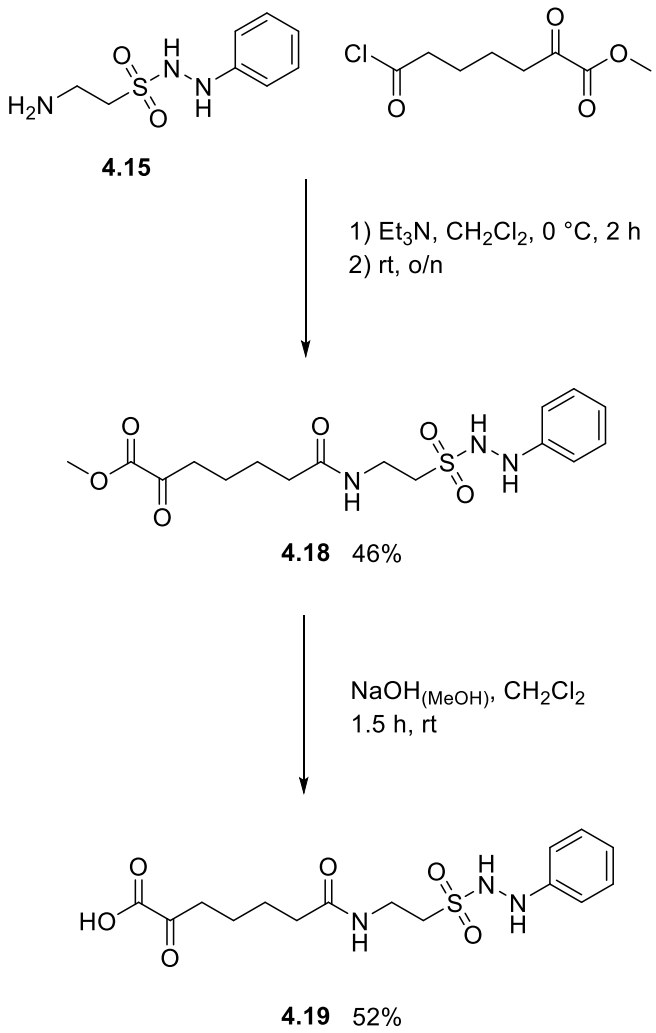


Scheme 4.18: Proposed mechanism of the chlorination of **4.2** with thionyl chloride.

The ^{1}H NMR spectra of the acid chloride had all of the CH_2 peaks appearing as one complex multiplet at 2.93 ppm and the methyl peak at 3.89 ppm, similar to the precursor. The HRMS of the **4.17** found *m/z* of 207.0417 and 209.0416, corresponding to the ($M[^{35}Cl]+H^+$) and ($M[^{37}Cl]+H^+$) molecular ions.

The chlorination reaction was performed just before the coupling reaction for the synthesis of the final compound as acid chlorides can be sensitive to moisture.

For the synthesis of the target compound (Scheme 4.8), coupling of CT4-27D with **4.17** was conducted and followed by ester hydrolysis as outlined in Scheme 4.19.



Scheme 4.19: Synthesis of **4.19**.

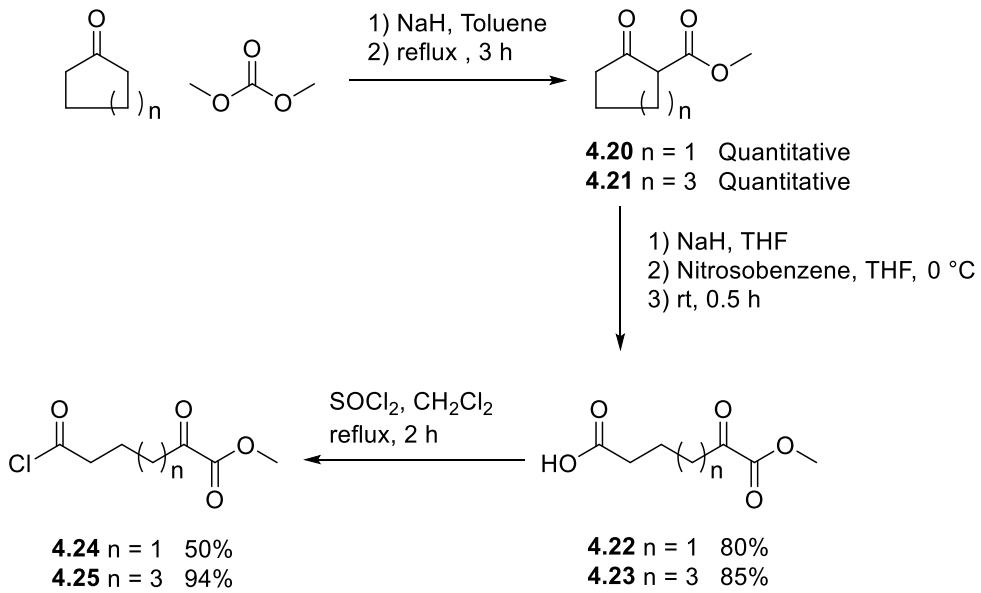
The coupling reaction was monitored by LRMS analysis in one hour intervals and as the reaction progression was slow, the reaction mixture was allowed to stir overnight. After the complete consumption of the starting material was observed, washing with acid and

water was followed by a trituration with ether to obtain the pure product **4.18** in a moderate yield (56%). ^{1}H NMR spectra showed the aromatic protons at 7.96, 7.66, and 7.60 ppm, with a collective integration of five protons and the methyl peak was observed at 3.89 ppm. The CH_2 protons of the acid chain appeared as a triplet at 3.51 ppm, 2.22 ppm as a triplet and at 1.54 ppm as a multiplet, with integrations of two, two, and four, respectively, in the ^{1}H NMR spectra. The CH_2 protons in the taurine linker appeared as separate multiplets with integrations of two each at 4.20 and 3.90 ppm. The HRMS of **4.18** had the *m/z* peak at 386.1379, corresponding to the $(M + H)^+$ ion.

Ester hydrolysis of **4.18** in methanolic sodium hydroxide yielded the product **4.19** in a 41% yield. The ^{1}H NMR spectra of **4.19** lacked the methyl protons as expected after hydrolysis, whereas the aromatic protons and the CH_2 protons appeared slightly shifted from where they appeared in the ^{1}H NMR spectra of **4.18**. Further confirmation of the product was conducted by detecting the molecular ion peak from the HRMS analysis at 370.1076, corresponding to $(M - H)^-$.

4.6 Changes to the α-keto acid terminal

Two further acid chlorides consisting of varied chain lengths were synthesised using the reaction scheme outlined in Scheme 4.20.



Scheme 4.20: Synthesis of acid chlorides for analogue generation.

Reaction of the cyclic ketones with dimethyl carbonate was quantitative in both cyclic systems with a new methyl peak appearing in the ^{1}H NMR spectra in the region of 3.72 – 3.75 ppm. The carbon NMR spectra contained two extra peaks corresponding to the carbonyl carbon and the methyl carbon in the region of 166 – 171 ppm and 52 – 56 ppm, respectively.

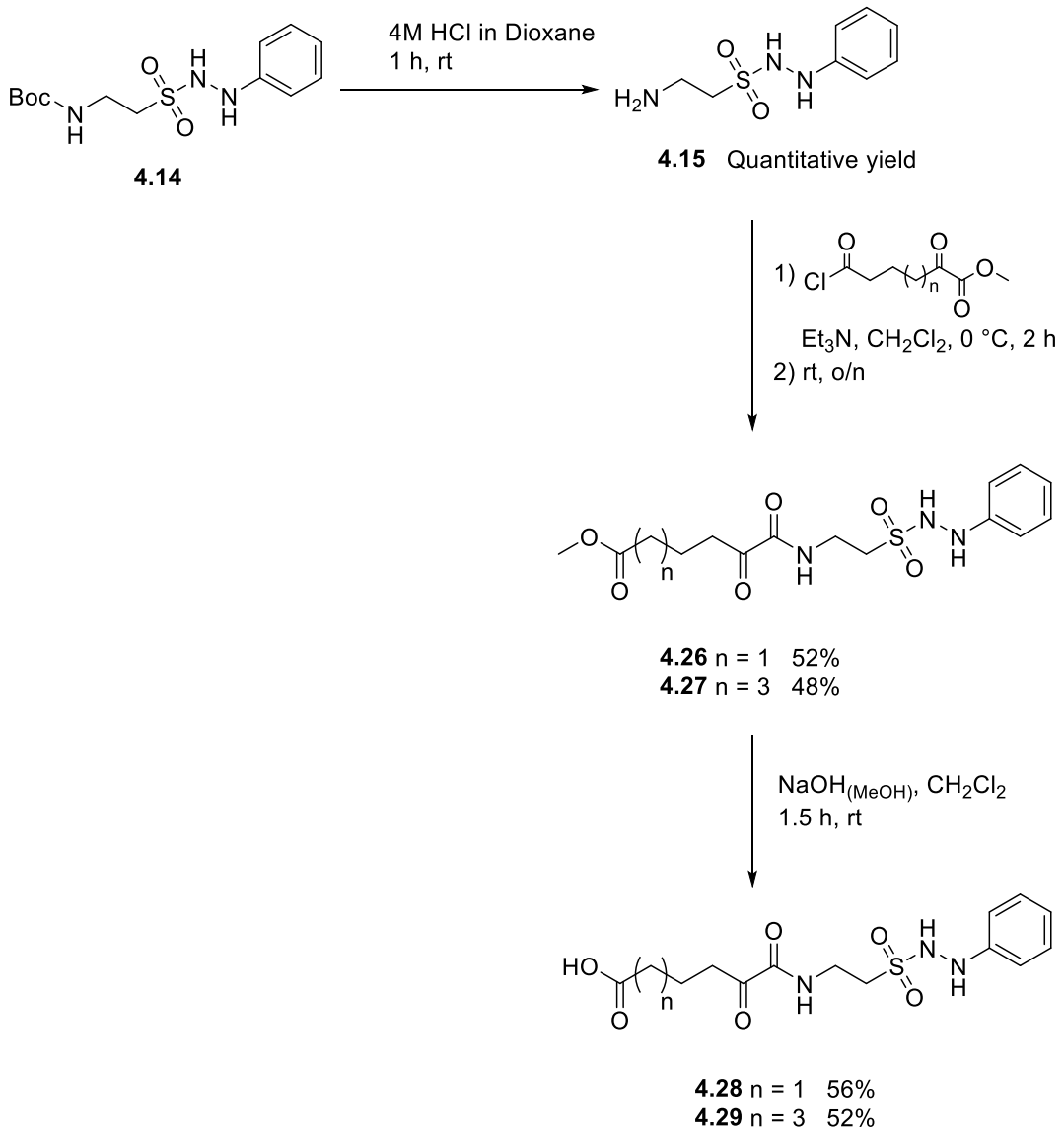
The ring opening was successful with products obtained in good yields (80 and 85%). The distinguishable peak for the new CH_2 group appeared in the region of 2.80 - 3.00 ppm on ^{1}H NMR spectra of the compounds, with an integration of two protons and the corresponding carbon was observed in the region of 38.2 – 39.0 ppm on ^{13}C NMR spectra.

Chlorination of the respective acid analogue generated oily acid chlorides. The noticeable difference between the acids and the acid chlorides in the ^{1}H NMR spectrum was the overlap or the close proximity of the CH_2 peaks when compared to the starting materials. The carbon NMR peaks were also slightly shifted. Further confirmation of the identity of the products were undertaken by HRMS, where ($M[^{35}Cl] + H^{+}$) ions were observed at 193.0262 and 221.0576 and ($M[^{37}Cl] + H^{+}$) were observed at 195.0261 and 223.0577, for **4.24** and **4.25** respectively.

The isolated acid chlorides were used in the coupling reaction as outlined in Scheme 4.21. Deprotection of the *tert*-butyloxycarbonyl group and preparation of the corresponding acid chloride were performed simultaneously, as the acid chloride can easily get converted to the corresponding acid and the unprotected amine group can decompose quickly. The ester protected linked compounds were semi-solids after purification and contained CH_2 peaks corresponding to the taurine skeleton in the region of 3.93 – 2.90 ppm as two triplets with integrations of two protons each. A clear set of peaks appeared in the aromatic region corresponding to the phenyl group in the region of 7.96 – 7.60 ppm with a collective integration of five protons. Observed peaks in the carbon NMR spectrum were comparable with that of the acid starting materials with an addition of an extra two carbons for the taurine linker in the region of 32.0 – 60.0 ppm and four new carbon peaks in the aromatic region of the ^{13}C NMR spectra.

Base hydrolysis of the esters by methanolic sodium hydroxide gave the products in a moderate yield. In the ^{1}H NMR spectra of the products, the peaks corresponding to the methyl esters were not observed. Furthermore, the identity of the target compounds was

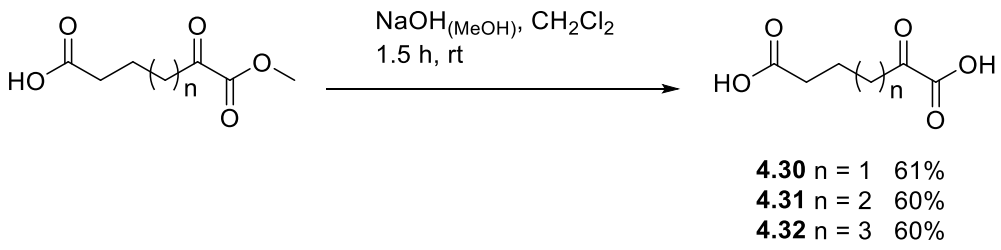
confirmed by HRMS where $(M - H)^-$ was observed at 356.0921 and 384.1234 for **4.28** and **4.49**, respectively.



Scheme 4.21: Synthesis of the linked compound analogues.

4.7 Synthesis of α-KPA and analogues

Synthesis of three different acids was achieved with the alkyl chain lengths of 3, 4 and 5 carbons using the previously synthesised ester analogues; **4.22**, **4.2** and **4.23** (Scheme 4.22). Due to the high polarity of the diacids, the NMR analysis was undertaken in deuterated water. In the HRMS analysis of the acids, the corresponding $(M - H)^{2-}$ ions were observed. On all of the proton and carbon NMR spectra of the final α-ketodiacids indicated that cleavage of the methyl group from the starting material ester had occurred.

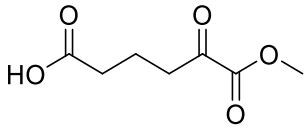
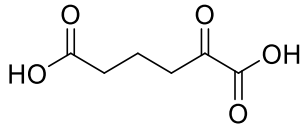
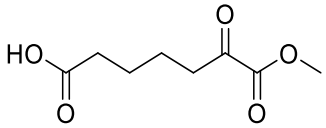
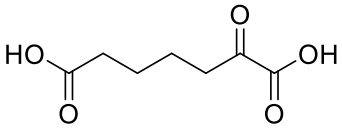
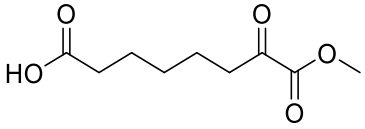
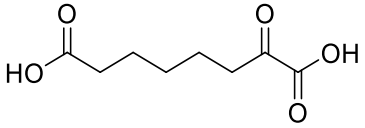


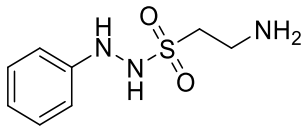
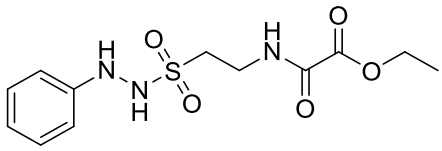
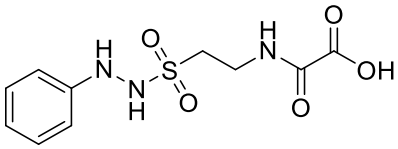
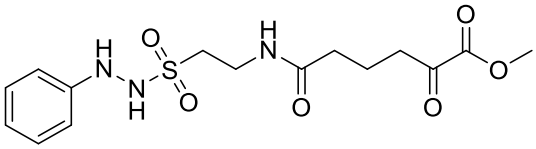
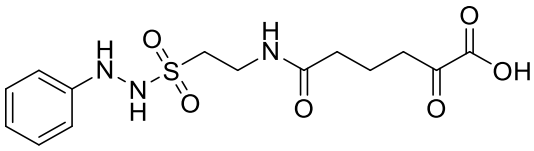
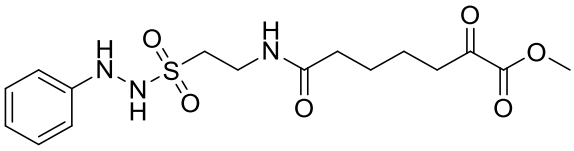
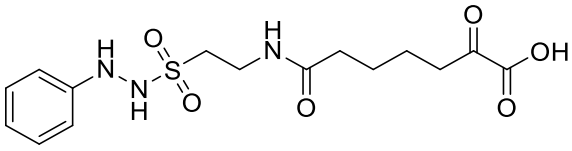
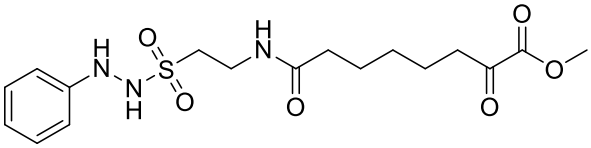
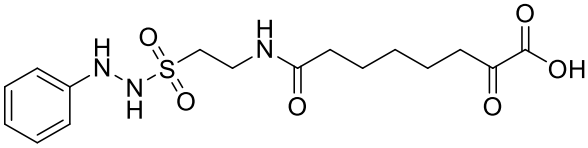
Scheme 4.22: Synthesis of α-KPA analogues.

4.8 Biological evaluation of the synthesised compounds

Using the DHPDS-DHDPR coupled assay IC_{50} values for the synthesised compounds were determined using isolated *E. coli* DHDPS. The data is tabulated in Table 4.1.

Table 4.1: IC_{50} values for the synthesised α-KPA analogues and analogues of HTS-14697 with extensions made at the sulfonyl sulphur.

Number	Compound	IC_{50}/ µM
4.22		298 ± 9
4.30		>500
4.2		>500
4.31 (*α*-KPA)		30.4 ± 3
4.23		71.1 ± 2
4.32		143 ± 4

4.15		>500
4.9		>500
4.16		>500
4.26		>500
4.28		>500
4.18		127 ± 3
4.19		>500
4.27		>500
4.29		>500

Some of the oxo acids and the corresponding esters showed promising inhibitory properties against *E. coli* DHDPS. Compound **4.31** (α-KPA) showed an IC_{50} of 30 µM which correlated well with what was indicated by Shrivastava and co-workers.[62] Compound **4.23**

showed some inhibitory properties with an IC_{50} of 71 µM. Moderate activity was observed from diacids **4.32** and **4.22** with IC_{50} values of 143 µM and 299 µM, respectively.

The intermediates of the α-oxoacids, **4.32**, **4.23**, and **4.22**, had activity against DHDPS enzyme. The first two compounds were the only instances where both the ester and the free acid were active with IC_{50} values of 143 µM and 71 µM, but coupling with the amine resulted in complete loss in activity, as observed for **4.27** and **4.29**.

Unfortunately, the only linked compound which showed any activity was **4.18** with an IC_{50} of 127 µM. This compound contained a chain length similar to that of **4.31** but, surprisingly, was active as the ester form but not as the acid. All of the other linked compounds did not inhibit DHDPS of *E. coli*, with IC_{50} results above 500 µM.

Three out of the five active compounds in the series were esters. Esters are susceptible to cleavage inside cellular environments which could be problematic due to the inactive nature of the acid counterparts (Table 4.1). It was not expected that any of these esters would have potency while the corresponding acids would be active.

4.9 Summary

An effective synthetic procedure was developed for the synthesis of a library of linked inhibitors. Changes could be made to the final compounds either via changing the hydrazine, the linker compound, or the α-oxoacid. These methods were used to synthesise a small library of eight compounds, with the most potent compound of the series, **4.18**, found to have an IC_{50} of 127 µM.

It was reinforced that the inhibitor α-KPA **4.31** is a potent inhibitor for the pyruvate binding site inside the active site with an IC_{50} of 30 µM.

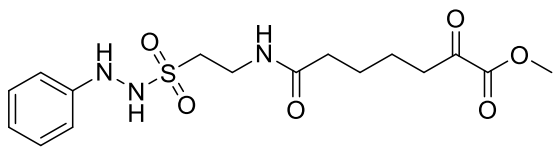
Even though most of the compounds synthesised in this chapter were not inhibitors of DHDPS, this work was found to be a useful step towards generating a wide variety of linked compounds. Further work in this area will be discussed in chapter 5.

Chapter 5 Generating sulfonylhydrazine analogues with extended alkyl functionality

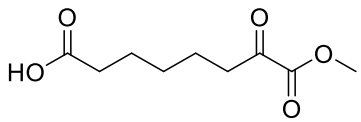
5.1 Introduction

From the preliminary data obtained in Chapter 4, further investigation was undertaken into the α-ketopimelic acid analogues to improve the biological activity.

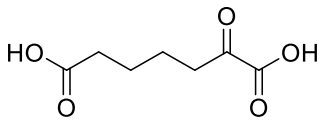
The best aromatic compound discussed in Chapter 4 was **4.18**, with an IC_{50} of 127 μM (Figure 5.1). Out of the other three compounds that were active, one was the β-oxoester (**4.23**) and two were β-oxoacids (**4.31** and **4.32**) with activities of 71 μM, 30 μM, and 143 μM, respectively (Figure 5.1).



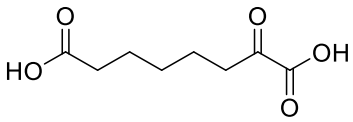
4.18
IC_{50} 127 μM



4.23
IC_{50} 71 μM



4.31
IC_{50} 30 μM



4.32
IC_{50} 143 μM

Figure 5.1: Best α-ketopimelic acid analogues from Chapter 4.

A library of compounds was generated by changing the unsubstituted aromatic ring to *p*-nitropyridine ring as the best inhibition in the methyl sulfonyl series was observed for this heteroaromatic substitution (**3.29**).

The position of the amide was also changed on the alkyl motif by changing the chain length on both sides of this functional group. These changes were made when the aromatic ring was either an unsubstituted aromatic ring or a *p*-nitropyridine.

The compounds were tested as both esters and respective acids to develop understanding the SAR further.

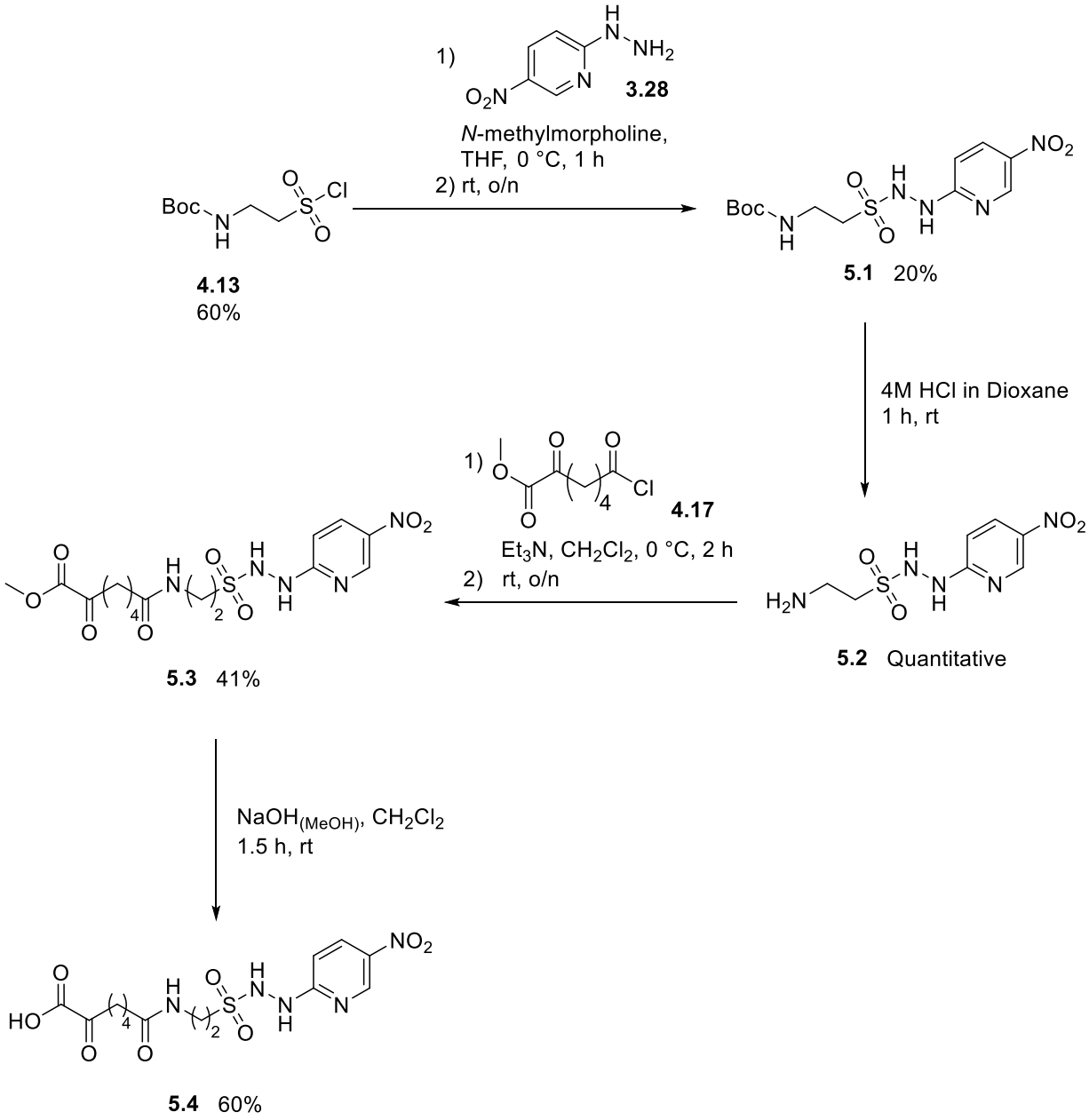
5.2 Analogue of 4.18; changes to the aromatic ring

Replacement of the aromatic ring to *p*-nitropyridine was attempted to generate the direct analogue of **4.18**. This alteration was implied due to the best inhibition of DHDPS at the ASA binding site was observed when the aromatic ring was a *p*-nitropyridine ring. It was hypothesised that this alteration would decrease the IC_{50} of the target compound relative to its phenyl ring analogue.

Synthesis of **4.13** was performed as discussed in Chapter 4.4 and Scheme 4.15. This compound was then coupled with 2-hydrazinyl-5-nitropyridine **3.28**, followed by *tert*-butyloxycarbonyl (Boc) deprotection, coupling with the corresponding β-keto acid chloride and ester deprotection under basic conditions to obtain the target compound (Scheme 5.1).

Compound **5.1** was synthesised by coupling the Boc-protected sulfonyl chloride **4.13** with **3.28**, and isolated using silica gel column chromatography in a 20% yield. In the ^{1}H NMR spectra, the aromatic protons were visible at 8.32, 7.29, and 7.21 ppm as a doublet, singlet and a doublet with integrations of one proton each, along with the Boc peak appearing at 2.01 ppm. The two CH_2 peaks for the taurine appeared at 3.73 ppm and 3.46 ppm as multiplets and the NH protons appeared at 9.89 and 8.53 ppm as broad singlets. Furthermore, the molecular ion peak observed at 384.0945 *m/z* corresponds to the $(M + Na)^+$ ion of **5.1**.

Upon deprotecting the Boc group, product **5.2** was obtained in a quantitative yield. In the ^{1}H NMR spectra, the sharp singlet for the Boc group was not observed whereas the aromatic peaks appeared at the same chemical shift. A downfield shift was observed for the alkyl protons compared to that of **5.1**. Identity of the product was confirmed by HRMS analysis where the *m/z* ion was observed at 284.0423 as expected.



Scheme 5.1: Direct analogue of **4.18**.

The coupling reaction of the acid chloride proceeded via S_N2 to generate the product **5.3**, in a 41% yield. The resulting spectra showed two additional peaks for the four CH_2 groups in the acid chain at 2.93 and 2.76 ppm as multiplets with integrations of four protons each, and a sharp singlet at 3.89 ppm for the methyl ester protons. In the carbon NMR spectra, the appearance of three carbonyl carbons at 194.2, 172.6, and 164.8 ppm was observed along with four extra CH_2 peaks at 38.5, 36.3, 27.6, and 22.6 ppm, with an additional methyl carbon at 51.6 ppm compared to that of the starting material **5.2**.

Methanolic sodium hydroxide cleaved the methyl ester group and the product was isolated after an extraction. In the ^{1}H NMR spectra of the product, the distinct methyl peak was not present, identifies that the generation of the desired product had been achieved. The peaks in the spectra was comparable to that of the starting material **5.3** where a slight shift of all of the other peaks were observed. In the HRMS analysis of **5.4**, a mass peak of 416.0883 *m/z* was observed for the $(M - H)^{-}$ ion as expected.

5.3 A library of compounds with alternate amide positioning

We have already optimised the chain length of the alkyl chain from the sulfonyl sulphur to the β-oxo carbonyl to be eight carbons in Chapter 4 via synthesising and biologically evaluating **4.18** (Figure 5.2). Therefore, a series of analogues were generated with retaining the chain length to the optimum but varying the positioning of the amide bond. This will introduce rigidity to the alkyl chain at different positions. Furthermore, analogues were generated to have an unsubstituted phenyl ring and/or *p*-nitropyridine ring.

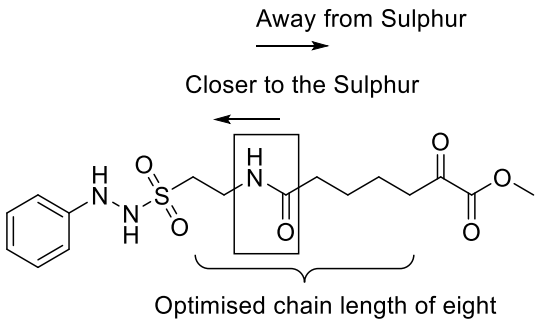


Figure 5.2: Summary of changes to shift the amide bond in the alkyl chain.

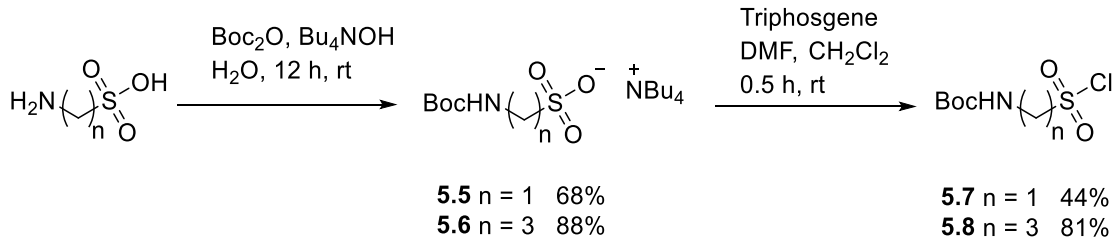
The steps taken in generating the series of analogues was to;

i) Generate Boc protected sulfonyl chlorides of different lengths
ii) Coupling sulfonyl chlorides with respective aromatic hydrazine
iii) Deprotecting the Boc group
iv) Coupling the ester protected β-oxo acid chloride
v) Ester deprotection

The intermediates from step 3 and 4 along with the final compounds will be subjected to biological evaluation against DHDPS.

5.3.1 Generation of sulfonyl chlorides with different alkyl chain lengths

Synthesis of sulfonyl chlorides with different alkyl chain lengths were undertaken (Scheme 5.2) using the same chemistry discussed in Chapter 4.4.



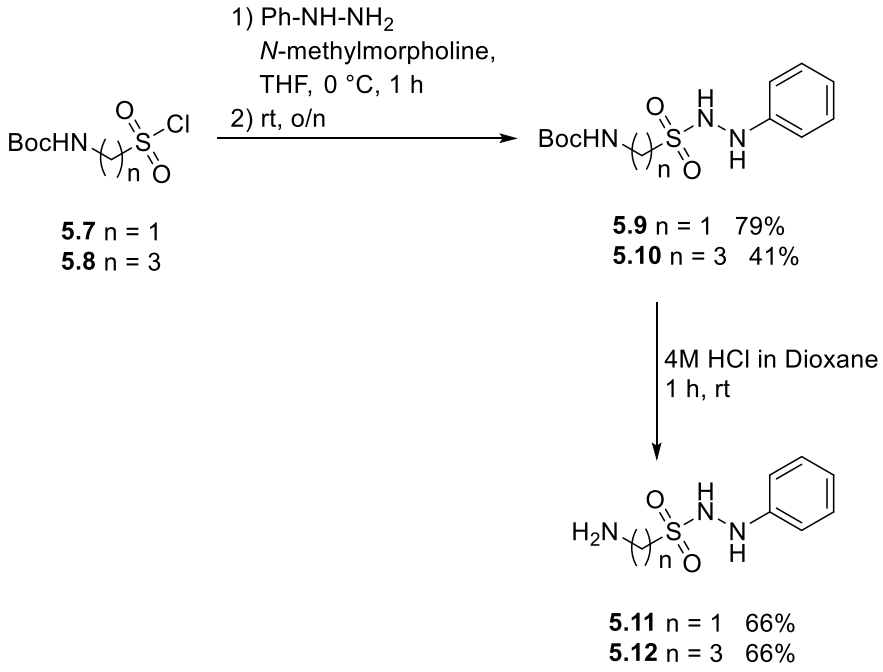
Scheme 5.2: Synthesis of sulfonyl chlorides with different chain lengths.

In the ^{1}H NMR spectra of the Boc protected tetrabutylammonium salt of the single carbon chain containing sulfonyl salt **5.5**, the CH_2 protons appeared at 4.17 ppm as a singlet whereas in the three carbon containing sulfonyl salt **5.6**, the CH_2 protons appeared at 2.87, 2.10, and 1.99 ppm as two triplets and a multiplet, respectively. The downfield effect of the CH_2 protons in **5.5** was due to its location, being between two electron-withdrawing atoms, nitrogen and sulphur. However, in compound **5.6** there are adjacent CH_2 groups which can donate electrons. This reduces the deshielding effect on the CH_2 groups resulting in the CH_2 protons appearing upfield in the ^{1}H NMR spectrum. The ^{1}H NMR peaks of the Boc group appeared at 1.26 ppm and 1.41 ppm for **5.5** and **5.6**, respectively to the tetrabutylammonium salt, the ^{1}H NMR signals appeared in the region of 3.28 – 1.00 ppm and 3.31 – 1.01 ppm for **5.5** and **5.6**, respectively, which are very similar.

