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Toxicodynamics of Methemoglobinemia Inducers

DANIEL CONOLE

A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY

THE UNIVERSITY OF AUCKLAND

2012
ABSTRACT

The pest control agent, para-aminopropiophenone (PAPP) (figure i), was recently registered in New Zealand for the humane control of stoats and feral cats. PAPP exhibits low toxicity towards most bird species and limited secondary poisoning risk. Moreover, a highly effective antidote is available. PAPP induces methemoglobinemia (MtHb), which acts to prevent red blood cells transporting oxygen. After an oral dose of PAPP, the supply of oxygen to the brain is reduced, causing animals to become lethargic, sleepy and unconscious prior to eventual death within one to two hours. Despite this knowledge, no previous study has comprehensively examined the effect of modifying the structure of PAPP on MtHb induction, and the potential for related compounds in pest control.

Broadly, this thesis is divided into three main parts, with an overall objective to enhance MtHb toxicity towards rats. Using the structure of PAPP as a platform, the initial part of this work (section 3.) will describe the design, synthesis and bioevaluation of PAPP-related analogues, in order to investigate SAR’s (structure-activity relationships) within this structural sub-class. Using the knowledge obtained from these studies, the second part of this work (section 4.) will describe the synthesis and bioevaluation of hydrolytically stable bioisosteres of benzocaine. In light of the results obtained from the first two sections, the final part of this work (section 5.) examines a prodrug approach towards a homologue of PAPP, PAVP (para-aminovalerophenone).

Part 1

Through examination of a number of alkyl aminophenones, it was discovered that a parabolic relationship between the partition coefficient of the analogue and MtHb induction existed, with an optimal lipophilicity for in vitro MtHb induction situated in the range of log $P = 3-3.5$ units, as demonstrated by PAHP (para-aminohexanoylphenone) ($n = 5$, log $P_{exp} = 3.49$) (figure ii). Extensive studies in this structural sub-class suggested that, besides lipophilicity, there were certain structural requirements for in vitro MtHb
activity, with both branched and cyclic alkyl chains appearing to be detrimental to MtHb induction (figure ii).

![Figure ii: Linear, branched and cyclic alkyl 4'-aminophenones.](image)

Investigation into various aryl aminophenones (such as aminochalcones) demonstrated that electronics play a significant role in MtHb induction (figure iii). Saturation of the double bond present in aminochalcone 55 (leading to aminodihydrochalcone 71) was found to lead to enhanced MtHb activity, suggesting that an increase in conformational flexibility is beneficial for MtHb induction (figure iii).

![Figure iii: Aryl 4'-aminophenone analogues.](image)

Three candidates from these series, PAVP, PAHP and compound 71 were evaluated in vivo, with PAVP exhibiting the greatest in vivo MtHb toxicity (figure iv).
Investigation into aminophenone isosteres revealed the importance of the ketone in PAPP with respect to MtHb induction (figure v). Ketone isosteres, including ketoximes, sulfoxides, sulfones and sulfonamides, appeared to possess similar electronic profiles to that of the parent ketone, however, closer examination of the Hammett constants of these substituents suggested that a particular combination of the field and resonance parameters may be required for potent MtHb induction.

**Part 2**

Benzocaine, a structurally similar compound to PAPP, has been reported to cause clinically relevant levels of MtHb. In a pursuit to circumnavigate the well documented susceptibility of benzocaine (figure vi) (another potent MtHb inducer) to *in vivo* hydrolysis, attention focused on a number of hydrolytically stable bioisosteres of benzocaine, including oxadiazoles and thiadiazoles (figure vii).
From these series, two candidates, 3-propyl-1,2,4-oxadiazole 146 and 5-propyl-1,3,4-oxadiazole 165, exhibited potent in vitro MtHb activity, however when examined in vivo, neither demonstrated sufficient in vivo MtHb toxicity to effect a lethal endpoint. This result was postulated to be a consequence of the extensive metabolism of these compounds in vivo (figure viii).

Structurally diverse heterocycles, including tetrazoles and diazines, were postulated to offer greater stability in vivo, however these compounds exhibited poor in vitro MtHb activity, and their failure was assumed to be the result of their suboptimal electronic profiles (figure ix).
Part 3

In an effort to access compounds with improved bioavailability and consequently greater \textit{in vivo} MtHb toxicity, a ketoxime prodrug strategy was employed for PAVP (figure x). When evaluated \textit{in vivo} at equimolar doses, the toxicity observed for PAVP ketoxime prodrugs was considerably less than that of the parent ketone, with the test species subjected to these compounds only revealing symptoms characteristic of sub-lethal MtHb induction.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{pavp_ketoxime_prodrugs.png}
\caption{PAVP ketoxime prodrugs.}
\end{figure}

In summary, through a systematic examination of the chemical space around the ketone of PAPP, the ester of benzocaine, and the ketone of PAVP, these three studies indicated that physicochemical parameters such as lipophilicity and electronics strongly influence the extent of \textit{in vitro} MtHb induced by these compounds.
I would like to dedicate this thesis to my late father, George John Conole. Thank you for always letting me know how proud of me you were.
ACKNOWLEDGEMENTS

Firstly, I would like to give glory to God, from whom I gain my strength, motivation and resolve. His grace is sufficient for me, for his power is made perfect in my weakness. Margaret, the success you have achieved in your field and beyond has been an inspiration to me. What a privilege it has been to learn from one of the best. By demanding a high standard from yourself and from your students, you have taught me to be disciplined and rigorous with my research. Thank you for the opportunity, it has been an honour to study under you.

To Dave, words probably cannot describe how grateful I am for your efforts over the last few years. Your supervision of my project has influenced my ideas, educated me how to think critically, and taught me many valuable techniques and skills in the lab. Thank you for your patience and kindness, your words of encouragement and constructive criticism have been invaluable. If you ever need help further with the Henderson-Hasselbach equation, don’t hesitate to give me a call.

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Last but certainly not least, I would like to acknowledge my wife Libby, who has been my rock, in particular during the final year of this project. You manage to always see the best in me when I only saw the worst, and you encouraged me to believe in myself. To Andrew and Jackie, your love and support for me over this time has been resolute, words cannot describe how much this has meant to me.
ABBREVIATIONS

\( \delta \) chemical shift

\( ^\circ \text{C} \) degree(s) Celsius

\( \sigma \) Hammett constant

\( \pi \) Hansch constant

\( \nu \) wavelength

6-PGA 6-phosphogluconate

A ataxia

Abs absorption

Ac acetyl

APCI atmospheric pressure chemical ionization

aq. aqueous

atm. atmosphere(s)

B blue paws, tail and nose

BNPP bis(4-nitrophenyl) phosphate

bp boiling point

BTI bis(trifluoroacetoxy)iodobenzene

Bu butyl

Bzl benzyl

c. concentrated

c. ca. approximately

cBu cyclobutyl

\( c\text{Ca}^{2+} \) concentration of calcium ions

\( c\text{Cl}^{-} \) concentration of chloride ions

cf. confer (compare or consult with)

cGlu concentration of D-glucose

cHx cyclohexyl

\( c\text{K}^{+} \) concentration of potassium ions

\( c\text{Lac} \) concentration of L-lactate

cm centimeter(s)
<table>
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<tr>
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<th>Description</th>
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<tr>
<td>cNa⁺</td>
<td>concentration of sodium ions</td>
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<td>carbon monoxide</td>
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<td>COHb</td>
<td>carboxyhemoglobin</td>
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<td>COSY</td>
<td>correlation spectroscopy</td>
</tr>
<tr>
<td>cPe</td>
<td>cyclopentyl</td>
</tr>
<tr>
<td>cPr</td>
<td>cyclopropyl</td>
</tr>
<tr>
<td>ctBil</td>
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</tr>
<tr>
<td>ctHb</td>
<td>concentration of total hemoglobin</td>
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<td>CYP</td>
<td>cytochrome P450</td>
</tr>
<tr>
<td>d</td>
<td>doublet</td>
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<td>1,2-dichloroethane</td>
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<td>doublet of doublets</td>
</tr>
<tr>
<td>ddt</td>
<td>doublet of doublet of triplets</td>
</tr>
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<td>DEPT</td>
<td>distortionless enhancement by polarization transfer</td>
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</tr>
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<td>electrospray ionization</td>
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<td>Et</td>
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<td>et al.</td>
<td>et alii (and others)</td>
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<td>F</td>
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<td>G-6-P</td>
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<td>G-6-P-D</td>
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</tr>
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<tr>
<td>HAS</td>
<td>hydroxylamine-O-sulfonic acid</td>
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<tr>
<td>HbF</td>
<td>fetal hemogobin</td>
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<tr>
<td>HCN</td>
<td>hydrogen cyanide</td>
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<td>HHb</td>
<td>deoxyhemoglobin</td>
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HMBC  heteronuclear multiple bond correlation
HPLC  high performance liquid chromatography
HSQC  heteronuclear single quantum coherence
Hz    hertz
i.m.  intramuscular
i.p.  intraperitoneal
i.v.  intravenous
IBX   2-iodoxybenzoic acid
iPr   isopropyl
IR    infra-red
IUCN  International Union for Conservation of Nature
J     coupling constant
kg    kilogram(s)
L     litre
L     lethargy
LD50  lethal dose to kill 50% of the test population
LDA   lithium diisopropylamide
lit.  literature
log $P_{\text{calc.}}$ calculated log $P$ value
log $P_{\text{exp.}}$ experimental log $P$ value
LR    Lawessons reagent
M     molar
M     molar
m     multiplet
$m$   meta
$m/z$ mass to charge ratio
$m$CPBA $meta$-chloroperoxybenzoic acid
Me    methyl
mg    milligram(s)
MHz   megahertz
min   minute(s)
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<td>mmHg</td>
<td>millimetre(s) of mercury</td>
</tr>
<tr>
<td>mp</td>
<td>melting point</td>
</tr>
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<td>mass spectroscopy</td>
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<td>methanesulfonic acid</td>
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<td>MtHb</td>
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<td>MW</td>
<td>microwave</td>
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<td>NADP</td>
<td>nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NADPH</td>
<td>reduced form of nicotinamide adenine dinucleotide phosphate</td>
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<td>para-aminoacetophenone</td>
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<tr>
<td>PAPP</td>
<td>para-aminopropiophenone</td>
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<tr>
<td>PAVP</td>
<td>para-aminovalerophenone</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>PB</td>
<td>phosphate buffer</td>
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<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
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<tr>
<td>$PCO_2$</td>
<td>partial pressure of carbon dioxide</td>
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<tr>
<td>Ph</td>
<td>phenyl</td>
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<tr>
<td>pKa</td>
<td>acid dissociation constant</td>
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<tr>
<td>PKC</td>
<td>protein kinase C</td>
</tr>
<tr>
<td>PLC</td>
<td>phospholipase C</td>
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<tr>
<td>$pO_2$</td>
<td>partial pressure of oxygen</td>
</tr>
<tr>
<td>PPA</td>
<td>polyphosphoric acid</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>psi</td>
<td>pounds per square inch</td>
</tr>
<tr>
<td>PVC</td>
<td>polyvinyl chloride</td>
</tr>
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<td>q</td>
<td>quartet</td>
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<td>R</td>
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<tr>
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<td>reverse phase high performance liquid chromatography</td>
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<td>RT</td>
<td>retention time</td>
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<td>tetrabutylammonium bromide</td>
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<tr>
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<td>temperature</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
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<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>tmp</td>
<td>tetramethylpiperidide</td>
</tr>
<tr>
<td>V</td>
<td>volts</td>
</tr>
<tr>
<td>v/v</td>
<td>volume per volume</td>
</tr>
<tr>
<td>W</td>
<td>watts</td>
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<td>w/</td>
<td>with</td>
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<td>μL</td>
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<td>μM</td>
<td>micromolar</td>
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1. INTRODUCTION
The introduction will be divided into three sections, with the first section (1.1.) discussing the history and importance of the elimination of vertebrate pests and the methods employed for their control. The second section (1.2.) will review, more specifically, the history of use, mechanism of action and benefits of a new agent for pest control, namely PAPP. The third and final section (1.3.) of the introduction will evaluate the factors needed to be considered for the design of PAPP-related analogues, concluding with an overview of the research strategy.

1.1. Importance, history and methods for the control of vertebrate pests

1.1.1. The importance of animal pest elimination

Cherret defined a pest animal as one which is noxious, destructive or troublesome to humans. Wood and Bedard qualified the definition to incorporate a requirement of causing economic damage. Broadly, pests can be defined as organisms that cause harm, whether through economic, environmental or epidemiological damage.

Today, vertebrates cause devastation on a global scale in a number of ways. Vertebrates cause problems in agriculture; to crops and livestock (e.g., European starlings (Sturnus vulgaris Linnaeus) in the United States), in and around buildings and other equipment (e.g., rabbits (Oryctolagus cuniculus Linnaeus) in Australia and New Zealand) and by spreading diseases (e.g., brushtail possums, (Trichosurus vulpecula), acting as reservoirs for bovine tuberculosis in New Zealand). Problems also occur in forestry, in conservation of plants (e.g. goats (Capra hircus Linnaeus) on the Galapagos Islands) and animals (e.g., brown tree snake (Boiga irregularis) on Guam). These vertebrate species can also pose a threat to our communities, in urban industry and in our own homes.