In the ^{1}H NMR spectra of the Boc-protected sulfonyl chlorides, **5.7** and **5.8**, the peaks corresponding to the tetrabutylammonium salt were not present whereas the BOC group appeared in the same region to that of the starting material at 2.46 ppm and 1.44 ppm in **5.7** and **5.8**, respectively. The CH_2 protons corresponding to **5.7** and **5.8** appeared at 4.67, 3.74, 3.32, and 2.23 ppm as a singlet, doublet, doublet, and a multipet respectively.

5.3.2 Coupling sulfonyl chlorides with aromatic hydrzines

Previously synthesised sulfonyl chlorides were then coupled with phenylhydrazine (Scheme 5.3) using the same chemistry as discussed in Chapter 4.3, Scheme 4.15.



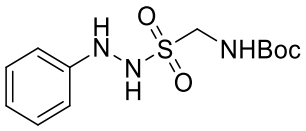
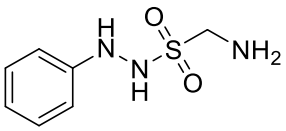
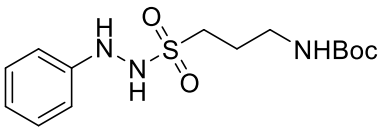
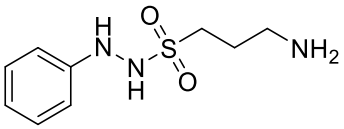
Scheme 5.3: Coupling sulfonyl chlorides with phenylhydrazine.

Coupling phenylhydrazine with sulfonyl chlorides resulted in additional protons in the ^{1}H NMR spectra in the aromatic region for **5.9** and **5.10** compared to the ^{1}H NMR spectra of **5.7** and **5.8**. The *m/z* ions obtained from HRMS analysis were the molecular ions of the corresponding compounds. This data confirms the identity of the products as the desired compounds.

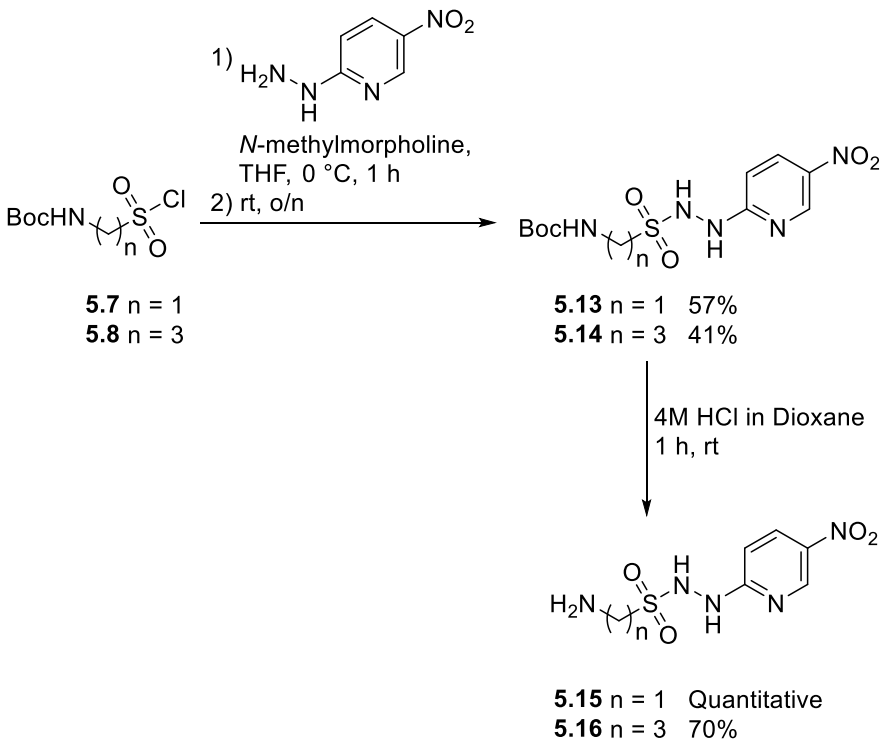
Upon deprotection of the Boc group, the sharp singlet in the region of 1.44 – 1.56 ppm observed for the Boc group was no longer visible. The other peaks were retained with slight shifts observed in the ^{1}H NMR spectrum. The HRMS analysis in which molecular ions were observed confirmed the identity of the products.

The characteristic ^{1}H NMR shifts, and the *m/z* ions obtained from the HRMS spectra of the phenylhydrazine coupled Boc protected sulfonyl chlorides are summarised in Table 5.1.

Table 5.1: Characterisation of **5.9** – **5.12** (from Scheme 5.3).

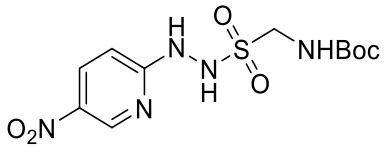
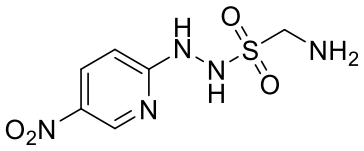
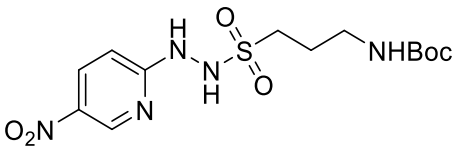
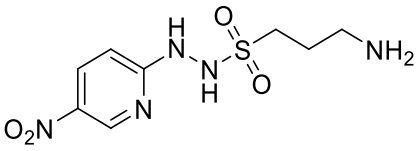
Compound	^{1}H NMR shifts / ppm			*m/z* (M + H)$^+$
	Aromatic protons	CH_2 protons	Boc group protons	
	7.76 7.49 6.83	3.75	1.56	324.0987
	7.89 7.58 6.78	4.15	-	202.0644
	7.94 7.58 6.85	3.75 3.13 2.52	1.44	330.1481
	7.90 7.57 6.80	3.70 3.13 2.55	-	230.0957

The sulfonyl chlorides were also coupled with 2-hydrazinyl-5-nitropyridine as shown in Scheme 5.4 followed by the analysis of the compounds as outlined in Table 5.2.



Scheme 5.4: Coupling sulfonyl chlorides with 2-hydrazinyl-5-nitropyridine.

Table 5.2: Characterisation of **5.13** – **5.16** (from Scheme 5.4).

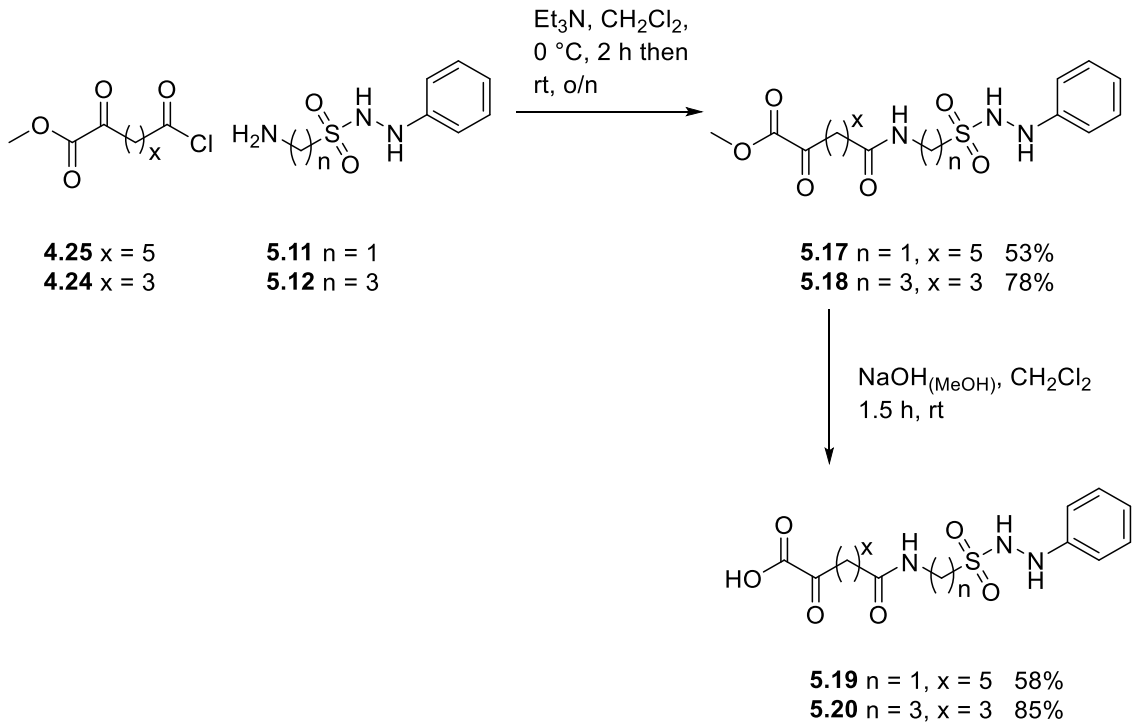
Compound	^{1}H NMR shifts / ppm			*m/z* (M + H)$^+$
	Aromatic protons	CH_2 protons	Boc group protons	
	8.04 7.76 7.48	5.57	1.56	348.0970
	8.04 7.76 7.48	5.59	-	248.0448
	8.97 8.36 7.35	4.25 3.38 2.79	1.45	376.1284
	8.98 8.34 7.32	4.23 3.35 2.81	-	276.0760

In the ^{1}H NMR analysis of the compounds generated after the coupling of the sulfonyl chlorides with **3.28**, appearance of the aromatic protons was observed as expected in the region of 9.00 – 7.30 ppm with an integration of three protons. In the ^{1}H NMR spectra of **5.13**, CH_2 protons appeared at 5.57 ppm as a singlet whereas in **5.14**, the CH_2 protons appeared in the region of 4.25 – 2.79 ppm as multiplets. The Boc group gave sharp singlets in both compounds.

The deprotection of the Boc group was achieved using trifluoroacetic acid to obtain products in good yields. The ^{1}H NMR spectra did not have the sharp singlet corresponding to the Boc group. Observation of the molecular ions corresponding to both compounds in the HRMS spectra confirmed the identity of the products (Table 5.2).

5.3.3 Coupling β-oxo acid chlorides

The synthesis was carried forward by coupling the previously synthesised compounds, **5.11**, **5.12** with β-oxo acid chlorides **4.25**, **4.24** respectively as shown in Scheme 5.5.

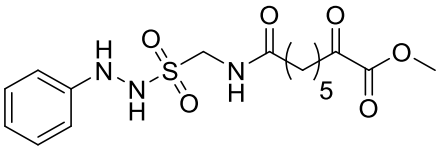
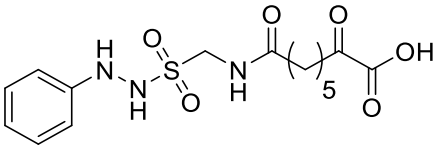
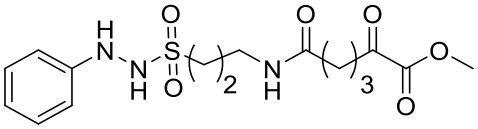
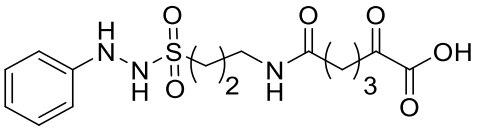


Scheme 5.5: Obtaining final compounds with a phenyl ring.

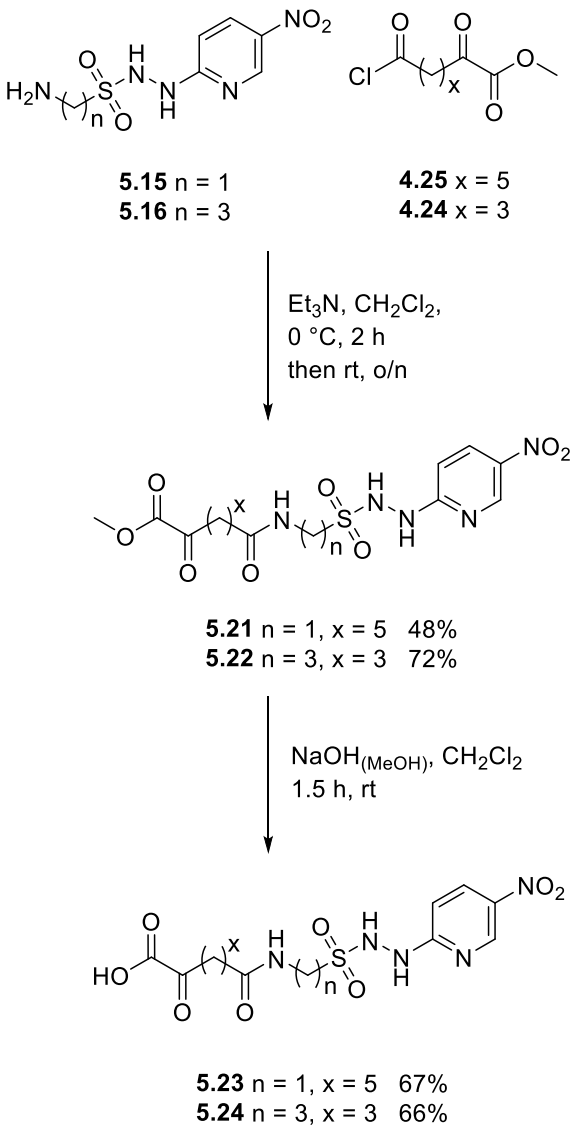
The coupling reaction with the phenylhydrazine connected linker to the acid chloride proceeded in good yields of 53% and 78% for both compounds (Scheme 5.5). Isolated ester protected compounds showed proton peaks in ^{1}H NMR studies where a sharp singlet was observed for the methyl protons and multiplets were observed for CH_2 protons additionally to the aromatic and CH_2 protons adjacent to the sulfonyl group as mentioned in Table 5.3. Notably, the CH_2 protons in the acid motif appeared as two multiplets, one with an integration of two protons and the other with an integration of four protons. This was observed in the respective acid chloride ^{1}H NMR spectra as well (Chapter 4.5).

The ester hydrolysis of both compounds was performed using methanolic sodium hydroxide to obtain the final compounds in good yields of 58% and 85% (Scheme 5.5). As expected, the methyl protons corresponding to the ester group were not present in the ^{1}H NMR spectra. The obtained *m/z* ions correspond to the $(M - H)^-$ further confirming the identity of the final products.

Table 5.3: Characterisation of **5.17** – **5.20** (Scheme 5.5).

Compound	^{1}H NMR shifts / ppm				*m/z*
	Aromatic protons	Alkyl protons adjacent to sulphonyl group	*β*-oxo acid protons	Terminal methyl ester protons	
	8.89 7.60 7.52	4.68	1.84 1.72 1.34	3.60	408.1199 $(M + H)^+$
	8.89 7.60 7.52	4.70	2.91 2.78 1.73 1.58 1.39	-	370.1077 $(M - H)^-$
	8.80 7.78 7.55	3.77 3.35 2.85	3.02 2.77	3.27	408.1201 $(M + H)^+$
	8.80 7.77 7.56	3.77 3.33 2.85	3.00 2.76	-	370.1077 $(M - H)^-$

Previously synthesised *p*-nitropyridine intermediates (**5.15** and **5.16**) were coupled with the corresponding acid chloride as outlined in Scheme 5.6.



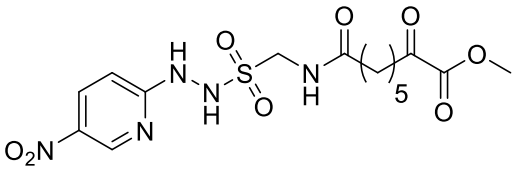
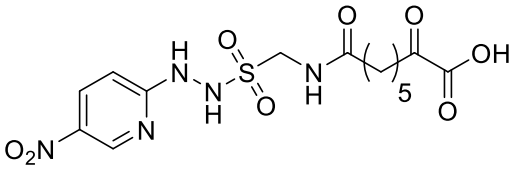
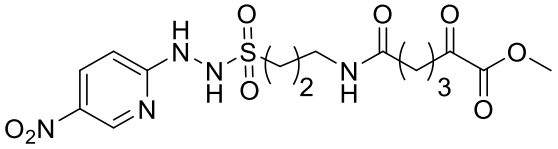
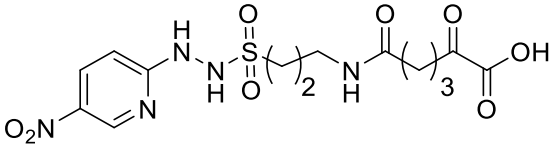
Scheme 5.6: Synthesis of compounds with a 2-hydrazinyl-5-nitropyridine core.

For the compounds discussed above, the ^{1}H NMR analysis along with the *m/z* ions obtained via HRMS was summarised in Table 5.4.

Amide bond formation between the terminal amide and acid chloride was achieved in dichloromethane using triethylamine to scavenge the resulting hydrochloric acid. ^{1}H NMR spectra of the coupled compounds showed proton peaks corresponding to methyl ester and the CH_2 groups in the alkyl chain of the β-ketoester in addition to the aromatic protons and the CH_2 protons adjacent to the sulfonyl sulphur. HRMS analysis showed the molecular ions of the corresponding compounds indicating the desired product formation.

Ester hydrolysis proceeded smoothly to generate final compounds, **5.23** and **5.24**, in good yields of 67% and 66%. The ^{1}H NMR spectra did not show the methyl ester protons, which show up as sharp singlets in the corresponding ester protected compounds. The molecular ions were observed from HRMS as expected, reinforcing the target compound generation (Table 5.4).

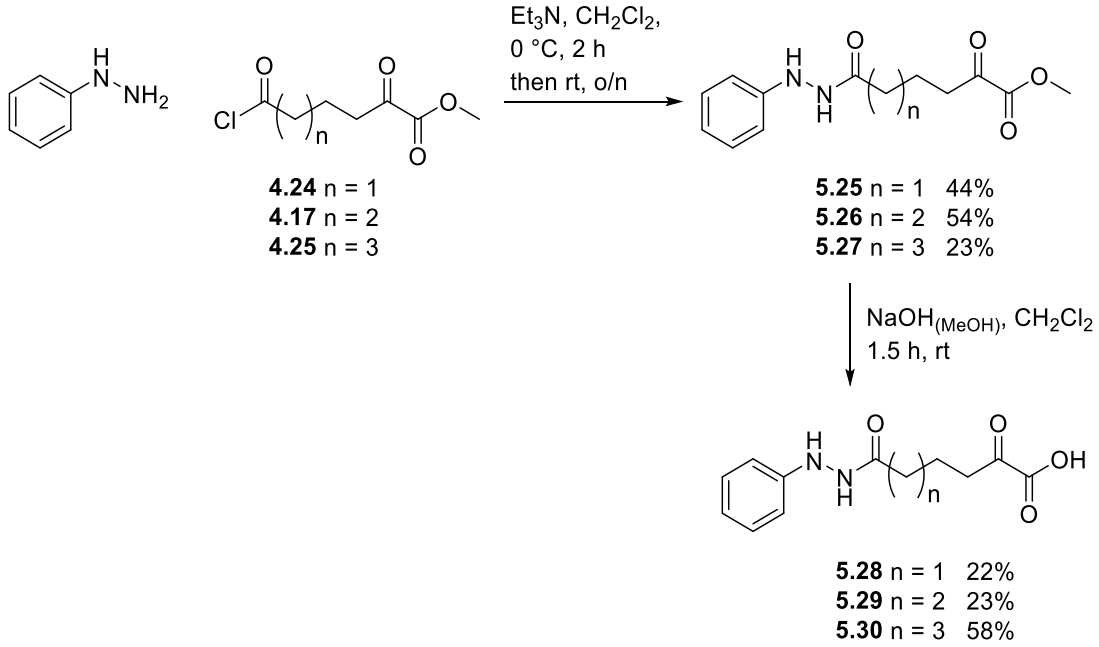
Table 5.4: Characterisation of **5.21** – **2.24** (Scheme 5.6).

Compound	^{1}H NMR shifts / ppm				*m/z*
	Aromatic protons	Alkyl protons adjacent to sulphonyl group	β-oxo acid protons	Terminal methyl ester protons	
	8.32 7.29 7.21	4.68	1.84 1.72 1.34	3.60	432.1182 $(M + H)^{+}$
	8.30 7.32 7.41	4.60	1.84 1.72 1.40	-	416.0881 $(M - H)^{-}$
	8.98 8.37 7.30	4.20 3.33 2.83	3.00 2.75	3.25	432.1184 $(M + H)^{+}$
	8.99 8.35 7.35	4.20 3.31 2.85	3.02 2.70	-	416.0881 $(M - H)^{-}$

5.4 Coupling phenylhydrazine with β-oxo acid chlorides

Furthermore, a small series of compounds were synthesised without the sulfonyl functionality (Scheme 5.7) by coupling phenylhydrazine directly with β-oxo acid chlorides (**4.24**, **4.17** and **4.25**). This was pursued as there was starting material remaining from previous steps.

This set of compounds was not expected to show any inhibitor activity against isolated DHDPS enzyme, as it has been established that having the sulfonylhydrazine functionality was essential for the observed inhibitory properties (Chapter 2 and 3).



Scheme 5.7: Keto-acid analogues with different chain lengths.

The coupling reaction with phenylhydrazine and **4.24** proceeded in a 44% yield (Scheme 5.7). The ^{1}H NMR spectra contained a sharp singlet for the methyl protons at 3.28 ppm, while the CH_2 protons appeared at 3.05 as a multiplet and at 2.76 ppm as a multiplet with an integration of two and four protons, respectively. In the carbon NMR spectrum, three carbonyl carbons were found at 194.2, 176.5, and 161.0 ppm, the methyl carbon from the ester group appeared at 51.6 ppm and the CH_2 carbons at 40.0, 38.2, and 20.2 ppm. This compound, upon hydrolysis with methanolic sodium hydroxide yielded the carboxylic acid in a 22% yield. The ^{1}H NMR spectra of the ester hydrolysed product **5.28** lacked the sharp singlet corresponded to the methyl ester. Apart from the methyl carbon, all the other carbons; carbonyl, aromatic and CH_2 carbons, were visible in the carbon NMR spectra confirming the target compound generation.

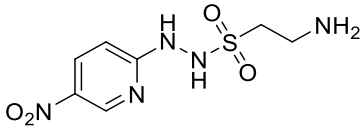
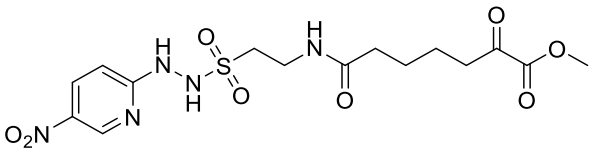
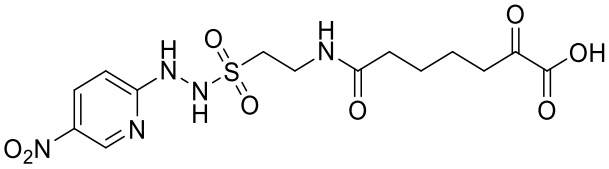
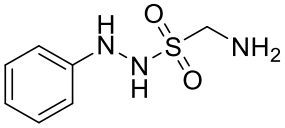
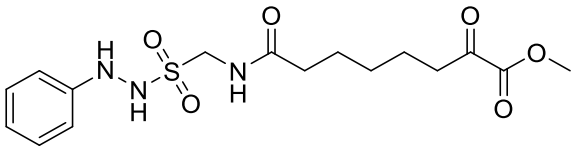
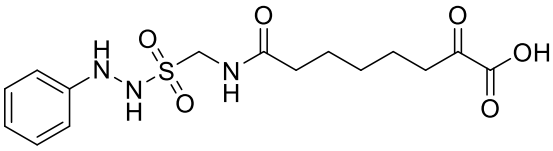
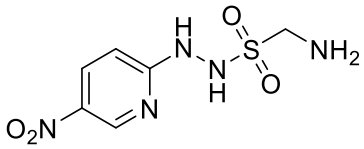
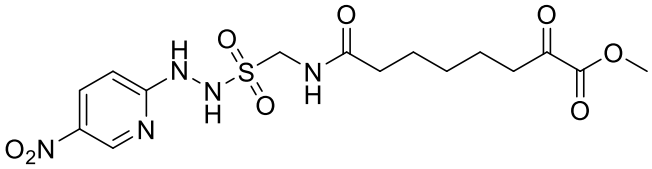
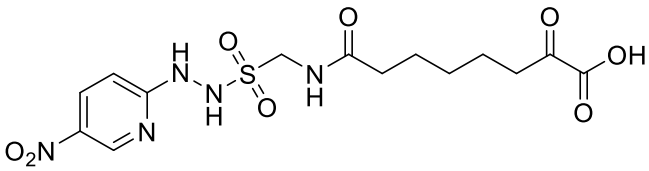
Coupling **4.17** with phenylhydrazine yielded the product in 54% yield. As expected in the ^{1}H NMR spectra the methyl ester gave a sharp singlet at 3.58 ppm, whereas the CH_2 protons gave peaks at 3.38 ppm and 2.85 ppm as multiplets with integration of four protons each. The HRMS spectra showed the molecular ion at 279.1338 *m/z* further confirming the identity of the product, **5.26**. Ester hydrolysis under basic conditions generated the final product **5.29** in 23% yield. The *m/z* ion was observed at 263.1036 as expected, which confirms the identity of the compound. In the ^{1}H NMR spectra, aromatic protons and the CH_2 protons are visible but the distinct sharp singlet was not visible confirming the ester hydrolysis.

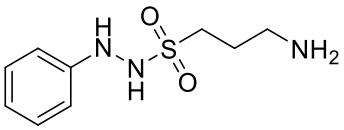
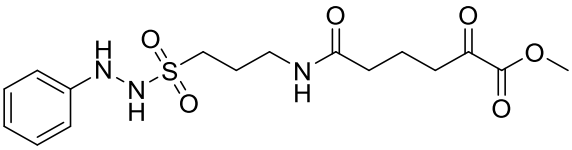
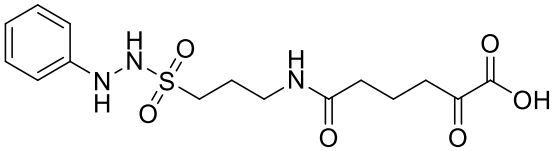
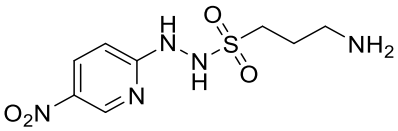
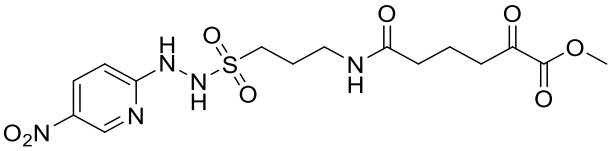
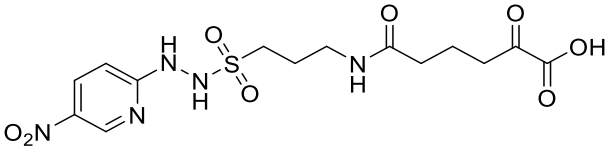
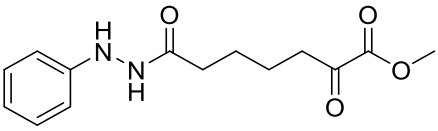
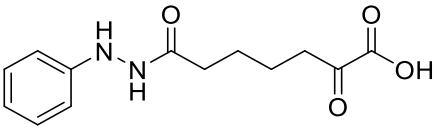
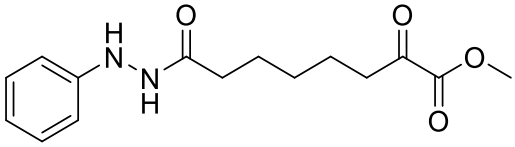
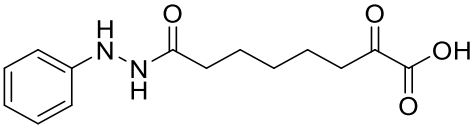
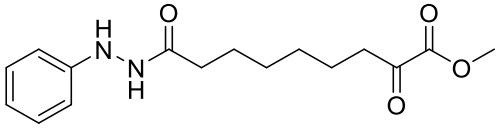
The final coupling reaction was between phenylhydrazine and **4.25**, which generated compound **5.27** in a 23% yield. The ^{1}H NMR spectrum showed peaks for methyl ester at 3.35 ppm and CH_2 groups at 3.65, 3.40, and 2.19 as multiplets and with integrations of four, four, and two, respectively. Similarly, in the carbon NMR spectrum three carbonyl carbons were observed at 195.0, 172.6, and 162.7 ppm, with the CH_2 carbons at 39.6, 38.6, 29.2, 25.6, and 25.1 ppm, and the methyl carbon at 51.2 ppm. Upon ester hydrolysis the compound **5.30** was obtained. The ^{1}H and carbon NMR spectra of **5.30** did not show the peak corresponding to the methyl ester, but the other peaks were observed with only slight shifts. Furthermore, the molecular ion obtained in the HRMS was at 277.1193 *m/z* as expected.

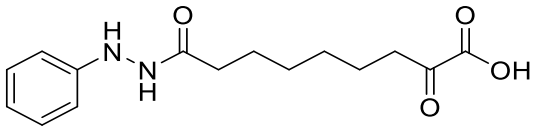
5.5 Biological evaluation of the synthesised compounds

Using the DHPDS-DHDPR coupled assay, IC_{50} values for the synthesised compounds were determined against isolated *E. coli* DHDPS. The data is tabulated in Table 5.5.

Table 5.5: IC_{50} values for the analogues of HTS-14697 with extended alkyl functionality at the sulphonyl sulphur.

Number	Compound	IC_{50}/ µM
5.2		> 500
5.3		> 500
5.4		> 500
5.11		> 500
5.17		> 500
5.19		> 500
5.15		> 500
5.21		> 500
5.23		373

5.12		> 500
5.18		65
5.20		> 500
5.16		> 500
5.22		59
5.24		337
5.25		~ 500
5.28		> 500
5.26		> 500
5.29		> 500
5.27		100 ± 3.9

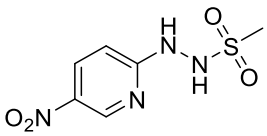
5.30		> 500

Disappointingly, **5.4** did not show any activity below 500 µM, nor its ester protected compound **5.3**.

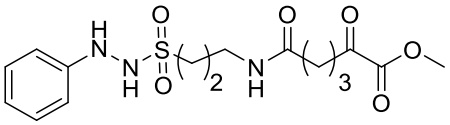
As expected, the compounds obtained after coupling aromatic hydrazines with different lengthened sulfonyl chlorides and deprotecting (**5.2**, **5.11**, **5.15**, **5.12**, and **5.16**) were inactive at or below 500 µM.

All of the analogues generated with single carbon chain containing sulfonyl chlorides were not active at or below 500 µM against *E.coli* DHDPS except for **5.23**, which had a *p*-nitropyridine ring and an IC_{50} of 373 µM.

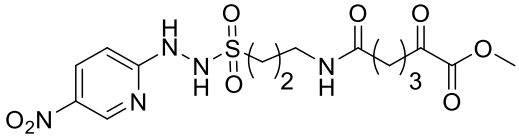
In the series where three carbon chain containing sulfonyl chlorides were used, two compounds, **5.20** and **5.22** showed low micromolar activity of 65 and 59 µM respectively, which was on par with the best inhibitor, **3.29** (Figure 5.3). Out of the two active compounds, **5.22** was slightly better at inhibiting DHDPS compared to **5.18** as hypothesised, due to the nitropyridine ring. Both these compounds exhibit greater than two-fold increase in activity compared to the High-throughput screened compound **CT1-2**. Interestingly, compound **5.24** was slightly active against isolated *E. coli* DHDPS with an IC_{50} of 337 µM. All of the other analogues in the series were inactive against isolated *E.coli* DHDPS.



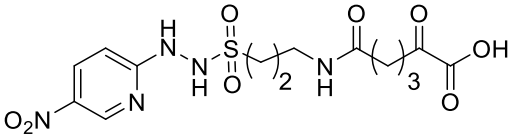
3.29
IC_{50} 67 µM



5.18
IC_{50} 65 µM



5.22
IC_{50} 59 µM



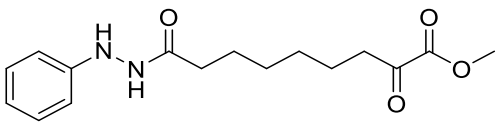
5.24
IC_{50} 337 µM

Figure 5.3: Best inhibitors of the series.

Notably, both compounds which were most active in this series are ester analogues and when the esters were hydrolysed, the resulting compounds were completely inactive against DHDPS at or below 500 µM. This trend was also observed in Chapter 4 where three out of five active compounds were esters.

Compounds **5.23**, **5.24**, **5.20**, and **5.22** were the result of shifting the amide bond while keeping the same chain length as of **4.18**. The most active compounds contained an amide group located four carbons away from the sulphur atom. This might be due to a favourable interaction with the binding site of the DHDPS enzyme.

In the small series of compounds discussed in section 5.4, to our surprise, one compound, **5.27**, showed moderate activity with an IC_{50} of 100 µM (Figure 5.4). The compound contained the longest β-keto ester in the series coupled to phenylhydrazine. The observed IC_{50} was interesting and needs further investigation as our previous studies had indicated that having the sulfonylhydrazine motif was essential for biological activity. This shows the potential of coupling long β-keto esters without the presence of the sulfonylhydrazine motif to gain further biological activity against DHDPS. Moreover, the trend of ester counterparts being the most active has continued where the acid analogue **5.30** showed complete loss in activity below 500 µM.



IC_{50} 100 µM

Figure 5.4: Compound **5.27**.

5.6 Summary

Further work on compounds containing long alkyl chains at the sulfonyl group were undertaken using the chemistry discussed in Chapter 4 to generate 21 novel compounds.

Unfortunately, all of the compounds obtained after coupling aromatic hydrazines with different length alkyl chain sulfonyl chlorides and deprotecting were inactive at or below 500 µM.

Most of the compounds that were synthesised by shifting the amide bond were also inactive. The best inhibitors from this series were **5.18** and **5.20**, which contained the *p*-nitropyridine ring. The IC_{50} was found to be in the low micromolar range and was similar to the best inhibitor for the ASA binding site **3.29**.

For all of these compounds, it was not possible to conclude a clear structure activity relationships. Co-crystallisation data would be very useful in order to determine how these compounds are interacting with the enzyme. Compounds discussed in this chapter are larger than compound **3.29**, where preliminary co-crystallisation data has been obtained. Therefore, it was not possible to predict the mode of binding and the site of binding of these compounds. Previous experience has shown that a slight change to the structure resulted in complete loss of activity or poor activity. Therefore, it is possible that these large compounds might have a different binding site and/or a different mode of binding compared to **3.29**.

It was discovered that the sulfonylhydrazine motif might not be crucial for the biological activity of compounds with a high lipophilicity. Direct substitution of a long β-ketoester to aromatic hydrazines can also result in low micromolar potency against the isolated DHDPS enzyme. Further work on optimisation of the alkyl chain length needs to be performed, preferably after obtaining a co-crystallised enzyme structure with **5.27**.

All of the active compounds with low micromolar activity are ester analogues. As discussed in Chapter 4.8, esters are susceptible to being cleaved and so the use of this functional group will need to be further optimised.

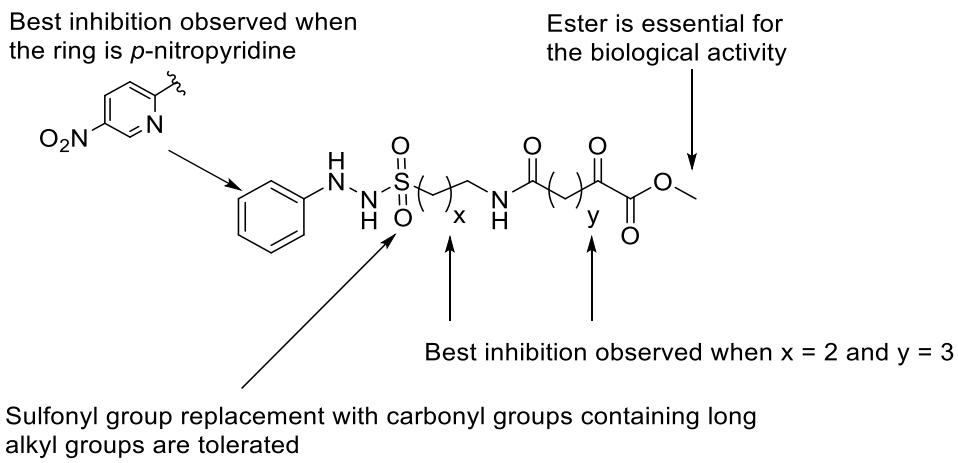


Figure 5.5: SAR summary of the sulfonyl hydrazides with extended alkyl functionality at the sulfonyl sulphur.

Chapter 6 Conclusions and Future Work

A traditional medicinal chemistry approach guided by biological data was utilized in this project to synthesise a library of sulfonylhydrazine compounds. The lead compound was broken down into four domains (Figure 6.1).

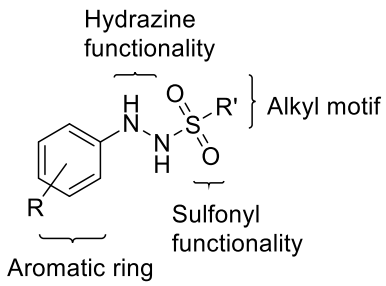
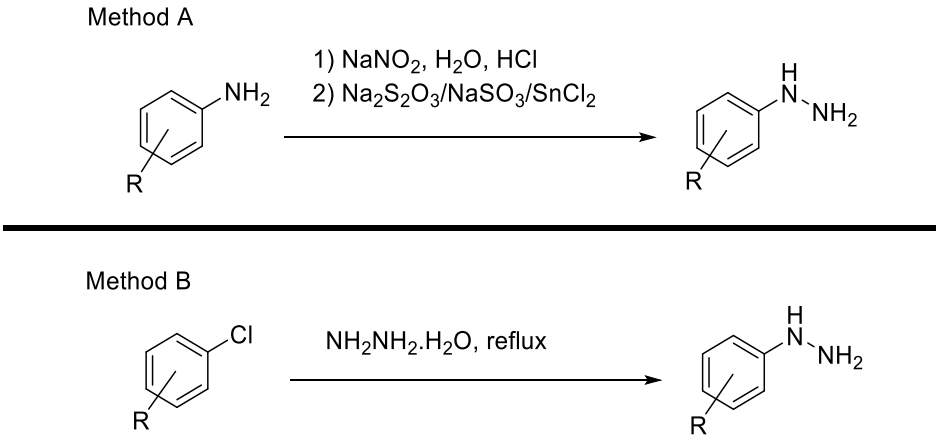


Figure 6.1: Domains of the target compound.

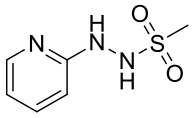
Initial optimisation of the lead compound was undertaken as discussed in Chapter 2 and 3. Variations were made at the aromatic moiety including ring expansion, ring contraction, and substitution with heterocyclic rings.

The two methods widely used in the literature were alternately used to synthesise the aromatic hydrazines depending on the functionalisation of the starting material (Scheme 6.1). Aromatic amines were diazotised using sodium nitrite under low temperature and acidic conditions, while the reduction of the diazonium salt was achieved using either sodium metabisulphite, sodium sulphite, or tin (II) chloride via method A to obtain the corresponding aromatic hydrazine. Halogenated aromatic starting materials were reacted with hydrazine hydrate under reflux conditions to generate the corresponding aromatic hydrazine following method B.



Scheme 6.1: Methods used to synthesise aromatic hydrazines.

Coupling of the synthesised aromatic hydrazines with methanesulfonyl chloride was carried out in dichloromethane to obtain the final products for testing. Analysis of the SAR of the 14 aromatic analogues synthesised revealed that the most active compound has a pyridine ring, where the pyridine nitrogen is *ortho* to the hydrazine functionality.



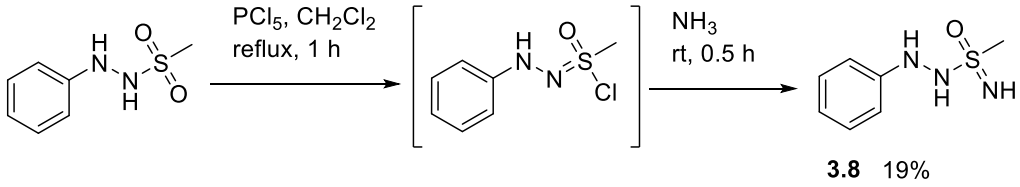
3.21
IC_{50} 109 μM

Figure 6.2: Compound **3.21**.

A total of five compounds were synthesised to investigate the importance of the hydrazine motif. Removal of one nitrogen from the hydrazine was undertaken by directly coupling aromatic amines with methanesulfonyl chloride. Direct coupling of benzylamine with methanesulfonyl chloride produced a compound with the same shape and size as the hydrazine bridge. The synthesis of a compound with a rigid azo bridge was also achieved; first by generating the diazo salt of aniline with tetrafluoroborate as the counter ion followed by coupling with sodium methanesulfinate. Absence of the hydrazine resulted in complete loss of biological activity against DHDPS, demonstrating the essential role of this functionality.

All of the three analogues generated via isostere replacement for the sulfonyl functionality (carbonyl group and sulfinimidamide), also resulted in the loss of biological activity illustrating the significance of the sulfonyl group. The synthesis of sulfinimidamides was challenging when the functional group at the sulphur did not contain an electron-

withdrawing group. In the synthesis of **3.8**, chlorination at the sulfonyl group was only possible with direct chlorination by phosphorus pentachloride after which dry ammonia gas was bubbled to generate the compound of interest (Scheme 6.2).



Scheme 6.2: Direct chlorination to synthesise sulfinimidamide.

Chain extensions were made at the terminal alkyl motif by synthesising different chain lengths; two and three carbon chains were introduced by coupling phenylhydrazine with ethanesulfonyl chloride and propylsulfonyl chloride. An isostere replacement was attempted with a trifluoromethyl group instead of a methyl group by coupling phenylhydrazine with triflic anhydride at –78 °C. None of the analogues had any activity against the isolated DHDPS enzyme.

Four compounds were synthesised combining the optimal features identified by previous SAR; *para* nitro functionality on a phenyl ring or on a pyridine ring with the nitrogen at the *ortho* position, and with varied alkyl chain lengths. All four analogues exhibited activity against isolated DHDPS enzyme, while the best inhibition was observed for compound **3.29** at 66.5 µM (Figure 6.3).

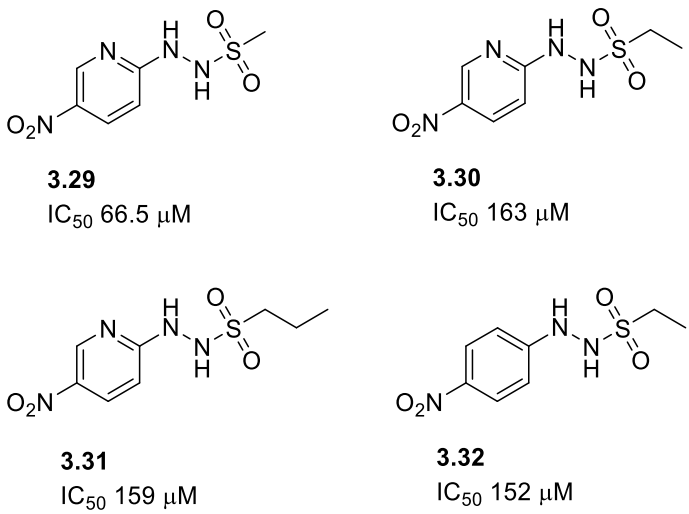


Figure 6.3: Best inhibitors of DHDPS obtained from the studies undertaken in chapter 2 and 3.

It was hypothesised that extension of compounds **CT1-2** and **3.29** may be possible. Preliminary crystal structure data (not yet available for publications) suggested that these compounds bind to the ASA binding pocket inside the active site of DHDPS. It was proposed that the chain extension may allow the pyruvate-like terminus (α-keto terminus) to bind at the pyruvate binding pocket covalently as discovered by Shrivastava and co-workers.[62] The initial target compound was set as shown in Figure 6.4. Three domains were identified in the target compound after retrosynthetic analysis; phenylhydrazine, sulfonyl chloride and α-keto acid terminus.

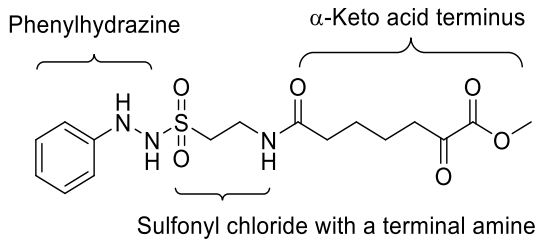


Figure 6.4: Target compound for Chapter 4.

The synthesis of the α-keto acid terminus was initiated through coupling of cyclohexane with dimethyl carbonate to obtain a β-keto ester, which upon oxidative ring opening using nitrosobenzene gave the desired compound of α-KPA **4.31**. A total of three α-keto esters and analogous acids with varied chain lengths were also synthesised and tested. These α-keto esters were coupled directly with phenylhydrazine after chlorination using thionyl chloride to obtain three compounds. The esters were then hydrolysed to obtain the acid analogues of the respective compound.

Three α-keto esters, three α-keto acids and six novel phenylhydrazine coupled compounds were tested against isolated *E. coli* DHDPS. The obtained IC_{50} for α-KPA was very close to the literature data.[62] Compounds **4.23** and **5.27** exhibited low micromolar activity. Both compounds are esters and contain five internal methylene groups at the α-keto ester.

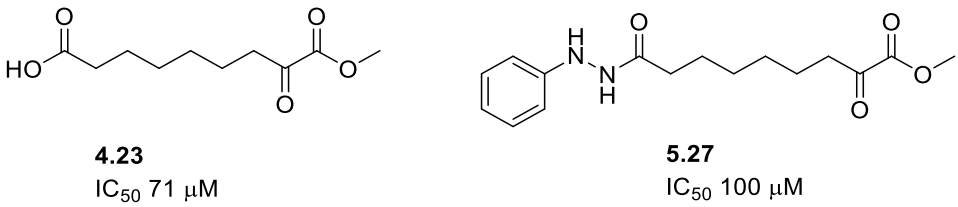
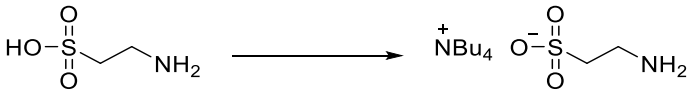


Figure 6.5: Compounds **4.23** and **5.27**.

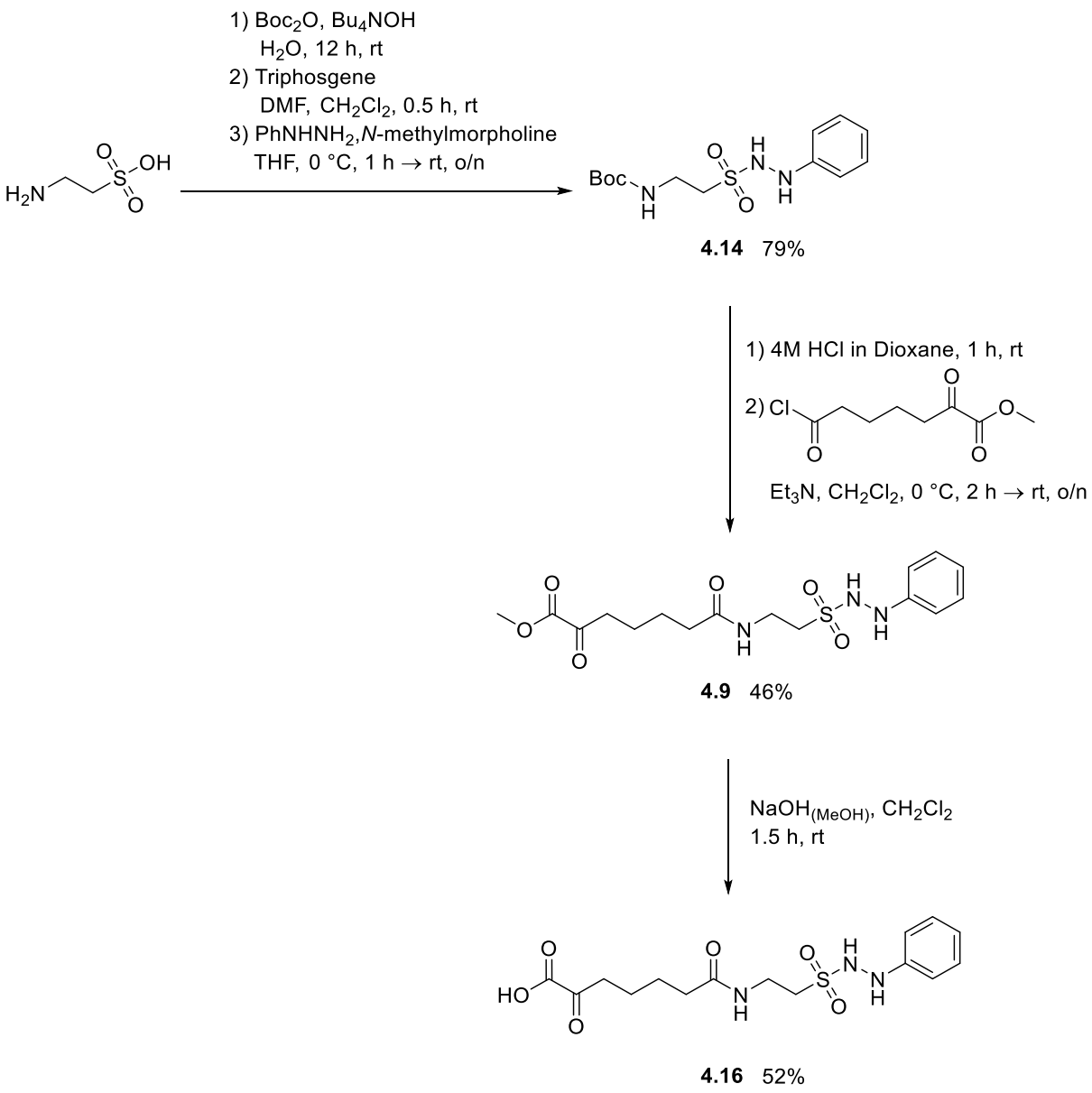
Initial attempts were made to couple the obtained α-keto ester directly with taurine via benzotriazole mediated coupling.[110, 111] Alterations of the reaction conditions did not yield the desired compound, it appeared that the solubility of taurine was creating problems. However, generation of a soluble taurine species was achieved via the synthesis of a tetrabutylammonium salt of taurine.



Scheme 6.3: Synthesis of tetrabutylammonium salt of taurine.

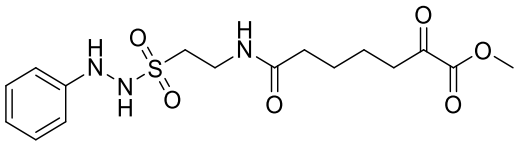
Successful synthesis of the soluble taurine species was followed by coupling of a simple α-keto acid of ethyl chlorooxoacetate. However, attempts to generate a sulfonyl chloride from the tetrabutylammonium salt were not successful regardless of the method employed.

A new approach was attempted to address the solubility issue; taurine was protected at both ends (amine and the hydroxyl groups with tertrabutylammonium salt and Boc protection respectively) (Scheme 6.4). The new taurine species were observed to be more soluble than the previously synthesised tetrabutylammonium salt of taurine. Synthesis of the sulfonyl chloride using triphosgene was successful, with the product immediately coupled with phenylhydrazine. Deprotection of the Boc group enabled coupling of the previously synthesised α-keto esters to obtain desired ester products.



Scheme 6.4: Successful synthesis of the target compound.

With the development of the robust methodology, generation of a library of compounds with extension at the sulfonyl sulphur was achieved. Three previously synthesised α-keto esters were coupled with **4.15** to generate three ester analogues. Upon hydrolysis, three acid analogues were obtained. Furthermore, the simplest α-keto ester, ethyl chlorooxoacetate was coupled with **4.15** to generate two more analogues; the ester and the acid. Compound **4.15** and the eight analogues were tested against *E. coli* DHDPS. Only one compound, **4.18**, was active with an IC_{50} of 127 µM, which nevertheless indicates that there is the possibility to include such extensions while retaining biological activity.

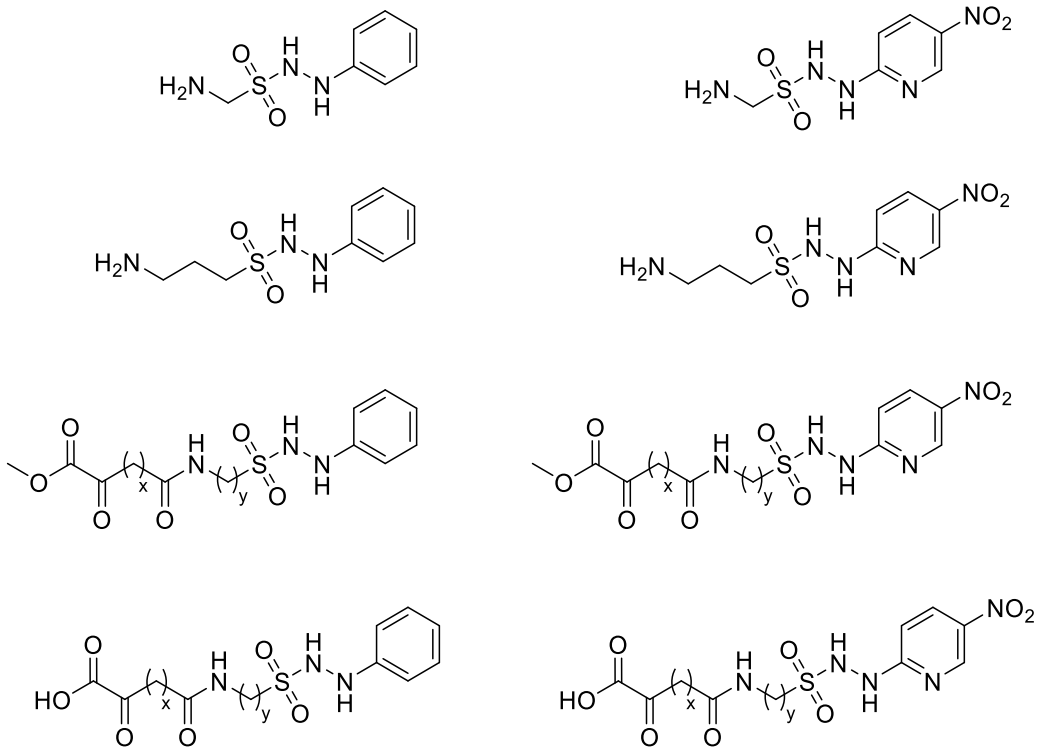


4.18
IC_{50} 127 ± 3 μM

Figure 6.6: Compound **4.18**.

It was hypothesised that the chain length of **4.18** is the optimum (eight carbons) and further analogues were generated to have the same chain length. Analogues of **4.18** were synthesised by varying the phenyl ring to 2-hydrazinyl-5-nitropyridine.

Two more sulfonyl chlorides were synthesised with varied chain lengths with one and three carbon chains. These were coupled with both phenylhydrazine and 2-hydrazinyl-5-nitropyridine to generate four compounds as intermediates, which enabled the coupling of the previously synthesised α-keto esters with different chain lengths (Figure 6.7). The ester analogues were hydrolysed to generate the respective acid analogue (Figure 6.7).



x and y were changed so that the sum was 6 i.e; x = 3, y = 3 and x = 5 and y = 1

Figure 6.7: Design of the extended analogues.

The discussed intermediates, ester and acid analogues were tested against DHDPS. Unfortunately, despite the challenging synthesis, only two compounds exhibited low micromolar potency, **5.18** and **5.22** (Figure 6.8). Both compounds have the same chain extension but with two different aromatic rings. These results suggest that this could be the optimal positioning of the amide bond, however due the lack of clear SAR in this series, confirming the hypothesis is challenging without an enzyme-bound crystal structure.

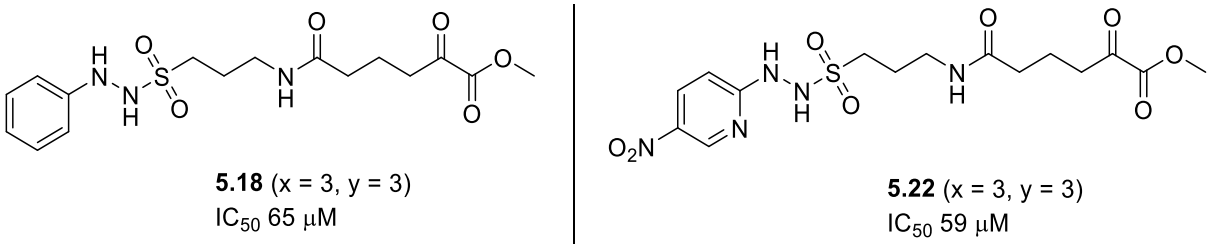


Figure 6.8: Active compounds from the library.

6.1 Conclusions for Chapter 2 and 3

The first half of the project developed a total of 30 compounds for testing against *E. coli* DHDPS with variations focused mainly at the aromatic core of **CT1-2**. A very narrow SAR was observed which is summarised in Figure 6.9, emphasising the selectivity of the binding pockets inside the active site of the enzyme.

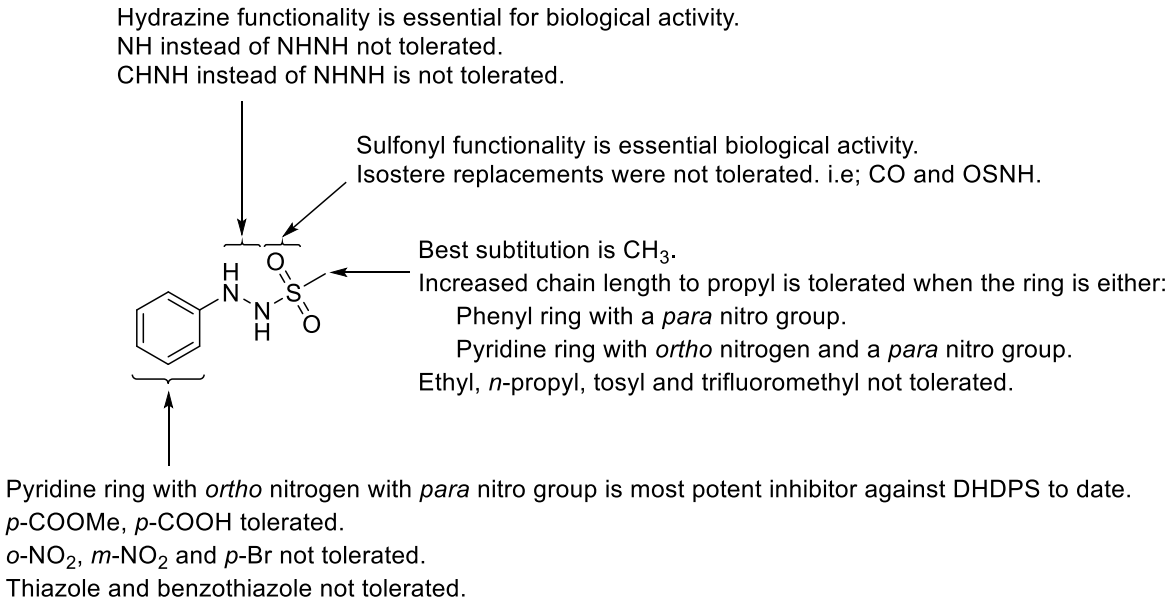


Figure 6.9: SAR of Chapter 2 and 3.

6.2 Future work related to Chapter 2 and 3

Synthesis of other heterocyclic cores is possible to further explore the SAR of this scaffold.

Thiophene is sterically and electronically similar to benzene compared to other aromatic heterocycles.[127] Utilising the chemistry discussed in Chapter 2 and 3, the synthesis of two analogues could possibly be achieved using commercially available 2-bromothiophene and 3-bromothiophene.

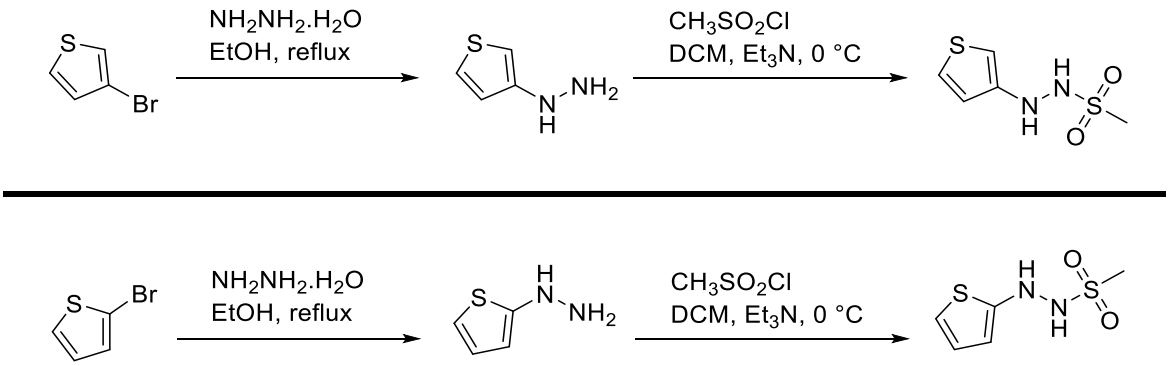


Figure 6.10: Proposed synthesis of compounds with thiophene core.

Similarly, other heterocyclic compounds (Figure 6.11) might also be synthesised using similar chemistry as discussed in Chapter 2 and 3 using commercially available bromofurans, bromopyrroles and 4-bromo-*1H*-imidazole.

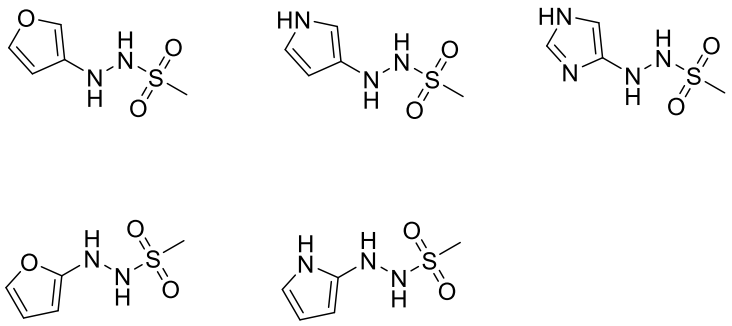


Figure 6.11: Heterocyclic analogues to understand SAR better.

Synthesis of aromatic compounds containing small electron-withdrawing groups, such as fluorine atoms could also be performed. Fluoro groups can act as an isostere for hydrogen, therefore synthesis of *ortho*, *meta* and *para* substituted fluoro analogues could improve the understanding of SAR (Figure 6.12).

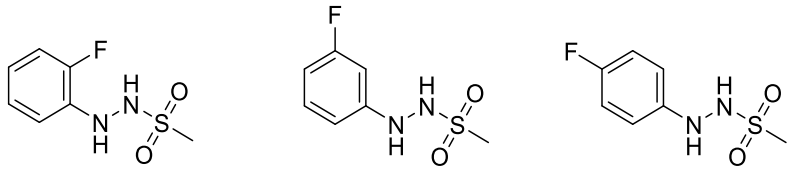


Figure 6.12: Fluoro analogues of **CT1-2**.

6.3 Conclusions for Chapter 4 and 5

The second part of the project generated 36 compounds for testing against *E. coli* DHDPS. This series was focused on extending the alkyl functionality at the sulphonyl group and the observed SAR is illustrated in Figure 6.13. Two of the best inhibitors synthesised in this project have chain extensions at the sulfonyl functionality, compounds **5.18** and **5.22**, affirming the possibility of further exploring this interesting chemistry and biochemical space.

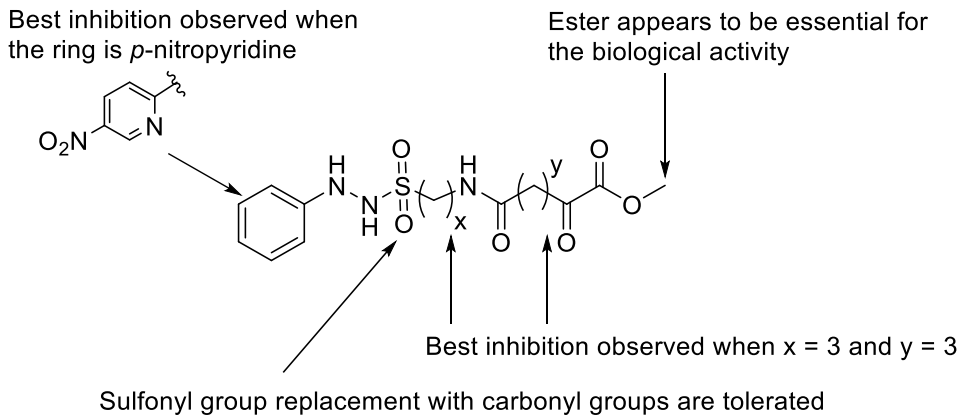


Figure 6.13: Summary of SAR of Chapter 4 and 5.

Predictions of the biological activity was difficult due to the lack of clear SAR from the extended series of compounds as discussed in Chapters 4 and 5. It is considered to be essential to understand the binding of either one or both of the compounds in the enzyme before beginning new synthetic approaches.

6.4 Future work related to Chapter 2 and 3

However, optimisation the compounds, **5.18** and **5.22** could be undertaken by replacing the terminal ester group due to its susceptibility to cleave in cellular environments. A one-pot conversion of esters to ketones has been developed by Jeon and co-workers which can be utilised as a method of removing the terminal ester group.[128]

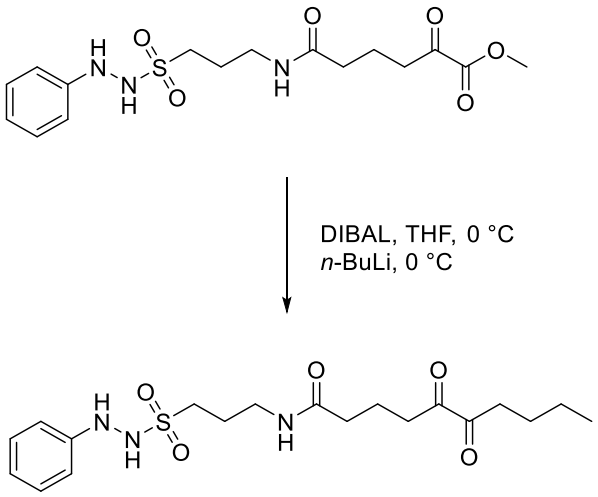


Figure 6.14: Replacing the terminal ester group with a terminal ketone functionality.

Furthermore, the internal amide group that is susceptible to amide cleavage, could be replaced by a rigid triazole ring via click chemistry. This compound could also demonstrate whether free rotation around amide group is crucial for the biological activity. A method for the synthesis of 2-azidoethane-1-sulfonylfluoride has been developed by Zhang and co-workers[129] which could be then used for the click reaction as illustrated in Figure 6.15.