With human settlement, many exotic species have been introduced legitimately with the intention to procure public benefit. Alien species such as farm stock and crops were introduced for fibres or furs, for sport, or as pets or ornaments. Other alien animals have been accidentally introduced with ships' cargoes, following shipwrecks or during military operations. The type and extent of damage by these introduced vertebrate pests is
varied, with impacts including effects on soil structure, soil erosion, water quality and runoff, alteration of plant species composition, changes in plant growth, biomass, reproduction and crop production, the spread of human livestock diseases, changes in ecosystem structure and even species extinction.³

A decline and extinction of endemic fauna and flora is a mode of damage observed particularly in New Zealand. Due to endemic fauna of New Zealand evolving in the absence of mammalian predators, an ecological holocaust has followed the colonisation of New Zealand.⁶

New Zealand has now lost over 40% of its pre-human land bird fauna, with no other country having a higher proportion of its surviving avifauna classified as threatened.⁷ Of the surviving 287 New Zealand bird species (150 of them endemic), 45 were classed as threatened in the 1996 IUCN Red List.⁷ Forty-one of these threatened species are endemic and several now exist only on mammal-free islands or in dwindling mainland populations due to the tide of predations caused by alien mammals.⁷

Several species of vertebrates, especially mammals such as possums (*Trichosurus vulpecula*), mice (*Mus musculus*), Norway rats (*Rattus norvegicus*), ship rats (*Rattus rattus*), rabbits (*Oryctolagus cuniculus* Linnaeus), cats (*Felis catus*), grey squirrels (*Sciurus carolinensis*) and stoats (*Mustela erminea*), have been successful invaders and colonizers of new territories, with particular infiltration of insular island ecosystems (e.g. New Zealand, Australia, Great Britain etc).⁴-⁸

It has been shown that rats *Rattus* spp. alone have reached 82% of the world's islands and island groups, and are implicated in the decline and extinction of birds from at least 31 islands.⁵ Moreover, rats cause substantial damage each year to agricultural interests worldwide. The World Health Organization estimates that 20% of all human food is destroyed or contaminated by rodents each year.⁹ More recently, the Food and Agriculture Organization of the United Nations reported that, worldwide, rats ruined more than 42 tons of food, worth an estimated US$30 billion.¹⁰ In addition to vast economic loss, rats are responsible for a number of health problems, acting as vectors for both viral and bacterial diseases, transmitting more than 35 types of disease to humans such as leptospirosis, cholera, salmonella and the bubonic plague.¹¹
1.1.2. Methods of pest control

Detailed reviews on the methods for the control of vertebrate pests have been published by Eason,\textsuperscript{12} Hone\textsuperscript{13} and Hoddle.\textsuperscript{4} Control methods used in New Zealand for the regulation of these vertebrate pests in the past have mainly relied on physical trapping, which is not only labour-intensive but also expensive.\textsuperscript{14} For the large-scale vertebrate control frequently required over the remote, rugged and forested land of New Zealand, the most viable cost-effective option is the use of toxic baits.\textsuperscript{14}

More recently, the use of increasingly unpopular poisons or poisons that are considered inhumane have been the mainstay of pest control; hence the development of safer, humane possum and predator toxins and/or delivery systems is highly desirable.\textsuperscript{6}

1.1.2.1. Toxicants used: their history, mode of action and their advantages/disadvantages

1.1.2.1.1. General list of substances used:

The chemicals used for controlling vertebrate pests in New Zealand fall into two general classes (i) non-anticoagulants, such as phosphorus, sodium fluoroacetate (1080), cyanide, cholecalciferol and norbormide (figure 1) and (ii) anticoagulants, which include pindone, diphacinone, coumatetralyl, brodifacoum, bromadiolone and flocoumafen (figure 2).\textsuperscript{6}
1. Introduction

1.1. Importance, history and methods for the control of vertebrate pests

Figure 1: Structures of non-anticoagulant compounds used in vertebrate pest control.

Figure 2: Structures of anticoagulant compounds used in vertebrate pest control.
1. Introduction

1.1. Importance, history and methods for the control of vertebrate pests

1.1.1. Non-anticoagulant poisons

A) Phosphorus (P₄)

Phosphorus (P₄) (alternatively named ‘white’ phosphorus) was introduced in the early 1920s and was the first agent used in Australia and New Zealand for rabbit control. Phosphorus became more effective as an acute toxicant in the 1950s when it was incorporated into a paste for rabbit control, however in the 1960s, phosphorus was taken off the market due to premature oxidation in the bait, rendering it ineffective.¹⁵

The exact mechanism by which phosphorus causes its toxic effect remains unclear, but it has often been referred to as a ‘protoplasmic poison’ which causes peripheral vascular dilation, which in turn results in anoxia in the liver, kidneys, heart and brain.¹⁵ This leads to structural damage of the vital organs and serious disruption of their metabolic function, causing disorders such as hypoglycemia, azotemia and inhibition of glycogen formation in the liver.¹⁵

Phosphorus is extremely effective, often achieving >90% kills. Phosphorus is also considered a broad spectrum toxicant, displaying high toxicity across a number of mammals, including LD₅₀ < 10 mg/kg for sheep, pigs, rabbits, dogs, cats, possums and poultry.¹⁵

This poison, however, is no longer in use by the Department of Conservation due to its animal welfare concerns, risk of fires and limited value of antidote. Additionally, phosphorus has also been known to cause secondary poisoning in dogs and birds.¹⁵
1. Introduction

1.1. Importance, history and methods for the control of vertebrate pests

B) 1080 (sodium fluoroacetate)

![Structure of 1080](image)

**Figure 3:** Structure of 1080.

1080 (figure 3) is the proprietary name for sodium fluoroacetate, the most widely used poison in New Zealand.\(^{16}\) It was first employed in the United States in the 1950s to control commensal rodents.\(^{15}\) In Australia, 1080 is widely used in baiting programmes for the control of vertebrate pest species.\(^{17}\) In New Zealand, 1080 has played a key role in the aerial control of possums, but has also proved successful in ground operations.\(^{18,19}\)

Chemically, it originates from plants such as the rat weed (*Palicourea margavii*) and ratsbane (*Dichapetalum*).\(^{20}\)

Following ingestion, the animal will condense 1080 with oxaloacetate to form fluorocitrate (scheme 1), which acts to inhibit the enzymes responsible for the metabolism of citrate and succinate in the Krebs cycle of the mitochondria (figure 4).\(^{16,21,22}\)

![Scheme 1: Production of fluorocitrate](image)

This subsequently causes an accumulation of citrate in tissues and plasma, leading to inhibition of cellular energy production and death.\(^{15}\)
1080 is also considered a broad spectrum toxin, however there is some notable variability in susceptibilities. Dogs are most susceptible, with most other carnivores also being highly sensitive. Herbivores are less sensitive, while birds and reptiles show greater resistance than mammals to 1080.\(^{15}\)

1080 is a well-established, highly effective and cheap toxin, however some severe drawbacks remain. Risk of secondary poisoning from possum carcasses especially in dogs is a problem, as there still remains no effective antidote for 1080. Of greater concern is the secondary poisoning of stock such as cattle; overseas restrictions have now been imposed, based on concerns of 1080 residues in exported meat.\(^{24}\) Moreover, 1080 is highly water-soluble, so it is dispensed easily into the environment by rain and stream water, although it is considered low risk for environmental contamination.\(^{15,25}\) 1080 has also been known to cause non-target bird deaths when incorporated into poor quality bait formulations.\(^{15}\)

More recently, there have been concerns with the humaneness of 1080. Potter \textit{et al.}\(^{26}\) suggested that, upon 1080 poisoning, stoats go through a 30 minute ataxic/hyperactive stage, which is likely to be associated with pain or distress.\(^{25}\) This notion has been somewhat supported by studies on humans who experience similar signs at the equivalent stage of 1080 poisoning.\(^{27}\)
C) Norbormide (NRB)

![Structure of norbormide (NRB)](image)

**Figure 5:** Structure of norbormide (NRB).

Norbormide (NRB) (figure 5) was first discovered as a rodenticide serendipitously in the 1960s during a routine screening of a variety of chemical agents for their pharmacological effects. Early studies showed that NRB toxicity was extremely selective towards the genus *Rattus* and uniquely non-toxic to other closely related species such as mice and guinea pigs. In addition, birds and rabbits can survive approximately fourteen times the lethal dose for rats.

The remarkable selectivity of NRB was demonstrated by Roszkowski *et al.*, who reported that cats, dogs, monkeys, pigs, chickens and ducks displayed no ill effects after a 1 g/kg dose, whereas Norwegian rats displayed an acute oral LD$_{50}$ between 5 and 15 mg/kg.

NRB exhibits species-selective vasoconstrictive activity confined only to the peripheral arteries of the rat. It has been proposed that the vasoconstriction involves disruption of the phospholipase C/protein kinase (PLC/PKC) pathway. NRB induces an influx of calcium ions, which causes smooth muscle contraction within the small blood vessels. Physiologically, this results in increased motor activity, loss of coordination, weakened hind extremities, heavy breathing and convulsions just before death.

Although NRB shows superior toxicity in pharmacological testing, field trials showed inferior efficacy when compared with standard agents such as zinc phosphide and warfarin. This was postulated to be as a result of a high level of absorption and...
subsequent vasoconstriction occurring in the mouth on first encounter with the bait.\textsuperscript{33} This can cause bait refusal and an increased chance of a sub-lethal dose intake. As a consequence, this reduces the efficiency of killing and can make formulation of an appropriate bait difficult.\textsuperscript{34}

D) Cholecalciferol (Vitamin D\textsubscript{3})

![Structure of cholecalciferol (Vitamin D\textsubscript{3}).](image)

**Figure 6**: Structure of cholecalciferol (Vitamin D\textsubscript{3}).

Cholecalciferol (figure 6) is a vitamin D-based poison developed in the 1980s initially as a rodenticide,\textsuperscript{35} and was introduced into New Zealand in 1995 for possum control.\textsuperscript{15} Once absorbed in the body, cholecalciferol undergoes metabolism to 25-hydroxycholecalciferol (scheme 2).\textsuperscript{35}

![Conversion of cholecalciferol to its active metabolite.](image)

**Scheme 2**: Conversion of cholecalciferol to its active metabolite.
The active metabolite liberates calcium from stores in the bones into the bloodstream. 25-Hydroxycholecalciferol also acts to decrease calcium excretion by the kidneys, with the net effect being an increased concentration of calcium in the blood, termed hypercalcaemia, and tissue calcification. Calcification occurs throughout the cardiovascular system, causing mineralisation, blockage of blood vessels and death due to heart failure.\textsuperscript{15}

Small vertebrates such as possums, rats and rabbits appear to be the most sensitive to cholecalciferol, while larger animals such as cats and dogs are less susceptible.\textsuperscript{24,36} LD\textsubscript{50} studies in ducks suggest that cholecalciferol is less toxic in birds.\textsuperscript{15,37}

Cholecalciferol can sufficiently act as an acute poison replacement for 1080, and also has a low risk of secondary poisoning.\textsuperscript{15} however it is very expensive in comparison to 1080, and is currently not registered for aerial application (as 1080 is). Sub-lethal poisoning of the target species can cause anorexia and wasting, which gives rise to animal welfare concerns.\textsuperscript{37} Moreover, in the occasion of accidental poisoning, only a complicated antidote is available for use.\textsuperscript{15}

**E) Cyanide**

\[
\begin{align*}
\text{M}&=\text{Na or K} \\
\text{M}&=\text{CEN} \\
\text{M}&=\text{CEN} \\
\end{align*}
\]

*Figure 7: Cyanide.*

Due to its fast mechanism of action with regards to toxicity, a number of countries considered cyanide (figure 7) to be too hazardous for use in pest control. In New Zealand however, potassium cyanide has been employed in various encapsulated forms for several decades for the control of possums.\textsuperscript{15}

Upon ingestion or inhalation, the toxicity of cyanide has been attributed mainly to a penetration of the outer mitochondrial membrane and a binding of the cyanide anion to the ferric heme portion of cytochrome \(a_3\) oxidase, itself an enzyme in the electron transport chain (scheme 3).\textsuperscript{15,21} Formation of this stable cytochrome \(a_3\) oxidase-cyanide
complex inhibits electron transfer from cytochrome $c$ oxidase to molecular oxygen. This causes a movement of H$^+$ ions across the inner membrane and a severe reduction in ATP production and resultant inhibition of cellular respiration. $^{21,38}$

**Scheme 3:** Mechanism of cyanide intoxication.$^{39}$

Physiologically, this results in a combination of cytotoxic hypoxia with lactate acidosis which depresses the central nervous system, causing respiratory arrest and death.$^{40}$

Cyanide is a broad spectrum toxin in mammals and birds. Cyanide is also effective as an insecticide, as hydrogen cyanide (HCN) gas is released when it is used as a fumigant.$^{15}$ When birds are exposed to a sub-lethal dose, they possess a greater ability to metabolise cyanide to thiocyanate, which aids excretion of the toxin,$^{40}$ however cold-blooded animals such as frogs are less susceptible to the toxicity of cyanide.$^{41}$

Cyanide as a pest control agent has a number of advantages. It is very cheap to make (1-2 cents per pellet) and is considered relatively effective and humane, as it has a very rapid mode of action (i.e. loss of consciousness and death within 6-18 min).$^{15}$ In addition, the pests are killed so quickly that they are usually found not far from the bait station. As a result, skin/carcass recovery is considerably easier for farmers who are carrying out large scale pest control. One of the other main benefits of cyanide is its relatively low secondary poisoning risk and biodegradability in the environment, as it is degraded to non-toxic cyanide salts by micro-organisms present in the soil.$^{15}$
Despite the clear advantages of cyanide, there are a number of serious drawbacks associated with its use. Cyanide is extremely dangerous to the user, due to the risk of inhalation of HCN gas. Cyanide is usually manufactured as an encapsulated paste, but these pellets deteriorate rapidly in wet weather, dissipating the toxic HCN by gaseous diffusion. One or two pellets of Feratox® (encapsulated potassium cyanide), contain a sufficient amount to be lethal to humans.\textsuperscript{15} Cyanide baits have been reported to kill a number of non-target species including kiwi, weka, and short-tailed bats.\textsuperscript{15} There are antidotes available for cyanide, however their use is controversial, as they themselves can cause tissue anoxia.\textsuperscript{38,42}

### 1.1.2.1.3. Anticoagulant poisons

Since the early 1950s, anticoagulants have been a mainstay in the development of rodenticides. They have been used to control commensal rodents worldwide, and also used in field treatments against crop pests.\textsuperscript{43} Due to the fact that most anticoagulants are very similar in nature and action, only a handful are discussed here. The main advantage of this class of compounds is that they consist of an effective antidote (vitamin K), which can ameliorate the effects of accidental poisoning.\textsuperscript{15}

**A) First generation anticoagulants**

Pindone is an early first generation anticoagulant rodenticide, first synthesized in 1937 and developed in the 1940s.\textsuperscript{44} Diphacinone, another first generation anticoagulant, was first introduced for use in New Zealand in the 1950s, also as a rodenticide. These compounds belong to the indandione class of anticoagulants (figure 8).\textsuperscript{15}
1. Introduction

1.1. Importance, history and methods for the control of vertebrate pests

![Structure of first generation anticoagulants, pindone and diphacinone and their parent compound, indandione.]