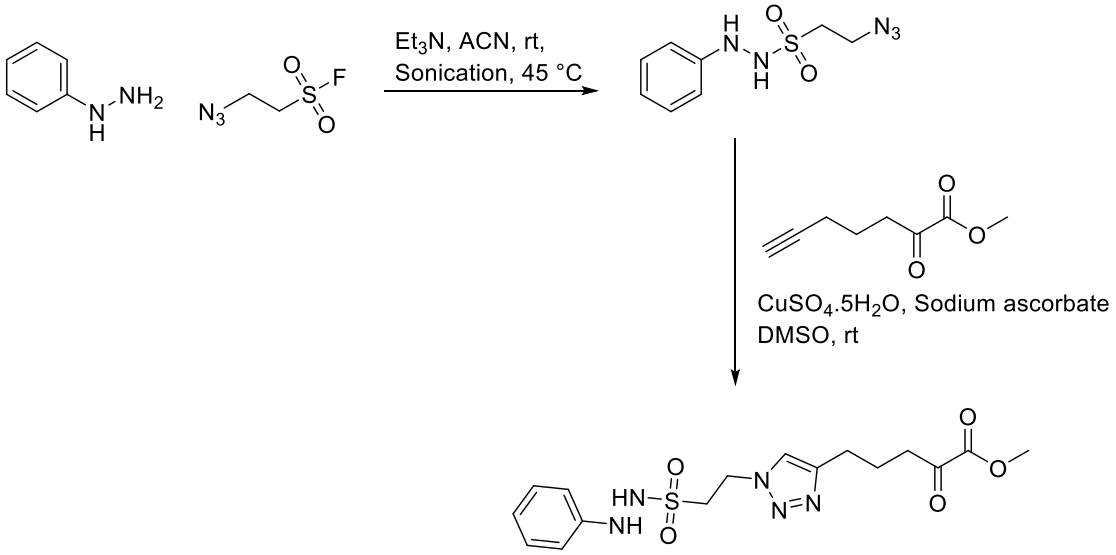


Figure 6.15: Proposed pathway to replace the amide with rigid triazole functionality.

Moreover, another analogue of an internal triazole ring could possibly be synthesised as shown in Figure 6.16 using different starting materials to what was used in Figure 6.15.

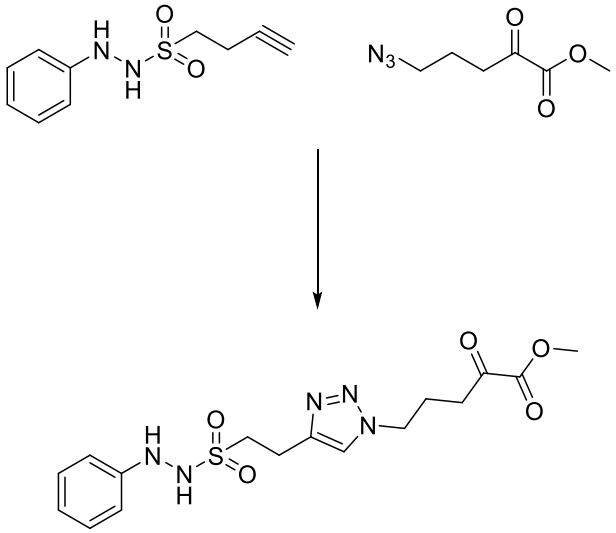


Figure 6.16: Synthesis of another analogue containing an internal triazole group.

6.5 Summary

In summary, reaction optimisation and development of robust synthetic methodologies were undertaken to synthesise a library of analogues of HTS-14697. This project led to the synthesis of 66 sulfonyl hydrazides with varied functionalities to yield 20 known compounds and 46 novel compounds. All of the synthesised compounds were subjected to testing against isolated *E.coli* DHDPS.

The biological results demonstrated the specificity of the binding site where a very narrow SAR was observed. A number of compounds were identified as low micro molar inhibitors of DHDPS, demonstrating sulfonyl hydrazides to be a promising scaffold in the generation of novel DHDPS inhibitors. The promising activity of the sulfonyl hydrazides against isolated *E. coli* DHDPS provides a lead for further studies to determine whether they possess antibacterial or herbicidal properties.

Chapter 7 Experimental

7.1 General

All commercial materials were used as received without further purification, unless otherwise specified. Purification of solvents and reagents, if required, was carried out by procedures described by Chai and Armarego.[130] Moisture sensitive reactions were performed under an atmosphere of nitrogen with all reactions carried out at room temperature, unless otherwise noted. Glassware was oven-dried and cooled under nitrogen prior to use.

Melting points were determined on a Reichert 'Thermopan' microscope hot stage apparatus and values were corrected by a 12% increase after calibration against known reference samples.

Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker Avance-400 spectrometer at 400.13 MHz for ^{1}H nuclei and 100.62 MHz for ^{13}C nuclei. Proton chemical shifts are reported in parts per million (ppm) from an internal standard of residual chloroform at δ 7.26 ppm or dimethylsulfoxide at δ 2.50 ppm. All chemical shifts were recorded as δ values in parts per million (ppm) and coupling constants (*J*) were recorded in hertz (Hz). For reporting of an NMR spectrum, the following terms were used; singlet (s), doublet (d), triplet (t), multiplet (m), broad (br).

Low-resolution electrospray ionisation (ESI) mass spectrometry was carried out using a Bruker Daltronics (Germany) Esquire^{6000} ion trap mass spectrometer at 140 °C with a flow rate of 4 µL/min, a mass range of 50 – 1000 *m/z* and a scan rate of 5500 *m/z/second* in positive/negative ion mode or using Low-resolution electrospray ionisation (ESI) Bruker Daltonics (Germany) HCT ultra ion trap mass spectrometry at 140 °C with a flow rate of 4 µL/min, a mass range of 50 – 1000 *m/z* and a scan rate of 5500 *m/z/second* in positive/negative ion mode (Standard- Enhanced mode) (Methanol was used as the mobile phase with 0.1% formic acid for positive mode or 0.1% ammonium chloride for negative mode).

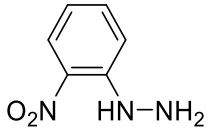
High-resolution electrospray ionisation (ESI) mass spectrometry was carried out using an Agilent Technologies Accurate Mass Q-TOF LC-MS 6530 using Autosampler 1260 Infinity II in positive mode. The samples were analysed using a flow rate of 1 mL/min, a mass range of 100 – 1,000 *m/z* and a scan rate of 10,000 *m/z/second*.

Thin layer chromatography (TLC) was used to monitor reactions and chromatographic fractions on Merck Kieselgel 60 F254 aluminium backed plates. Darasil® LC60A (40 – 63 micron) F254 was used as the stationary phase to perform flash chromatography. Gradient elution using ethyl acetate and hexane, analytical grade were used unless otherwise stated.

Analytical reverse phase high performance liquid chromatography (RP-HPLC) was performed on a Shimadzu LC-20AB Prominence system fitted with a Phenomenex® Jupiter C18 300 Å column (250 mm x 4.6 mm, 10 μm), using a buffered binary solvent system; Solvent A: Milli Q water; Solvent B: acetonitrile. Gradient elution was performed using a gradient of 5 % solvent A to 95% solvent B over 25 minutes with a flow rate of 1 mL/min, monitored at 254 nm.

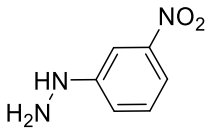
7.2 Syntheses

(2-Nitrophenyl)hydrazine hydrochloride, 2.1



2-Nitroaniline (3.62 mmol, 500 mg) was dissolved in concentrated hydrochloric acid (20 mL) and water (10 mL) and cooled to 5 °C. A cold solution of sodium nitrite (5.43 mmol, 375 mg) in 3 mL of water was added dropwise maintaining the temperature. The reaction mixture was stirred for 5 minutes. Dropwise addition of a cold solution of tin (II) chloride dehydrate (9.05 mmol, 2.04 g) in concentrated hydrochloric acid (10 mL) was undertaken at -1 °C. The reaction mixture was stirred for 2 hours in the salt-ice bath and stored in the freezer overnight. The solid dropped was collected via suction filtration and washed with cold 1 M hydrochloric acid to yield pure compound **2.1** (318.4 mg, 46%) as a bright yellow solid, m.p. 92 - 94 °C (lit. 90)[131]. δ_H (400 MHz, DMSO) 9.21 (br, 1H, NH), 8.14 (d, *J* = 8 Hz, 1H, ArH), 7.74 (t, *J* = 8 Hz, 1H, ArH), 7.31 (d, *J* = 7.2 Hz, 1H, ArH), 7.08 (t, *J* = 8 Hz, 1H, ArH), 3.66 (br, 2H, NH_2). δ_C (100 MHz, DMSO) 141.6, 136.6, 134.3, 126.5, 130.8, 115.6. LRMS (ESI): *m/z* 177.0 $(M + Na)^+$ HRMS-ESI (*m/z*): $(M + Na)^+$ calcd for $C_6H_7N_3NaO_2^+$, 176.0430; found, 176.0431.

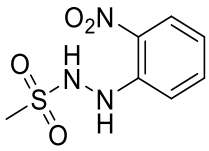
(3-Nitrophenyl)hydrazine, 2.2



3-Nitroaniline (1.45 mmol, 200 mg) was dissolved in 1:1 mixture of concentrated hydrochloric acid and water (10 mL) and cooled to 5 °C. A cold solution of sodium nitrite (1.45 mmol, 99 mg) in 2 mL of water was added dropwise maintaining the temperature. The reaction mixture was stirred for 5 minutes. Dropwise addition of a cold solution of sodium sulphite (7.25 mmol, 611 mg) in water (4 mL) was undertaken while maintaining the temperature. The reaction mixture was stirred for 1.5 hours in the ice bath and warmed to room temperature and stirred for another 0.5 hours. The solid dropped was collected via

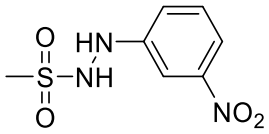
suction filtration to yield crude (3-nitrophenyl)hydrazine (92.8 mg, 42%), m.p. 93-95 °C (lit. 92 - 95 °C)[71] . Upon purification on a column with 30% ethyl acetate: hexane pure compound was yielded (71.7 mg, 33%), m.p. 92 - 96 °C. δ_H (400 MHz, DMSO) 9.21 (s, NH), 7.84 (d, *J* = 6 Hz, 1H, ArH), 7.46 (d, *J* = 6 Hz, 1H, ArH), 7.40 (d, *J* = 8 Hz, 2H, ArH). δ_C (100 MHz, DMSO) 149.9, 148.5, 130.5, 118.8, 112.5, 106.3. LRMS (ESI): *m/z* 154.1 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_6H_8N_3O_2^+$, 154.0611; found, 154.0610.

_N'_-(2-Nitrophenyl)methanesulfonohydrazide, 2.3



(2-Nitrophenyl) hydrazine hydrochloride (0.370 mmol, 70 mg) was dissolved in dichloromethane (10 mL) and triethylamine (0.74 mmol, 103.2 µL) under nitrogen environment. To the mixture, dropwise addition of methanesulfonyl chloride (0.370 mmol, 28.7 µL) was performed and the reaction mixture was stirred in room temperature overnight. The reaction mixture was washed with water, brine and concentrated in vacuum to yield crude product. Upon purification on a column with 30% ethyl acetate: hexane pure compound was yielded as a dark orange colour solid (13.1 mg, 14%), m.p. 138 - 142 °C. δ_H (400 MHz, DMSO) 9.40 (br, 1H, NH), 9.36 (br, 2H, NH_2), 8.06 (d, *J* = 8 Hz, 1H, ArH), 7.62 (t, *J* = 8 Hz, 1H, ArH), 7.53 (d, *J* = 8 Hz, 1H, ArH), 6.89 (t, *J* = 8 Hz, 1H, ArH), 3.06 (s, 3H, CH_3). δ_C (100 MHz, DMSO) 145.5, 136.7, 132.6, 126.1, 118.9, 116.4, 38.8. LRMS (ESI): *m/z* 232.1 $(M + H)^+$, 121.9, 109.2, 153.2. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_7H_{10}N_3O_4S^+$, 232.0387; found, 232.0386.

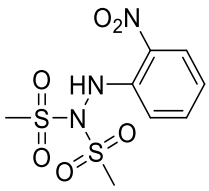
_N'_-(3-Nitrophenyl)methanesulfonohydrazide, 2.4



(3-nitrophenyl) hydrazine hydrochloride (0.370 mmol, 70 mg) was dissolved in dichloromethane (10 mL) and triethylamine (0.74 mmol, 103 µL) under nitrogen

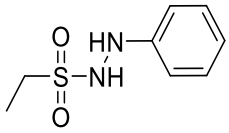
environment. To the mixture, dropwise addition of methanesulfonyl chloride (0.370 mmol, 28.7 μL) was performed and the reaction mixture was stirred in room temperature overnight. The reaction mixture was washed with water, brine and concentrated in vacuum to yield crude product. Upon purification on a column with 30% ethyl acetate: hexane pure compound was yielded as a dark orange colour solid (10 mg, 10%) m.p. 189-191 °C. δ_H (400 MHz, DMSO) 8.23 (d, *J* = 8 Hz, 1H, ArH), 7.85 (t, *J* = 8 Hz, 1H, ArH), 7.69 (s, ArH, 1H), 7.67 (t, *J* = 6 Hz, 1H, ArH), 3.37 (s, 3H, CH_3). δ_C (100 MHz, $CDCl_3$) 151.6, 148.6, 130.0, 119.2, 114.3, 104.2, 42.6. LRMS (ESI): *m/z* 232.0 $(M + H)^+$, 122.9, 1519. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_7H_{10}N_3O_4S^+$, 232.0387; found, 232.0388.

_N_-(Methylsulfonyl)-_N'_-(2-nitrophenyl)methanesulfonohydrazide, 2.9



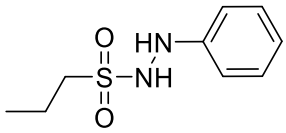
(2-Nitrophenyl) hydrazine hydrochloride (1.31 mmol, 200 mg) was dissolved in dichloromethane (10 mL) and triethylamine (3.93 mmol, 548 μL) under a nitrogen environment. To the mixture, dropwise addition of methanesulfonyl chloride (2.61 mmol, 202 μL) was performed and the reaction mixture was stirred at room temperature for 2 hours. The reaction mixture was washed with water, brine and concentrated in vacuum to yield the crude product as a bright orange solid. The crude product was triturated with chloroform for 2 hours to yield the pure compound as a bright yellow solid (57 mg, 18%), m.p. 146 - 150 °C. δ_H (400 MHz, DMSO) 9.72 (s, 1H, NH), 8.24 (d, *J* = 7.2 Hz, 1H, ArH), 7.65 (t, *J* = 7.2 Hz, 1H, ArH), 7.42 (d, *J* = 8 Hz, 1H, ArH), 7.09 (t, *J* = 8 Hz, 1H, ArH), 3.44 (s, 6H, CH_3). δ_C (100 MHz, DMSO) 143.1, 136.4, 135.4, 126.6, 121.6, 115.8, 43.1. LRMS (ESI): *m/z* 332.0 $(M + Na)^+$, 187.2, 152.9, 122.5. HRMS-ESI (*m/z*): $(M + Na)^+$ calcd for $C_8H_{11}N_3NaO_6S_2^+$, 331.9981; found, 331.8951.

***N'*-Phenylethanesulfonohydrazide, 2.10**



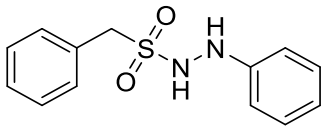
Phenylhydrazine (5 mmol, 500 μL) and ethanesulfonylchloride (10 mmol, 960 μL) were mixed in water (5 mL) at room temperature, overnight. The resulting solid was filtered and washed thoroughly with water to yield pure compound as a pale pink solid (440 mg, 45%), m.p. 76 – 78 °C. δ_H (400 MHz, $CDCl_3$) 7.27 (t, *J* = 4 Hz, 3H, ArH), 6.95 (d, *J* = 4 Hz, 2H, ArH), 5.95 (br, 1H, NH), 3.00 (q, *J* = 8 Hz 2H, CH_2), 1.36 (t, *J* = 8 Hz, 3H, CH_3). δ_C (100 MHz, $CDCl_3$) 146.3, 129.5, 121.7, 113.7, 46.4, 7.7. LRMS (ESI): *m/z* 223.1 $(M + Na)^+$. HRMS-ESI (*m/z*): $(M + Na)^+$ calcd for $C_8H_{12}N_2NaO_2S^+$, 223.0512; found, 223.0510.

***N'*-Phenylpropane-1-sulfonohydrazide, 2.11**



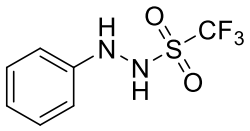
Phenylhydrazine (5 mmol, 500 μL) and propanesulfonylchloride (10 mmol, 1.2 mL) were mixed in water (5 mL) at room temperature overnight. The resulting solid was filtered and washed thoroughly with water to yield the crude product (714 mg). A portion from the crude product (300 mg) was stirred in water (50 mL) for 4 hours, filtered and dried to obtain pure compound as a white solid (137 mg, 70%), m.p. 98 – 100 °C. δ_H (400 MHz, $CDCl_3$) 7.27 (t, *J* = 4 Hz, 3H, ArH), 6.93 (d, *J* = 4 Hz, 2H, ArH), 6.03 (br, 1H, NH), 2.96 (t, *J* = 8 Hz, 2H, CH_2), 1.91 – 1.82 (m, 2H, CH_2), 0.99 (t, *J* = 8 Hz, 3H, CH_3). δ_C (100 MHz, $CDCl_3$) 146.4, 129.4, 121.6, 113.6, 53.4, 16.8, 12.4. LRMS (ESI): *m/z* 215.2 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_9H_{15}N_2O_2S^+$, 215.0849; found, 215.0850.

N',1-Diphenylmethanesulfonohydrazide, 2.12



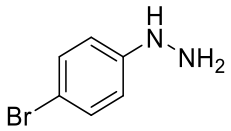
To a solution of sodium carbonate (4 mmol, 424 mg) in water (20 mL) phenylhydrazine (2 mmol, 200 µL) was added and stirred vigorously. Addition of phenylmethanesulfonyl chloride (2 mmol, 382 mg) was done slowly. The reaction mixture was stirred overnight at room temperature. The precipitate was collected to yield pure compound as a white solid (160 mg, 30%), m. p. 119- 121 °C (decomp 173 °C)[132]. δ_H (400 MHz, $CDCl_3$) 9.10 (br, 1H, NH), 7.96 (br, 1H, NH), 7.43 – 7.35 (m, 5H, ArH), 7.16 (t, J = 8 Hz, 2H, ArH), 6.90 (d, J = 4 Hz, 2H, ArH), 6.73 (t, J = 8 Hz, 1H, ArH), 4.39 (s, 2H, CH_2). δ_C (100 MHz, $CDCl_3$) 149.2, 131.5, 130.0, 129.1, 128.9, 128.5, 119.5, 113.2, 55.6. LRMS (ESI): *m/z* 263.1 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_{13}H_{15}N_2O_2S^+$, 263.0849; found, 263.0850.

1,1,1-Trifluoro-N'-phenylmethanesulfonohydrazide, 2.13



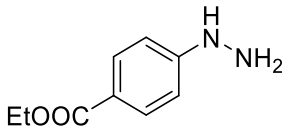
Phenylhydrazine (300 µL, 0.328 mmol) and triethylamine (70 µL, 0.492) was dissolved in dry dichloromethane (10 mL) and the mixture was cooled to 0 °C. Dropwise addition of triflic anhydride (140 µL, 0.822 mmol) was performed while maintaining the temperature. Then the reaction mixture was warmed to room temperature and stirred for 1 hour. The reaction mixture was washed with water, 5% $NaHCO_3$ and water. The organic layer was dried and concentrated to obtain the product as an orange oil (38 mg, 47%). δ_H (400 MHz, $CDCl_3$) 7.35 (t, J = 8 Hz, 2H, ArH), 7.14 (t, J = 8 Hz, 1H, ArH), 7.03 (d, J = 8 Hz, 2H, ArH). δ_C (100 MHz, $CDCl_3$) 140.0, 129.7, 124.9, 119. 0. δ_F (376 MHz, $CDCl_3$) -210.1. LRMS (ESI): *m/z* 241.3 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_7H_8F_3N_2O_2S^+$, 241.0253; found, 241.0252.

(4-Bromophenyl)hydrazine, 2.14



4-Bromoaniline (1.16 mmol, 200 mg) was dissolved in 12 M hydrochloric acid (1 mL) and cooled to 0 °C in an ice bath. Dropwise addition of a cold solution of sodium nitrite (1.16 mmol, 80 mg) in water (1 mL) was performed maintaining the temperature. Formed diazonium salt solution was added dropwise to a cold stannous (II) chloride (3.5 mmol, 660 mg) in 12 M hydrochloric acid (10 mL). The reaction mixture was stirred at 0 °C for 2 hours and stored in the freezer overnight. Formed solid was filtered, poured on to water and neutralized until pH 10 using 1 M sodium hydroxide. The resulting solution was extracted using diethyl ether (three times), dried over magnesium sulphate and concentrated to obtain pure product as a brown solid (192 mg, 88%), m.p. 68 - 70 °C (lit. 105- 106 °C)[133]. δ_H (400 MHz, DMSO) 9.95 (br, 2H, NH_2), 8.31 (br, 1H, NH), 7.45 (d, *J* = 8 Hz, 2H, ArH), 6.89 (d, *J* = 8 Hz, 2H, ArH). δ_C (100 MHz, DMSO) 152.2, 131.5, 113.8, 107.3. LRMS (ESI): *m/z* 187.1 $(M[^{79}Br] + H)^+$, 189.1 $(M[^{81}Br] + H)^+$. HRMS-ESI (*m/z*): $(M[^{79}Br] + H)^+$ calcd for $C_6H_8[^{79}Br]N_2$, 186.9865; found, 186.9866, $C_6H_8[^{81}Br]N_2$, 188.9865; found, 188.9865.

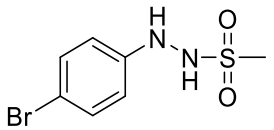
Ethyl 4-hydrazinylbenzoate, 2.15



A cold solution of sodium nitrite (3 mmol, 207 mg) in water (3 mL) was added dropwise to a cold solution of ethyl 4-benzoate (2 mmol, 302 mg) in concentrated hydrochloric acid (9 mL) while maintaining the temperature at 0 °C. The formed diazonium salt solution was added dropwise to a slurry of tin (II) chloride (5.56 mmol, 1.05 g) maintaining the temperature at 0 °C. The reaction was stirred at room temperature for 10 minutes and the formed solid was collected, washed with diethyl ether and dried to yield pure compound as a white solid (212 mg, 59%), m.p. 114-116 °C (lit. 113-115 °C)[134]. δ_H (400 MHz, DMSO) 8.22 (d, *J* = 7.2 Hz, 2H, ArH), 8.06 (d, *J* = 7.2 Hz, 2H, ArH), 4.36 (q, *J* = 7.2 Hz,

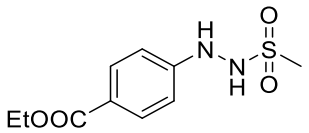
2H, CH_2), 1.34 (t, J = 6.8 Hz, 3H, CH_3). δ_C (100 MHz, DMSO) 165.9, 150.3, 130.9, 122.3, 113.6, 60.7, 14.7. LRMS (ESI): *m/z* 203.1 $(M + Na)^+$. HRMS-ESI (*m/z*): $(M + Na)^+$ calcd for $C_9H_{12}N_2NaO_2^+$, 203.0791; found, 203.0790.

N'-(4-Bromophenyl)methanesulfonohydrazide, 2.16



4-Bromoaniline (0.44 mmol, 100 mg) was dissolved in a mixture of dichloromethane (5 mL) and triethylamine (1.32 mmol, 184 µL) under nitrogen at room temperature. Methanesulfonyl chloride (0.44 mmol, 36 µL) was added dropwise to the reaction mixture and continued stirring overnight under nitrogen. The reaction mixture was washed with water, brine, dried over anhydrous $MgSO_4$ and concentrated in vacuum. Silica gel column was performed on 30% ethyl acetate: hexane to purify the pure compound as a beige powder (47 mg, 33%), m.p. 109 – 111 °C. δ_H (400 MHz, $CDCl_3$) 7.40 (d, J = 8 Hz, 2H, ArH), 6.87 (d, J = 8 Hz, 2H, ArH), 6.02 (br, 1H, NH), 5.98 (br, 1H, NH), 3.02 (s, 3H, CH_3). δ_C (100 MHz, $CDCl_3$) 145.3, 132.4, 115.2, 113.7, 39.9. LRMS (ESI): *m/z* 265.0 $(M[^{79}Br] + H)^+$, 267.1 $(M[^{81}Br] + H)^+$. HRMS-ESI (*m/z*): $(M[^{79}Br] + H)^+$ calcd for $C_7H_{10}[^{79}Br]N_2O_2S$, 264.9641; found, 264.6940, $C_7H_{10}[^{81}Br]N_2O_2S$, 266.9641; found, 266.6939.

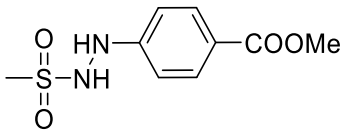
Ethyl 4-(2-(methanesulfonyl) hydrazinyl) benzoate, 2.17



Triethylamine (1.8 mmol, 272 µL) followed by methanesulfonyl chloride (1.2 mmol, 93.2 µL) was added to ethyl 4-hydrazinylbenzoate (0.6 mmol, 100 mg) in anhydrous dichloromethane (7 mL). The reaction mixture was stirred at room temperature for overnight. Dichloromethane reaction mixture was washed with three portions of water, followed by a portion of brine. Upon concentration orange colour oil was obtained which was purified by silica gel column chromatography to yield pure compound as a beige

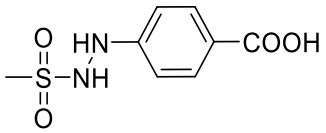
colour solid (25 mg, 17%), m.p. 62-64 °C. δ_H (400 MHz, DMSO) 8.21 (d, *J* = 6.8 Hz, 2H, ArH), 8.04 (d, *J* = 6.8 Hz, 2H, ArH), 4.36 (q, *J* = 7.2 Hz, 2H, CH_2), 3.47 (s, 3H, CH_3), 1.34 (t, *J* = 6.8 Hz, 3H, CH_3). δ_C (100 MHz, DMSO) 165.1, 151.5, 135.5, 131.4, 124.7, 61.9, 14.5. LRMS (ESI): *m/z* 260.0 $(M + H)^+$, 215.2, 149.8, 109.1. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_{10}H_{15}N_2O_4S^+$, 259.0747; found, 259.0747.

Methyl 4-(2-(methylsulfonyl)hydrazinyl)benzoate, 2.18



Ethyl 4-(2-(methanesulfonyl) hydrazinyl) benazoate) (0.113 mmol, 30 mg) was dissolved in a 1:10 mixture of methanol:dichloromethane (3 mL). To this mixture sodium hydroxide (0.124 mmol, 5 mg) was added. The reaction was monitored by TLC using 30% hexane: ethyl acetate. Dichloromethane (7 mL) was added to the reaction mixture and washed with water, brine, dried with anhydrous magnesium sulphate and concentrated to obtain the pure product as an orange solid (12 mg, 38%), m. p. 185 – 188 °C. δ_H (400 MHz, $CDCl_3$) 11.09 (br, 1H, NH), 9.50 (br, 1H, NH), 7.70 (d, *J* = 8 Hz, 2H, ArH), 7.15 (d, *J* = 8 Hz, 2H, ArH), 4.01 (s, 3H, OCH_3), 2.93 (s, 3H, CH_3). δ_C (100 MHz, $CDCl_3$) 165.6, 155.0, 130.6, 113.6, 51.6, 42.9. LRMS (ESI): *m/z* 245.2 $(M - H)^+$, 214.0, 136.2, 109.8. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_9H_{13}N_2O_4S^+$, 245.0591; found, 245.0590.

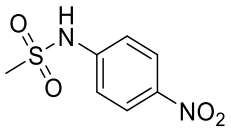
4-(2-(Methylsulfonyl)hydrazinyl)benzoic acid, 2.19



Ethyl 4-(2-(methanesulfonyl) hydrazinyl) benzoate (0.27 mmol, 258 mg) was dissolved in 10% methanol, dichloromethane (v/v) (6 mL) and addition of 2M aqueous sodium hydroxide (0.3 mmol, 150 μL) was followed. Instant colour change was observed from bright orange to reddish brown. The reaction mixture was refluxed while monitoring by TLC. After 1.5 hours complete consumption of the starting material was observed and a solid had separated out. The solid was filtered out and washed with the solvent and dried

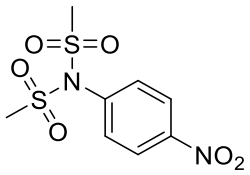
under vacuum to obtain the pure product as a beige powder (67 mg, 91%), m.p.> 250 °C. δ_H (400 MHz, $CDCl_3$) 8.52 (br, 1H, NH), 8.03 (d, *J* = 8 Hz, 2H, ArH), 7.84 (d, *J* = 8 Hz, 2H, ArH), 7.23 (br, 1H, NH), 3.39 (s, 3H, CH_3). δ_C (100 MHz, $CDCl_3$) 167.1, 130.8, 129.5, 127.5, 123.9, 35.2. LRMS (ESI): *m/z* 230.0 $(M - H)^-$, 121.3. HRMS-ESI (*m/z*): $(M - H)^-$ calcd for $C_8H_9N_2O_4S^-$, 229.0289; found, 229.0290.

_N_-(4-Nitrophenyl)methanesulfonamide, 2.20



4-Nitroaniline (1.45 mmol, 200 mg) was dissolved in pyridine (520 µL) and cooled to 0 °C. Dropwise addition of cold methanesulfonyl chloride (1.73 mmol, 112 µL) was performed and the reaction mixture was allowed to warm to room temperature and stirring was continued overnight. The reaction mixture was washed with water, brine, dried over anhydrous $MgSO_4$ and concentrated in vacuum. Silica gel column was performed on 40% ethyl acetate: hexane to purify the pure compound as a yellow powder (80 mg, 26%), m.p. 139 – 141 °C (lit. 180-182 °C)[135]. δ_H (400 MHz, DMSO) 10.72 (s, 1H, NH), 8.21 (d, *J* = 8 Hz, 2H, ArH), 7.36 (d, *J* = 8 Hz, 2H, ArH), 3.17 (s, 3H, CH_3). δ_C (100 MHz, DMSO) 145.5, 142.6, 125.9, 118.0, 40.6. LRMS (ESI): *m/z* 217.1 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_7H_9N_2O_4S^+$, 217.0278; found, 217.0277.

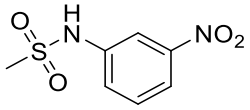
_N_-(Methylsulfonyl)-_N_-(4-nitrophenyl)methanesulfonamide, 2.21



4-Nitroaniline (1.45 mmol, 200 mg) was dissolved in a solution of triethylamine (2.9 mmol, 406 µL) in dichloromethane (10 mL). The reaction mixture was washed with water, brine and dried over anhydrous magnesium sulphate. Upon concentration pure compound was obtained as a yellow solid (156 mg, 35%), m.p. 112 – 116 °C. δ_H (400 MHz, DMSO) 8.31 (d, *J* = 8 Hz, 2H, ArH), 7.85 (d, *J* = 8 Hz, 2H, ArH), 3.31 (s, 6H, CH_3). δ_C (100 MHz,

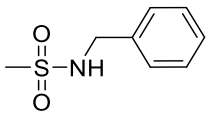
DMSO) 148.7, 139.9, 132.9, 125.1, 43.7. LRMS (ESI): *m/z* 316.8 $(M + Na)^+$. HRMS-ESI (*m/z*): $(M + Na)^+$ calcd for $C_8H_{10}N_2NaO_6S_2^+$, 316.9872; found, 316.9869.

_N_-(3-Nitrophenyl)methanesulfonamide, 2.22



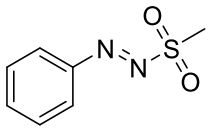
Dropwise addition of methanesulfonyl chloride (1.5 mmol, 112 µL) was performed to a cold solution of 3-nitroaniline (1.45 mmol, 200 mg) in pyridine (760 µL) at 0 °C. The reaction mixture was warmed to room temperature gradually and stirred overnight and concentrated under vacuum. Obtained residue was triturated with water (5 mL) to obtain a bright yellow solid. The solid was washed with 2M hydrochloric acid to obtain pure compound as a pale yellow solid (227 mg, 72%), m.p. 126 – 130 °C (lit. 150 -152 °C)[136]. δ_H (400 MHz, DMSO) 10.36 (s, 1H, NH), 8.04 (s, 1H, ArH), 7.92 (d, *J* = 8 Hz, 1H, ArH), 7.61 (m, 2H, ArH), 3.60 (br, 2H,NH_2), 3.09 (s, 3H, CH_3). δ_C (100 MHz, DMSO) 148.8, 140.2, 131.3, 125.6, 118.5, 113.5, 40.2. LRMS (ESI): *m/z* 217.1 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_7H_9N_2O_4S^+$, 217.0278; found, 217.1042. HRMS-ESI (*m/z*): $(M + Na)^+$ calcd for $C_7H_8N_2NaO_4S^+$, 239.0097; found, 239.0096.

_N_-Benzylmethanesulfonamide, 2.23



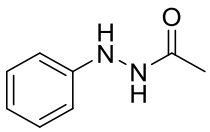
Dropwise addition of methanesulfonyl chloride (2.75 mmol, 144 µL) was performed to a solution containing benzylamine (2.75 mmol, 300 µL) in water (10 mL) with sodium carbonate (5.5 mmol, 583 mg). The reaction mixture was stirred overnight at room temperature. Precipitated solid was collected by suction filtration to obtain pure compound as a white fluffy solid (153 mg, 30%), m. p. 110-113 °C. δ_H (400 MHz, $CDCl_3$) 7.38 – 7.26 (m, 5H, ArH), 4.72 (br, 1H, NH), 4.35 (d, J= 16, 2H, CH_2), 2.87 (s, 3H, CH_3). δ_C (100 MHz, $CDCl_3$) 128.9, 128.7, 128.2, 127.9, 127.5, 127.4, 47.2, 41.2. LRMS (ESI): *m/z* 208.1 $(M + H)^+$. HRMS-ESI (*m/z*): (M + H)+ calcd for $C_8H_{12}NO_2S^+$, 208.0403; found, 207.9852.

1-(Methylsulfonyl)-2-phenyldiazene, 2.25



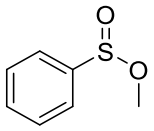
Aniline (2 mmol, 180 µL) was dissolved in tetrafluoroboric acid (48% w/v, 381 µL) and water (3 mL) followed by cooling to 0°C. Addition of a solution of $NaNO_2$ (2.17 mmol, 150 mg) was performed dropwise maintaining the temperature. The mixture was stirred for 30 minutes and the thick precipitate was collected. Obtained diazo salt (0.29 mmol, 56 mg) was suspended in DCM (3 mL) and cooled to 0 °C. To this sodium methanesulfinate (0.29 mmol, 30 mg) was added in one portion. The temperature of the mixture was allowed to warm to room temperature slowly and stirred overnight. The resulted mixture was filtered, and the filtrate was concentrated. Obtained crude solid was subjected to column chromatography in 10% ethyl acetate: hexane to obtain the product (7 mg, 13%), m. p . 72 – 75 °C (lit. 73 -74)[137]. δ_H (400 MHz, $CDCl_3$) 7.95 (d, *J* = 8 Hz, 2H, ArH), 7.67 (t, *J* = 8 Hz, 1H, ArH), 7.58 (t, *J* = 8 Hz, 2H, ArH), 3.22 (s, 3H, CH_3). δ_C (100 MHz, $CDCl_3$) 128.9, 128.7, 128.5, 43.9. LRMS (ESI): *m/z* 185.3 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_7H_9N_2O_2S^+$, 185.0379; found, 185. 0285.

***N'*-Phenylacetohydrazide, 3.1**



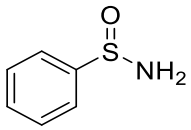
Phenylhydrazine (5 mmol, 500 µL) and acetic acid (125 mmol, 7 mL) was mixed and heated for 1.5 hours in an oil bath. Remaining acetic acid was removed by distillation and the residue was diluted with diethyl ether (10 mL). The reaction mixture was dried and evaporated to obtain the pure product as a white solid (284 mg, 38 %), m.p. 86 -88 °C (lit. 128 -129 °C)[138]. NMR showed the compound to be a mixture of 2 rotamers (ratio: 2.2/1). δ_H (400 MHz, $CDCl_3$) 7.50 (br, 1H, NH), 7.31 – 7.21 (m, 3H, ArH), 6.83 (d, *J* = 8 Hz, 2H, ArH), 6.76 (d, 1H, NH), 2.06 (s, 3H, CH_3). δ_C (100 MHz, $CDCl_3$) 147.3, 129.5, 121.4, 113.5, 112.5, 20.9. LRMS (ESI): *m/z* 151.2 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_8H_{11}N_2O^+$, 151.0866; found, 151.0861.

Methyl benzenesulfinate, 3.2



(In a two neck round bottom flask connected to a septum and a nitrogen gas adaptor) Sodium benzenesulfinate (3.04 mmol, 500 mg) was mixed with dichloromethane (8 mL) and the suspension was cooled to 0 °C, followed by the addition of $SOCl_2$ (9.14 mmol, 666 µL) while maintaining the temperature. After 20 minutes, the reaction was warmed to room temperature and stirred for 3 hours. The reaction mixture was concentrated in vacuum to obtain a white powdery solid which was dissolved in dry dichloromethane (5 mL) added to dry methanol (20 mL). The mixture was cooled to 0 °C and dropwise addition of triethylamine (9.14 mmol, 1.3 mL) was performed. The reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was concentrated in vacuum to obtain the crude product which was diluted with dichloromethane and washed with water and brine to obtain the pure product as a light-yellow oil (345 mg, 73%). δ_H (400 MHz, DMSO) 7.70 (d, *J* = 4 Hz 2H, ArH), 7.63 (t, *J* = 4 Hz, 3H, ArH), 3.42 (s, 3H, CH_3). δ_C (100 MHz, DMSO) 144.2, 133.0, 129.8, 125.6, 50.6. LRMS (ESI): *m/z* 158.0 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_7H_9O_2S^+$, 157.0318; found, 157.0317.

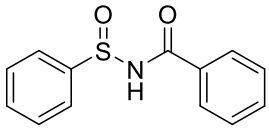
Benzenesulfinamide, 3.3



Methyl benzenesulfinate (2.2 mmol, 345 mg) was dissolved in dry THF (6 mL) and the mixture was cooled to –78 °C followed by the dropwise addition of 1M LiHMDS (3.3 mmol, 3.3 mL). After complete addition, the reaction mixture was allowed to warm to room temperature and stirred for 1.5 h. Addition of saturated ammonium chloride solution (8 mL) to the reaction mixture was performed and the reaction was stirred overnight. The reaction mixture was extracted with ethyl acetate. The combined organic layers were dried and concentrated under vacuum and the crude product was recrystallized from 2:1, hexane: ethyl acetate to obtain product as colourless crystals (223 mg, 72%), m.p. 117- 119 °C (lit.

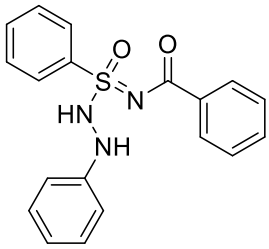
112 – 115 °C)[139]. δ_H (400 MHz, DMSO) 7.75 (d, *J* = 8 Hz, 2H, ArH), 7.50 (t, *J* = 8 Hz, 3H, ArH), 4.35 (br, 2H, NH_2). δ_C (100 MHz, DMSO) 146.5, 131.1, 128.9, 125.4. LRMS (ESI): *m/z* 142.0 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_6H_8NOS^+$, 142.0321; found, 142.0322.

_N_-(Phenylsulfinyl)benzamide, 3.4



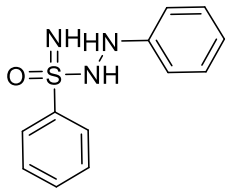
Benzenesulfinamide (1.06 mmol, 150 mg) was dissolved in dry THF (5 mL) and the reaction mixture was cooled to –78 °C and dropwise addition of 2.5 M *n*-butyllithium solution (2.68 mmol, 1.7 mL) was performed maintaining the temperature below –65 °C. The reaction was stirred at –78 °C for 10 minutes and rapid addition of benzoic anhydride (1.29 mmol, 293 mg) was performed and stirred for 10 minutes. The reaction was warmed to room temperature and and stirred overnight. The reaction mixture was diluted with saturated sodium bicarbonate solution (20 mL). The reaction mixture was extracted with dichloromethane. The combined organic layers were dried and concentrated under vacuum and the crude product was recrystallized from 2:1, hexane: ethyl acetate to obtain the product as light orange crystals (138 mg, 53%), m.p. 138 -140 °C (lit. 141 -143 °C)[140]. δ_H (400 MHz, $CDCl_3$) 8.20 (br, 1H, NH), 7.80 (m, 4H, ArH), 7.58 (m, 4H, ArH), 7.46 (t, *J* = 8 Hz, 2H, ArH). δ_C (100 MHz, $CDCl_3$) 167.3, 134.2, 133.6, 132.1, 131.7, 129.6, 128.9, 127.9, 124.8. LRMS (ESI): *m/z* 268.2 $(M + Na)^+$. HRMS-ESI (*m/z*): $(M + Na)^+$ calcd for $C_{13}H_{11}NNaO_2S^+$, 268.0403; found, 268.0402.

***N*-(Oxo(phenyl)(2-phenylhydrazinyl)-λ^6-sulfanylidene)benzamide, 3.5**



N-(Phenylsulfinyl)benzamide **55** (0.407 mmol, 100 mg) was dissolved in dry ACN (7.5 mL) and *N*-chlorosuccinimide (1.26 mmol, 168.7 mg) was added. After for 30 minutes phenylhydrazine (1.22 mmol, 120 µL) was added dropwise. After 3 hours, the reaction was filtered to remove solids and the filtrate was diluted with water and extracted with ethyl acetate. The combined organic layers were dried and concentrated under vacuum and the crude product was washed with ether. The filtrate form ether wash was concentrated to obtain the product as a beige solid (105 mg, 73%), m.p. 117 – 119 °C. δ_H (400 MHz, $CDCl_3$) 7.93 (d, 1H, NH), 7.82 (d, *J* = 8 Hz, 2H, ArH), 7.65 (m, 1H, NH), 7.55 (m, 3H, ArH), 7.45 (m, 5H, ArH), 7.33 (m, 5H, ArH). δ_C (100 MHz, $CDCl_3$) 177.8, 136.7, 132.3, 131.2, 129.6, 129.5, 129.3, 128.9, 128.8, 127.7, 127.5, 127.2, 125.0. LRMS (ESI): *m/z* 352.3 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_{19}H_{18}N_3O_2S^+$,352.1114; found, 352.1111.

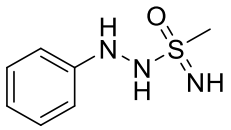
***N'*-Phenylbenzenesulfonimidhydrazide, 3.6**



To a solution of *N*-(oxo(phenyl)(2-phenylhydrazinyl)-λ^6-sulfanylidene)benzamide **56** (30 mg, 0.085 mmol) in methanol (200 µL), 2M HCl in ether (0.255 µL) was added and stirred for 2 hours. The reaction was diluted with 15 mL of water and extracted with chloroform. The combined organic layers were dried and concentrated under vacuum and the crude product was purified by column chromatography on 10% ethyl acetate: hexane to obtain the product as a pale yellow solid (14 mg, 66%), m.p. 112 – 115 °C. δ_H (400 MHz, $CDCl_3$) 7.57 (m, 3H, ArH), 7.47 (m, 1H, ArH), 7.41 (m, 2H, ArH), 7.36 (m, 4H, ArH). δ_C (100

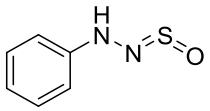
MHz, $CDCl_3$) 143.0, 136.6, 133.6, 131.4, 131.0, 129.6, 129.4, 129.1, 128.8, 127.9, 127.6, 122.8. LRMS (ESI): *m/z* 248.3 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_{12}H_{14}N_3OS^+$, 248.0852; found, 248.0850.

_N'_-Phenylmethanesulfonimidhydrazide, 3.8



N'-Phenylmethanesulfonohydrazide (50 mg, 0.268 mmol) and PCl_5 (62 mg, 0.295 mmol) was mixed in dichloromethane (10 mL) and refluxed for 30 minutes. The reaction mixture was cooled to room temperature and dry ammonia gas was made in a separate apparatus and bubbled though the reaction mixture for 30 minutes which created a yellow solid. The solid was filtered out and triturated with ether. After filtration, the solid was filtered and triturated with ether. Column chromatography using 20% ethyl acetate: hexane obtained the product as a purple solid (9.7 mg, 19%), m. p. 42 – 45 °C. δ_H (400 MHz, $CDCl_3$) 7.89 (d, 1H, NH), 7.83 (d, *J* = 8 Hz, 2H, ArH), 7.59 (d, 1H, NH), 7.50 (m, 3H, ArH), 3.15 (s, 3H, CH_3). δ_C (100 MHz, $CDCl_3$) 141.8, 130.2, 125.8, 34.9. LRMS (ESI): *m/z* 186.4 $(M + H)^+$, 107.2. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_7H_{12}N_3OS^+$, 186.0696; found, 186.0698.

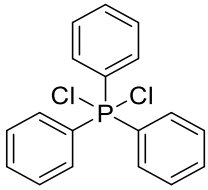
(2-Phenylhydrazono)-λ^4-sulfanone , 3.10



Imidazole (500 mg, 7.34 mmol) was dissolved in dichloromethane and cooled to -10 °C. To this addition of thionyl chloride (220 µL, 1.8×10^3 mmol) was added and warmed to 20 °C. Formed solid was filtered out and the filtrate was transferred to a clean round bottom flask and cooled to -10 °C. Addition of another portion of thionyl chloride (135 µL, 1.8×10^3) was performed and kept for 10 minutes before dropwise addition of phenylhydrazine (4 mL, 3.67 mmol) after further cooling to –40 °C. The reaction was kept cold for 5 hours and at room temperature overnight. The solid was collected and washed with dichloromethane to obtain the pure product (200 mg, 35%), 108-110 °C (lit. 103 – 105

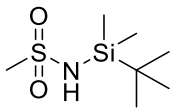
°C)[141]. δ_H (400 MHz, DMSO) 7.23 (t, *J* = 8 Hz, 2H, ArH), 6.93 (d, *J* = 8 Hz, 2H, ArH), 6.86 (t, *J* = 8 Hz, 1H, ArH). δ_C (100 MHz, DMSO) 147.1, 129.3, 121.2, 114.4. LRMS (ESI): *m/z* 155.1 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_6H_7N_2OS^+$,155.0274; found, 155.0268.

Dichlorotriphenyl-λ^5-phosphane, 3.12



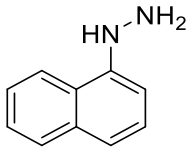
Triphenylphosphine (500 mg, 1.9 mmol) and hexachloroethane (452 mg, 1.9 mmol) was stirred in chloroform (18 mL) at 70 °C for six hours. The resulted solution was concentrated in vacuum to obtain the product as a white sold (519 mg, 82%), m.p. 170 – 173 °C (lit. 176 °C)[142]. δ_H (400 MHz, DMSO) 7.61 ppm (m, 3H, ArH), 7.53 (m, 2H, ArH). δ_c (100 MHz, DMSO) 137.5, 136.8, 129.5, 128.5. LRMS (ESI): *m/z* 355.2 $(M + Na)^+$. HRMS-ESI (*m/z*): $(M + Na)^+$ calcd for $C_{18}H_{15}Cl_2NaP^+$, 355.0181; found, 355.1249.

***N*-(*tert*-Butyldimethylsilyl)methanesulfonamide, 3.13**



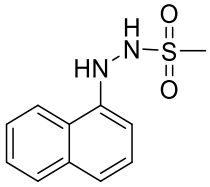
In a dry two neck flask methanesulfonamide (500 mg, 5.25 mmol) and triethylamine (1.47 mL, 10.51 mmol) was stirred in tetrahydrofuran (8 mL) at room temperature. Dropwise addition of a solution of *tert*-butyldimethylsilyl chloride (927 mg, 6.15 mmol) in toluene (3 mL) was performed over five minutes. The mixture was stirred for 72 hours and the formed suspension was filtered and ether was used to precipitate the impurities and isolate the product from the filtrate by concentrating under vacuum to obtain pure product (989 mg, 96%), m.p. 72 -74 °C. δ_H (400 MHz, DMSO) 7.03 (s, 1H, NH), 2.89 (s, 3H, CH_3), 0.87 (s, 9H, 3×CH_3), 0.15 (s, 6H, 2×CH_3). δ_c (100 MHz, DMSO) 44.5, 26.2, 17.7, -3.9. LRMS (ESI): *m/z* 210.1 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_7H_{20}NO_2SSi^+$, 210.0979; found, 210.0975.

Naphthalen-1-ylhydrazine, 3.14



α-Naphthylamine (1.4 mmol, 200 mg) was dissolved in concentrated hydrochloric acid (10 mL) and cooled to -5 °C in a salt-ice bath. Dropwise addition of a cold solution of sodium nitrite (1.4 mmol, 960 mg) in water (2 mL) was performed maintaining the temperature. Formed diazonium salt solution was added dropwise to a cold stannous (II) chloride (7 mmol, 1.6 g) in hydrochloric acid (10 mL). The reaction mixture was stirred at -5 °C for 2 hours and stored in the freezer overnight. Formed solid was filtered, washed with ether and dried to obtain pure product as a light brown solid (79 mg, 36%), m.p. 112 – 114 °C (lit. 118 -119 °C)[143]. δ_H (400 MHz, DMSO) 10.25 (br, 2H, NH_2), 8.78 (br, 1H, NH), 8.02 – 7.89 (m, 3H, ArH), 7.57 – 7.51 (m, 2H, ArH), 7.45 (t, J = 8 Hz, 1H, ArH), 6.93 (d, J = 8 Hz, 1H, ArH). δ_C (100 MHz, DMSO) 133.9, 128.6, 126.9, 126.4, 126.1, 126.0, 123.6, 122.1. LRMS (ESI): *m/z* 158.2 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_{10}H_{11}N_2^+$, 158.0844; found, 158.0838.

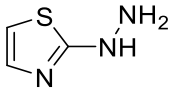
***N'*-(Naphthalen-1-yl)methanesulfonohydrazide, 3.15**



In a solution of dichloromethane (5 mL) and triethylamine (0.96 mmol, 134 µL), napthylen-1-ylhydrazine (0.48 mmol, 77 mg) was dissolved under nitrogen. Methanesulfonyl chloride (0.48 mmol, 38 µL) was added dropwise to the reaction mixture and stirred overnight at room temperature, under nitrogen. The reaction mixture was washed with water (three times), brine (once), and dried over magnesium sulphate. Obtained crude product was purified using silica gel column chromatography on 30% ethyl acetate: hexane to obtain the pure product as a yellow solid (35 mg, 31%), 178 – 181 °C. δ_H (400 MHz, DMSO) 10.25 (br, 2H, NH_2), 8.78 (br, 1H, NH), 8.02 – 7.89 (m, 3H, ArH),

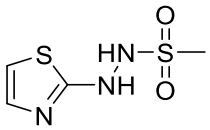
7.57 – 7.51 (m, 2H, ArH), 7.45 (t, J = 8 Hz, 1H, ArH), 6.93 (d, J = 8 Hz, 1H, ArH), 3.14 (s, 3H, CH_3). δ_C (100 MHz, $CDCl_3$) 142.1, 134.5, 128.9, 127.6, 126.3, 125.2, 124.9, 121.3, 119.3, 109.3, 42.6. LRMS (ESI): *m/z* 237.1 $(M + H)^+$, 157.2, 126.9, 108.2. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_{11}H_{13}N_2O_2S^+$, 237.0692; found, 237.0693.

2-Hydrazinyl-4,5-dihydrothiazole, 3.16



2-Aminothiazole (1 mmol, 100 mg) was dissolved in 25% aqueous hydrochloric acid (3 mL) and cooled to -10 °C. A cold solution of sodium nitrite (1 mmol, 69 mg) in water (1 mL) was added dropwise to the thiazole mixture maintaining the temperature. To this diazonium salt mixture, a cold solution of tin chloride dihydrate (2 mmol, 452 mg) in concentrated hydrochloric acid (1 mL) was added dropwise maintaining the temperature. The reaction mixture was stirred for 1 hour at 0 °C and basified with 50% (w/v) sodium hydroxide solution until pH 11. The resulting mixture was extracted three times with dichloromethane. Combined organic layers were dried over anhydrous magnesium sulphate and concentrated to obtain the pure product as a pale yellow solid (49 mg, 43%), m.p. 97–98 °C (96 -98 °C)[144]. δ_H (400 MHz, DMSO) 6.90 (d, J = 8 Hz, 1H, ArH), 6.84 (br, 2H, NH & NH_2), 6.53 (d, J = 8 Hz, 1H, ArH). δ_C (100 MHz, DMSO) 169.3, 139.1, 103.9, 40.2. LRMS (ESI): *m/z* 116.2 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_3H_6N_3S^+$, 116.0277; found, 116.0275.

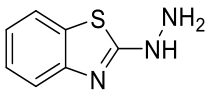
***N'*-(4,5-Dihydrothiazol-2-yl)methanesulfonohydrazide, 3.17**



2-Hydrazinyl-4,5-dihydrothiazole (1.03 mmol, 119 mg) was dissolved in pyridine (5 mL) and cooled to -10 °C. Dropwise addition of methanesulfonyl chloride (1.03 mmol, 108 μL) was performed with vigorous stirring to obtain a clear orange solution. The reaction mixture was warmed to 20 °C and stirred for 1 hour, then the reaction mixture was poured

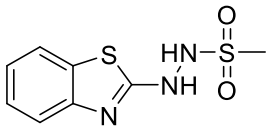
to a solution containing concentrated HCl (1 mL) in water (10 mL) with stirring. The resulting mixture was cooled to 4 °C and stirred for 1 hour. The reaction mixture was warmed to room temperature and extracted with ethyl acetate, dried over anhydrous $MgSO_4$ and concentrated in vacuum to yield crude product. Purification was performed on silica gel column chromatography on 5% methanol: dichloromethane to yield pure compound as a beige colour solid (46 mg, 31%), m.p. 188 - 190 °C. δ_H (400 MHz, DMSO) 7.23 (d, *J* = 4 Hz, 1H, ArH), 6.79 (d, *J* = 4 Hz, 1H, ArH), 2.87 (s, 3H, CH_3). δ_C (100 MHz, DMSO) 168.9, 139.1, 124.7, 40.9. LRMS (ESI): *m/z* 193.8 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_4H_8N_3O_2S_2^+$, 194.0052; found, 194.0048.

2-Hydrazinylbenzo*[d]*thiazole, 3.18



2-Chlorobenzothiazole (3.8 mmol, 652 mg) and hydrazine hydrate (12.3 mmol, 596 μL) was refluxed in ethanol (3 mL) for 40 minutes. Cooling the reaction mixture to room temperature yielded pure product as white needle shaped crystals (833 mg, 98%), m.p. 148 – 150 °C (lit. 150 – 152 °C)[145]. δ_H (400 MHz, DMSO) 8.98 (br, 1H, NH), 7.65 (d, *J* = 8 Hz, 1H, ArH), 7.30 (d, *J* = 8 Hz, 1H, ArH), 7.18 (t, *J* = 8 Hz, 1H, ArH), 6.96 (t, *J* = 8 Hz, 1H, ArH). δ_C (100 MHz, DMSO) 174.3, 153.9, 130.9, 125.7, 121.4, 120.7, 118.3. LRMS (ESI): *m/z* 166.1 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_7H_8N_3S^+$, 166.0433; found, 166.0432.

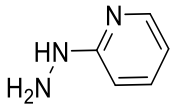
***N'*-(Benzo*[d]*thiazol-2-yl)methanesulfonohydrazide, 3.19**



2-Hydrazinylbenzo*[d]*thiazole (0.6 mmol, 100 mg) was dissolved in pyridine (1 mL) and cooled to -10 °C. Dropwise addition of methanesulfonyl chloride (0.6 mmol, 47 μL) was performed with vigorous stirring to obtain a clear orange solution. The reaction mixture was warmed to 20 °C and stirred for 1 hour, then the reaction mixture was poured into a

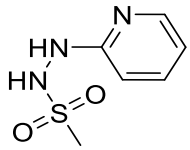
solution containing concentrated hydrochloric acid (1 mL) in water (10 mL) with stirring. The resulting mixture was cooled to 4 °C and stirred for 1 hour. The reaction mixture was warmed to room temperature and extracted with ethyl acetate, dried over anhydrous $MgSO_4$ and concentrated in vacuum to yield crude product. Purification was performed on silica gel column chromatography to yield pure compound as a beige colour solid (46 mg, 31%), m.p. 190 - 192 °C. δ_H (400 MHz, DMSO) 7.75 (d, *J* = 8 Hz, 1H, ArH), 7.42 (d, *J* = 8 Hz, 1H, ArH), 7.27 (t, *J* = 8 Hz, 1H, ArH), 7.09 (t, *J* = 8 Hz, 1H, ArH), 2.89 (s, 3H, CH_3). δ_C (100 MHz, DMSO) 171.8, 159.5, 126.3, 126.2, 122.2, 121.9, 121.8, 40.0. LRMS (ESI): *m/z* 244.1 $(M + H)^+$, 164.2, 134.9. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_8H_{10}N_3O_2S_2^+$, 244.0209; found, 244.0201.

2-Hydrazinylpyridine, 3.20



2-Chloropyridine (10.28 mmol, 1 mL) was mixed with hydrazine hydrate (157 mmol, 7.65 mL) and the mixture was refluxed in an oil bath for 6 hours. The reaction mixture was cooled down to room temperature and extracted with ether. Ethereal layers were dried over anhydrous magnesium sulphate and concentrated under vacuum to obtain pure product as a dark orange oil (846 mg, 74%). δ_H (400 MHz, $CDCl_3$) 8.11 (d, *J* = 8 Hz, 1H, ArH), 7.47 (t, *J* = 6 Hz, 1H, ArH), 6.69 (d, *J* = 6 Hz, 1H, ArH), 6.65 (d, *J* = 8 Hz, 1H, ArH), 6.04 (br, 1H, NH), 3.72 (br, 2H, NH_2). δ_C (100 MHz, $CDCl_3$) 161.2, 147.7, 137.5, 114.4, 107.0. LRMS (ESI): *m/z* 110.1 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_5H_8N_3^+$, 110.0713; found, 110.0712.

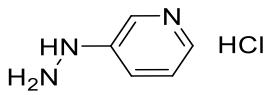
***N'*-(Pyridin-2-yl)methanesulfonohydrazide, 3.21**



2-Hydrazinylpyridine (2.75 mmol, 300 mg) was dissolved in pyridine (3 mL) and cooled to -10 °C. Addition of methanesulfonyl chloride (2.75 mmol, 213 μL) was performed while

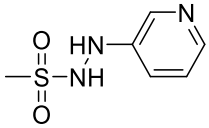
maintaining the temperature. After stirring for 0.5 hours, the reaction mixture was warmed to 20 °C and continued stirring for 1 hour. To this reaction mixture, 1:1 mixture of hydrochloric acid and water (1 mL) was poured. The reaction mixture was cooled to 4 °C and stirred for 1 hour. The yellow solid dropped was collected via suction filtration and dried under vacuum to obtain pure compound (380 mg, 73%), 158 – 160 °C. δ_H (400 MHz, DMSO) 9.10 (s, 1H, NH), 8.62 (s, 1H, NH), 8.04 (d, *J* = 8 Hz, 1H, ArH), 7.55 (t, *J* = 6 Hz, 1H, ArH), 6.81 (d, *J* = 6 Hz, 1H, ArH), 6.71 (t, *J* = 8 Hz, 1H, ArH), 2.96 (s, 3H,CH_3). δ_C (100 MHz, DMSO) 160.1, 147.8, 138.1, 115.4, 107.2, 38.9. LRMS (ESI): *m/z* 188.1 $(M + H)^+$, 109.2, 107.9. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_6H_{10}N_3O_2S^+$, 188.0488; found, 188.0484.

3-Hydrazinylpyridine hydrochloride, 3.22



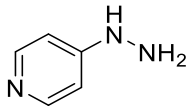
3-Pyridinamine (2 mmol, 188 mg) was dissolved in concentrated hydrochloric acid (2 mL) and cooled to -10 °C in a salt-ice bath. Dropwise addition of a cold solution of sodium nitrite (2 mmol, 138 mg) in water (1.2 mL) was performed maintaining the temperature. Formed diazonium salt solution was added dropwise to a cold stannous (II) chloride (5 mmol, 1.2 g) in hydrochloric acid (1.2 mL). The reaction mixture was stirred at -10 °C for 2 hours and stored in the freezer overnight. Formed solid was filtered and dried to obtain pure product as an off-white solid (168 mg, 77%), m. p. 143-145 °C. δ_H (400 MHz, DMSO) 8.72 (d, 1H, ArH), 8.65 (s, 1H, ArH), 7.74 (d, *J* = 8 Hz, 1H, ArH), 7.48 (d, *J* = 8 Hz, 1H, ArH). δ_C (100 MHz, $CDCl_3$) 145.0, 138.6, 137.5, 124.6, 122.9. LRMS (ESI): *m/z* 110.1 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_5H_8N_3^+$,110.0713; found, 110.0710.

***N'*-(Pyridin-3-yl)methanesulfonohydrazide, 3.23**



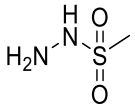
3-Hydrazinylpyridine hydrochloride (1.15 mmol, 167 mg) was dissolved in dichloromethane (5 mL) and triethylamine (3.45 mmol, 520 μL). To the reaction mixture, dropwise addition of methanesulfonyl chloride (1.15 mmol, 90 μL) was performed and stirring was continued overnight. The reaction mixture was washed with water and brine, dried over anhydrous magnesium sulphate and concentrated under vacuum. Obtained crude was purified using a silica gel column on 30% ethyl acetate: hexane to yield pure compound as a bright yellow powder (40 mg, 15%), m. p. 98 -100 °C. δ_H (400 MHz, $CDCl_3$) 8.71 (d, *J* = 6 Hz, 1H, ArH), 8.63 (d, *J* = 6 Hz, 1H, ArH), 7.71 (d, *J* = 6 Hz, 1H, ArH), 7.44 (d, *J* = 6 Hz, 1H, ArH), 3.43 (s, 3H, CH_3). δ_C (100 MHz, $CDCl_3$) 145.2, 138.6, 137.8, 124.6, 122.5, 42.6. LRMS (ESI): *m/z* 188.0 $(M + H)^+$, 108.2, 107.5. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_6H_{10}N_3O_2S^+$, 188.0488; found, 188.0485.

4-Hydrazinylpyridine, 3.24



Hydrazine hydrate (100 mmol, 4.9 mL) and 4-chloropyridine (25 mmol, 3.75 g) was refluxed in ethanol (20 mL) for 20 hours. The mixture was cooled to room temperature and further in a freezer and the resulting needle crystals were washed with ethanol and collected to obtain the product as white needles (2.07 g, 75%), m.p. 66 – 68 °C (lit. 30 °C)[99]. δ_H (400 MHz, DMSO) 8.01 (d, *J* = 8 Hz, 2H, ArH), 6.79 (d, *J* = 8 Hz, 2H, ArH), 7.08 (br, 2H, NH_2). δ_C (100 MHz, DMSO) 158.7, 143.4, 106.0. LRMS (ESI): *m/z* 110.2 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_5H_8N_3^+$, 110.0713; found, 110.0710.

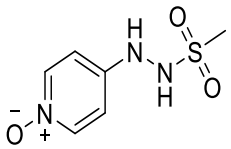
Methanesulfonohydrazide, 3.25



Hydrazine monohydrate (10 mmol, 774 μL) was dissolved in water (1.5 mL) and cooled to 0 °C. Addition of methanesulfonyl chloride (10 mmol, 485 μL) was performed dropwise followed by the addition of 2M sodium hydroxide solution (0.01 mmol, 5 mL) while

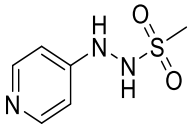
maintaining the temperature at 0 °C. The reaction mixture was warmed to room temperature and then concentrated in vacuum. Ether (30 mL) was added to the concentrate and the resulting solid removed. The filtrate was concentrated to obtain the product as a white solid (808 mg, 74%), m.p. 79-81 °C (lit. 50 – 52 °C)[146]. δ_H (400 MHz, $CDCl_3$) 5.55 (br, 1H, NH), 3.85 (br, 2H, NH_2), 3.00 (s, 3H, CH_3). δ_C (100 MHz, $CDCl_3$) 43.2. LRMS (ESI): *m/z* 111.0 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $CH_7N_2O_2S^+$, 111.0223; found, 111.0220.

4-(2-(Methylsulfonyl)hydrazinyl)pyridine 1-oxide, 3.26



4-Chloropyridine-*N*-oxide (100 mg) and methanesulfonohydrazide (78.6 mg) was dissolved in DMF (10 mL). The mixture was refluxed for 3 days. The brown solid obtained was isolated after cooling the reaction mixture to room temperature. The solid was recrystallized with MeOH to obtain a beige solid as the pure product (40 mg, 28%), m.p. 153 -155 °C. δ_H (400 MHz, DMSO) 8.68 (d, *J* = 8 Hz, 2H, ArH), 7.92 (d, *J* = 8 Hz, 2H, ArH), 2.48 (s, 3H, CH_3). δ_C (100 MHz,DMSO) 141.2, 139.8, 128.3, 40.1. LRMS (ESI): *m/z* 204.1 $(M + H)^+$, 110.5, 96.2. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_6H_{10}N_3O_3S^+$, 204.0437; found, 204.0430.

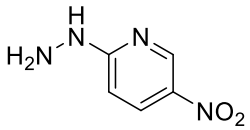
***N'*-(Pyridin-4-yl)methanesulfonohydrazide, 3.27**



A suspension of 4-(2-(methylsulfonyl)hydrazinyl)pyridine 1-oxide (100 mg, 0.5 mmol), acetic acid (4.6 µL, 0.09 mmol), Pd/C (10% w/w, 10 mg) in ethanol (15 mL) was placed under a hydrogen atmosphere. The reaction mixture was heated at 70 °C for 24 hours and filtered through a bed of Celite. The filterate was concentrated to obtain a white solid as the final compound (91 mg, quantitative yield), m.p. 78 -81 °C. δ_H (400 MHz, DMSO)

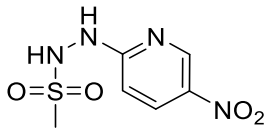
8.94 (d, J = 8 Hz, 2H, ArH), 8.24 (d, J = 8 Hz, 2H, ArH), 2.50 (s, 3H, CH3). δ_C (100 MHz, $CDCl_3$) 155.9, 150.0, 190.5, 43.0. LRMS (ESI): *m/z* 188.2 $(M + H)^+$, 109.9. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_6H_{10}N_3O_2S^+$, 188.0488; found, 188.0488.

2-Hydrazinyl-5-nitropyridine, 3.28



2-Chloro-5-nitropyridine (1.89 mmol, 300 mg) was dissolved upon heating in ethanol (11 mL) until a clear solution was obtained. A solution of hydrazine hydrate (1.89 mmol, 92 μL) was created with ethanol (1 mL). Later was added dropwise to the 2-chloro-5-nitropyridine solution to obtain a yellow solution. Upon refluxing this mixture for 1 hour, addition of potassium hydroxide (1.89 mmol, 107 mg) was followed and refluxing was continued for 2 hours. Obtained green paste was stored at -20 °C overnight. The resulting precipitate was collected via vacuum filtration, washed with cold ethanol and dried. The solid was recrystallized from acetonitrile to obtain pure compound (120 mg, 41%), m.p. 120 -124 °C. δ_H (400 MHz, DMSO) 9.17, (br, 1H, ArH), 8.86 (br, 1H, ArH), 8.15 (d, J = 8 Hz, 1H, ArH), 6.81 (d, J = 8 Hz, 1H, NH), 4.66 (br, 2H, NH_2). δ_C (100 MHz, $CDCl_3$) 164.8, 143.6, 140.2, 133.6, 108.6. LRMS (ESI): *m/z* 155.2 $(M - H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_5H_7N_4O_2^+$, 155.0564; found, 155.0566.