**Figure 8:** Structure of first generation anticoagulants, pindone and diphacinone and their parent compound, indandione.

In New Zealand and Australia, pindone has been used to control wallabies and possums, however it has proven most effective in the control of rabbits. Diphacinone is considered to be more toxic than pindone. Toxicity data on both pindone and diphacinone display marked species variation, with rabbits needing a dose in the range of only 2-18 mg/kg, whereas non-target species such as horses, cattle, goats, chicken, dogs and cats need a dose of 35-100 mg/kg for these compounds to be lethal.

Although these compounds have been proven to be highly effective for rodent and rabbit control, they are generally considered to be of low toxicity to a range of species. Pindone has not been successful for possum control in New Zealand, and has been found to be highly persistent in the environment, which raises the risk of secondary poisoning. When anticoagulants such as pindone have been used in the field control of pests, it has resulted in wildlife contamination. In the USA, golden eagles showed signs of...
haemorrhages after eating meat from animals poisoned with diphacinone. Furthermore, the low potency of such anticoagulants has led to sub-lethal doses in target species, which has resulted in increased resistance towards this class of compounds.15

B) Second generation anticoagulants

Brodifacoum is a widely used, potent, second-generation anticoagulant rodenticide developed in the mid-1970s. This compound has experienced success in aerial applications in island rodent eradication programmes and has been used to control possums and rats in New Zealand since the 1990s. Bromadiolone, another second-generation anticoagulant, was patented in 1968 and introduced to the market as a rodenticide in New Zealand in 1980, however it is not as widely used as brodifacoum.15,43 These compounds are synthetic products, structurally related to the naturally occurring coumarin (figure 9).

Second generation anticoagulants, like their predecessors, act by interfering with the normal synthesis of vitamin K-dependent clotting factors in the liver, however brodifacoum and bromadiolone exhibit greater potency than their first generation counterparts. This is likely to be due to their greater affinity for the vitamin K-epoxide.

Figure 9: Structure of 2nd generation anticoagulants and their parent compound, coumarin.
reductase, and subsequent accumulation and persistence in the liver and kidneys after absorption.\textsuperscript{51}

Brodifacoum and bromadiolone are extremely toxic to a number of animal species.\textsuperscript{15} The majority of mammals display LD\textsubscript{50} values < 1 mg/kg and most birds showing LD\textsubscript{50} values < 3 mg/kg. Second generation anticoagulants are unlikely to affect invertebrates, which have different blood-clotting systems from vertebrates.\textsuperscript{52}

Although second generation anticoagulants possess advantages over their first generation predecessors such as greater potency and efficacy in possum control, their synthetic complexity mean they are much more expensive than their non-anticoagulant competitors (1080 or cyanide).\textsuperscript{15} As with most anticoagulants, secondary poisoning is a real concern, with a number of species of non-target birds being affected during use of brodifacoum for rodent control.\textsuperscript{15} In 2000, the NZ Department of Conservation ordered the reduction of brodifacoum in mainland field use due to concerns relating to the contamination of bird and game.\textsuperscript{53} Studies conducted on predators (mainly foxes and buzzards) exposed to bromadiolone via contaminated prey showed elevated liver concentrations of the anticoagulant poison.\textsuperscript{54}

Even though second generation anticoagulants are considered to be of greater potency than their precursors, these compounds still require 2-4 weeks for fatality to occur in possums, hence they are not considered to be humane.\textsuperscript{15}
1.2. Para-aminopropiophenone (PAPP) – a new agent for pest control

1.2.1. History, development and use of PAPP

Para-aminopropiophenone (PAPP) (1) was first synthesized in 1900 and first generated interest in World War II as an antidote for cyanide poisoning (figure 10). Research in the 1950s and 60s discovered that PAPP possessed radioprotective properties which became useful as a treatment for radiation poisoning in the 1970s in the UK.

![Structure of PAPP (1).](image)

Figure 10: Structure of PAPP (1).

In the 1980s, the United States Fish and Wildlife Services investigated PAPP as a toxin for coyote (Canis latrans) control and found that it was highly toxic to canids, however, vomiting was a complicating factor, which could not initially be solved, and so the research was discontinued. In the meantime, 1080 has continued to be the main toxin in use, however more recently, PAPP has been revisited and investigated in both New Zealand and Australia for the humane control of introduced predators such as feral cats and stoats. In 2011, PAPP’s registration phase was completed, with the toxin currently marketed in New Zealand for the control of stoats and feral cats under the trade name PredaSTOP®.

1.2.2. Mode of action of PAPP

The toxic effects of PAPP are attributed to the formation of methemoglobin (MtHb), which acts to impair the red blood cells (RBC’s) ability to carry oxygen, resulting in cerebral hypoxia, central nervous system depression and eventual death. For the
purposes of the present research, a closer look at the mechanism by which PAPP induces methemoglobinemia was required.

1.2.3. **The normal function of hemoglobin in the RBC**

Hemoglobin is a globular protein situated in the red blood cell that is responsible for oxygen transport from the lungs to the tissues, which operates within a physiological range of oxygen partial pressures in arterial and venous blood (30-100 mmHg).\(^6^7\) Hemoglobin is comprised of a tetramer of four subunits, two α and two β (figure 11), each containing a porphyrin heme group, that can reversibly combine with a molecule of oxygen.

![Tetrameric structure of hemoglobin and chemical structure of porphyrin heme group.](image)

*Figure 11:* Tetrameric structure of hemoglobin and chemical structure of porphyrin heme group.\(^6^8\)

A sigmoidal relationship exists between the affinity of the heme groups for oxygen, and the partial pressure of oxygen in the blood (figure 12). The relationship is sigmoidal due to an interaction present between the four heme groups called ‘cooperativity’. These interactions involve stereochemical changes in both the tertiary structure of each subunit and in the quaternary structure of the overall tetramer, acting to alter the oxygen affinity of each of the heme groups.\(^6^7,6^9\)
Hemoglobin usually exists in one of two conformations; oxy- and deoxy-hemoglobin. In the oxygen free, deoxy-conformation, the tetramer is stabilized by salt bridges formed between oppositely charged amino acid residues of adjacent subunits. In this quaternary structure, the relative affinity of the heme groups for oxygen is low.\textsuperscript{67}

The binding of oxygen to a heme group results in a change in the tertiary structure of the subunit and a breakage of the salt bridge to its neighbouring subunit. This breakage of the salt bridge removes the constraint that stabilizes the deoxy conformation, resulting in an increased affinity of the adjacent heme group to oxygen. This process is then repeated like a chain reaction until eventually all the salt bridges are broken, and all four hemes are bound to oxygen, resulting in a shift towards the oxy-conformation or ‘liganded form’.\textsuperscript{71,72}

The sigmoidal relationship between oxygen affinity of hemoglobin and partial pressure of oxygen is significant when fully oxygenated hemoglobin reaches the tissue capillaries. The sigmoidal oxygen dissociation curve means that release of one oxygen molecule in tissue capillaries results in a decreased affinity for the residually bound oxygen, which subsequently results in delivery of large amounts of oxygen to the tissue.\textsuperscript{67}
1.2.4. PAPP and its induction of methemoglobinemia

Methemoglobinemia (MtHb) is the phenomenon by which PAPP causes its toxic effect. MtHb occurs when the central iron atom of a heme group situated on the hemoglobin protein is oxidized from the ferrous (Fe$^{2+}$) to the ferric (Fe$^{3+}$) form (scheme 4). 67, 73, 74

Scheme 4: Oxidation of porphyrin heme group.

With its hydrophobic structure, the heme pocket shields the ferrous iron against oxidation to the ferric state, however a small amount of methemoglobin is formed continuously in the red cells regardless. 75 Several endogenous reduction systems exist in the RBC to restore methemoglobin (Fe$^{3+}$) to the reduced state (Fe$^{2+}$), and in normal individuals, only about 1% of total hemoglobin is methemoglobin at any given time. 76

The oxidation of the heme iron from ferrous (Fe$^{2+}$) to ferric (Fe$^{3+}$) causes a 'left shift' in the oxygen dissociation curve, resulting in a higher affinity for oxygen by the remaining heme iron, moreover, the hemoglobin loses its ability to combine reversibly with molecular oxygen. 77 As a result of the cooperativity observed in hemoglobin, the irreversible binding of the heme to oxygen subsequently leads to the other hemoglobin subunits holding on to oxygen more tightly, ultimately impairing the ability of hemoglobin to transport oxygen and carbon dioxide to tissues, leading to tissue hypoxia, cyanosis, impaired aerobic respiration, metabolic acidosis, and in severe cases, death. 74, 76, 78
The most common form of methemoglobinemia is ‘acquired’ or ‘drug-induced’ methemoglobinemia.\textsuperscript{56} This type of MtHb is predominantly caused by ingestion or skin exposure to indirect oxidizing agents\textsuperscript{79} such as aryl amines,\textsuperscript{42,58} which act to cause MtHb formation without themselves being the causative agents (figure 13). The MtHb inducing properties of the majority of these aryl amines were discovered as side effects of marketed drugs such as analgesics (acetaminophen), anaesthetics (benzocaine), antibiotics (sulphanilamide and sulfamethoxazole) and anti-malarials (dapsone).

![Figure 13: Aryl amines known to induce MtHb.](image)

Upon ingestion, aryl amines (such as PAPP) are oxidized by hepatic mixed function oxidases into aryl hydroxylamines, which constitute the active metabolite with regards to induction of MtHb.\textsuperscript{42}
1. Introduction

1.2. *Para*-aminopropiophenone – a new agent for pest control

![Scheme 5: Metabolism of aromatic amines in the liver.](image)

Once passively diffused into the red blood cell, and in the presence of oxygen, aryl hydroxylamines undergo a coupled redox reaction with hemoglobin, forming methemoglobin ($\text{Fe}^{3+}$), the toxic endpoint, and aryl nitroso derivatives (scheme 5).

The coupled redox reaction first involves the oxidation of a free radical aryl hydroxylamine to an aryl nitroso derivative, and the compliment reduction of molecular oxygen to the hydrogen peroxide. Secondly, the free radical hydrogen peroxide subsequently reacts *in situ* to oxidize hemoglobin to methemoglobin, forming a hydroxide anion and a hydroxyl radical as by-products (scheme 6). To counter the effect of this xenobiotic-induced coupled redox reaction, NADH-cytochrome $b$ reductase oxidase, (also referred to as methemoglobin reductase) acts to reduce methemoglobin back to hemoglobin. MtHb induction is an NADPH-dependent reaction (using NADPH generated from the glucose-6-phosphate pathway), with reductive enzymes present in the red cells such a diaphorase, NADP flavin reductase and glutathione which act to drive MtHb formation through back-reduction of aryl nitroso derivatives to aryl hydroxylamines (scheme 6).

As a result of a number of enzymatic pathways available to back-reduce aryl nitroso derivatives, the overall net effect is a cycle that drives the transformation of hemoglobin into methemoglobin, until the aryl hydroxylamine and aryl nitroso derivatives are eventually eliminated by side reactions, or until endogenous reducing enzyme co-factors (NADPH) become depleted.
1. Introduction

1.2. Para-aminopropiophenone – a new agent for pest control

**Scheme 6:** Formation of methemoglobin (MtHb) by aryl amines.\(^{83}\)
1. Introduction

The cyclic nature of MtHb induction caused by indirect oxidizing agents demonstrates how the uptake of a relatively small amount of aryl hydroxylamine can transform a large number of hemoglobin equivalents into methemoglobin, suggesting that these compounds are toxicologically very potent.

1.2.5. Effectiveness and species variation of PAPP

The effectiveness of PAPP is dependent on strain, species, sex and route of administration. Via the oral route, PAPP has been found to exhibit good species selectivity, being extremely toxic to stoats, cats, bobcats, kit foxes, coyotes and dogs, with LD$_{50}$ values all under 50 mg/kg, whereas most non-target species such as birds demonstrate LD$_{50}$ values above 100 mg/kg.

The variations between species have been attributed mainly to differences in hepatic and erythrocyte enzyme expression. Species with greater MtHb reductase activity (mice, rats) show less susceptibility to the toxic effects of PAPP. Deficiency in glucose-6-phosphate-dehydrogenase (G-6-P-D) or glutathione reductase enzymes has also been shown to increase the level of MtHb formation. Differences in the metabolic pathways responsible for the detoxification and excretion of PAPP also affect its overall toxicity. It has been reported that PAPP is metabolized by N-acetylation in rats, whereas ring and aliphatic oxidation occurs in dogs. In monkeys, both N-acetylation and oxidation take place.

One other factor known to affect the toxicity of PAPP is genetic abnormalities within an individual of a species, which act to alter the structure of hemoglobin. These abnormalities cause the hemoglobin to be more susceptible to oxidation and thus MtHb formation, however this type of susceptibility is of rare incidence.
1.2.6. Benefits of PAPP use as a pesticide

PAPP has been proven to achieve a high number of kills in the field, with hydrochloride bait formulation of PAPP in pen trials exhibiting an LD$_{50}$ = 9.3 mg/kg and 15.5 mg/kg to stoats and ferrets, respectively.$^{6,61,62}$ Once PAPP has induced its toxic effect, it is readily metabolized to non-toxic intermediates, so it is not present in carcasses at levels which could cause secondary poisoning of domestic animals such as dogs, and so it will not persist and move through the food chain.$^{60}$

One of the main advantages of PAPP as a vertebrate pesticide is its relatively humane mode of action.$^{61}$ PAPP induces its lethal effect by reducing the oxygen supply to the brain, leading to symptoms such as progressive lethargy, drowsiness, stupor and eventually unconsciousness just before death.$^{61,63,90}$ Moreover, the time to death is short, usually within 1-2 h following ingestion.$^{26,25}$ PAPP is considered more humane in comparison to 1080, which, although quick to induce its toxic effect, causes the animal to enter an ataxic/hyperactive stage involving muscular spasms, and it has been suggested that this phase is most likely to be associated with pain or distress for the animal.$^{25}$ PAPP is also considered more humane than anticoagulants such as diphacinone, which, although less acute in their mode of action in comparison to 1080, are known to consist of an average time to death of 10.5 days in ferrets.$^{91}$ In summary, PAPP is postulated to provide a more suitable balance between humane vertebrate pest control markers such as time to death and level of distress.

A further advantage of PAPP as a pest control agent is that there exists a simple antidote, methylene blue, for non-target species in case of accidental poisoning of pets or farm dogs. Methylene blue acts as an electron donor for the non-enzymatic reduction of methemoglobin. The NADPH methemoglobin reductase enzyme converts methylene blue (oxidized form) to leucomethylene blue (reduced form), using NADPH (scheme 7).$^{42}$ Leucomethylene blue then subsequently reduces methemoglobin (Fe$^{3+}$) to hemoglobin (Fe$^{2+}$), with the methylene blue regenerated from this reaction then recycled to undergo another redox reaction with NADPH methemoglobin reductase (scheme 7).$^{42}$
Scheme 7: Methylene blue as an antidote for PAPP-induced MtHb toxicity.  

In summary, PAPP, possesses an antidote, is relatively humane, exhibits low toxicity to most bird species, is not persistent in the food chain, and has a low chance of secondary poisoning. In light of these properties, PAPP is considered to represent a pest control agent that is more acceptable than conventional poisons, embodying the most significant advance in the field since the 1970s.
1.3. Factors to be considered in the design of MtHb inducers

1.3.1. Aim of current research

Although prior reviews have provided some insight into the nature of toxicity of MtHb inducers in mammals, a detailed study that has systematically investigated structure-activity relationships with regards to PAPP-induced MtHb has been lacking. Using PAPP as a platform, the aim of this research was to design, synthesise and evaluate structurally-related analogues of PAPP for their MtHb inducing properties, with the aim of enhancing toxicity towards rats. In order to design potent MtHb inducers, a consideration of not only structural but also the physicochemical parameters significant to MtHb toxicity (such as lipophilicity and electronics) was required.

1.3.2. Lipophilicity and log $P$

In 1997, Lipinski and co-workers proposed the ‘rule of five’, which essentially stated the requirements for a drug to be orally active. Lipinski’s ‘rule of five’ states that, in general, an orally active drug has no more than one violation of the following criteria:

- Not more than five hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms)
- Not more than ten hydrogen bond acceptors (nitrogen or oxygen atoms)
- A molecular mass not greater than 500 Daltons
- An octanol-water partition coefficient (log $P$) not greater than five

With regards to the design of MtHb inducers, the last restriction in the list of criteria concerning the partition coefficient, is of particular importance. The partition coefficient is a ratio of concentrations of the unionized solute between two phases (usually octanol and water) with the logarithmic ratio called ‘log $P$’ (figure 14). The log $P$ value is also known as a measure of lipophilicity for a specific molecule.
Lipophilicity, expressed as log $P$, appears in some form in almost every analysis of physicochemical properties related to absorption.\textsuperscript{95} In 1964, Hansch\textsuperscript{96,97} reported that the relationship between lipophilicity and biological activity was frequently parabolic, suggesting that this correlation often leads to an optimum log $P$ value for a desired activity or selective distribution. Lipinski\textsuperscript{93} demonstrated that the lipophilic profile for a number of successful orally active drugs was, more often than not, situated near log $P = 2$.

Experimentally measured log $P$ values available from the literature, supplemented by \textit{in silico} predictions,\textsuperscript{98} will be used to determine the structural analogues of PAPP that will be synthesized. An emphasis will be placed on probing the effect of compound lipophilicity on drug absorption, uptake into red blood cells, and ultimately MtHb toxicity, \textit{in vivo}.

1.3.3. Electronics and the Hammett constant

In the late 1930s, Hammett\textsuperscript{99,100} proposed that there was a relationship between the structure of compounds and their chemical reactivity. Firstly, Hammett elucidated that, in acid-base reactions, the acid strengths of various benzoic acids were strongly influenced by the electronic nature of their substituents.\textsuperscript{101} Further to this, Hammett discovered that this relationship was also present in a number of other reactions involving benzoic acids and benzoic acid derivatives (e.g. hydrolysis, esterification).\textsuperscript{99} These observations led Hammett to propose a quantitative relationship between the nature of the substituent R, and the reactivity of the side chain Y, of the benzoic acid or benzoic acid derivative (figure 15). This relationship is now known as the Hammett equation (figure 15).
In this equation, $k$ and $k^\circ$ represent the rate or equilibrium constants for reactions of the substituted and the unsubstituted benzene derivatives, respectively. Sigma, or $\sigma$, represents the substituent (or Hammett) constant, which depends solely on the nature and position of the substituent R, while $\rho$ represents the reaction constant, which depends on the reaction, the conditions under which it takes place, and the nature of the side chain Y. The equation was tested by Hammett on fifty-two reaction series\textsuperscript{102} and was found to express considerable agreement with respect to the influence of the substituent R on reactivity. The validity of this equation, however, is restricted to substituents in the meta- and para-positions of the benzene ring\textsuperscript{103}.

Currently, the Hammett equation is generally accepted as a valid SAR development tool, and is relevant when considering the biochemical mechanism of action by which PAPP causes MtHb induction (scheme 8).

When ingested, PAPP undergoes a number of key biotransformations, the first being oxidation of PAPP through to its hydroxylamine derivative 2 (scheme 8). Once in the red cell, further biotransformation occurs through the coupled redox reaction of hydroxylamine derivative 2, as described in detail in section 1.2.4. (scheme 8).
Mahmud et al. demonstrated that, in the presence of rat liver microsomes, erythrocytes and NADPH, there was significant correlation between the MtHb induction and Hammett constants ($\sigma$) of a number of para-substituted aryl amines and dapsone analogues, suggesting that it is the electronic nature of the para-substituent that influences the biotransformations that play a role in MtHb induction (figure 16).
Figure 16: Correlation between the Hammett substituent constant ($\sigma$) and metabolism-dependent methemoglobin induction for para-substituted aryl amines in the presence of rat liver microsomes in a 1-compartment model.\textsuperscript{104}

By consultation of Hammett substituent constants available from the literature, supported by \textit{in silico} predictions,\textsuperscript{98} structural analogues of PAPP will be designed with an emphasis on probing the effect that the electronic nature of the substituent (attached to the \textit{para} position of the aryl amine ring), exhibits on the biotransformations that take in MtHb induction.
1.3.4. Experimental design rationale

The overall approach towards the development of novel toxins for MtHb induction will start with the design of PAPP-related analogues, based on a consideration of the relevant physicochemical parameters (scheme 9). Once potential structural candidates have been put forward, compounds will be synthesized and subsequently characterized by standard spectroscopic techniques (NMR, IR, and MS) as described in section 7.2. Following this, experimental evaluation of the physicochemical parameters relevant to MtHb induction will be performed on the aforementioned analogues (scheme 9). Subsequently, compounds will then be screened via an in-house in vitro MtHb assay to determine their toxicity towards red blood cells (scheme 9). Further to this, candidates will be nominated for synthetic scale-up and subsequent in vivo evaluation in rats using a pilot cage trial (scheme 9). Depending on the outcome of the in vivo results, compounds may be further subjected to in vitro stability experiments, in an effort to gather additional information with respect to their metabolic profile (scheme 9).

Scheme 9: Design rationale for biological testing and structure-activity development.
This experimental design will facilitate a feedback loop, whereby physicochemical evaluation, \textit{in vitro} bioevaluation, \textit{in vivo} bioevaluation and stability studies will be considered in the subsequent design and optimization of further analogues. Ultimately, a structure-activity profile of potential MtHb inducers will be constructed (scheme 9).
2. ESTABLISHMENT OF EXPERIMENTAL TECHNIQUES FOR THE EVALUATION OF SYNTHESIZED ANALOGUES
2.1. Experimental appraisal of relevant physicochemical parameters

2.1.1. Lipophilicity, log P and RP-HPLC technique

Traditionally, log P is measured via the ‘shake-flask method’ as the partition coefficient between octanol and water.\textsuperscript{94,97,105} Despite a large library of experimental data being available for validation, this method\textsuperscript{106} is often slow, tedious, and wasteful, due to its requirement of relatively large amount of pure compound.\textsuperscript{107} Furthermore, emulsions may form easily when the analyte is shaken,\textsuperscript{108} and complications can arise when compounds exhibit instability in aqueous media.\textsuperscript{109} These complications result in difficulty in determining accurate log P values for desired compounds.

Over the years, there have been many attempts to develop alternative methods for log P measurement. Such developments include modified shake-flask methods using PVC (polyvinyl chloride),\textsuperscript{110} stir-flask methods,\textsuperscript{111} filter-probe measurements,\textsuperscript{112} filter-chamber measurements,\textsuperscript{113} solid-phase microextraction,\textsuperscript{114} and various potentiometric methods.\textsuperscript{113,115,116}

One technique developed that is of interest to the current research involves the use of reversed phase high performance liquid chromatography (RP-HPLC). In this technique, the retention factor, or time taken to pass through a column, is used to measure the lipophilic properties of each compound. The retention factors of literature compounds can be graphed against their experimental log P values.\textsuperscript{117} The retention factors of compounds with unknown log P values can subsequently be measured, with these values used to calculate their corresponding experimental log P values.\textsuperscript{117}

With respect to the current research, our main concerns were how to deal with very small quantity of compound (< 5 mg) and how to measure a large number of compounds quickly. RP-HPLC has long been recognized as a reliable and reproducible method for log P determination,\textsuperscript{118-121} and offers advantages such as ease of sample preparation, high sensitivity, and computer automation to allow relatively high throughput of compounds.\textsuperscript{106} Ultimately, RP-HPLC represented a quick, easy, reliable and reproducible method for evaluation of log P in the present work.\textsuperscript{122}
The RP-HPLC assay for log $P$ assignment employed a method adapted from that of Acanski et al. as described in detail in section 7.1.2. The primary hurdle in the development of the RP-HPLC log $P$ assay was the construction of a reliable calibration curve. The standards for this curve were selected based on a number of considerations (table 1).

<table>
<thead>
<tr>
<th>Standard</th>
<th>log $P$ Lit. Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aniline (4)</td>
<td>0.94$^{94,124}$</td>
</tr>
<tr>
<td>Acetanilide (5)</td>
<td>1.16$^{125}$</td>
</tr>
<tr>
<td>Benzonitrile (6)</td>
<td>1.56$^{124}$</td>
</tr>
<tr>
<td>Acetophenone (7)</td>
<td>1.62$^{124}$</td>
</tr>
<tr>
<td>Benzyl Acetate (8)</td>
<td>1.96$^{124}$</td>
</tr>
<tr>
<td>Propiophenone (9)</td>
<td>2.19$^{124}$</td>
</tr>
<tr>
<td>4-Chloroacetophenone (10)</td>
<td>2.51$^{126}$</td>
</tr>
<tr>
<td>Butryophenone (11)</td>
<td>2.66$^{127}$</td>
</tr>
<tr>
<td>Valerophenone (12)</td>
<td>3.11$^{128}$</td>
</tr>
<tr>
<td>Benzophenone (13)</td>
<td>3.18$^{128}$</td>
</tr>
<tr>
<td>Naphthalene (14)</td>
<td>3.41$^{94,129}$</td>
</tr>
<tr>
<td>Hexanophenone (15)</td>
<td>3.64$^{130}$</td>
</tr>
</tbody>
</table>

Table 1: Log $P$ standards chosen for the calibration curve.

The RP-HPLC log $P$ assay is known to be inconsistent when dealing with ionizable compounds, hence only compounds that are neutral at pH 7 were chosen. The interaction of each compound with the packed C$_{18}$ stationary phase is strongly influenced by its structural motifs, hence compounds with similar functional groups to that of PAPP (such as aniline (4) and phenones 7, 9-13 and 15) were selected. With consideration that the favourable partition coefficient for successful orally active drugs is log $P$ ca. 2, standards were chosen to represent a range of log $P$ values centred around this value, with increments in either direction of 0.5 log $P$ units (e.g. ~ 0.5, ~1.0...
~3.5). In an effort to increase the reliability of the method further, a compound with a different structural motif but similar log $P$ to that of each phenone (7, 9-13 and 15) and/or aniline (4) was also measured (table 1). Compounds were measured in triplicate to confirm reproducibility, with a very low standard error obtained (graph 1). The retention times for the standards showed good correlation ($R^2 = 0.9802$) with their corresponding experimental shake-flask log $P$ values (graph 1). The constructed standard curve was subsequently used to calculate the corresponding log $P_{\text{exp.}}$ values for prepared analogues.

Graph 1: Log $P$ standards calibration curve.

The reliable, accurate RP-HPLC log $P$ assay established within this work provided a useful indicator for the lipophilic properties of prepared analogues prior to their in vitro screening.
2.1.2. Electronics, the Hammett constant and the ipso $^{13}$carbon NMR shift technique

Hammett constants are traditionally calculated by measuring the acid dissociation constant of a particular ring-substituted benzoic acid. For the purpose of this research, preparing the corresponding benzoic acid of each MtHb inducer analogue, in order to measure its acid dissociation constant by such a method would be time consuming and wasteful. An alternative, more rapid and efficient method was preferred.