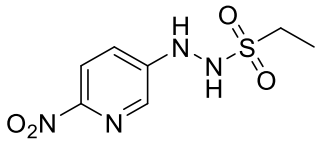
***N*'-(5-Nitropyridin-2-yl)methanesulfonohydrazide, 3.29**



5-Hydrazinyl-2-nitropyridine (0.32 mmol, 50 mg) was dissolved in pyridine (1 mL) and cooled to -10 °C. Dropwise addition of methanesulfonyl chloride (0.32 mmol, 25 μL) was performed with vigorous stirring to obtain a cleat orange solution. The reaction mixture was warmed to 20 °C and stirred for 1 hour, then the reaction mixture was poured to a solution containing concentrated HCl (1 mL) in water (10 mL) with stirring. The resulting mixture was cooled to 4 °C and stirred for 1 hour. The reaction mixture was warmed to

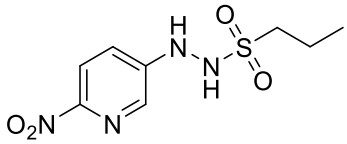
room temperature and extracted with ethyl acetate, dried over anhydrous $MgSO_4$ and concentrated in vacuum to yield pure compound as a beige colour solid (57 mg, 76%), m.p. 68 – 74 °C. δ_H (400 MHz, DMSO) 10.01 (s, 1H, NH), 9.64 (s, 1H, NH), 8.94 (s, 1H, ArH), 8.34 (d, *J* = 8 Hz, 2H, ArH), 6.93 (d, *J* = 8 Hz, 1H, ArH), 2.92 (s, 3H, CH_3). δ_C (100 MHz, DMSO) 146.2, 137.2, 133.8, 106.2, 39.2. LRMS (ESI): *m/z* 233.0 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_6H_9N_4O_4S^+$, 233.0339; found, 233.0338, $(M + Na)^+$ calcd for $C_6H_8N_4NaO_4S^+$, 255.0158; found, 255.0127.

_N'_-(6-Nitropyridin-3-yl)ethanesulfonohydrazide, 3.30



2-Hydrazinyl-5-nitropyridine (100 mg, 0.648 mmol) was dissolved in pyridine (2 mL) and cooled to -10 °C. Addition of ethanesulfonyl chloride (62 µL, 0.648 mmol) was performed. The reaction mixture was warmed to room temperature and stirred for 1 hour. Addition of a mixture of concentrated HCl (10µL) in water (0.5 mL) followed by cooling the mixture in the fridge for 1 hour. The reaction mixture was warmed to room temperature, filtered to give a filterate which was concentrated to obtain the product as a yellow solid (140 mg, 88%), m.p. 78 -80 °C. δ_H (400 MHz, DMSO) 9.98 (br, 1H, NH), 9.68 (br, 1H, NH), 8.94 (s, 1H, ArH), 8.33 (d, *J* = 12 Hz, 1H, ArH), 6.92 (d, *J* = 8 Hz, 1H, ArH), 3.15 (q, *J* = 8 Hz, 2H, CH_2), 1.24 (t, *J* = 8 Hz, 3H, CH_3). δ_C (100 MHz, DMSO) 146.2, 137.2, 133.9, 124.4, 106.4, 106.1, 44.9. LRMS (ESI): *m/z* 247.1 $(M + H)^+$, 155.1, 125.2. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_7H_{11}N_4O_4S^+$, 247.0496; found, 247.0496.

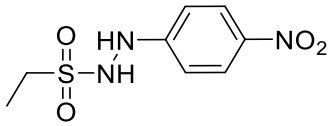
_N'_-(6-Nitropyridin-3-yl)propane-1-sulfonohydrazide, 3.31



2-Hydrazinyl-5-nitropyridine (100 mg, 0.648 mmol) was dissolved in pyridine (2 mL) and cooled to -10 °C. Addition of 1-propanesulfonyl chloride (73 µL, 0.648 mmol) was

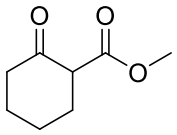
performed. The reaction mixture was warmed to room temperature and stirred for 1 hour. Addition of a mixture of concentrated HCl (10µL) in water (0.5 mL) and followed by cooling the mixture in the fridge for 1 hour. The reaction mixture was warmed to room temperature, filtered and the filtrate concentrated to obtain the product as a yellow solid (154 mg, 91%), m. p. 99 – 102 °C. δ_H (400 MHz, $CDCl_3$) 10.00 (br, 1H, NH), 9.66 (br, 1H, NH), 8.94 (s, 1H, ArH), 8.34 (d, *J* = 8 Hz, 1H, ArH), 6.93 (d, *J* = 8 Hz, 1H, ArH), 3.13 (m, 2H, CH_2), 1.74 (m, 2H, CH_2), 0.97 (t, *J* = 8 Hz, 3H, CH_3). δ_C (100 MHz, $CDCl_3$)162.0, 146.3, 137.2, 133.9, 106.3, 40.2, 17.1, 13.1. LRMS (ESI): *m/z* 261.2 $(M + H)^+$, 154.5, 123.5, 110.0. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_8H_{13}N_4O_4S^+$, 261.0652; found, 261.0650.

N'-(4-Nitrophenyl)ethanesulfonohydrazide, 3.32



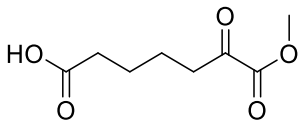
(4-Nitrophenyl)hydrazine (0.65 mmol, 100 mg) was dissolved in dichloromethane (5 mL) and triethylamine (1.3 mmol, 182 µL). To the reaction mixture, dropwise addition of methanesulfonyl chloride (0.65 mmol, 62 µL) was performed and stirring was continued overnight. The reaction mixture was washed with water, saturated sodium bicarbonate and brine, dried over anhydrous magnesium sulphate and concentrated under vacuum. Obtained crude was purified using a silica gel column on 30% ethyl acetate: hexane to yield pure compound as an orange powder (48 mg, 30%), m.p. 90-92 °C. δ_H (400 MHz, $CDCl_3$) 8.45 (d, *J* = 12 Hz, 2H, ArH), 8.11 (d, *J* = 12 Hz, 2H, ArH), 3.54 (q, *J* = 6 Hz, 2H, CH_2), 1.54 (t, *J* = 6 Hz, 3H, CH_3). δ_C (100 MHz, $CDCl_3$) 125.0, 125.1, 43.2, 7.5. LRMS (ESI): *m/z* 268.2 $(M + Na)^+$, 154.1, 123.1. HRMS-ESI (*m/z*): $(M + Na)^+$ calcd for $C_8H_{11}N_3NaO_4S^+$, 268.0362; found, 268.0363.

Methyl 2-oxocyclohexane-1-carboxylate, 4.1



(In a three neck flask quipped with a dropping funnel, a condenser connected to nitrogen gas inlet and a stopper), Sodium hydride (60% dispersion in mineral oil) (212.5 mmol, 5.10 g) and toluene (80 mL) were mixed, dimethyl carbonate (90 mmol, 7.6 mL) in toluene (8 mL) was added and the reaction was refluxed for 1 hour. Dropwise addition of a solution of cyclohexanone (36 mmol, 3.8 mL) in toluene (8 mL) was performed via the dropping funnel. A suspension formed immediately. The mixture was refluxed for 3 hours. The reaction was cooled to room temperature slowly after the reflux and further cooling was performed on a salt-ice bath. Reaction was quenched by slow addition of acetic acid (200 mL) and water (100 mL) upon which the solid got dissolved. The mixture was then extracted with ethyl acetate (3 times), dried over magnesium sulphate and concentrated to obtain the crude which had the product and mineral oil. Colum chromatography was performed using 30% ethyl acetate: hexane to isolate the product as a brown oil as a dark brown oil in quantitative yield (5.6 g). δ_H (400 MHz, $CDCl_3$) 3.75 (s, 3H, CH_3), 2.27 (t, *J* = 8 Hz, 2H, CH_2), 2.22 (t, *J* = 8 Hz, 2H, CH_2), 1.65 (m, 5H, CH_2, CH). δ_C (100 MHz, $CDCl_3$) 173.4, 172.1, 57.2, 51.3, 41.5, 29.1, 22.4, 21.9. LRMS (ESI): *m/z* 179.1 $(M + Na)^+$. HRMS-ESI (*m/z*): $(M + Na)^+$ calcd for $C_8H_{12}NaO_3^+$, 179.0679; found, 179.0675.

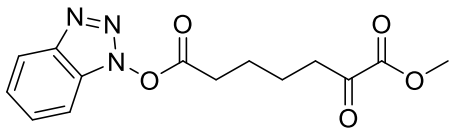
7-Methoxy-6,7-dioxoheptanoic acid, 4.2



In a three-neck flask quipped with a dropping funnel, gas adaptor connected to nitrogen gas and a thermometer, sodium hydride (60% dispersion in mineral oil) (35.98 mmol, 0.86 g) and tetrahydrofuran (36 mL) were mixed. Dropwise addition of a solution of methyl 2-oxocyclohexane-1-carboxylate (35.98 mmol, 5.62 g) in tetrahydrofuran (144 mL) was performed at room temperature. The reaction mixture was cooled to 0 °C, followed by dropwise addition of a solution of nitrosobenzene (37.78 mmol, 4.04 g) in tetrahydrofuran (39 mL) while maintaining the temperature at 0 °C. The reaction mixture was slowly

warmed to room temperature after the complete addition of nitrosobenzene solution. The reaction mixture was stirred for 30 minutes at room temperature. Reaction was quenched by adding 1M HCl (200 mL) dropwise to the reaction mixture at room temperature. The resulting mixture was extracted with ethyl acetate and combined organic layer were dried. Column chromatography was performed with 20% ethyl acetate and hexane to obtain pure product as a brown oil (5.4 g, 79%). δ_H (400 MHz, $CDCl_3$) 3.87 (s, 3H, CH_3), 2.88 (t, *J* = 4 Hz, 2H, CH_2), 2.39 (t, *J* = 4 Hz, 2H, CH_2), 1.69 (t, *J* = 4 Hz, 4H, CH_2). δ_C (100 MHz, $CDCl_3$) 193.6, 178.3, 165.1, 52.9, 38.8, 33.5, 29.4, 23.8. LRMS (ESI): *m/z* 187.2 $(M - H)^-$. HRMS-ESI (*m/z*): $(M - H)^-$ calcd for $C_8H_{11}O_5^-$, 187.0612; found, 187.0610.

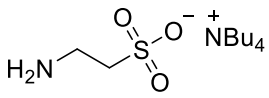
7-(*1H*-benzo[*d*][1,2,3]triazol-1-yl) 1-methyl 2-oxoheptanedioate, 4.3



Benzothiazole (3.79 g, 31.88 mmol) was dissolved in dichloromethane (270 mL) and addition of thionyl chloride (1 mL, 11.69 mmol) was performed and stirred at room temperature for 1 hour under nitrogen. Dropwise addition of triethylamine (3 mL, 21.25 mmol) was performed after cooling the reaction mixture to –70 °C and stirred for 0.5 hours followed by the addition of a solution of 7-methoxy-6,7-dioxoheptanoic acid (2 g, 10.62 mmol) in dichloromethane (3 mL). Reaction temperature was maintained for four hours and then the reaction mixture was allowed to warm to room temperature. After which the reaction mixture was washed with water, saturated sodium hydrogen carbonate and brine. The organic layer was dried and concentrated to obtain the pure product as a brown oil (2.8 g, 84%). δ_H (400 MHz, $CDCl_3$) 8.30 (d, *J* = 8 Hz, 1H, ArH), 8.13 (d, *J* = 8 Hz, 1H, ArH), 7.66 (t, *J* = 8 Hz, 1H, ArH), 7.25 (t, *J* = 8 Hz, 1H, ArH), 3.88 (s, 3H, CH_3), 3.47 (t, *J* = 8 Hz, 2H, CH_2), 2.97 (t, *J* = 8 Hz, 2H, CH_2), 1.97 (m, 2H, CH_2), 1.85 (m, 2H, CH_2). δ_C (100 MHz, $CDCl_3$) 190.6, 172.0, 161.4, 130.5, 128.8, 128.7, 123.2, 120.2, 114.4, 53.0, 38.9,

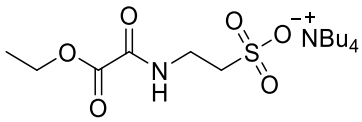
35.1, 23.6, 22.3. LRMS (ESI): *m/z* 306.2 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_{14}H_{16}N_3O_5^+$, 306.1084; found, 306.1083.

Tetrabutylammonium 2-aminoethane-1-sulfonate, 4.6



Taurine (2.3 g, 19 mmol) was added to a stirring solution of a tetrabutylammonium hydroxide in water (2M, 95 mL) and stirred overnight at room temperature. The reaction mixture was extracted with dichloromethane to obtain the pure product as a viscous oil (6.65 g, Quantitative yield). δ_H (400 MHz, DMSO) 3.17 (t, *J* = 8 Hz, 8H, 4×CH_2), 2.76 (t, *J* = 8 Hz, 2H, CH_2), 2.48 (t, *J* = 8 Hz, 2H, CH_2), 1.56 (m, 8H, 4×CH_2), 1.30 (m, 8H, 4×CH_2), 0.93 (t, *J* = 8 Hz, 12H, 4×CH_3). δ_C (100 MHz, DMSO) 58.0, 55.1, 39.0, 23.5, 19.7, 14.0. LRMS-ESI (*m/z*): $(C_{16}H3_6N)^+$ 242.19, $(C_2H_6NO_3S)^-$ 490.24.

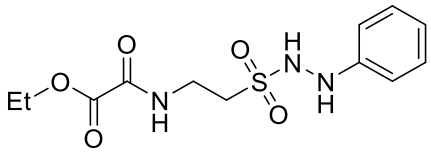
Tetrabutylammonium 2-(2-ethoxy-2-oxoacetamido)ethane-1-sulfonate, 4.7



Tetrabutylammonium 2-aminoethane-1-sulfonate (240 mg, 0.65 mmol) was dissolved in acetonitrile (10 mL) followed by addition of ethyl 2-chloro-2-oxoacetate (80 µL, 072 mmol) under nitrogen. The reaction was stirred at room temperature for 18 hours. The resulted solid was filtered out and the filtrate was concentrated to obtain the product as an orange oil (266 mg, 88%). δ_H (400 MHz, DMSO) 4.22 (m, 2H, CH_2), 3.40 (t, *J* = 8 Hz, 2H, CH_2), 3.16 (t, *J* = 8 Hz, 8H, 4×CH_2), 2.59 (t, *J* = 8 Hz, 2H, CH_2), 1.57 (m, 8H, 4×CH_2), 1.30 (m, 8H, 4×CH_2), 1.25 (m, 2H, CH_2), 0.93 (t, *J* = 4 Hz, 12H, 4×CH_3). δ_C (100 MHz,

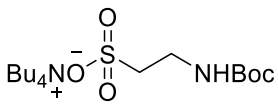
DMSO) 159.5, 158.7, 62.86, 62.81, 59.1, 49.8, 36.1, 24.2, 20.0, 13.8. LRMS-ESI (*m/z*): $(C_{16}H3_6N)^+$ 242.28, $(C_6H_{10}NO_6S)^-$ 123.83.

Ethyl 2-oxo-2-((2-((2-phenylhydrazinyl)sulfonyl)ethyl)amino)acetate, 4.9



2-Amino-*N'*-phenylethane-1-sulfonohydrazide (30 mg, 0.135 mmol) was dissolved in dry dichloromethane (3 mL) and the mixture was cooled on an ice bath. Addition of triethylamine (40 µL, 0.278 mmol) was followed by dropwise addition of a solution of ethylchlorooxoacetate (16 µL, 0.135 mmol). The reaction mixture was warmed to room temperature slowly and stirred at room temperature overnight. The reaction mixture was washed with water, 2M HCl, saturate sodium bicarbonate and brine. The organic layer was dried and concentrated. The crude product was washed with ether to obtain the product as a pale-yellow oil (10 mg, 22%). δ_H (400 MHz, $CDCl_3$) 7.96 (t, *J* = 8 Hz, 2H, ArH), 7.66 (d, *J* = 8 Hz, 1H, ArH), 7.60 (t, *J* = 8 Hz, 2H, ArH), 4.31 (m, 2H, CH_2), 3.79 (m, 2H, CH_2), 3.01 (m, 2H, CH_2), 1.35 (m, 3H, CH_3). δ_C (100 MHz, $CDCl_3$) 161.0, 158.9, 150.9, 113.5, 128.6, 122.6, 61.5, 58.6, 33.0, 13.6. LRMS (ESI): *m/z* 316.1 $(M + H)^+$, 138.2, 107.5, 101.8. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_{12}H_{18}N_3O_5S^+$, 316.0962; found, 316.0960.

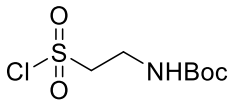
Tetrabutylammonium 2-((*tert*-butoxycarbonyl)amino)ethane-1-sulfonate, 4.12



To a solution of taurine (300 mg, 2.39 mmol) in water (2.4 mL), tetrabutylammonium hydroxide (0.2 M, 12 mL) was added followed by the dropwise addition of a solution of di-*tert*-butyl dicarbonate (523 mg, 2.39 mmol) in acetone (8 mL). The mixture was stirred overnight at room temperature. The reaction mixture was then concentrated to remove acetone and the remaining water mixture was extracted 3 times with dichloromethane. The organic layers were combined, dried and concentrated to obtain a pale-yellow oil (1.11 g, quantitative yield). δ_H (400 MHz, $CDCl_3$) 3.53 (m, 2H, CH_2), 3.28 (m, 8H, 4 CH_2), 2.91

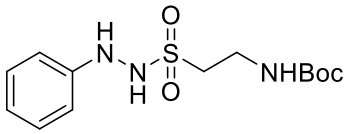
(m, 2H, CH_2), 1.64 (m, 8H, 4 CH_2), 1.44 (m, 8H, 4 CH_2), 1.39 (s, 9H, 3 CH_3), 1.00 (t, *J* = 8 Hz, 12H, 4 CH_3). δ_C (100 MHz, $CDCl_3$) 153.1, 58.8, 53.3, 50.8, 30.9, 28.5, 24.0, 19.7, 13.7. LRMS-ESI (*m/z*): $(C_{16}H3_6N)^+$ 242.19, $(C_7H_{14}NO_5S)^-$ 224.06.

***tert*-Butyl (2-(chlorosulfonyl)ethyl)carbamate, 4.13**



Tetrabutylammonium 2-((*tert*-butoxycarbonyl)amino)ethane-1-sulfonate (770 mg, 1.65 mmol) was dissolved in dry tetrahydrofuran (5 mL) in a two neck flask under nitrogen. Addition of triphosgene (795 mg, 0.65 mmol) performed and the reaction mixture was stirred for 30 minutes. The reaction mixture was concentrated under vacuum and the resulting yellow oil was purified by column to obtain the product as a white solid (240 mg, 60%, m.p. 62 – 64 °C) after drying under vacuum overnight. δ_H (400 MHz, $CDCl_3$) 5.10 (br, 1H, NH), 3.90 (m, 2H, CH_2), 3.77 (m, 2H, CH_2), 1.44 (s, 9H, 3 CH3). δ_C (100 MHz, $CDCl_3$) 155.7, 80.7, 65.0, 38.0, 28.3. LRMS (ESI): *m/z* 244.2 $(M + H)^+$. HRMS-ESI (*m/z*): $(M[^{35}Cl]+H^+)$ calcd for $C_7H_{15}[^{35}Cl]NO_4S^+$, 244.0405; found, 244.0405, $(M[^{37}Cl]+H^+)$ calcd for $C_7H_{15}[^{37}Cl]NO_4S^+$, 246.0405; found, 246.0404.

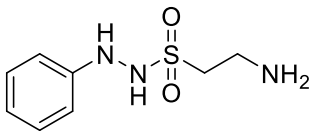
***tert*-Butyl (2-((2-phenylhydrazinyl)sulfonyl)ethyl)carbamate, 4.14**



To a stirred solution of phenylhydrazine (48 µL, 0.48 mmol) in dry tetrahydrofuran (5 mL) 4-methylmorpholine (140 µL, 0.92 mmol) was added and the solution was cooled to 0 °C. Dropwise addition of a solution of *tert*-butyl (2-(chlorosulfonyl)ethyl)carbamate (100 mg, 0.41 mmol) in dry tetrahydrofuran (10 mL) was performed while maintaining the temperature. The reaction mixture was stirred for 1 hour at 0 °C and then at room temperature overnight. The reaction mixture was concentrated under vacuum and the resulting crude was diluted with ethyl acetate and washed consecutively with 1M $KHSO_{4(aq)}$, brine, 5% $NaHCO_{3(aq)}$ and brine. The organic layer was dried and concentrated under vacuum to obtain the product as a red-orange oil (129 mg, 79%). δ_H (400 MHz, $CDCl_3$)

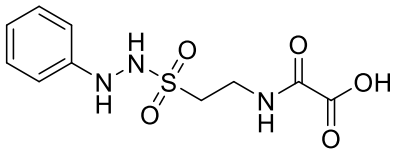
9.13 (s, 1H, NH), 7.26 (t, J = 8 Hz, 2H, ArH), 6.99 (d, J = 8 Hz, 2H, ArH), 6.93 (t, J = 8 Hz, 1H, ArH), 6.30 (s, 1H, NH), 6.04 (br, 1H, NH), 4.05 (t, J = 8 Hz, 2H, CH_2), 3.29 (t, J = 8 Hz, 2H, CH_2), 1.54 (s, 9H, 3 CH_3). δ_C (100 MHz, $CDCl_3$) 163.0, 146.6, 129.5, 121.8, 113.9, 85.5, 48.5, 34.9, 28.1. LRMS (ESI): *m/z* 316.4 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_{13}H_{22}N_3O_4S^+$, 316.3955; found, 316.3955.

2-Amino-*N'*-phenylethane-1-sulfonohydrazide, 4.15



Tert-butyl (2-((2-phenylhydrazinyl)sulfonyl)ethyl)carbamate (30 mg, 0.09 mmol) was dissolved in dichloromethane (2 mL) and cooled to 0 ^oC, followed by addition of trifluoacetic acid (20 µL, 0.19 mmol) and the reaction was warmed to room temperature and stirred for 3 hours. The reaction mixture was washed with saturated sodium bisulfite solution. The organic layer was dried and concentrated to obtain the product in quantitative yield as an orange oil (20 mg). δ_H (400 MHz, $CDCl_3$) 9.14 (s, 2H, NH_2), 7.96 (t, J = 8 Hz, 2H, ArH), 7.66 (d, J = 8 Hz, 1H, ArH), 7.60 (t, J = 8 Hz, 2H, ArH), 4.04 (m, 2H, CH_2), 3.15 (m, 2H, CH_2). δ_C (100 MHz, $CDCl_3$) 150.8, 128.7, 121.7, 112.8, 62.5, 30.8. LRMS (ESI): *m/z* 216.2 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_8H_{14}N_3O_2S^+$, 216.0801; found, 216.0803.

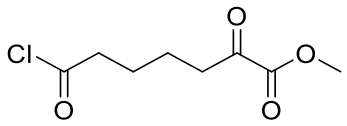
2-Oxo-2-((2-((2-phenylhydrazinyl)sulfonyl)ethyl)amino)acetic acid, 4.16



Ethyl 2-oxo-2-((2-((2-phenylhydrazinyl)sulfonyl)ethyl)amino)acetate (25 mg, 0.079 mmol) was dissolved in ethanol. To the solution, addition of ethanolic sodium hydroxide (2M, 80 µL) was performed and the mixture was heated to 50 °C for 2 hours. The resulted mixture was concentrated, and the residue was diluted with ethyl acetate. Resulting solution was washed with 2M HCl, water and dried prior to concentrating under vacuum

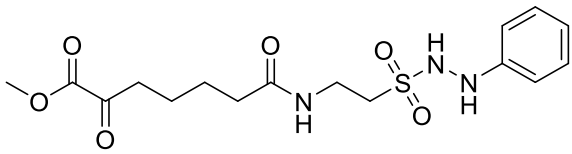
to obtain the pure compound as a pale-yellow oil (10 mg, 44%). δ_H (400 MHz, $CDCl_3$) 7.88 (t, *J* = 4 Hz, 2H, ArH), 7.56 (d, *J* = 4 Hz, 1H, ArH), 7.50 (t, *J* = 4 Hz, 2H, ArH), 4.25 (m, 2H, CH_2), 3.98 (m, 2H, CH_2). δ_C (100 MHz, $CDCl_3$) 160.5, 157.9, 150.9, 130.5, 128.5, 122.9, 60.5, 33.9. LRMS (ESI): *m/z* 286.1 $(M - H)^-$, 179.5. HRMS-ESI (*m/z*): (M - H)- calcd for $C_{10}H_{12}N_3O_5S^-$, 286.0503; found, 286.0501.

Methyl 7-chloro-2,7-dioxoheptanoate, 4.17



7-Methoxy-6,7-dioxoheptanoic acid (200 mg, 0.98 mmol) was dissolved in dichloromethane (5 mL) and addition of $SOCl_2$ (80 µL, 1.08 mmol) was followed. The reaction mixture was refluxed for 2 hours and excess $SOCl_2$ was distilled out and the residue was obtained as a brown oil (513 mg, 94%). δ_H (400 MHz, $CDCl_3$) 3.89 (s, 3H, CH_3), 2.93 (m, 8H, $4CH_2$). δ_C (100 MHz, $CDCl_3$) 195.5, 170.9, 160.5, 52.3, 47.0, 40.5, 24.6, 22.5. LRMS (ESI): *m/z* 206.9 $(M + H)^+$. HRMS-ESI (*m/z*): $(M[^{35}Cl]+H^+)$ calcd for $C_8H_{12}[^{35}Cl]O_4^+$, 207.0419; found, 207.0417, $(M[^{37}Cl]+H^+)$ calcd for $C_8H_{12}[^{37}Cl]O_4^+$, 209.0419; found, 209.0419.

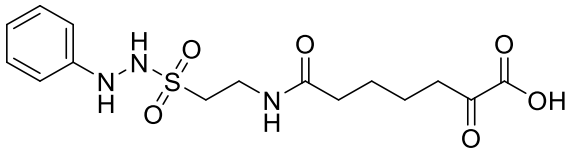
Methyl 2,7-dioxo-7-((2-((2-phenylhydrazinyl)sulfonyl)ethyl)amino)heptanoate, 4.18



2-Amino-*N'*-phenylethane-1-sulfonohydrazide (100 mg, 0.464 mmol) was dissolved in dry dichloromethane (10 mL) and the mixture was cooled on an ice bath. Addition of triethylamine (130 µL, 0.929 mmol) was followed by dropwise addition of a solution of methyl 6-chloro-2,6-dioxohexanoate (92 mg, 0.464 mmol). The reaction mixture was warmed to room temperature slowly and stirred overnight then washed with water, 2M HCl, saturate sodium bicarbonate and brine. The organic layer was dried and concentrated. The crude product was washed with ether to obtain the product as an orange oil (83 mg,

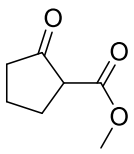
46%). δ_H (400 MHz, $CDCl_3$) 7.96 (t, *J* = 8 Hz, 2H, ArH), 7.66 (d, *J* = 8 Hz, 1H, ArH), 7.60 (t, *J* = 8 Hz, 2H, ArH), 4.20 (m, 2H, CH_2), 3.90 (m, 2H, CH_2), 3.89 (s, 3H, CH_3), 3.51 (t, *J* = 8 Hz, 2H, CH_2), 2.22 (t, *J* = 8 Hz, 2H, CH_2), 1.54 (m, 4H, 2×CH_2). δ_C (100 MHz, $CDCl_3$) 194.0, 172.3, 161.2, 150.0, 129.8, 123.6, 112.9, 60.2, 50.9, 39.1, 36.5, 33.6, 27.8, 22.6. LRMS (ESI): *m/z* 387.0 $(M + H)^+$, 280.5, 177.9, 140.2. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_{16}H_{24}N_3O_6S^+$, 386.1380; found, 386.1379.

2,7-Dioxo-7-((2-((2-phenylhydrazinyl)sulfonyl)ethyl)amino)heptanoic acid, 4.19



Methyl 2,7-dioxo-7-((2-((2-phenylhydrazinyl)sulfonyl)ethyl)amino)heptanoate (50 mg, 0.129 mmol) was dissolved in methanol and addition of methanolic sodium hydroxide (2M, 130 µL) was performed. The resulted mixture was concentrated, and the residue was diluted with ethyl acetate. Resulted solution was washed with 2M HCl, water and dried prior to concentrating under vacuum for obtaining the pure compound as a pale-yellow oil (20 mg, 41%). δ_H (400 MHz, $CDCl_3$) 7.88 (t, *J* = 8 Hz, 2H, ArH), 7.75 (d, *J* = 8 Hz, 1H, ArH), 7.48 (t, *J* = 8 Hz, 2H, ArH), 4.22 (m, 2H, CH_2), 3.88 (m, 2H, CH_2) 2.10 (m, 4H, 2 CH_2), 1.48 (m, 4H, 2 CH_2). δ_C (100 MHz, $CDCl_3$) 194.9, 172.6, 162.3, 151.3, 129.3, 122.9, 113.6, 60.2, 39.6, 36.3, 33.6, 27.9, 23.1. LRMS (ESI): *m/z* 370.0 $(M - H)^-$, 264.1, 157.5. HRMS-ESI (*m/z*): $(M - H)^-$ calcd $C_{15}H_{20}N_3O_6S^-$, 370.1078; found, 370.1076.

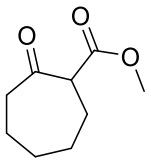
Methyl 2-oxocyclopentane-1-carboxylate, 4.20



(In a three neck flask quipped with a dropping funnel, a condenser connected to nitrogen gas inlet and a stopper), Sodium hydride (60% dispersion in mineral oil) (140 mmol, 5.6 g) and toluene (80 mL) were mixed, dimethyl carbonate (125 mmol, 10.5 mL) in toluene (13 mL) was added and the reaction was refluxed for 1 hour. Dropwise addition of a

solution of cyclopentanone (50 mmol, 4.5 mL) in toluene (6 mL) was performed via the dropping funnel. A suspension formed immediately. The mixture was refluxed for 3 hours. The reaction was cooled to room temperature slowly after the reflux and further cooling was performed on a salt-ice bath. Reaction was quenched by slow addition of acetic acid (200 mL) and water (100 mL) upon which the solid was dissolved. The mixture was then extracted with ethyl acetate (3 times), dried over magnesium sulphate and concentrated to obtain the crude which had the product and mineral oil. Upon standing in the vacuum pump, the product solidified which was washed thoroughly with hexane to remove mineral oil and to obtain the product as a dark brown solid in quantitative yield (7.1 g), m. p. 110 – 112 °C (lit. 109 – 111 °C)[147]. δ_H (400 MHz, $CDCl_3$) 3.75 (s, 3H, CH_3), 3.17 (t, J = 8 Hz, 1H, CH), 1.87 (m, 2H, CH_2), 1.70 (m, 4H, 2×CH_2). δ_C (100 MHz, $CDCl_3$) 199.3, 166.7, 77.0, 55.8, 34.8, 25.2, 24.0. LRMS (ESI): *m/z* 164.8 $(M + Na)^+$. HRMS-ESI (*m/z*): $(M + Na)^+$ calcd for $C_7H_{10}NaO_3^+$, 165.0522; found, 165.0521.

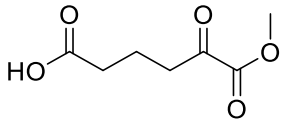
Methyl 2-oxocycloheptane-1-carboxylate, 4.21



In a three-neck flask quipped with a dropping funnel, a condenser connected to nitrogen gas inlet and a stopper, sodium hydride (60% dispersion in mineral oil) (213 mmol, 5.10 g) and toluene (80 mL) were mixed. Then dimethyl carbonate (90 mmol, 7.6 mL) in toluene (8 mL) was added and the reaction was refluxed for 1 hour. Dropwise addition of a solution of cycloheptanone (36 mmol, 4.3 mL) in toluene (6 mL) was performed via a dropping funnel. A suspension formed immediately. The mixture was refluxed for 3 hours. The reaction was then cooled to room temperature slowly and further cooling was performed on a salt-ice bath. Reaction was quenched by the slow addition of acetic acid (200 mL) and water (100 mL) upon which the solid got dissolved. The mixture was then extracted with ethyl acetate (3 times), dried over magnesium sulphate and concentrated to obtain the crude material which had the product and mineral oil. Upon standing in the vacuum pump, the product solidified which was washed thoroughly with hexane to remove mineral oil and to give the product as a dark brown solid in quantitative yield (6.62 g), m. p. 25 – 28 °C. δ_H

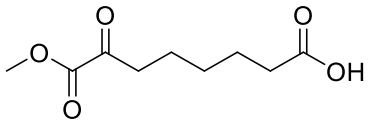
(400 MHz, $CDCl_3$) 3.72 (s, 3H, CH_3), 3.55 (d, 1H, CH_2), 2.61 (m, 2H, CH_2), 2.41 (m, 4H, CH_2), 1.86 (m, 2H, CH_2), 1.44 (m,2H, CH_2). δ_C (100 MHz, $CDCl_3$) 208.9, 171.0, 58.9, 52.2, 43.1, 29.7, 28.0, 27.6, 24.4. LRMS (ESI): *m/z* 171.1 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_9H_{15}O_3^+$, 171.1016; found, 171.1015.

6-Methoxy-5,6-dioxohexanoic acid, 4.22



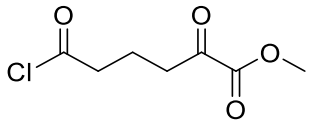
In a three-neck flask quipped with a dropping funnel, gas adaptor connected to nitrogen gas and a thermometer, sodium hydride (60% dispersion in mineral oil) (25.0 mmol, 0.59 g) and tetrahydrofuran (25 mL) were mixed. Dropwise addition of a solution of methyl 2-oxocyclopentane-1-carboxylate (25.0 mmol, 3.5 g) in tetrahydrofuran (100 mL) was performed at room temperature. The reaction mixture was cooled to 0 °C, followed by dropwise addition of a solution of nitrosobenzene (25.8 mmol, 2.21 g) in tetrahydrofuran (25 mL) while maintaining the temperature at 0 °C. The reaction mixture was slowly warmed to room temperature after the complete addition of nitrosobenzene solution. The reaction mixture was stirred for 30 minutes at room temperature. Reaction was quenched by adding 1M HCl (50 mL) dropwise to the reaction mixture at room temperature. The resulting mixture was extracted with ethyl acetate and combined organic layer were dried. Column chromatography was performed with 20% ethyl acetate and hexane to obtain the pure product as an orange brown oil (3.4 g, 80%). δ_H (400 MHz, $CDCl_3$) 3.87 (s, 3H, CH_3), 2.96 (t, J = 8 Hz, 2H, CH_2), 2.45 (t, J = 8 Hz, 2H, CH_2), 1.98 (t, J = 8 Hz, 2H, CH_2). δ_C (100 MHz, $CDCl_3$) 193.2, 178.2, 161.2, 53.0, 38.2, 32.4, 17.8. LRMS (ESI): *m/z* 173.1 $(M - H)^-$. HRMS-ESI (*m/z*): $(M - H)^-$ calcd for $C_7H_9O_5^-$, 173.0455; found, 173.0453.