In 1961, Spiesecke et al. proposed that there was a correlation between the Hammett constant ($\sigma$) of substituents on mono-substituted benzenes and the $^{13}$C NMR chemical shift ($\delta$) of the para carbon isotope (figure 17).

![Figure 17: Relationship between the NMR chemical shift ($\delta$) of the para $^{13}$carbon isotope and the Hammett constant ($\sigma$) of substituents attached to benzene.](image)

Spiesecke et al. reported that the $^{13}$C NMR chemical shift ($\delta$) was governed by the electron density on the carbon atom (itself influenced by field and resonance effects) and anisotropic-induced magnetic field effects. More specifically, Spiesecke et al. demonstrated that the general trend observed for para-$^{13}$C NMR chemical shifts ($\delta$) was consistent with the expected change in $\pi$-electron density at the para-$^{13}$C isotope, which is attributed to the electronic contribution of the para-substituent. Therefore, for a mono-substituted benzene system, electron-donating substituents cause an increased shielding at the para-$^{13}$C isotope (causing a decrease in chemical shift ($\delta$)), while electron-withdrawing substituents cause a decreased shielding at the para-$^{13}$C isotope (causing an increase in chemical shift ($\delta$)), relative to the para-$^{13}$C isotope in the unsubstituted benzene. Of the compounds measured in this study, the largest variation
was observed between nitrobenzene and \(N,N\)-dimethylaniline, with their \(para\)-\(^{13}\text{C}\) chemical shifts differing by about 18 ppm (figure 18).\(^{132}\)

![Figure 18: Linear correlation between \(para\)-\(^{13}\text{C}\) chemical shift (\(\delta\)) and Hammett constant (\(\sigma\)) of various substituents attached to benzene.\(^{132}\)](image)

Over the years, many further studies have supported the aforementioned correlation between the Hammett constant and the \(^{13}\text{C}\) NMR chemical shift (\(\delta\)).\(^{133-137}\) In 1994, Mezzina \textit{et al.}\(^{138}\) demonstrated similar correlations to that of Spiesecke\(^{132}\) for a series of \(para\)-substituted phenylhydrazines and anilines. Given the substantial data provided by the literature, it was proposed that for the purpose of this research, the observed relationship between the Hammett constant and the \(^{13}\text{C}\) NMR chemical shift (\(\delta\)) for the carbon \(ipso\) to nitrogen of the aryl amine could be used to determine the relative electronic contribution of various \(para\)-substituents in analogues of PAPP (figure 19).
Measurement of the ipso $^{13}$C NMR chemical shift for the synthesized MtHb analogues was postulated to provide insight into the role that electronic effects play in the biotransformation and ultimately the toxicity of these molecules. Advantages of this particular technique include speed and ease of measurement (5-10 mins), minimal processing, and the potential for high-throughput. Standardization of deuterated solvent was preferred, in order to minimize variability with respect to the solvent environment. Parr et al.\textsuperscript{139} and Axenrod et al.\textsuperscript{140} demonstrated that the NMR chemical shifts of substituted anilines were significantly affected by the hydrogen bonding character of the solvent. In light of this, deuterated chloroform (CDCl$_3$) was chosen over deuterated dimethyl sulfoxide (d$_6$-DMSO). Ipso $^{13}$C NMR chemical shifts were measured in parts per million (ppm) in reference to the same carbon in PAPP, which was set at $\delta = 0$ ppm.
2.2. In vitro evaluation

2.2.1. MtHb determination using a spectrophotometric assay

Historically, MtHb has been measured by a spectrophotometric method first established by Evelyn and Malloy in 1938, and a number of investigators have since adopted and/or modified this method for their own research. For the purpose of this research, this technique was explored as a method for determining the MtHb induced by synthesized analogues, by adoption of the modified procedure reported by Harrison and Jollow.

Incubation conditions were adapted from Coleman et al. and Mahmud et al., with the initial conditions as follows: washed red blood cells (100 μL) were incubated (37 °C, 1 h, n = 4) with compound (100 μM) in DMSO (5% v/v), microsomes (1 mg/ml) and NADPH (1 mM), with a final volume of 200 μL, diluted with PBS (phosphate buffered saline) (0.1 M, pH 7.4).

After the 1 h incubation, samples were then assayed for MtHb induction. Firstly, blood samples (100 μL) were hemolyzed using Na+/K+ phosphate buffer (0.02 M, pH 7.8) containing 0.05% v/v Triton X-100 (4 mL). From the hemolyzed solution were taken four 0.80 mL aliquots and one drop of aqueous 10% w/v potassium cyanide added to samples 2 and 4, and one drop of aqueous 10% w/v potassium ferricyanide added to samples 3 and 4. The absorbance of each sample (S1, S2, S3, S4) was subsequently measured spectrophotometrically at a wavelength of 635 nm, and the MtHb percentage then calculated from the absorbances as follows (figure 20).

\[
\text{MtHb} (\%) = \frac{S1_{ABS} - S2_{ABS}}{S3_{ABS} - S4_{ABS}} \times 100
\]

**Figure 20:** MtHb(%) calculation based on the methods of Harrison and Jollow.
Background MtHb levels were accounted for by a negative control, consisting of 5% DMSO and no compound. Incubations were performed in triplicate to reduce variability and identify any outliers.

The initial aim was to establish a suitable practical working concentration of PAPP for the MtHb assay. This was conducted through incubation with a range (0.001-100mM) of PAPP concentrations (graph 2).

**Graph 2:** Concentration-response curve for MtHb induction of PAPP in rat blood, using the Harrison and Jollow\textsuperscript{146} method.

Measurements obtained from the concentration-response curve (graph 2) exhibited a standard error that was unacceptably high (±10-20% MtHb). In light of this, a series of investigatory experiments was conducted in an attempt to identify the cause of the observed variation in an effort to minimize this error.

The observed variability could to be attributed to a number of sources of error. One area that difficulties could arise from is the *in vitro* bioactivation of PAPP to its hydroxylamine active metabolite 2. To investigate whether the bioactivation process was playing a role in the observed variability, sodium nitrite, a direct oxidant of hemoglobin which does not require bioactivation, was assayed for MtHb induction. It was hypothesized that sodium nitrite-induced MtHb would permit a simplified version of the MtHb assay, allowing examination as to whether the rat liver microsomes and/or NADPH
(components of the assay responsible for bioactivation of PAPP) were implicated in the variability of the results. In order to reduce the variability, the incubations were performed in quadruplicate. Unfortunately, the relative standard error obtained from the MtHb measurements for sodium nitrite was no less than that of PAPP (table 2). Nevertheless, sodium nitrite was employed for further preliminary experiments, in an effort to rule out rat liver microsomes and NADPH as factors associated with error.

<table>
<thead>
<tr>
<th>Sodium nitrite</th>
<th>PAPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run No.</td>
<td>MtHb(%)</td>
</tr>
<tr>
<td>1</td>
<td>8.8</td>
</tr>
<tr>
<td>2</td>
<td>11.8</td>
</tr>
<tr>
<td>3</td>
<td>15.4</td>
</tr>
<tr>
<td>4</td>
<td>17.4</td>
</tr>
<tr>
<td>Mean</td>
<td>13.3</td>
</tr>
<tr>
<td>SD</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Table 2: MtHb of sodium nitrite (100μM) vs. PAPP (100μM) obtained by the Harrison and Jollow\textsuperscript{146} method.

With preliminary investigations suggesting that the bioactivation process was not the source for the observed variability, attention next turned to the time taken to complete particular components of the assay procedure. The labour intensive nature of the MtHb assay resulted in a considerable standing time for samples while the quenching, work up and plate-loading was taking place. Moreover, this time-lag was amplified when the number of analogues being assayed increased. In order to determine whether the time-delay was affecting MtHb measurements, samples were measured at time = 0 and 15 mins post-incubation. While MtHb measurements with relatively low variation could be obtained at time = 0, those observed 15 mins post-incubation were considerably larger, and subsequently led to a nonsensical MtHb result. This suggested that in the time taken for quenching, working up and loading of numerous samples onto the 96-well plate,
considerable drift in absorbance readings for samples was occurring, which perhaps contributed to the observed variation (graph 3).

Graph 3: MtHb levels for sodium nitrite (100μM) at time = 0 and 15 mins post-incubation.

As a result of the considerable standing time associated with the MtHb assay procedure, the temperature of the samples during this time was likely to equilibrate to room temperature (ca. 25 °C), potentially allowing enzymes and co-factors present in the samples to continue to catalyze biochemical reactions, such as the reduction of MtHb, thus altering the level of MtHb measured. In an effort to arrest any potential enzymatic activity present in the RBC post-incubation, an experiment was conducted whereby the samples were placed on ice (ca. 4 °C) immediately after the 1 hour incubation period, for the duration of the quench, work up and MtHb measurement. Promisingly, this appeared to reduce the observed variation for sodium nitrite-induced MtHb, compared with that at 25 °C (graph 4). As a consequence, the same procedure was conducted using PAPP (including rat liver microsomes and NADPH), however the observed standard error, while less than that at room temperature, was still approximately ±10% (graph 5).
2. Establishment of experimental techniques

2.2. In vitro evaluation

Graph 4: Temperature-dependency of MtHb induced by sodium nitrite (100 μM) post-incubation.

Graph 5: Temperature-dependency of MtHb induced by PAPP (100 μM) post-incubation.

Despite a reduction in the variability observed when samples were put on ice post-incubation, the standard error remained unacceptably high. The main advantage of the Harrison and Jollow method\textsuperscript{146} was the potential for a relatively high-throughput screening of compounds through the use of a 96-well plate. Unfortunately, however, the MtHb measurements obtained from samples loaded onto the 96-well plate reader were considerably different to that of single quartz and plastic cuvettes (graph 6). The results indicated that only the quartz cuvette offered readings at an acceptable standard error, suggesting that MtHb results obtained from the 96-well plate reader were unreliable (graph 6).
Another complication associated with this method consisted of the requirement to use cyanides, which are known to thermally decompose to produce toxic fumes of hydrogen cyanide, carbon monoxide, carbon dioxide, and nitrogen oxide. As a result, extra safe handling procedures were required, adding further time to the assay procedure.

Further to the matter of cyanides, the wording of the method discussing their use instructed the addition of a ‘drop’ of a particular cyanide compound to each aliquot. This is a vague and inexact instruction, highly dependent on what is used to prepare the ‘drop’ (e.g. Pasteur pipette). Moreover, due to the yellow/brown colour of potassium ferricyanide, it was postulated that the error associated with the addition of this cyanide compound may play a role in interfering with the absorption reading on S3 at 635 nm, which in turn could affect MtHb determination. In light of this, an experiment was conducted whereby the cyanide added was standardized to a number of fixed volumes, using an autopipette to measure the volume. The results demonstrated an inverse relationship between absorbance reading for S3 and the volume of potassium ferricyanide added, suggesting that addition of excess potassium ferricyanide altered the MtHb determination (graph 7). In light of this, the volume of potassium ferricyanide was, from this point forward, standardized to 20μL per sample.

**Graph 6:** Discrepancy of MtHb measurements for a single sodium nitrite (100μM) sample in different spectrophotometric cells.
2. Establishment of experimental techniques

2.2. *In vitro* evaluation

**Graph 7:** Effect on MtHb formed by sodium nitrite (100μM) when standardizing the addition volume of potassium ferricyanide.

Despite efforts to identify and address potential sources of variability such as the bioactivation of PAPP, standing time and temperature-dependency of samples, cyanide addition volumes and spectrophotometric cell type for absorbance readings, the lowest standard error that could be obtained was still unacceptably high. This was of particular concern in light of the objective to compare the *in vitro* activity of prepared structurally-related PAPP analogues, some of which may demonstrate potentially similar levels of MtHb induction. Moreover, this procedure was not amenable to the relatively high throughput, reliable and reproducible assay desired for this project. In light of these complications, an alternative, simpler method for MtHb determination was sought.
2.2. MtHb determination using a blood-gas analyzer

Faced with a lack of success in accurately determining MtHb using spectrophotometric methods, attention next turned to the employment of an automated device. In the 1990s, automated methods of MtHb determination became common practice in biomedical research. Courtesy of Radiometer®, an ABL700 blood-gas analyzer was obtained in order to investigate whether an automatic device could enable accurate determination of MtHb induction. Put simply, a blood-gas analyzer is a ‘point of care’ instrument, used primarily in hospitals, designed for use by phlebotomists and nurses for the analysis of patients’ blood. Quick and easy to use, these machines measure a number of blood parameters such as blood gas (pO₂, pCO₂ and pH), electrolytes (cK⁺, cNa⁺, cCa²⁺ and cCl⁻), metabolites (cGlu and cLac) and CO-oximetry (sO₂, ctHb, O₂Hb%, COHb%, MtHb%, HHb%, HbF% and ctBil). Of relevance to this research were the blood-gas analyzer’s CO-oximetry capabilities, more specifically, its ability to measure MtHb. The CO-oximetry unit also uses a spectrophotometric method to determine MtHb, however it does so with greater sophistication. The ABL series of instruments are widely regarded as the gold standard for CO-oximeters, due to their possession of an optical measuring system based on a 128-wavelength spectrophotometer. Their basic manifold consists of the spectrophotometer connected via an optical fibre to a combined hemolyzer and cuvette (figure 21).

![Figure 21: The set-up of the ABL700 CO-oximetry unit.](image)
When measuring a sample, the blood is transported to the cuvette in the hemolyzer unit, for which the temperature is regulated to 37 °C. The sample is then ultrasonically hemolyzed in the cuvette in order to rupture the walls of the red blood cells to give an optically clear solution. Air bubbles are eliminated and hemolysis is enhanced by the exertion of an extra atmosphere of positive pressure maintained through hemolysis and measurement. Light from the lamp unit is transmitted through the cuvette and is guided via the optical fibre to the spectrophotometer, which tranduces the light signal into an absorption spectrum. From this spectrum, the analyzer’s computer calculates the blood samples CO-oximetry parameters. The CO-oximeter’s 128-wavelength spectrophotometer automatically corrects interferences that can impair the accuracy of the CO-oximetry measurements. 152

The cumulative effect of the meticulous control of these variables is an unparalleled level of accuracy and precision. Despite this method not having the ability to measure samples simultaneously (as with the 96-well plate), whole blood samples can be loaded by means of a capillary, without the requirement for a work-up procedure. Moreover, no more than two minutes are required for the combined measurement and rinse time. In light of these advantages, the blood-gas analyzer was considered an appropriate tool for the determination of MtHb induction for PAPP and its structurally-related analogues.
With a reliable MtHb determination method now in hand, the development of a working concentration for PAPP was revisited. This was required in order to find the concentration at which the synthesized analogues would show the greatest variability with respect to their MtHb inducing properties. The concentration-response curve established from the new method exhibited a considerable decrease in variation across replicates (n = 4) (≤±5%) (graph 8).

Graph 8: Concentration-response curve for PAPP.

Moreover, the data points displayed a sigmoidal relationship between concentration and MtHb induction, which was in agreement with concentration-response curves exhibited by compounds demonstrating a similar mechanism of MtHb toxicity. As a result of it being situated on the steepest point of the concentration-response curve, a working concentration of 100μM was nominated (graph 8). It was postulated that this concentration would be most likely to facilitate the greatest spread of data when evaluating structurally-related analogues of PAPP which may also exhibit similar MtHb inducing properties. Conversely, if concentrations 10μM or 1mM were adopted, then a ‘bottoming out’ or ‘saturation’ of MtHb induction may have been observed, respectively, potentially making a relative ranking of the PAPP analogues exhibited MtHb induction difficult.
With a working concentration now established, efforts next turned to examining the effect of changing the variables associated with the incubation conditions. The first variable under investigation was the source of blood, with an investigatory experiment undertaken that was primarily focused on determining the role that the rat blood played on influencing the inter-assay variability across a number of days. The results suggested that freshly collected rat blood would be required to reduce this inter-assay variability, due to a steady decrease in the observed MtHb levels as the blood became older (graph 9).

Another variable under investigation was the termination time for the incubation. Generally, it is not advised that incubations including co-factors such as NADPH go for longer than a single hour, as it has been demonstrated that the aqueous half-life of NADPH in buffer (at pH 7.4 and at 37 °C) is ca. 60 mins. With this in mind, attention next turned to evaluating the MtHb induction profile every 15 min over the standard incubation period, in an effort to elucidate whether a one hour time point was an appropriate stopping point to measure MtHb. The MtHb induction time profile demonstrated that peak MtHb levels occur around the 45 min interval, and by the one hour mark, the levels of MtHb were beginning to decline (graph 10).
Graph 10: MtHb induction profile for three PAPP incubations over a single hour period.

These findings suggest that, the forward reaction \((\text{Fe}^{2+} \rightarrow \text{Fe}^{3+})\) and the reverse reaction \((\text{Fe}^{3+} \rightarrow \text{Fe}^{2+}, \text{catalyzed by MtHb reductase})\) adopted an equilibrium position between 45-60 minutes. As a result, the one hour incubation period was deemed appropriate for the assay, on the grounds that the level of MtHb observed appears to plateau before this point, suggesting sufficient time had been permitted for a maximum MtHb level to be reached.

A variable also of importance with regards to the biochemical pathways taking place during the incubation is the activity exhibited by the microsomes employed for bioactivation. Microsomal activity can vary from day to day and batch to batch, depending on the number of freeze-thaw cycles and rigor of preparation, respectively.\(^{155}\) To explore the effect of this variable, an investigatory experiment was conducted employing a number of microsomal batches, of which the number of freeze-thaw cycles and the date of preparation were unknown. The results showed considerable variation in the observed MtHb induction when different batches of microsomes were employed (graph 11).
Graph 11: MtHb(%) induced by PAPP (100μM), bioactivated by different batches of microsomes.

Due to the variation observed across these batches, the following measures were implemented in an attempt to reduce the variability associated with the microsomes. A large, fresh batch of microsomes was prepared,\textsuperscript{156} and the protein content and CYP1A, CYP2B and CYP2E1 activity of the microsomes determined by standard methods.\textsuperscript{157-159} Microsomes were then split into one hundred aliquots, each with sufficient microsomal content for one assay, to avoid any complications associated with freeze-thaw cycles. This batch that was prepared was the only batch of microsomes used for the remainder of the \textit{in vitro} work.

Further to the issue of microsomes was their concentration in the assay. Due to the mechanism of MtHb toxicity being metabolism-dependent,\textsuperscript{80} it was considered that increasing the microsomal concentration from the recommended 1 mg/mL could also increase the bioactivation of PAPP to its hydroxylamine derivative, causing elevated levels of MtHb and thus conceivably enhancing the sensitivity of the assay. To investigate this, an experiment was conducted whereby a series of microsomal concentrations were examined, with the results demonstrating that, counterintuitively, MtHb induction decreased in relation to an increase in microsomal concentration (graph 12).
2. Establishment of experimental techniques

2.2. *In vitro* evaluation

Graph 12: Relationship between microsomal concentration (mg/mL) and levels of MtHb.

Explanations for the observed trend may be the binding of the substrate to phospholipid, due to the lipophilic nature of PAPP, or protein binding. Such binding would reduce free substrate concentration. More likely perhaps, is a greater extent of non-productive metabolism at higher microsomal concentrations, whereby the biotransformations on PAPP are predominantly not only *N*-hydroxylation, but instead further oxidation of the hydroxylamine derivative of PAPP to its nitroso, nitro and azoxy derivatives. Studies by Yang *et al.*\(^{160}\) suggest that the *in vitro* metabolic profile of PAPP is complex, and so for the purposes of this research, the original 1 mg/mL microsomal concentration was retained, due to its comparatively superior levels of MtHb induction.

The final variable explored was the vehicle in which MtHb inducing compounds were introduced into the *in vitro* system. Dimethyl sulfoxide (DMSO) was the solvent of choice for dissolution of PAPP and its related analogues, due to its ability to dissolve most organic compounds.\(^{161}\) However, DMSO is known to affect the metabolic activities of various cytochrome P450s found in rat microsomes.\(^{162}\) To explore whether this property of DMSO potentially affects the level of MtHb induction observed, an experiment was undertaken whereby a series of concentrations of DMSO were examined while maintaining the drug concentration constant. Concentration of DMSO was indeed found to affect the level of MtHb induction, with MtHb induction decreasing in relation to an increase in concentration of DMSO (graph 13).
2. Establishment of experimental techniques  

2.2. In vitro evaluation

As a result, a concentration of 1% v/v DMSO was subsequently adhered to for the evaluation of analogues, in an attempt to minimize the effects of the solvent on the metabolic activity of the cytochrome P450s in rat microsomes, and ultimately the levels of observed in vitro MtHb induction.

Further to the aforementioned modifications, additional measures included the preparation of a fresh solution of NADPH in buffer for each assay, in an effort to minimize aqueous-mediated degradation of the co-factor prior to use. Additionally, novel compounds of unknown stability in organic solvents were dissolved in DMSO only just prior to the commencement of the MtHb assay.

Despite these modifications, a small amount of inter-assay variation was still observed. This error was hypothesized to be due to inherent variation of the blood collected from individual Sprague-Dawley rats, and thus was quite difficult to avoid. In order to negate this error, positive and negative controls were included (PAPP and DMSO, respectively) for each assay.

Ultimately, these investigatory experiments facilitated the establishment of a standardized, relatively high-throughput in vitro assay, whereby synthesized PAPP-like analogues were able to be evaluated and ranked according to their respective levels of MtHb induction. The standard assay procedure employed for in vitro bioevaluation from this stage forward is described in section 7.3.1.

**Graph 13:** Relationship between concentration of DMSO and levels of MtHb.
2. Establishment of experimental techniques

2.3. In vivo evaluation

Based on information obtained from the experimental physicochemical measurements and in vitro MtHb assays, candidates were nominated for further in vivo evaluation. The in vivo experiments were carried out using the procedure reported in Fisher et al.\textsuperscript{163} as described in section 7.3.6.

A major hurdle to overcome during this stage of evaluation involved the development of an appropriate dosing vehicle for PAPP and its related analogues. In past studies,\textsuperscript{163} PAPP has been administered via oral gavage using a polypropylene glycol (or polyethylene glycol) and triethanolamine mixture, however, due to concerns with viscosity, this vehicle proved problematic. Alternatively, solubility studies demonstrated that, due to its partial ionization character (pKa \textit{ca}. 2.8), PAPP could be solubilized in a formulation of DMSO (5\% v/v) and hydrochloric acid (0.2 M), up to a concentration of 24 mg/mL. For the purpose of this research, this dilute aqueous acid vehicle, similar to that used in studies by Rennison et al.\textsuperscript{164} was employed for the oral gavage.

The data obtained from these cage trials allowed determination of the toxicity profile of the prepared analogues, with this information being considered with the respect to the design subsequent analogues.

2.4. Further stability studies

In an extension of the in vivo studies, biological stability studies (as described in sections 7.3.6.-7.3.9.) were conducted on selected compounds, in an effort to identify metabolic pathways that potentially played a role in the clearance and/or detoxification of these compounds in vivo. Information gathered from these studies also offered valuable insight with regards to the overall strategy towards the design of potent in vivo MtHb inducers.
3. DESIGN, SYNTHESIS AND BIOEVALUATION OF PAPP-RELATED ANALOGUES

With the overall approach towards the development of novel MtHb inducers now established, the specific objectives for the design, synthesis and bioevaluation of PAPP-related analogues were:

A) To examine the significance of the lipophilic and structural parameters of the alkyl side-chain of PAPP, with regards to its influence on both \textit{in vitro} MtHb induction and \textit{in vivo} acute oral toxicity, with comparisons made between the two.

- It was envisaged that this could be achieved through the preparation and bioevaluation of a series of linear, branched and cyclic alkyl chain aminophenones (figure 22).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure22.png}
\caption{Alkyl chain 4'-aminophenones.}
\end{figure}

B) In parallel, a second objective was to examine the apparent link between the radioprotective and MtHb inducing properties of PAPP, and whether this relationship was potentially useful in the design of MtHb inducers.

- It was postulated that this objective could be achieved through the evaluation of known radioprotective aryl analogues of PAPP, and related compounds (figure 23).
C) The final goal within this series was to investigate the importance of the ketone functionality in PAPP and its role in MtHb induction.

- It was envisaged that this could be achieved through the synthesis and bioevaluation of a select group of ketone isosteres (figure 24).

\[
\text{Ar = Ph, CH=CHAr}
\]

**Figure 23:** Radioprotective aryl analogues of PAPP.

**Figure 24:** PAPP analogues incorporating ketone isosteres.
3.1. Alkyl 4'-aminophenones

3.1.1. Linear alkyl chain 4'-aminophenones 1 and 16-19

Initial attention focused on a homologous series of PAPP analogues, with the objective being to examine the effect of alkyl chain length on MtHb toxicity. In previous studies, it was demonstrated that for a homologous series of aminophenones 1, 16-19 (n = 0, 1, 2, 3 and 4), acute oral toxicity in rats was elevated for \( p \)-aminobutyrophenone (PABP) 17 (n = 2) and \( p \)-aminovalerophenone (PAVP) 18 (n = 3) compared to \( p \)-aminoacetophenone (PAAP) 16 (n = 0), PAPP (n = 1) and \( p \)-aminocaprophenone (PACP) 19 (n = 4) (table 3).

Table 3: Acute oral toxicity of alkyl 4'-aminophenones 1 and 16-19 in rats.

<table>
<thead>
<tr>
<th></th>
<th>Acute toxicitya</th>
<th>p.o. LD50 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 PAAP n = 0</td>
<td>381 ± 22</td>
<td></td>
</tr>
<tr>
<td>1  PAPP n = 1</td>
<td>221 ± 26</td>
<td></td>
</tr>
<tr>
<td>17 PABP n = 2</td>
<td>85 ± 31</td>
<td></td>
</tr>
<tr>
<td>18 PAVP n = 3</td>
<td>85 ± 31</td>
<td></td>
</tr>
<tr>
<td>19 PACP n = 4</td>
<td>216 ± 43</td>
<td></td>
</tr>
</tbody>
</table>

aAdverse effect of aminophenones 1 and 16-19 on rats, resulting from a single oral dose, measured as the dose required for lethality in 50% of the test population (LD50, mg/kg).

In accordance with general partition coefficient theory, for each extension of the alkyl chain by a single carbon unit within such a series, it is generally accepted that there is a corresponding stepwise increase in the overall lipophilicity of the molecule. The acute oral toxicity, assumed to be largely due to \( \textit{in vivo} \) MtHb induction, suggests that there is a potential correlation between lipophilicity and the MtHb inducing properties of these compounds. More importantly, this study suggests that there may be an optimal log \( P \) value for MtHb toxicity. The synthesis, physicochemical and \( \textit{in vitro} \) evaluation of a
series of linear alkyl chain aminophenones 1 and 16-19 was now undertaken in order to provide a comparison with the reported experimental in vivo data.\textsuperscript{165}

A) Synthesis of linear alkyl chain 4'-aminophenones 1 and 16-19

Efforts to prepare the linear alkyl chain aminophenones 1 and 16-19 adopting literature methods focused initially on the synthesis of PAAP 16 as a model compound. PAAP has previously been prepared via a direct Friedel-Crafts acylation of aniline (4) with acetyl chloride in the presence of zinc under microwave (MW) irradiation.\textsuperscript{166} Unfortunately, in our hands, this route failed to afford PAAP, leading only to the recovery of unreacted starting materials (scheme 10). Likewise, the Friedel-Crafts acylation of acetanilide (5) (employed as a protected aniline equivalent), using aluminum chloride and acetyl chloride,\textsuperscript{167} also proved unsuccessful, despite an array of conditions being evaluated (scheme 10) (table 4). An alternative strategy employing the Fries rearrangement of acetanilide (5) in the presence of titanium(IV) chloride\textsuperscript{168} also failed to afford PAAP, resulting in the decomposition of starting materials (scheme 10).

\begin{center}
\includegraphics[width=0.5\textwidth]{chem.png}
\end{center}

\textit{Reagents and conditions:} (a) Zn, AcCl, MW, 300 W, 50 °C (sealed-tube), 30 s (R = H); (b) AcCl, AlCl₃, CH₂Cl₂, r.t., 18 h (R = Ac); (c) TiCl₄, r.t., 2 h (R = Ac).