8-Methoxy-7,8-dioxooctanoic acid, 4.23



In a three-neck flask quipped with a dropping funnel, gas adaptor connected to nitrogen gas and a thermometer), sodium hydride (60% dispersion in mineral oil) (43.5 mmol, 1.04 g) and tetrahydrofuran (44 mL) were mixed. Dropwise addition of a solution of methyl 2-oxocycloheptane-1-carboxylate **35** (43.5 mmol, 7.4 g) in tetrahydrofuran (174 mL) was performed at room temperature. The reaction mixture was cooled to 0 °C, followed by dropwise addition of a solution of nitrosobenzene (43.9 mmol, 4.7 g) in tetrahydrofuran (47 mL) while maintaining the temperature at 0 °C. The reaction mixture was slowly warmed to room temperature after the complete addition of nitrosobenzene solution. The reaction mixture was stirred for 30 minutes at room temperature. Reaction was quenched by adding 1M HCl (200 mL) dropwise to the reaction mixture at room temperature. The resulting mixture was extracted with ethyl acetate and combined organic layer were dried and concentrated in vacuum to yield crude product which was purified by washing with hexane to result in compound as a brown solid (7.4 g, 85%), m. p. 45 – 48 °C. δ_H (400 MHz, $CDCl_3$) 3.86 (s, 3H, CH_3), 2.85 (t, *J* = 8 Hz, 2H, CH_2), 2.36 (t, *J* = 8 Hz, 2H, CH_2), 1.66 (m, 4H, CH_2), 1.41 (m, 2H, CH_2). δ_C (100 MHz, $CDCl_3$) 193.9, 178.3, 161.5, 53.0, 39.0, 33.5, 28.3, 24.3, 22.5. LRMS (ESI): *m/z* 201.1 $(M - H)^-$. HRMS-ESI (*m/z*): $(M - H)^-$ calcd for $C_9H_{13}O_5^-$, 201.0768; found, 201.0766.

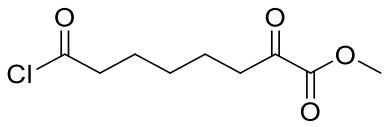
Methyl 6-chloro-2,6-dioxohexanoate, 4.24



6-Methoxy-5,6-dioxohexanoic acid (500 mg, 2.87 mmol) was dissolved in dichloromethane (5 mL) and addition of $SOCl_2$ (462 µL, 3.58 mmol) was followed. The reaction mixture was refluxed for 2 hours and excess $SOCl_2$ was distilled out and the residue was obtained as a brown oil (278 mg, 50%). δ_H (400 MHz, $CDCl_3$) 3.93 (s, 3H, CH_3), 2.99 (m, 2H, CH_2), 2.58 (m, 4H, 2×CH_2). δc (100 MHz, $CDCl_3$) 193.8, 172.5, 161.0, 50.9, 42.9, 37.0, 18.5. LRMS (ESI): *m/z* 193.0 $(M + H)^+$. HRMS-ESI (*m/z*): $(M[^{35}Cl]+H^+)$

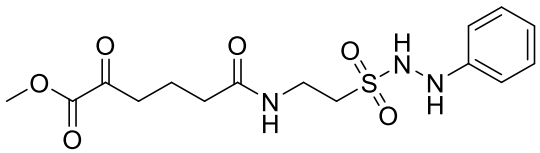
calcd for $C_7H_{10}[^{35}Cl]O_4^+$, 193.0262; found, 193.0261, ($M[^{37}Cl]+H^+$) calcd for $C_7H_{10}[^{37}Cl]O_4^+$, 195.0262; found, 195.0263.

Methyl 8-chloro-2,8-dioxooctanoate, 4.25



8-Methoxy-7,8-dioxooctanoic acid (500 mg, 24 mmol) was dissolved in dichloromethane (5 mL) and addition of $SOCl_2$ (2.3 mL, 30.9 mmol) was followed. The reaction mixture was refluxed for 2 hours and excess $SOCl_2$ was distilled out and the residue was obtained as a brown oil (513 mg, 94%). δ_H (400 MHz, $CDCl_3$) 3.87 (s, 3H, CH_3), 2.90 (t, 2H. CH_2), 2.86 (t, J = 4 Hz, 2H. CH_2), 1.74 (m, 2H, CH_2), 1.67 (m, 2H, CH_2), 1.40 (m, 2H, CH_2). δ_C (100 MHz, $CDCl_3$) 193.7, 173.6, 161.7, 53.0, 46.8, 38.9, 27.6, 24.7, 22.4. LRMS (ESI): *m/z* 220.9 $(M + H)^+$. HRMS-ESI (*m/z*): ($M[^{35}Cl]+H^+$) calcd for $C_9H_{14}[^{35}Cl]O_4^+$, 221.0575; found, 221.0576, ($M[^{37}Cl]+H^+$) calcd for $C_9H_{14}[^{37}Cl]O_4^+$, 223.0575; found, 223.0577.

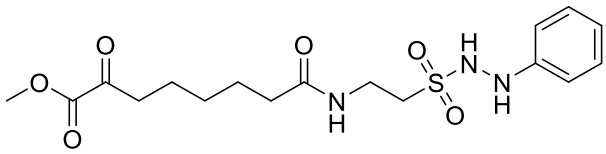
Methyl 2,6-dioxo-6-((2-((2-phenylhydrazinyl)sulfonyl)ethyl)amino)hexanoate, 4.26



2-amino-*N'*-phenylethane-1-sulfonohydrazide (70 mg, 0.325 mmol) was dissolved in dry dichloromethane (5 mL) and the mixture was cooled on an ice bath. Addition of triethylamine (90 µL, 0.650 mmol) was followed by dropwise addition of a solution of methyl 6-chloro-2,6-dioxohexanoate (63 mg, 0.325 mmol). The reaction mixture was warmed to room temperature slowly and stirred at room temperature overnight. The reaction mixture was washed with water, 2M HCl, saturate sodium bicarbonate and brine. The organic layer was dried and concentrated. The crude product was washed with ether to obtain the product as a yellow oil (74 mg, 56%). δ_H (400 MHz, $CDCl_3$) 7.96 (t, J = 4 Hz, 2H, ArH), 7.66 (d, J = 4 Hz, 1H, ArH), 7.60 (t, J = 4 Hz, 2H, ArH), 3.93 (s, 3H, CH_3), 3.73 (m, 2H, CH_2), 3.46 (m, 2H, CH_2), 2.99 (m, 2H, CH_2), 2.58 (m, 4H, 2×CH_2). δ_C (100

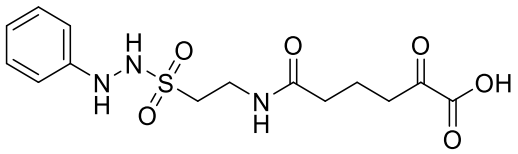
MHz, $CDCl_3$) 195.3, 172.6, 160.9, 150.6, 129.5, 122.6, 113.2, 58.6, 50.6, 37.7, 37.5, 33.6, 20.5. LRMS (ESI): *m/z* 372.3 $(M + H)^+$, 234.1, 159.6, 107.3. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_{15}H_{22}N_3O_6S^+$, 372.1224; found, 372.01222.

Methyl 2,8-dioxo-8-((2-((2-phenylhydrazinyl)sulfonyl)ethyl)amino)octanoate, 4.27



2-Amino-*N'*-phenylethane-1-sulfonohydrazide (100 mg, 0.464 mmol) was dissolved in dry dichloromethane (10 mL) and the mixture was cooled on an ice bath. Addition of triethylamine (130 µL, 0.929 mmol) was followed by dropwise addition of a solution of methyl 8-chloro-2,8-dioxooctanoate (103 mg, 0.464 mmol). The reaction mixture was warmed to room temperature slowly and stirred at room temperature overnight. The reaction mixture was washed with water, 2M HCl, saturate sodium bicarbonate and brine. The organic layer was dried and concentrated. The crude product was washed with ether to obtain the product as an orange oil (96 mg, 52%). δ_H (400 MHz, $CDCl_3$) 7.96 (t, *J* = 8 Hz, 2H, ArH), 7.66 (d, *J* = 8 Hz, 1H, ArH), 7.60 (t, *J* = 8 Hz, 2H, ArH), 3.87 (s, 3H, CH_3), 3.77 (m, 2H, CH_2), 3.65 (m, 2H, CH_2), 2.90 (t, *J* = 4 Hz, 2H. CH_2), 2.86 (t, *J* = 4 Hz, 2H. CH_2), 1.74 (m, 2H, CH_2), 1.67 (m, 2H, CH_2), 1.40 (m, 2H, CH_2). δ_C (100 MHz, $CDCl_3$) 193.6, 172.6, 161.2, 151.3, 129.3, 122.9, 113.6, 60.3, 52.3, 40.2, 36.9, 33.5, 28.6, 25.6, 24.6. LRMS (ESI): *m/z* 400.2 $(M + H)^+$, 185.9, 214.2. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_{17}H_{26}N_3O_6S^+$, 400.1537; found, 400.1536.

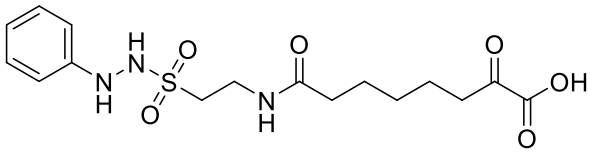
2,6-Dioxo-6-((2-((2-phenylhydrazinyl)sulfonyl)ethyl)amino)hexanoic acid, 4.28



Methyl 2,6-dioxo-6-((2-((2-phenylhydrazinyl)sulfonyl)ethyl)amino)hexanoate (50 mg, 0.134 mmol) was dissolved in methanol and addition of methanolic sodium hydroxide (2M, 140 µL) was performed. The resultant mixture was concentrated, and the residue was

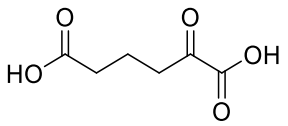
diluted with ethyl acetate. resultant solution was washed with 2M HCl, water and dried prior to concentrating under vacuum for obtaining the pure compound as a pale-yellow oil (25 mg, 52%). δ_H (400 MHz, $CDCl_3$) 7.96 (t, *J* = 8 Hz, 2H, ArH), 7.66 (d, *J* = 8 Hz, 1H, ArH), 7.60 (t, *J* = 8 Hz, 2H, ArH), 3.76 (m, 2H, CH_2), 3.59 (m, 2H, CH_2), 2.66 (t, *J* = 4 Hz, 2H, CH_2), 2.10 (t, *J* = 4 Hz, 4H, CH_2), 1.49. δ_C (100 MHz, $CDCl_3$) 195.2, 172.5, 160.9, 151.3, 129.8, 122.3, 113.2, 60.3, 37.7, 36.2, 33.6, 20.5. LRMS (ESI): *m/z* 355.8 $(M - H)^-$, 249.5, 144.2. HRMS-ESI (*m/z*): $(M - H)^-$ calcd for $C_{14}H_{18}N_3O_6S^-$, 356.0922; found, 356.0921.

2,8-Dioxo-8-((2-((2-phenylhydrazinyl)sulfonyl)ethyl)amino)octanoic acid, 4.29



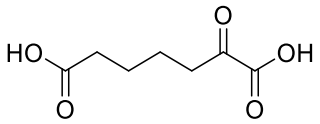
Methyl 2,8-dioxo-8-((2-((2-phenylhydrazinyl)sulfonyl)ethyl)amino)octanoate (50 mg, 0.125 mmol) was dissolved in methanol and addition of methanolic sodium hydroxide (2M, 125 µL) was performed. The resultant mixture was concentrated, and the residue was diluted with ethyl acetate. resultant solution was washed with 2M HCl, water and dried prior to concentrating under vacuum for obtaining the pure compound as a pale-yellow oil (23 mg, 48%). δ_H (400 MHz, $CDCl_3$) 7.96 (t, *J* = 8 Hz, 2H, ArH), 7.66 (d, *J* = 8 Hz, 1H, ArH), 7.60 (t, *J* = 8 Hz, 2H, ArH), 3.98 (m, 2H, CH_2), 3.85 (m, 2H, CH_2), 2.85 (t, *J* = 4 Hz, 2H, CH_2), 2.36 (t, *J* = 4 Hz, 2H, CH_2), 1.66 (m, 2H, CH_2), 1.41 (m, 2H, CH_2). δ_C (100 MHz, $CDCl_3$) 193.5, 172.3, 162.9, 151.3, 129.3, 122.6, 113.3, 60.3, 40.2, 36.6, 33.6, 28.6, 25.6, 25.0. LRMS (ESI): *m/z* 384.2 $(M - H)^-$, 278.9, 170.9. HRMS-ESI (*m/z*): $(M - H)^-$ calcd $C_{16}H_{22}N_3O_6S^-$, 384.1235; found, 384.1234.

2-Oxohexanedioic acid, 4.30



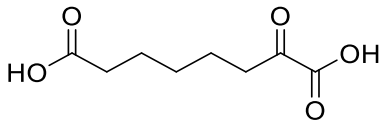
6-Methoxy-5,6-dioxohexanoic acid (175 mg, 1.12 mmol) was dissolved in 10% (v/v) methanol: dichloromethane (5 mL) and addition of methanolic NaOH (2.5 M, 538 µL) was added dropwise. The yellow solid was collected by filtration after 1.5 hours in sufficient purity (110 mg, 60%), m. p. 122 – 125 °C (lit. 126 -127 °C)[148]. δ_H (400 MHz, D_2O) 2.66 (t, 2H, CH_2), 2.10 (t, 4H, CH_2), 1.49. δ_C (100 MHz, D_2O) 194.8, 178.5, 162.9, 38.0, 33.1, 19.6. LRMS (ESI): *m/z* 79.1 $(M - H)^{2-}$. HRMS-ESI (*m/z*): $(M - H)^{2-}$ calcd for $C_6H_6O_5^{2-}$, 79.0113; found, 79.0111.

2-Oxoheptanedioic acid, 4.31



Methyl 7-chloro-2,7-dioxoheptanoate (200 mg, 1.06 mmol) was dissolved in 10% methanol: dichloromethane (5 mL) and addition of methanolic NaOH (2.5 M, 510 µL) was added dropwise. The solid was collected after 1.5 hours to obtain the pure compound as a yellow solid (110 mg, 60%), m. p. 52 – 55 °C. δ_H (400 MHz, D_2O) 2.10 (m, 4H, 2 CH_2), 1.48 (m, 4H, 2 CH_2). δ_C (100 MHz, D_2O) 183.9, 172.0, 171.1, 37.4, 37.3, 25.6. LRMS (ESI): *m/z* 88.0 $(M - H)^{2-}$. HRMS-ESI (*m/z*): $(M - H)^{2-}$ calcd for $C_7H_8O_5^{2-}$, 86.0192; found, 86.0190.

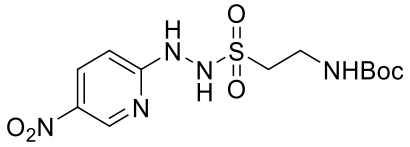
2-Oxooctanedioic acid, 4.32



8-Methoxy-7,8-dioxooctanoic acid (150 mg, 0.74 mmol) was dissolved in 10% (v/v) methanol: dichloromethane (5 mL) and addition of methanolic NaOH (2.5 M, 360 µL) was added dropwise. The solid was collected after 1.5 hours to obtain the pure compound as an

orange-brown solid (85 mg, 61%), m. p. 45 – 48 °C. δ_H (400 MHz, D_2O) 2.66 (t, *J* = 8 Hz, 2H, CH_2), 2.08 (m, 4H, 2 CH_2), 1.47 (m, 4H, 2 CH_2). δ_C (100 MHz, D_2O) 196.0, 177.9, 162.9, 40.1, 33.5, 28.9, 25.1, 24.9. LRMS (ESI): *m/z* 92.9 $(M - H)^{2-}$. HRMS-ESI (*m/z*): $(M - H)^{2-}$ calcd for $C_8H_{10}O_5^{2-}$, 93.0269; found, 93.0266.

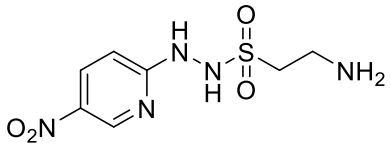
***tert*-Butyl (2-((2-(5-nitropyridin-2-yl)hydrazinyl)sulfonyl)ethyl)carbamate, 5.1**



To a stirred solution of 2-hydrazinyl-5-nitropyridine (486 mg, 2.87 mmol) in dry tetrahydrofuran (10 mL) 4-methylmorpholine (983 µL, 8.93 mmol) was added and the solution was cooled to 0 °C. Dropwise addition of a solution of *tert*-butyl ((chlorosulfonyl)methyl)carbamate (700 mg, 2.87 mmol) in dry tetrahydrofuran (20 mL) was performed while maintaining the temperature. The reaction mixture was stirred for 1 hour at 0 °C and then at room temperature overnight. The reaction mixture was concentrated under vacuum and the resulting crude was diluted with ethyl acetate and washed consecutively with 1M $KHSO_{4(aq)}$, brine, 5% $NaHCO_{3(aq)}$, brine. The organic layer was dried and concentrated under vacuum. Obtained crude product was purified through column chromatography to obtain the product as a brown oil (600 mg, 20%). δ_H (400 MHz, $CDCl_3$) 8.98 (s, 1H, NH_2), 8.53 (s, 1H, NH), 8.32 (d, *J* = 8 Hz, 1H, ArH), 7.29 (s, 1H, ArH), 7.21 (d, *J* = 8 Hz, 1H, ArH), 5.11 (d, 1H, NH), 3.73 (m, 2H, CH_2), 3.46 (m, 2H, CH_2), 2.01 (s, 9H, 3 CH_3). δ_C (100 MHz, $CDCl_3$) 151.9, 146.9, 155.3, 118.6, 125.9, 79.6,

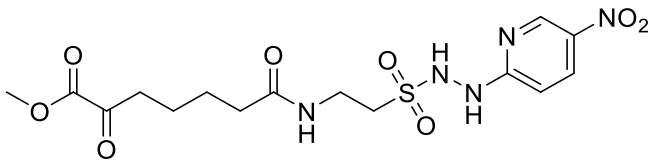
60.2, 34.9, 28.9. LRMS (ESI): *m/z* 384.2 $(M + Na)^+$. HRMS-ESI (*m/z*): $(M + Na)^+$ calcd for $C_{12}H_{19}N_5NaO_6S^+$, 384.0948; found, 384.0945.

2-Amino-*N'*-(5-nitropyridin-2-yl)ethane-1-sulfonohydrazide, 5.2



tert-Butyl (2-((2-(6-nitropyridin-3-yl)hydrazinyl)sulfonyl)ethyl)carbamate (200 mg, 0.553 mmol) was dissolved in dichloromethane (15 mL) and cooled to 0 °C, followed by addition of trifluoacetic acid (850 µL, 1.10 mmol) and the reaction was warmed to room temperature and stirred for 3 hours. The reaction mixture was washed with saturated sodium bisulfite solution. The organic layer was dried and concentrated to obtain the product in quantitative yield as an orange oil (66 mg). δ_H (400 MHz, $CDCl_3$) 9.00 (s, 2H, NH_2), 8.53 (s, 1H, NH), 8.32 (d, *J* = 8 Hz, 1H, ArH), 7.29 (s, *J* = 8 Hz, 1H, ArH), 7.21 (d, *J* = 8 Hz, 1H, ArH), 5.11 (d, 1H, NH), 3.87 (m, 2H, CH_2), 3.73 (m, 2H, CH_2). δ_C (100 MHz, $CDCl_3$) 151.5, 146.1, 136.5, 125.5, 118.9, 62.5, 32.6. LRMS (ESI): *m/z* 284.3 $(M + Na)^+$, 155.1, 137.8, 108.2. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_7H_{11}N_5NaO_4S^+$, 284.0424; found, 284.0423.

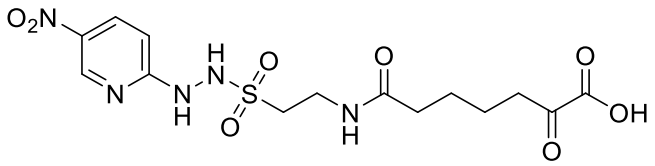
Methyl-7-((2-((2-(5-nitropyridin-2-yl)hydrazinyl)sulfonyl)ethyl)amino)-2,7-dioxoheptanoate, 5.3



Freshly prepared methyl 7-chloro-2,7-dioxoheptanoate (118 mg, 0.57 mmol) was dissolved in dichloromethane. To the cold solution addition of triethylamine (310 µL, 2.22 mmol) followed by the addition of a solution of 2-amino-*N'*-(5-nitropyridin-2-yl)ethane-1-sulfonohydrazide (150 mg, 0.57 mmol) in dichloromethane (3 mL) was performed maintaining the temperature. The resulting reaction mixture was kept at 0 °C for 1 hour and overnight at room temperature. Reaction mixture was washed successively with water,

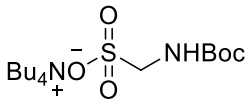
brine and the dried dichloromethane layer was concentrated under vacuum. The crude product was subjected to column chromatography in 20% EtOAc: Hex to obtain the product as a red oil (102 mg, 41%). δ_H (400 MHz, $CDCl_3$) 8.32 (d, *J* = 8 Hz, 1H, ArH), 7.29 (s, 1H, ArH), 7.21 (d, *J* = 8 Hz, 1H, ArH), 4.20 (m, 2H, CH_2), 3.99 (m, 2H, CH_2), 3.89 (s, 3H, CH_3), 2.93 (m, 4H, $4CH_2$), 2.76 (m, 4H, $4CH_2$). δ_C (100 MHz, $CDCl_3$) 194.2, 172.6, 164.8, 161.3, 143.5, 140.2, 133.3, 108.9, 60.0, 51.6, 38.5, 36.3, 33.5, 27.6, 22.6. LRMS (ESI): *m/z* 423.3 $(M + H)^+$, 278.4, 171.9, 155.3. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_{15}H_{22}N_5O_8S^+$, 432.1184; found, 432.1182.

7-((2-((2-(5-Nitropyridin-2-yl)hydrazinyl)sulfonyl)ethyl)amino)-2,7-dioxoheptanoic acid, 5.4



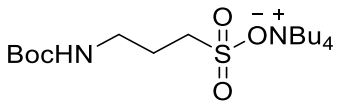
Methyl 7-((2-((2-(5-nitropyridin-2-yl)hydrazinyl)sulfonyl)ethyl)amino)-2,7-dioxoheptanoate (100 mg, 0.23 mmol) was dissolved in dichloromethane (5 mL) and methanol (2 mL). Addition of methanolic sodium hydroxide (2M, 750 µL) was performed and the reaction mixture was stirred at room temperature for 4 hours. The resulting solution was concentrated and diluted with ethyl acetate and washed with 2M HCl, water and brine. The dried organic layer was concentrated to obtain the pure product as a pale brown oil (59 mg, 60%). δ_H (400 MHz, $CDCl_3$) 8.30 (d, *J* = 8 Hz, 1H, ArH), 7.30 (s, 1H, ArH), 7.23 (d, *J* = 8 Hz, 1H, ArH), 4.20 (m, 2H, CH_2), 3.89 (m, 2H, CH_2), 2.90 (m, 4H, $4CH_2$), 2.70 (m, 4H, $4CH_2$). δ_C (100 MHz, $CDCl_3$) 195.0, 172.5, 164.5, 161.2, 143.5, 140.3, 133.2, 108.6, 60.5, 40.2, 36.9, 33.6, 27.4, 22.8. LRMS (ESI): *m/z* 416.1 $(M - H)^-$, 262.8, 156.9. HRMS-ESI (*m/z*): $(M - H)^-$ calcd for $C_{14}H_{18}N_5O_8S^-$, 416.0882; found, 416.0883.

Tetrabutylammonium ((*tert*-butoxycarbonyl)amino)methanesulfonate, **5.5**



Aminomethanesulfonic acid (300 mg, 2.69 mmol) was dissolved in water (2 mL) and addition of tetra-butylammonium hydroxide (0.2 M, 13.5 mL) was performed. To the reaction mixture, dropwise addition of a solution of di-*tert*-butyl dicarbonate (700 mg, 2.69 mmol) in acetone (8 mL) was performed. The reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated to remove acetone, and the remaining aqueous mixture was extracted with dichloromethane and the combined organic layers were dried and concentrated under vacuum to obtain the product as an oil (1 g, 68%). δ_H (400 MHz, $CDCl_3$) 4.17 (d, J = 8 Hz, 2H, CH_2), 3.28 (m, 8H, 4 CH_2), 1.45 (m, 16H, 8 CH_2), 1.26 (s, 9H, 3CH_3), 1.00 (t, J = 8 Hz, 12H, 3 CH_3). δ_C (100 MHz, $CDCl_3$) 156.5, 80.2, 66.2, 58.0, 28.6, 23.6, 20.0, 14.0. LRMS-ESI (*m/z*): $(C_{16}H_{36}N)^+$ 242.22, $(C_6H_{12}NO_5S)^-$ 210.06.

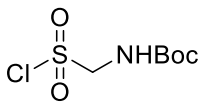
Tetrabutylammonium 3-((*tert*-butoxycarbonyl)amino)propane-1-sulfonate, 5.6



3-Amino-1-propanesulfonic acid (300 mg, 2.15 mmol) was dissolved in water (3 mL) and of *tetra*-butylammonium hydroxide (0.2 M, 11 mL) was performed. To the reaction mixture dropwise addition of a solution of Boc_2O (759 mg, 2.15 mmol) in acetone (8 mL) was performed. The reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated to remove acetone, and the remaining aqueous mixture was extracted with dichloromethane and the combined organic layers were dried and concentrated under vacuum to obtain the product as an oil (912.6 mg, 88%). δ_H (400 MHz, $CDCl_3$) 3.31 (m, 8H, CH_2), 2.87 (t, J = 8 Hz, 2H, CH_2), 2.10 (t, J = 8 Hz, 2H,CH_2), 1.99 (m, 2H, CH_2), 1.65 (m, 8H, CH_2), 1.45 (m, 8H, CH_2), 1.41 (s, 9H, CH_3), 1.01 (t, J = 8 Hz,

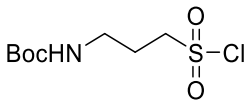
12H, CH_3). δ_C (100 MHz, $CDCl_3$) 156.2, 80.3, 57.6, 54.2, 41.1, 29.0, 24.6,23.6, 19.5, 13.5. LRMS-ESI (*m/z*): $(C_{16}H_{36}N)^+$, 242.22, $(C_8H_{16}NO_5S)^-$, 238.09.

***tert*-Butyl ((chlorosulfonyl)methyl)carbamate, 5.7**



Tetrabutylammonium ((*tert*-butoxycarbonyl)amino)methanesulfonate (1 g, 2.20 mmol) was dissolved in dry tetrahydrofuran (25 mL). Triphosgene (262 mg, 0.883 mmol) was added while stirring and the reaction mixture was stirred for 30 minutes. The reaction mixture was concentrated under vacuum. The resulting yellow oil was purified by column to obtain the product as an orange-brown oil (229 mg, 44%). δ_H (400 MHz, $CDCl_3$) 4.67 (d, J = 8 Hz, 2H, CH_2), 2.46 (s, 9H, 3 CH_3). δ_C (100 MHz, $CDCl_3$) 156.1, 82.6, 80.0, 29.0. LRMS (ESI): *m/z* 230.0 $(M + H)^+$. HRMS-ESI (*m/z*): $(M[^{35}Cl]+H^+)$ calcd for $C_6H_{13}[^{35}Cl]NO_4S^+$, 230.0248; found, 230.0247, $(M[^{37}Cl]+H^+)$ calcd for $C_6H_{13}[^{37}Cl]NO_4S^+$, 232.0248; found, 232.0247.

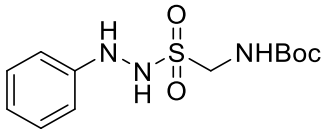
***tert*-Butyl (3-(chlorosulfonyl)propyl)carbamate, 5.8**



Tetrabutylammonium 3-((*tert*-butoxycarbonyl)amino)propane-1-sulfonate (912 mg, 1.89 mmol) was dissolved in dry dichloromethane (5 mL). To the solution, addition of DMF (63 µL, 0.81 mmol) and triphosgene (225 mg, 0.758 mmol) was performed. The reaction was stirred for 30 minutes and concentrated under vacuum. The crude reaction mixture was purified from column chromatography using 50% EtOAc: Hexane to obtain the product as a pale yellow oil (400 mg, 81%). δ_H (400 MHz, $CDCl_3$) 3.74 (m, 2H, CH_2), 3.32 (m, 2H, CH_2), 2.23 (m, 2H, CH_2), 1.44 (s, 9H, CH_3). δ_C (100 MHz, $CDCl_3$) 156.0, 80.2, 65.2, 41.0, 29.0, 27.6. LRMS (ESI): *m/z* 258.1 $(M + H)^+$. HRMS-ESI (*m/z*): $(M[^{35}Cl]+H^+)$

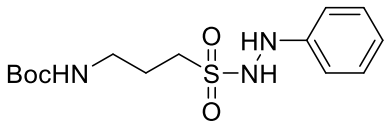
calcd for $C_8H_{17}[^{35}Cl]NO_4S^+$, 258.0561; found, 258.0560, ($M[^{37}Cl]+H^+$) calcd for $C_8H_{17}[^{37}Cl]NO_4S^+$, 260.0561; found, 260.0560.

***tert*-Butyl (((2-phenylhydrazinyl)sulfonyl)methyl)carbamate, 5.9**



To a stirred solution of phenylhydrazine (56 µL, 0.57 mmol) in dry tetrahydrofuran (5 mL) 4-methylmorpholine (167 µL, 1.52 mmol) was added and the solution was cooled to 0 °C. Dropwise addition of a solution of *tert*-butyl ((chlorosulfonyl)methyl)carbamate (112 mg, 0.48 mmol) in dry tetrahydrofuran (10 mL) was performed while maintaining the temperature. The reaction mixture was stirred for 1 hour at 0 °C and at room temperature overnight. The reaction mixture was concentrated under vacuum and the resulting crude was diluted with ethyl acetate and washed consecutively with 1M $KHSO_4$, brine, 5% $NaHCO_3$ and brine. The organic layer was dried and concentrated under vacuum to obtain the product as a red-orange oil (129 mg, 79). δ_H (400 MHz, $CDCl_3$) 7.76 (d, J = 8 Hz, 2H, ArH), 7.49 (m, 2H, ArH), 6.83 (d, J = 8 Hz, 1H, ArH), 4.13 (m, 1H, CH_2), 3.75 (m, 1H, CH_2), 1.56 (s, 9H, 3 CH_3). δ_C (100 MHz, $CDCl_3$) 156.1, 151.3, 130.0, 121.3, 113.6, 80.0, 72.1, 28.3. LRMS (ESI): *m/z* 324.2 $(M + Na)^+$. HRMS-ESI (*m/z*): $(M + Na)^+$ calcd for $C_{12}H_{19}N_3NaO_4S^+$, 324.0988; found, 324.0987.

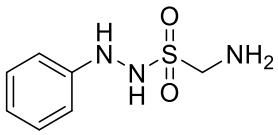
***tert*-Butyl (3-((2-phenylhydrazinyl)sulfonyl)propyl)carbamate, 5.10**



To a stirred solution of phenylhydrazine (214 µL, 2.17 mmol) in dry tetrahydrofuran (5 mL), 4-methylmorpholine (636 µL, 5.79 mmol) was added and the solution was cooled to 0 °C. Dropwise addition of a solution of *tert*-butyl (3-(chlorosulfonyl)propyl)carbamate (480 mg, 1.86 mmol) in dry tetrahydrofuran (10 mL) was performed while maintaining the temperature. The reaction mixture was stirred for 1 hour at 0 °C and at room temperature overnight. The reaction mixture was concentrated under vacuum and the resulting crude

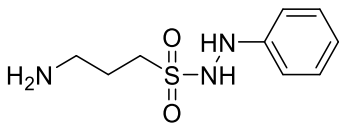
was diluted with ethyl acetate and washed consecutively with 1M $KHSO_{4(aq)}$, brine, 5% $NaHCO_{3(aq)}$ and brine. The organic layer was dried and concentrated under vacuum to obtain the product as an orange oil (256 mg, 41%). δ_H (400 MHz, $CDCl_3$) 7.94 (d, *J* = 8 Hz, 2H, ArH), 7.58 (d, *J* = 8 Hz, 2H, ArH), 6.85 (d, *J* = 8 Hz, 1H, ArH), 3.75 (t, *J* = 8 Hz, 2H, CH_2), 3.13 (t, *J* = 4 Hz, 2H, CH_2), 2.52 (t, *J* = 4 Hz, 2H, CH_2), 1.44 (s, 9H, CH_3). δ_C (100 MHz, $CDCl_3$) 155.2, 151.2, 130.0, 122.6, 113.0, 80.1, 55.0, 40.1, 28.5, 20.6. LRMS (ESI): *m/z* 330.5 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_{14}H_{24}N_3O_4S^+$, 330.1482; found, 330.1481.