\textbf{Scheme 10:} Attempted Friedel-Crafts acylation of aniline (4) and acetanilide (5), and Fries rearrangement of acetanilide (5).
3. Design, synthesis and bioevaluation of PAPP-related analogues

### Table 4: Attempted Friedel-Crafts acylation of acetanilide (5).

<table>
<thead>
<tr>
<th>Entry No.</th>
<th>Eqv. AcCl</th>
<th>Eqv. AlCl₃</th>
<th>Solvent</th>
<th>Temp. (°C)</th>
<th>Time</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.5</td>
<td>2.5</td>
<td>CH₂Cl₂</td>
<td>r.t.</td>
<td>18 h</td>
<td>no prod.</td>
</tr>
<tr>
<td>2</td>
<td>2.5</td>
<td>2.5</td>
<td>PhMe</td>
<td>reflux</td>
<td>3 h</td>
<td>by-product</td>
</tr>
<tr>
<td>3</td>
<td>2.5</td>
<td>2.5</td>
<td>1,2-DCE</td>
<td>r.t.</td>
<td>18 h</td>
<td>no prod.</td>
</tr>
<tr>
<td>4</td>
<td>1.1</td>
<td>5</td>
<td>1,2,-DCE</td>
<td>r.t.</td>
<td>18 h</td>
<td>no prod.</td>
</tr>
<tr>
<td>5</td>
<td>1.1</td>
<td>5</td>
<td>1,2,-DCE</td>
<td>reflux</td>
<td>1 h</td>
<td>decomp.</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>10</td>
<td>1,2,-DCE</td>
<td>r.t.</td>
<td>18 h</td>
<td>no prod.</td>
</tr>
</tbody>
</table>

Ultimately, aminophenones PABP and PACP were prepared by a two-step procedure. Firstly, Friedel-Crafts acylation\(^{169}\) of bromobenzene (21) with the corresponding acid chloride, using aluminum chloride under solvent free conditions, afforded bromophenones 22 and 23 as an inseparable mixture of ortho and para regioisomers (scheme 11).

**Reagents and conditions:** RCOCl, AlCl₃, r.t., 3-6 h;

**Scheme 11:** Synthesis of bromophenones 22 and 23.
With the partially purified bromophenone precursors 22 and 23 in hand, a range of conditions were evaluated in order to effect the copper(I)-catalyzed amination of 23 in an endeavour to access PABP, PACP and further aminophenones (table 5).

Table 5: Copper(I)-catalyzed amination of 4'-bromophenone 23.

<table>
<thead>
<tr>
<th>Entry No.</th>
<th>Eqv. NH₃</th>
<th>Solvent</th>
<th>Cu(I) Cat.</th>
<th>Temp. (°C)</th>
<th>Time</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ca. 2</td>
<td>H₂O/DMSO</td>
<td>CuI (20 mol%)</td>
<td>70</td>
<td>5 h</td>
<td>S.M.</td>
</tr>
<tr>
<td>2</td>
<td>ca. 40</td>
<td>EG</td>
<td>Cu₂O (5 mol%)</td>
<td>80</td>
<td>16 h</td>
<td>2%</td>
</tr>
<tr>
<td>3</td>
<td>ca. 40</td>
<td>EG</td>
<td>Cu₂O (10 mol%)</td>
<td>80</td>
<td>16 h</td>
<td>8%</td>
</tr>
<tr>
<td>4</td>
<td>ca. 2.5</td>
<td>DMSO</td>
<td>Cu₂O (10 mol%)</td>
<td>80</td>
<td>16 h</td>
<td>3%</td>
</tr>
<tr>
<td>5</td>
<td>ca. 2</td>
<td>H₂O/DMSO</td>
<td>Cu₂O (10 mol%)</td>
<td>80</td>
<td>16 h</td>
<td>16%</td>
</tr>
<tr>
<td>6</td>
<td>ca. 2</td>
<td>H₂O/DMSO</td>
<td>Cu₂O (25 mol%)</td>
<td>80</td>
<td>16 h</td>
<td>22%</td>
</tr>
<tr>
<td>7</td>
<td>ca. 10</td>
<td>H₂O/DMSO</td>
<td>Cu₂O (25 mol%)</td>
<td>80</td>
<td>16 h</td>
<td>31%</td>
</tr>
</tbody>
</table>

*Lit. procedure;¹⁷⁰ Bromophenone 23 (1 eqv.), K₂CO₃ (3 eqv.), L-proline (40 mol%); ²Lit. procedure;¹⁷¹ bromophenone 23 (1 eqv.), sealed-tube; ³Bromophenone 23 (1 eqv.), sealed-tube; ⁴Ammonia source, saturated in solvent; ⁵Yield calculated over two steps; ⁶Ethylene glycol.

By modification of the methods of Kim et. al.¹⁷⁰ and Lang et al.,¹⁷¹ the optimized system for the copper(I)-catalyzed amination of 22 and 23 involved using aqueous ammonia in dimethyl sulfoxide, in the presence of 25 mol% of copper(I) oxide, under sealed-tube conditions (table 5). Subsequent purification by flash chromatography enabled the
isolation of desired *para*-isomers PABP and PACP, in 16\% and 31\% yield respectively, over two steps (scheme 12).

**Scheme 12:** Synthesis of linear alkyl 4′-aminophenones PABP and PACP.

PAVP was accessed via acid-hydrolysis\textsuperscript{172} of the commercially available acetamide \textit{26}\textsuperscript{173} at elevated temperature (scheme 13). PAAP and PAPP were obtained from commercial sources.\textsuperscript{173,174}

**Scheme 13:** Synthesis of linear alkyl 4′-aminophenone PAVP.

\textbf{B) Physicochemical evaluation of linear alkyl chain 4′-aminophenones 1 and 16-19}

Evaluation of the partition coefficient of alkyl chain aminophenones 1 and 16-19 using RP-HPLC methods confirmed that their lipophilic properties increased in a stepwise
fashion, by approximately 0.4 log $P$ units per carbon, as the length of the alkyl chain increased (table 6). Moreover, the $ipso$ $^{13}$C shifts of aminophenone homologues 1 and 16-19 were demonstrated to be similar to that of the lead compound, PAPP (table 6), suggesting that the electronic properties of these compounds were largely analogous. With this knowledge in hand, it was postulated such a series could provide a valuable insight into the role of lipophilicity upon the $in vitro$ MtHb inducing properties of aminophenone homologues 1 and 16-19.

**Table 6: Electronic and lipophilic parameters of alkyl 4'-aminophenones 1 and 16-19.**

<table>
<thead>
<tr>
<th></th>
<th>NH$_2$ $ipso$ $^{13}$C shift (ppm, relative to PAPP)</th>
<th>log $P_{exp.}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAAP n = 0</td>
<td>+0.11</td>
<td>1.34</td>
</tr>
<tr>
<td>PAPP n = 1</td>
<td>0.00</td>
<td>1.65</td>
</tr>
<tr>
<td>PABP n = 2</td>
<td>-0.03</td>
<td>2.06</td>
</tr>
<tr>
<td>PAVP n = 3</td>
<td>-0.03</td>
<td>2.48</td>
</tr>
<tr>
<td>PACP n = 4</td>
<td>-0.10</td>
<td>3.01</td>
</tr>
</tbody>
</table>

**C) $In vitro$ evaluation of linear alkyl chain 4'-aminophenones 1 and 16-19**

Interestingly, the $in vitro$ MtHb induction data obtained for aminophenones 1 and 16-19 did not parallel the trend observed in the $in vivo$ study. On the contrary, aminophenones 1 and 16-19 exhibited increasing $in vitro$ MtHb induction as the alkyl chain length increased within the series (graph 14). Moreover, PACP (rather than PAVP) demonstrated the greatest MtHb induction ($69.1 \pm 1.4\%$) (graph 14).
Graph 14: *In vitro* MtHb induction of linear alkyl 4'-aminophenones 1 and 16-19.

The results from this initial *in vitro* MtHb assay suggested that, while PAVP may possess an optimal log $P$ value for *in vivo* MtHb induction, the same may not be true for *in vitro* MtHb induction. To determine whether an optimal lipophilicity for *in vitro* MtHb induction existed at an elevated log $P$ value, the synthesis, physicochemical and *in vitro* evaluation of further elongated linear alkyl chain aminophenones ($p$-aminoheptanoylphenone (PAHP, $n = 5$) 27, $p$-aminooctanoylphenone (PAOP, $n = 6$) 28, $p$-aminodecanoylphenone (PADP, $n = 8$) 29 and $p$-aminododecanoylphenone (PADOP, $n = 10$ 30) was next undertaken (figure 25).

Figure 25: Further elongated linear alkyl 4'-aminophenones 27-30.
D) Synthesis of further elongated linear alkyl chain 4'-aminophenones 27-30

Based on available starting materials, it was determined that further elongated linear alkyl chain aminophenones 27-30 could be accessed directly via a one-step Ru(II)-catalyzed $\alpha$-alkylation,\textsuperscript{175} rather than the previously described two-step acylation/amination procedure (section 3.1.1.A). PAAP was alkylated with the appropriate alcohol, in the presence of dichlorotris(triphenylphosphine)ruthenium(II) and potassium hydroxide at elevated temperature, to afford aminophenone homologues, PAHP, PAOP, PADP and PADOP, in 17%, 9%, 11% and 9% yields, respectively (scheme 14).

\[
\text{Reagents and conditions: ROH, RuCl}_3(PPh_3)_3, \text{KOH, 1,4-dioxane, 90°C (sealed-tube), 42 h.}
\]

**Scheme 14:** Synthesis of further elongated linear alkyl 4'-aminophenones PAHP, PAOP, PADP and PADOP.

E) Physicochemical evaluation of further elongated linear alkyl chain 4'-aminophenones 27-30

Measurement of the partition coefficients of aminophenones 27-30 by RP-HPLC established that an increase in their respective lipophilicities was again correlated with each extension of the alkyl chain (table 7). The differences between the ipso $^{13}$C shifts of individual aminophenones 27-30 were again found to be small, suggesting that, with respect to their electronic properties, these compounds were very similar. This provided a good platform to further probe the relationship between lipophilicity and \textit{in vitro} MtHb induction (table 7).
Table 7: Electronic and lipophilic parameters of further elongated linear alkyl 4'-aminophenones 27-30.

<table>
<thead>
<tr>
<th></th>
<th>NH$_2$ ipso $^{13}$C shift (ppm, relative to PAPP)</th>
<th>log $P_{exp.}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP n = 1</td>
<td>0.00</td>
<td>1.65</td>
</tr>
<tr>
<td>PAHP n = 5</td>
<td>-0.11</td>
<td>3.49</td>
</tr>
<tr>
<td>PAOP n = 6</td>
<td>-0.14</td>
<td>3.91</td>
</tr>
<tr>
<td>PADP n = 8</td>
<td>-0.04</td>
<td>4.58</td>
</tr>
<tr>
<td>PADOP n = 10</td>
<td>-0.10</td>
<td>5.15</td>
</tr>
</tbody>
</table>

F) In vitro evaluation of further elongated linear alkyl chain 4'-aminophenones 1, 16-19 and 27-30

The in vitro MtHb induction properties for the combined series of aminophenones 1, 16-19 and 27-30 (n = 0 → n = 10) were examined concurrently. The linear chain homologues 1, 16-19 and 27-30 exhibited a steady increase in in vitro MtHb induction until the alkyl chain reached 6 carbons in length (PAHP, n = 5, MtHb = 74.1 ± 2.8%) after which further extension caused MtHb induction to decline considerably (graph 15).

From these preliminary studies, it became apparent that in vitro MtHb induction was strongly influenced by the lipophilic properties of the analogue. Upon comparing the log $P_{exp.}$ values for this series of compounds with the corresponding in vitro MtHb induction data, the optimal lipophilicity for in vivo MtHb induction would appear to sit between 3.0-3.5 log $P$ units.
When neutral molecules enter the blood stream they are predominantly transported into the erythrocyte via passive diffusion down a concentration gradient, thus the greater levels of *in vitro* MtHb induction observed for PAHP, compared to PAVP, may be a result of enhanced passive diffusion across the red blood cell membrane. At log $P$ values greater than 3.5, one explanation for the decline in activity within this series could be the potential ‘trapping’ of these compounds within the phospholipid bilayer of cell membranes. A second explanation for the observed tail off in MtHb induction at log $P$ values greater than 3.5 may be that these compounds simply begin to lose their solubility under assay conditions, leading to precipitation and consequently reduced uptake. Interestingly, the degree of lipophilicity required for maximum *in vitro* MtHb induction did not appear to correlate with that observed for the *in vivo* toxicity in rats. This observed *in vitro/in vivo* discrepancy could potentially result from PAVP exhibiting
greater bioavailability than PAHP, resulting in PAVP exhibiting superior *in vivo* MtHb induction. With these complicating factors in mind, synthesis of MtHb inducers from this point forward focused on the design of analogues possessing a log $\log P$ value close to that of PAVP, a known potent *in vivo* MtHb inducer, despite PAHP exhibiting superior *in vitro* MtHb induction.