1-Amino-*N'*-phenylmethanesulfonohydrazide, 5.11



tert-Butyl (((2-phenylhydrazinyl)sulfonyl)methyl)carbamate (100 mg, 0.331 mmol) was dissolved in dichloromethane (10 mL) and cooled, followed by the addition of trifluoacetic acid (50 µL, 0.663 mmol). Thereaction was warmed to room temperature and stirred for 3 hours. The reaction mixture was washed with saturated sodium bisulfite solution. The organic layer was dried and concentrated to obtain the product in quantitative yield as an orange oil (66 mg, Quantitative yield). δ_H (400 MHz, $CDCl_3$) 7.89 (d, *J* = 8 Hz, 2H, ArH), 7.58 (m, 2H, ArH), 6.78 (d, *J* = 8 Hz, 1H, ArH), 4.15 (m, 2H, CH_2). δ_C (100 MHz, $CDCl_3$) 150.6, 130.2, 121.9, 113.2, 70.5. LRMS (ESI): *m/z* 202.1 $(M + H)^+$, 126.2, 107.5. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_7H_{12}N_3O_2S^+$, 202.0645; found, 202.0644.

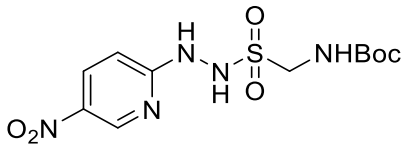
3-Amino-*N'*-phenylpropane-1-sulfonohydrazide, 5.12



tert-Butyl (3-((2-phenylhydrazinyl)sulfonyl)propyl)carbamate (125 mg, 0.379 mmol) was dissolved in dichloromethane (10 mL) and cooled, followed by the addition of trifluoacetic acid (58 µL, 0.758 mmol). The reaction was allowed to warm to room temperature and stirred for 3 hours. The reaction mixture was washed with saturated sodium bisulfite

solution, then the organic layer was dried and concentrated to obtain the product an orange oil (61 mg, 70%). δ_H (400 MHz, $CDCl_3$) 7.90 (d, *J* = 8 Hz, 2H, ArH), 7.57 (d, *J* = 8 Hz, 2H, ArH), 6.80 (d, *J* = 8 Hz, 1H, ArH), 3.70 (t, *J* = 4 Hz, 2H, CH_2), 3.13 (t, *J* = 4 Hz, 2H, CH_2), 2.55 (t, *J* = 4 Hz, 2H, CH_2). δ_C (100 MHz, $CDCl_3$) 151.3, 130.2, 122.9, 113.6, 60.0, 40.2, 23.3. LRMS (ESI): *m/z* 230.2 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_9H_{16}N_3O_2S^+$, 230.0958; found, 230.0957.

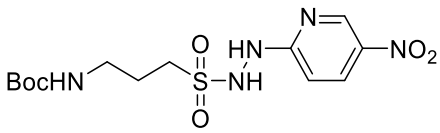
***tert*-Butyl (((2-(5-nitropyridin-2-yl)hydrazinyl)sulfonyl)methyl)carbamate, 5.13**



To a stirred solution of 2-hydrazinyl-5-nitropyridine (78 mg, 0.50 mmol) in dry tetrahydrofuran (5 mL) 4-methylmorpholine (157 µL, 1.42 mmol) was added and the solution was cooled to 0 °C. Dropwise addition of a solution of *tert*-butyl (2-(chlorosulfonyl)ethyl)carbamate (112 mg, 0.46 mmol) in dry tetrahydrofuran (10 mL) was performed while maintaining the temperature. The reaction mixture was stirred for 1 hour at 0 °C and then at room temperature overnight. The reaction mixture was concentrated under vacuum and the resulting crude was diluted with ethyl acetate and washed consecutively with 1M $KHSO_4$, brine, 5% $NaHCO_3$ and brine. The organic layer was dried and concentrated under vacuum to obtain the product as an orange oil (88 mg, 57%). δ_H (400 MHz, $CDCl_3$) 8.35 (s, 1H, NH), 8.04 (s, 1H, ArH), 7.76 (m, 1H, ArH), 7.48 (m, 2H, ArH), 5.57 (d, *J* = 8 Hz, 1H, CH_2), 1.56 (s, 9H, 3 CH_3). δ_C (100 MHz, $CDCl_3$) 156.1, 151.2,

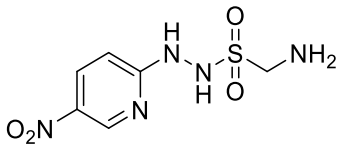
146.0, 136.9, 126.2, 118.2, 80.2, 72.3, 28.4. LRMS (ESI): *m/z* 348.2 $(M + Na)^+$. HRMS-ESI (*m/z*): $(M + Na)^+$ calcd for $C_{11}H_{18}N_5O_6S^+$, 348.0972; found, 348.0970.

tert-Butyl (3-((2-(5-nitropyridin-2-yl)hydrazinyl)sulfonyl)propyl)carbamate, 5.14



To a stirred solution of 2-hydrazinyl-5-nitropyridine (330 mg, 1.83 mmol) in dry tetrahydrofuran (5 mL), 4-methylmorpholine (577 µL, 5.07 mmol) was added and the solution was cooled to 0 °C. Dropwise addition of a solution of *tert*-butyl (3-(chlorosulfonyl)propyl)carbamate (473 mg, 1.83 mmol) in dry tetrahydrofuran (10 mL) was performed while maintaining the temperature. The reaction mixture was stirred for 1 hour at 0 °C and at room temperature overnight. The reaction mixture was concentrated under vacuum and the resulting crude was diluted with ethyl acetate and washed consecutively with 1M $KHSO_{4(aq)}$, brine, 5% $NaHCO_{3(aq)}$ and brine. The organic layer was dried and concentrated under vacuum to obtain the product as an orange oil (256 mg, 41%). δ_H (400 MHz, $CDCl_3$) 8.97 (s, 1H, ArH), 8.36 (d, *J* = 8 Hz, 1H, ArH), 7.35 (d, *J* = 8 Hz, 1H, ArH), 4.25 (t, *J* = 8 Hz, 2H, CH_2), 3.38 (m, 2H, CH_2), 2.79 (m, 2H, CH_2), 1.45 (s, 9H, CH_3). δ_C (100 MHz, $CDCl_3$) 165.0, 155.6, 140.2, 143.8, 133.3, 108.6, 80.0, 55.0, 40.5, 28.6, 20.5. LRMS (ESI): *m/z* 376.4 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_{13}H_{22}N_5O_6S^+$, 376.1285; found, 376.1284.

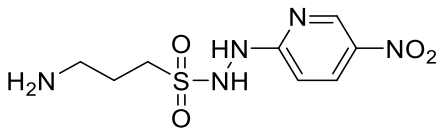
1-amino-N'-(5-nitropyridin-2-yl)methanesulfonohydrazide, 5.15



tert-Butyl (((2-(6-nitropyridin-3-yl)hydrazinyl)sulfonyl)methyl)carbamate (50 mg, 0.143 mmol) was dissolved in dichloromethane (5 mL) and cooled, followed by addition of trifluoacetic acid (22 µL, 0.287 mmol) and the reaction was warmed to room temperature and stirred for 3 hours. The reaction mixture was washed with saturated sodium bisulfite

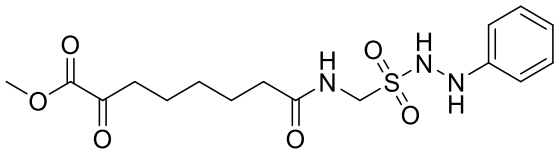
solution. Washed organic layer was dried and concentrated to obtain the product in quantitative yield as an orange oil (35 mg, Quantitative yield). δ_H (400 MHz, $CDCl_3$) 8.40 (s, 1H, NH), 8.04 (s, 1H, ArH), 7.76 (m, 1H, ArH), 7.48 (m, 1H, ArH), 5.59 (d, *J* = 12 Hz, 2H, CH_2). δ_C (100 MHz, $CDCl_3$) 151.6, 146.0, 135.9, 124.2, 118.4, 70.9. LRMS (ESI): *m/z* 248.3 $(M + H)^+$, 124.8, 123.5, 93.7. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_6H_{10}N_5O_4S^+$, 248.0448; found, 248.0448.

3-Amino-*N'*-(5-nitropyridin-2-yl)propane-1-sulfonohydrazide, 5.16



tert-Butyl (3-((2-(5-nitropyridin-2-yl)hydrazinyl)sulfonyl)propyl)carbamate (100 mg, 0.266 mmol) was dissolved in dichloromethane (10 mL) and cooled, followed by the addition of trifluoacetic acid (41 µL, 0.532 mmol). The reaction was allowed to warm to room temperature and stirred for 3 hours. The reaction mixture was washed with saturated sodium bisulfite solution, then the organic layer was dried and concentrated to obtain the product in quantitative yield as an orange oil (52 mg, 70%). δ_H (400 MHz, $CDCl_3$) 8.98 (d, *J* = 8 Hz, 1H, ArH), 8.34 (d, *J* = 8 Hz, 1H, ArH), 7.32 (d, *J* = 8 Hz, 1H, ArH), 4.23 (t, *J* = 4 Hz, 2H, CH_2), 3.35 (m, 2H, CH_2), 2.81 (m, 2H, CH_2). δ_C (100 MHz, $CDCl_3$) 164.6, 143.5, 140.5, 133.5, 108.9, 55.0, 40.5, 23.5. LRMS (ESI): *m/z* 276.3 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_8H_{14}N_5O_4S^+$, 276.0761; found, 276.0760.

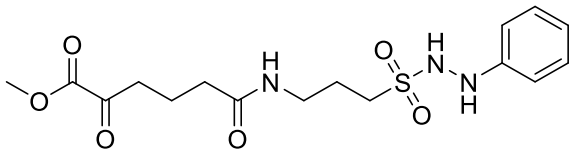
Methyl 2,8-dioxo-8-((((2-phenylhydrazinyl)sulfonyl)methyl)amino)octanoate, 5.17



Freshly prepared methyl 8-chloro-2,8-dioxooctanoate (163 mg, 0.74 mmol) was dissolved in dichloromethane (10 mL) and cooled. To the cold solution addition of triethylamine (310 µL, 2.22 mmol) followed by the addition of a solution of 1-amino-*N'*-(5-nitropyridin-2-yl)methanesulfonohydrazide (150 mg, 0.74 mmol) in dichloromethane (3 mL) was

performed maintaining the temperature. The resulting reaction mixture was kept at 0 °C for 1 hour and overnight at room temperature. Reaction mixture was washed successively with water, brine and the dried dichloromethane layer was concentrated under vacuum. The crude product was subjected to column chromatography in 30% EtOAc: Hex to obtain the product as an orange oil (152 mg, 53%). δ_H (400 MHz, $CDCl_3$) 8.89 (t, *J* = 8 Hz, 2H, ArH), 7.60 (d, *J* = 8 Hz, 1H, ArH), 7.52 (t, *J* = 8 Hz, 2H, ArH), 4.68 (s, 2H, CH_2), 3.60 (s, 3H, CH_3), 1.84 (m, 4H, CH_2), 1.72 (m, 4H, CH_2), 1.34 (m, 2H, CH_2). δ_C (100 MHz, $CDCl_3$) 194.0, 172.6, 161.4, 151.0, 130.1, 122.6, 113.6, 70.5, 50.9, 40.2, 36.2, 28.5, 25.6, 24.6. LRMS (ESI): *m/z* 408.3 $(M + Na)^+$, 278.5, 187.0, 124.6. HRMS-ESI (*m/z*): $(M + Na)^+$ calcd for $C_{16}H_{23}N_3NaO_6S^+$, 408.1200; found, 408.1199.

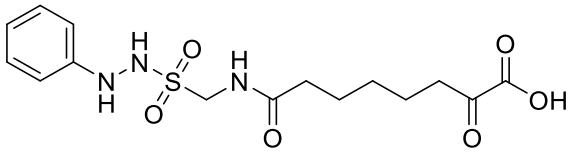
Methyl 2,6-dioxo-6-((3-((2-phenylhydrazinyl)sulfonyl)propyl)amino)hexanoate, 5.18



Freshly prepared methyl 6-chloro-2,6-dioxohexanoate (46 mg, 0.264 mmol) was dissolved in dichloromethane (5 mL). To the cold solution addition of triethylamine (110 µL, 0.79 mmol) followed by the addition of a solution of 3-amino-*N'*-phenylpropane-1-sulfonohydrazide (60 mg, 0.26 mmol) in dichloromethane (2 mL) was performed maintaining the temperature. The resulting reaction mixture was kept at 0 °C for 1 hour and overnight at room temperature. Reaction mixture was washed successively with water, brine and the dried dichloromethane layer was concentrated under vacuum. The crude product was subjected to column chromatography in 40% EtOAc: Hex to obtain the product as a red oil (75 mg, 78%). δ_H (400 MHz, $CDCl_3$) 8.80 (t, *J* = 8 Hz, 2H, ArH), 7.78 (d, *J* = 8 Hz, 1H, ArH), 7.55 (t, *J* = 8 Hz, 2H, ArH), 3.77 (m, 2H, CH_2), 3.35 (m, 2H, CH_2), 3.27 (s, 3H, CH_3), 3.02 (m, 2H, CH_2), 2.85 (t, *J* = 4 Hz, 2H, CH_2), 2.77 (m, 4H,CH_2). δ_C (100 MHz, $CDCl_3$) 194.2, 172.6, 161.2, 151.2, 130.0, 122.6, 113.2, 60.0, 51.3, 38.6, 37.6,

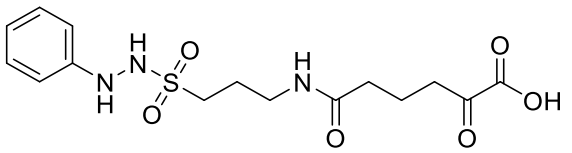
36.2, 20.2, 19.3. LRMS (ESI): *m/z* 408.2 $(M + Na)^+$. HRMS-ESI (*m/z*): $(M + Na)^+$ calcd for $C_{16}H_{23}N_3NaO_6S^+$, 408.1200; found, 408.1201.

2,8-Dioxo-8-((((2-phenylhydrazinyl)sulfonyl)methyl)amino)octanoic acid, 5.19



Methyl 2,8-dioxo-8-((((2-phenylhydrazinyl)sulfonyl)methyl)amino)octanoate (100 mg, 0.260 mmol) was dissolved in methanol and addition of methanolic sodium hydroxide (2M, 250 µL) was performed. Reaction mixture was stirred at room temperature for 3 hours and the resulting mixture was concentrated, and the residue was diluted with ethyl acetate. Resulted solution was washed with 2M HCl, water and dried prior to concentrating under vacuum for obtaining the pure compound as a pale orange oil (56 mg, 58%). δ_H (400 MHz, $CDCl_3$) 8.89 (t, *J* = 8 Hz, 2H, ArH), 7.60 (d, *J* = 8 Hz, 1H, ArH), 7.52 (t, *J* = 8 Hz, 2H, ArH), 4.70 (s, 2H, CH_2), 2.91 (t, *J* = 4 Hz, 2H. CH_2), 2.78 (t, *J* = 4 Hz, 2H. CH_2), 1.73 (m, 2H, CH_2), 1.58 (m, 2H, CH_2), 1.39 (m, 2H, CH_2). δ_C (100 MHz, $CDCl_3$) 195.1, 172.6, 162.8, 151.2, 129.3, 122.6, 113.6, 70.6, 40.2, 36.9, 28.5, 25.2, 24.5. LRMS (ESI): *m/z* 370.2 $(M - H)^-$, 263.1. HRMS-ESI (*m/z*): $(M - H)^-$ calcd for $C_{15}H_{20}N_3O_6S^-$, 370.1078; found, 370.1077.

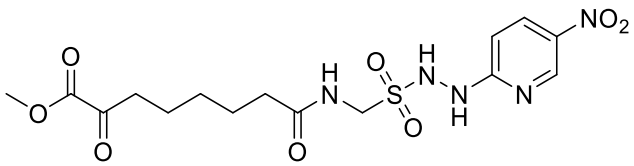
2,6-Dioxo-6-((3-((2-phenylhydrazinyl)sulfonyl)propyl)amino)hexanoic acid, 5.20



Methyl 2,6-dioxo-6-((3-((2-phenylhydrazinyl)sulfonyl)propyl)amino)hexanoate (50 mg, 0.129 mmol) was dissolved in methanol and addition of methanolic sodium hydroxide (2M, 250 µL) was performed. Reaction mixture was stirred at room temperature for 3 hours and the resulting mixture was concentrated, and the residue was diluted with ethyl acetate. Resulted solution was washed with 2M HCl, water and dried prior to concentrating under vacuum for obtaining the pure compound as a pale orange oil (40 mg, 85%). δ_H (400 MHz,

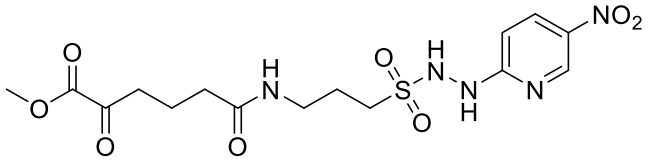
$CDCl_3$) 8.80 (t, *J* = 8 Hz, 2H, ArH), 7.77 (d, *J* = 8 Hz, 1H, ArH), 7.56 (t, *J* = 8 Hz, 2H, ArH), 3.77 (m, 2H, CH_2), 3.33 (m, 2H, CH_2), 3.00 (m, 2H, CH_2), 2.85 (t, *J* = 4 Hz, 2H, CH_2), 2.76 (m, 4H, 2×CH_2). δ_C (100 MHz, $CDCl_3$) 195.1, 172.6, 162.6, 151.2, 130.1, 122.6, 113.2, 54.8, 51.2, 37.2, 35.9, 20.1, 19.3. LRMS (ESI): *m/z* 370.2 $(M - H)^-$. HRMS-ESI (*m/z*): $(M - H)^-$ calcd for $C_{15}H_{20}N_3O_6S^-$, 370.1078; found, 370.1077.

Methyl 8-((((2-(5-nitropyridin-2-yl)hydrazinyl)sulfonyl)methyl)amino)-2,8-dioxooctanoate, 5.21



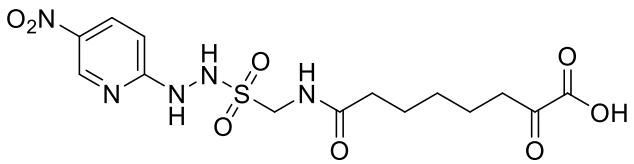
Freshly prepared methyl 8-chloro-2,8-dioxooctanoate (163 mg, 0.74 mmol) was dissolved in dichloromethane (10 mL) and cooled. To the cold solution addition of triethylamine (310 µL, 2.22 mmol) followed by the addition of a solution of 1-amino-*N'*-(5-nitropyridin-2-yl)methanesulfonohydrazide (163 mg, 0.74 mmol) in dichloromethane (3 mL) was performed maintaining the temperature. The resulting reaction mixture was kept at 0 °C for 1 hour and overnight at room temperature. Reaction mixture was washed successively with water, brine and the dried dichloromethane layer was concentrated under vacuum. The crude product was subjected to column chromatography in 20% EtOAc: Hex to obtain the product as a bright red oil (153 mg, 48%). δ_H (400 MHz, $CDCl_3$) 8.32 (d, *J* = 8 Hz, 1H, ArH), 7.29 (s, 1H, ArH), 7.21 (d, *J* = 8 Hz, 1H, ArH), 4.68 (s, 2H, CH_2), 3.60 (s, 3H, CH_3), 1.84 (m, 4H, CH_2), 1.72 (m, 2H, CH_2), 1.34 (m, 4H, CH_2). δ_C (100 MHz, $CDCl_3$) 194.2, 180.0, 164.2, 161.2, 143.2, 140.2, 133.6, 108.9, 70.6, 51.6, 40.2, 36.2, 28.6, 25.6, 24.9. LRMS (ESI): *m/z* 432.3 $(M + H)^+$, 278.4, 186.2, 155.3, 124.6 HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_{15}H_{22}N_5O_8S^+$, 432.1184; found, 432.1182.

Methyl 6-((3-((2-(5-nitropyridin-2-yl)hydrazinyl)sulfonyl)propyl)amino)-2,6-dioxohexanoate, 5.22



Freshly prepared methyl 6-chloro-2,6-dioxohexanoate (44 mg, 0.252 mmol) was dissolved in dichloromethane (5 mL). To the cold solution addition of triethylamine (105 µL, 0.75 mmol) followed by the addition of a solution of 3-Amino-N'-(5-nitropyridin-2-yl)propane-1-sulfonohydrazide (70 mg, 0.25 mmol) in dichloromethane (3 mL) was performed maintaining the temperature. The resulting reaction mixture was kept at 0 °C for 1 hour and overnight at room temperature. Reaction mixture was washed successively with water, brine and the dried dichloromethane layer was concentrated under vacuum. The crude product was subjected to column chromatography in 30% EtOAc: Hex to obtain the product as an orange red oil (78 mg, 72%). δ_H (400 MHz, $CDCl_3$) 8.98 (d, *J* = 8 Hz, 1H, ArH), 8.37 (d, *J* = 8 Hz, 1H, ArH), 7.30 (d, *J* = 8 Hz, 1H, ArH), 4.20 (t, *J* = 12 Hz, 2H, CH_2), 3.33 (m, 2H, CH_2), 3.25 (s, 3H, CH_3), 3.00 (m, 2H, CH_2), 2.83 (t, *J* = 12 Hz, 2H, CH_2), 2.75 (m, 4H,CH_2). δ_C (100 MHz, $CDCl_3$) 194.0, 172.5, 164.9, 161.2, 143.6, 140.2, 133.6, 108.6, 55.0, 51.2, 38.5, 37.6, 36.2, 20.3, 16.5. LRMS (ESI): *m/z* 432.3 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_{15}H_{22}N_5O_8S^+$, 432.1184; found, 432.1184.

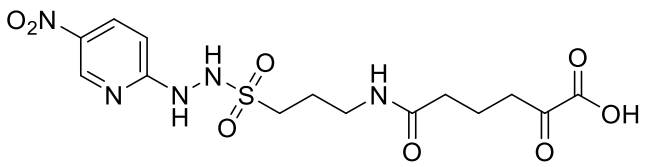
8-((((2-(5-Nitropyridin-2-yl)hydrazinyl)sulfonyl)methyl)amino)-2,8-dioxooctanoic acid, 5.23



Methyl 8-((((2-(5-nitropyridin-2-yl)hydrazinyl)sulfonyl)methyl)amino)-2,8-dioxooctanoate (76 mg, 177 mmol) was dissolved in dichloromethane (5 mL) and methanol (2 mL). Addition of methanolic sodium hydroxide (2M, 500 µL) was performed and the reaction mixture was stirred at room temperature for 4 hours. The resulting solution was concentrated and diluted with ethyl acetate and washed with 2M HCl, water and brine.

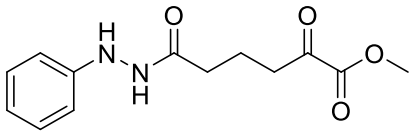
The dried organic layer was concentrated to obtain the pure product as a pale brown oil (50 mg, 67%). δ_H (400 MHz, $CDCl_3$) 8.30 (d, *J* = 8 Hz, 1H, ArH), 7.32 (s, 1H, ArH), 7.41 (d, *J* = 8 Hz, 1H, ArH), 4.60 (s, 2H, CH_2), 1.84 (m, 4H, CH_2), 1.72 (m, 2H, CH_2), 1.40 (m, 4H, CH_2). δ_C (100 MHz, $CDCl_3$) 195.1, 172.6, 164.8, 162.9, 143.6, 140.2, 133.1, 108.9, 70.5, 39.2, 36.2, 28.3, 25.1, 24.9. LRMS (ESI): *m/z* 416.3 $(M + H)^+$. HRMS-ESI (*m/z*): $(M - H)^-$ calcd for $C_{14}H_{18}N_5O_8S^-$, 416.0882; found, 416.0881.

6-((3-((2-(5-nitropyridin-2-yl)hydrazinyl)sulfonyl)propyl)amino)-2,6-dioxohexanoic acid, 5.24



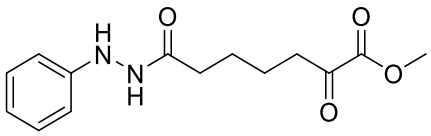
Methyl 6-((3-((2-(5-nitropyridin-2-yl)hydrazinyl)sulfonyl)propyl)amino)-2,6-dioxohexanoate (50 mg, 0.115 mmol) was dissolved in methanol and addition of methanolic sodium hydroxide (2M, 250 µL) was performed. Reaction mixture was stirred at room temperature for 3 hours and the resulting mixture was concentrated, and the residue was diluted with ethyl acetate. Resulted solution was washed with 2M HCl, water and dried prior to concentrating under vacuum for obtaining the pure compound as a pale orange oil (32 mg, 66%). δ_H (400 MHz, $CDCl_3$) 8.99 (d, *J* = 8 Hz, 1H, ArH), 8.35 (d, *J* = 8 Hz, 1H, ArH), 7.35 (d, *J* = 8 Hz, 1H, ArH), 4.20 (t, *J* = 12 Hz, 2H, CH_2), 3.31 (m, 2H, CH_2), 3.02 (m, 2H, CH_2), 2.85 (t, *J* = 12 Hz, 2H, CH_2), 2.70 (m, 4H,CH_2). δ_C (100 MHz, $CDCl_3$) 195.2, 172.6, 164.5, 162.0, 143.6, 140.1, 133.0, 108.6, 54.8, 38.2, 37.5, 36.2, 20.6, 19.6. LRMS (ESI): *m/z* 416.3 $(M - H)^-$. HRMS-ESI (*m/z*): $(M - H)^-$ calcd for $C_{14}H_{18}N_5O_8S^-$, 416.0882; found, 416.0881.

Methyl 2,6-dioxo-6-(2-phenylhydrazinyl)hexanoate, 5.25



Freshly prepared methyl 6-chloro-2,6-dioxohexanoate (110 mg, 0.57 mmol) was dissolved in dichloromethane (10 mL) and cooled. Triethylamine (240 µL, 1.72 mmol) and phenylhydrazine (57 µL, 0.57 mmol) were then added. The reaction mixture was stirred on an ice bath for 1 hour, allowed to warm to room temperature then stirred overnight. The resulting solution was washed with water and brine, then dried and concentrated *in vacuo* to obtain the crude material. Column chromatography using 20% ethyl acetate:hexane gave the product as a brown oil (250 mg, 44%). δ_H (400 MHz, $CDCl_3$) 7.88 (m, 3H, ArH), 7.72 (m, 2H, ArH), 3.28 (s, 3H, CH_3), 3.05 (m, 2H, CH_2), 2.76 (m, 4H, 2×CH_2). δ_C (100 MHz, $CDCl_3$) 194.2, 176.5, 161.0, 148.6, 130.0, 122.6, 113.6, 51.6, 40.0, 38.2, 20.2. LRMS (ESI): *m/z* 265.2 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_{13}H_{17}N_2O_4^+$, 265.1183; found, 256.1180.

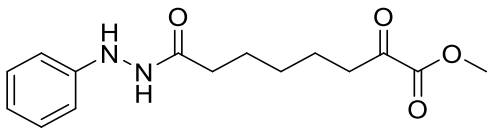
Methyl 2,7-dioxo-7-(2-phenylhydrazinyl)heptanoate, 5.26



Freshly prepared methyl 7-chloro-2,7-dioxoheptanoate (108 mg, 0.53 mmol) in was dissolved in dichloromethane (10 mL) and cooled. Triethylamine (222 µL, 1.59 mmol) and phenylhydrazine (52 µL, 0.531 mmol) were then added. The reaction mixture was stirred on an ice bath for 1 hour, allowed to warm to room temperature then stirred overnight. The resulting solution was washed with water and brine, then dried and concentrated *in vacuo* to obtain the crude material. Column chromatography using 30% ethyl acetate:hexane gave the product as a brown oil (285 mg, 54%). δ_H (400 MHz, $CDCl_3$) 7.75 (t, J = 8 Hz, 2H, ArH), 7.68 (d, J = 8 Hz, 1H, ArH), 7.52 (t, J = 8 Hz, 2H, ArH), 3.58 (s, 3H, CH_3),3.38 (m, 4H, 2×CH_2), 2.85 (m, 4H, 2×CH_2). δ_C (100 MHz, $CDCl_3$) 193.8, 176.2, 161.2, 149.2,

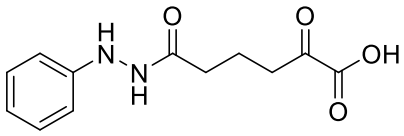
130.0, 122.0, 113.6, 51.0, 40.0, 38.2, 27.8, 23.6. LRMS (ESI): *m/z* 279.3 $(M + H)^+$. HRMS-ESI (*m/z*): $(M + H)^+$ calcd for $C_{14}H_{19}N_2O_4^+$, 279.1339; found, 279.1338.

Methyl 2,8-dioxo-8-(2-phenylhydrazinyl)octanoate, 5.27



Freshly prepared methyl 8-chloro-2,8-dioxooctanoate (36 mg, 0.161 mmol) was dissolved in dichloromethane (5 mL) and cooled. Triethylamine (67 µL, 0.43 mmol) and phenylhydrazine (20 µL, 0.177 mmol) were then added. The reaction mixture was stirred on an ice bath for 1 hour, allowed to warm to room temperature then stirred overnight. The resulting solution was washed with water and brine, then dried and concentrated *in vacuo* to obtain the crude material. Column chromatography using 30% ethyl acetate:hexane gave the product as a brown oil (15 mg, 23%). δ_H (400 MHz, $CDCl_3$) 8.32 (d, 1H, NH), 8.17 (d, 1H, NH), 7.51 (m, 3H, ArH), 7.39 (m, 2H, ArH), 3.35 (s, 3H, CH_3), 3.65 (m, 4H, 2×CH_2), 3.40 (m, 4H, 2×CH_2), 2.19 (m, 2H, CH_2). δ_C (100 MHz, $CDCl_3$) 194.2, 161.2, 176.5, 150.0, 129.6, 122.6, 113.3, 51.2, 39.6, 38.6, 29.2, 25.6, 25.1. LRMS (ESI): *m/z* 315.6 $(M + Na)^+$, 215.4, 187.2, 108.6. HRMS-ESI (*m/z*): $(M + Na)^+$ calcd for $C_{15}H_{20}N_2NaO_4^+$, 315.1315; found, 315.1315.

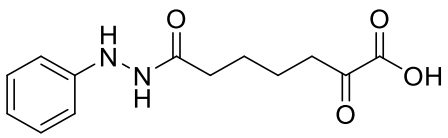
2,6-Dioxo-6-(2-phenylhydrazinyl)hexanoic acid, 5.28



Freshly prepared 6-chloro-2,6-dioxohexanoic acid (153 mg, 0.861 mmol) was dissolved in dichloromethane (15 mL) and cooled. Triethylamine (360 µL, 2.58 mmol) and phenylhydrazine (93 µL, 0.947 mmol) was added and the reaction mixture was stirred on the ice bath for 1 hour, allowed to warm to room temperature and then stirred overnight. The resulting solution was washed with water, brine, then dried and concentrated. Column chromatography using 30% ethyl acetate:hexane gave the product as a brown oil (60 mg, 22%). δ_H (400 MHz, $CDCl_3$) 7.96 (m, 3H, ArH), 7.66 (m, 2H, ArH), 3.01 (m, 2H, CH_2),

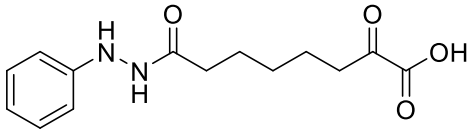
2.67 (m, 4H, 2×CH_2). δ_C (100 MHz, $CDCl_3$) 195.0, 176.2, 162.7, 149.2, 129.3, 122.3, 113.2, 39.1, 38.1, 19.8. LRMS (ESI): *m/z* 249.2 $(M - H)^-$. HRMS-ESI (*m/z*): $(M - H)^-$ calcd for $C_{12}H_{13}N_2O_4^-$, 249.0881; found, 249.0880.

2,7-Dioxo-7-(2-phenylhydrazinyl)heptanoic acid, 5.29



Freshly prepared 7-chloro-2,7-dioxoheptanoic acid (190 mg, 0.98 mmol) was dissolved in dichloromethane (15 mL) and cooled. Triethylamine (413 µL, 2.97 mmol) and phenylhydrazine (107 µL, 1.08 mmol) were added and the reaction mixture was stirred on an ice bath for 1 hour. After allowing the reaction to warm to room temperature, the reaction was then stirred at overnight. The reaction was washed with water and then brine, then dried and concentrated. Column chromatography using 30% ethyl acetate:hexane gave the product as a brown oil (15 mg, 23%). δ_H (400 MHz, $CDCl_3$) 7.89 (t, *J* = 8 Hz, 2H, ArH), 7.72 (d, *J* = 8 Hz, 1H, ArH), 7.58 (t, *J* = 8 Hz, 2H, ArH), 3.48 (m, 4H, 2×CH_2), 2.91 (m, 4H, 2×CH_2). δ_C (100 MHz, $CDCl_3$) 195.2, 176.5, 162.3, 149.3, 130.2, 122.7, 113.2, 38.7, 38.0, 27.5, 23.6. LRMS (ESI): *m/z* 263.5 $(M - H)^-$, 187.3, 156.9. HRMS-ESI (*m/z*): $(M - H)^-$ calcd for $C_{13}H_{15}N_2O_4^-$, 263.1037; found, 263.1036.

2,8-Dioxo-8-(2-phenylhydrazinyl)octanoic acid, 5.30



Methyl 2,8-dioxo-8-(2-phenylhydrazinyl)octanoate (20 mg, 0.068 mmol) was dissolved in methanol, then methanolic sodium hydroxide (2M, 70 µL) was added. The reaction mixture was heated to 50 °C for 2 hours then concentrated. The residue was diluted with ethyl acetate and washed with 2M HCl, water and then dried. Concentration under vacuum gave the pure compound as a pale-yellow oil (11 mg, 58%). δ_H (400 MHz, $CDCl_3$) 7.48 (m, 3H, ArH), 7.21 (m, 2H, ArH), 2.64 (m, 4H, 2×CH_2), 2.35 (m, 4H, 2×CH_2), 2.11 (m, 2H, CH_2). δ_C (100 MHz, $CDCl_3$) 195.0, 176.6, 162.7, 149.2, 129.3, 122.7, 113.1, 39.0,

38.5., 29.2, 25.6, 25.0. LRMS (ESI): *m/z* 277.5 $(M - H)^-$, 200.5, 172.0. HRMS-ESI (*m/z*): $(M - H)^-$ calcd for $C_{14}H_{17}N_2O_4^-$, 277.1194; found, 277.1193.

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