### 3.1.2. Branched alkyl chain 4'-aminophenones 31-37

Building on the established relationship between the alkyl chain length of aminophenones and MtHb toxicity, further exploration through branched alkyl chain aminophenones 31-37 was now undertaken in order to determine the effect of alkyl chain branching on MtHb induction (figure 26).

![Figure 26: Branched alkyl 4'-aminophenones 31-37.](image)

**A) Synthesis of branched alkyl chain 4'-aminophenones 31-37**

Branched alkyl chain aminophenones 31-36 were similarly prepared in variable yield by Friedel-Crafts acylation of bromobenzene (21) with the appropriate acid chloride, with subsequent amination of the resultant bromophenones 38-43 (scheme 15).
3. Design, synthesis and bioevaluation of PAPP-related analogues

3.1. Alkyl 4'-aminophenones

Due to unsuccessful attempts to access pivaloyl aminophenone 37 using the above Friedel-Crafts acylation route, an alternative strategy was adopted whereby pivaloyl bromophenone 44 was synthesized through a Cu(I)-promoted acylation of 4-bromobenzoyl chloride (45) with tert-butyllithium in the presence of copper(I) bromide dimethyl sulfide complex (scheme 16). Subsequent amination of bromophenone 44 afforded pivaloyl aminophenone 37 in 14% yield (scheme 16).

**Reagents and conditions:** (a) rBuLi, CuBr, CH₅SCH₃, THF, -78 °C, 4 h; (b) aq. NH₃, CuO, DMSO, 90 °C (sealed-tube), 16 h.

**Scheme 16:** Synthesis of pivaloyl 4'-aminophenone 37.

B) Physicochemical evaluation of branched alkyl chain 4'-aminophenones 31-37

Determination of the partition coefficient of branched aminophenones 31-37 by RP-HPLC demonstrated a similar trend to that observed for linear alkyl chain...
amino phenones 1 and 16–19, with the lipophilicity of each amino phenone dependent on the number of carbons present in the alkyl chain (table 8). In general, the ipso $^{13}$C shifts of the amino phenones 31–36 were similar to that of the parent compound, PAPP, however pivaloyl amino phenone 37 exhibited an ipso $^{13}$C shift that was upfield (-1.18 ppm) from that of PAPP (table 8). The observation was in agreement with the reported Hammett constant for the pivaloyl substituent ($\sigma = +0.32$), compared to that of PAPP ($\sigma = +0.50$). An explanation for this observation has been proposed by Bowden et al. who suggested that the electronic effect of the pivaloyl group is less than that of its branched counterparts due to its steric bulk interacting with the aromatic ring, which results in a significant twisting and deconjugation of the carbonyl group relative to the plane of the aryl amine ring.

Table 8: Electronic and lipophilic parameters of branched alkyl 4'-aminophenones 31–37.

<table>
<thead>
<tr>
<th>Compound</th>
<th>NH$_2$ ipso $^{13}$C Shift (ppm, relative to PAPP)</th>
<th>log $P_{exp.}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>R = CH$_2$CH$_3$ (PAPP)</td>
<td>0.00</td>
<td>1.65</td>
</tr>
<tr>
<td>31 R = CH(CH$_3$)$_2$</td>
<td>-0.10</td>
<td>2.02</td>
</tr>
<tr>
<td>32 R = CH(CH$_3$)CH$_2$CH$_3$</td>
<td>-0.15</td>
<td>2.44</td>
</tr>
<tr>
<td>33 R = CH$_2$CH(CH$_3$)$_2$</td>
<td>+0.06</td>
<td>2.46</td>
</tr>
<tr>
<td>37 R = C(CH$_3$)$_3$</td>
<td>-1.18</td>
<td>2.46</td>
</tr>
<tr>
<td>R = (CH$_2$)$_3$CH$_3$ (PAVP)</td>
<td>-0.03</td>
<td>2.48</td>
</tr>
<tr>
<td>34 R = CH(CH$_3$)CH$_2$CH$_2$CH$_3$</td>
<td>+0.17</td>
<td>2.84</td>
</tr>
<tr>
<td>35 R = CH$_2$CH(CH$_3$)CH$_2$CH$_3$</td>
<td>+0.25</td>
<td>2.85</td>
</tr>
<tr>
<td>36 R = CH$_2$CH$_2$CH(CH$_3$)$_2$</td>
<td>-0.08</td>
<td>2.95</td>
</tr>
</tbody>
</table>
3.1.1. Alkyl 4'-aminophenones

C) In vitro evaluation of branched alkyl chain 4'-aminophenones 31-37

In consideration of the complicating factors previously described for the in vitro MtHb assay (section 3.1.1.F), branched aminophenones 31-37 were compared to the lead compound, PAPP, and the most potent in vivo MtHb inducer within this series to date, PAVP. Despite possessing similar partition coefficients, branched aminophenones 32 (log $P_{\text{exp.}} = 2.44$, MtHb = 31.1 ± 1.2%) and 33 (log $P_{\text{exp.}} = 2.46$, MtHb = 36.9 ± 1.7%) exhibited lower levels of in vitro MtHb induction when compared directly to PAVP (log $P_{\text{exp.}} = 2.48$, MtHb = 58.1 ± 1.5%) (graph 16).

Graph 16: In vitro MtHb induction of branched alkyl 4'-aminophenones 31-37.
Again, possessing perceived ‘near optimal’ lipophilic properties (log $P_{\text{exp.}}$ ca. 3) for in vitro MtHb induction (section 3.1.1.F), aminophenones 34 (log $P_{\text{exp.}} = 2.84$, MtHb = 49.5 ± 0.7%), 35 (log $P_{\text{exp.}} = 2.85$, MtHb = 49.0 ± 1.0%) and 36 (log $P_{\text{exp.}} = 2.95$, MtHb = 52.7 ± 1.6%) likewise exhibited inferior in vitro MtHb induction compared to PAVP (graph 16). These results imply that, in general, branching of the alkyl chain substituent of the aminophenone is, for whatever reason, detrimental to in vitro MtHb induction within this series, suggesting that, in addition to the lipophilic parameter, there may also be structural requirements for optimal MtHb induction. Counterintuitively, pivaloyl aminophenone 37, constituting the greatest extent of branching and ‘sub-optimal’ lipophilicity (log $P_{\text{exp.}} = 2.46$), exhibited the highest MtHb toxicity within the series (54.2 ± 1.6%) (graph 16). This unusual result may have been influenced by the slightly lower electron-withdrawing nature of the pivaloyl substituent, when compared with other branched alkyl chain aminophenones, as evidenced by the larger than expected drift of its ipso $^{13}$C shift (-1.18 ppm), compared to the same carbon in PAPP.

### 3.1.3. Cyclic alkyl chain 4'-aminophenones 46-49

In order to further investigate the significance of the nature of the alkyl chain on MtHb induction within the PAPP series, a selection of cyclic alkyl chain aminophenones 46-49 were prepared (figure 27).

![Figure 27: Cyclic alkyl 4'-aminophenones 46-49.](image-url)
A) Synthesis of cyclic alkyl chain 4'-aminophenones 46-49

Cyclic alkyl chain aminophenones 46-49 were likewise prepared by Friedel-Crafts acylation\textsuperscript{169} of bromobenzene (21) using the appropriate acid chloride, followed by copper(I)-catalyzed amination of the resultant bromophenones 50-53 (scheme 17).\textsuperscript{171}

![Scheme 17: Synthesis of cyclic alkyl 4'-aminophenones 46-49.](image)

**Reagents and conditions:** (a) RCOCl, AlCl$_3$, r.t., 3-6 h; (b) aq. NH$_3$, CuO, DMSO, 90 °C (sealed-tube), 16 h.

Scheme 17: Synthesis of cyclic alkyl 4'-aminophenones 46-49.

B) Physicochemical evaluation of cyclic alkyl chain 4'-aminophenones 46-49

The partition coefficients of cyclic alkyl chain aminophenones 46-49 followed the same trend observed within the earlier linear alkyl chain aminophenone series (section 3.1.1.B), with a stepwise increase in lipophilicity noted upon expansion of the carbocycle (table 9). The ipso $^{13}$C shifts of the cyclic aminophenones 46-49 were again comparable to that of PAPP, hence it was assumed that electronics would again have a negligible impact on MtHb induction within this series, with a focus on compound lipophilicity and structure (table 9).
Table 9: Electronic and lipophilic parameters of cyclic alkyl 4’-aminophenones 46-49.

<table>
<thead>
<tr>
<th></th>
<th>NH$_2$ ipso $^{13}$C shift (ppm, relative to PAPP)</th>
<th>log $P_{\text{exp.}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP</td>
<td>0.00</td>
<td>1.65</td>
</tr>
<tr>
<td>46 n = 1</td>
<td>-0.07</td>
<td>1.82</td>
</tr>
<tr>
<td>47 n = 2</td>
<td>-0.12</td>
<td>2.29</td>
</tr>
<tr>
<td>PAIVP</td>
<td>-0.03</td>
<td>2.48</td>
</tr>
<tr>
<td>48 n = 3</td>
<td>-0.25</td>
<td>2.71</td>
</tr>
<tr>
<td>49 n = 4</td>
<td>-0.18</td>
<td>3.07</td>
</tr>
</tbody>
</table>

C) *In vitro* evaluation of cyclic alkyl chain 4'-aminophenones 46-49

*In vitro* evaluation of cyclic aminophenones 46-49 suggested that MtHb induction was again strongly influenced by the lipophilicity of each aminophenone, with the ‘near optimally’ lipophilic cyclohexyl aminophenone 49 (n = 4, log $P_{\text{exp.}}$ = 3.07) displaying the greatest *in vitro* MtHb toxicity (40.5 ± 1.6%) within this series (graph 17). Cyclic aminophenones 48 (n = 3, log $P_{\text{exp.}}$ = 2.71) and 49 (n = 4, log $P_{\text{exp.}}$ = 3.07) exhibited a more favourable lipophilic profile for *in vitro* MtHb induction compared with PAIVP (log $P_{\text{exp.}}$ = 2.48), and thus would possibly be expected to elicit superior levels of activity (table 9). On the contrary, cyclic aminophenones 48 (n = 3, MtHb = 33.5 ± 1.6%) and 49 (n = 4, MtHb = 40.5 ± 1.1%) induced levels of *in vitro* MtHb that were largely inferior to that of PAIVP (58.1 ± 1.5%), suggesting that the introduction of cyclic alkyl chain substituent was generally unfavorable for *in vitro* MtHb induction (graph 17).
3. Design, synthesis and bioevaluation of PAPP-related analogues

3.1. Alkyl 4'-aminophenones

In conclusion, *in vitro* MtHb evaluation of cyclic aminophenones 46-49 suggest a similar observation to that made for branched aminophenones 46-49, in that there are certain structural requirements for optimal *in vitro* MtHb induction, with cyclic structures being less favourable.
3.2. Aryl 4'-aminophenones

It has long been established that PAPP exhibits radioprotective properties,\textsuperscript{181,182} which have been attributed to anoxia (extremely low levels of oxygen) that is associated with high levels of MtHb observed following administration to animals.\textsuperscript{183} While there are a number of studies that support the claim that there is a link between the radioprotective and MtHb inducing properties of PAPP,\textsuperscript{59,181,184-186} there are also numerous studies that have disputed this claim.\textsuperscript{187-192} With the radioprotective properties of PAPP being of interest to the field of medicine, a number of structure-activity relationship studies have been conducted,\textsuperscript{187-190,193,194} hence the apparent link between these phenomena was considered potentially useful in the design of PAPP-related analogues as MtHb inducers. In light of this, in parallel to work on alkyl aminophenones, attention focused on a series of radioprotective aryl analogues of PAPP.

3.2.1. 4'-Aminobenzophenone (54) and 4'-aminochalcone (55)

In a structure-activity relationship study focused on radioprotection, Blickenstaff et al.\textsuperscript{187} demonstrated that aminobenzophenone 54, an aryl analogue of PAPP, exhibited radioprotective properties comparable to PAPP (table 10).

\textbf{Table 10:} Radioprotective properties of PAPP,\textsuperscript{187} 4'-aminobenzophenone (54)\textsuperscript{187,188} and 4'-aminochalcone (55).\textsuperscript{188}

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Radiation survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP</td>
<td>116</td>
<td>20/20, 100%\textsuperscript{187}</td>
</tr>
<tr>
<td>4’-Aminobenzophenone (54)</td>
<td>154</td>
<td>39/40, 97%\textsuperscript{188}</td>
</tr>
<tr>
<td>4’-Amino Chalcone (55)</td>
<td>174</td>
<td>10/11, 91%\textsuperscript{188}</td>
</tr>
</tbody>
</table>
As a result of the promising radioprotective properties observed for aminobenzophenone 54, Blickenstaff et al.\textsuperscript{188} extended their structure-activity relationship study to other aryl aminophenones, demonstrating that aminochalcone 55 was of similar activity to aminobenzophenone 54 with regards to radioprotection (table 10). In order to investigate the relationship between the radioprotective and MtHb inducing properties of PAPP, the synthesis, physicochemical and \textit{in vitro} evaluation of radioprotective aryl analogues 54 and 55 was undertaken (figure 28).

**Figure 28**: PAPP and radioprotective aryl 4'-aminophenones, 4'-aminobenzophenone (54) and 4'-aminochalcone (55).

**A) Synthesis of 4'-aminobenzophenone (54) and 4'-aminochalcone (55)**

Aminobenzophenone 54 was prepared in 22\% yield over two steps, via 4'-bromobenzophenone (56), through the Friedel-Crafts benzoylation\textsuperscript{195} of benzene, using 4-bromobenzoyl chloride (45) and aluminum chloride, with subsequent amination as described previously (scheme 18).\textsuperscript{171}

![Scheme 18: Synthesis of 4'-aminobenzophenone (54).](image)

**Reagents and conditions**: (a) AlCl\textsubscript{3}, C\textsubscript{6}H\textsubscript{6}, reflux, 36 h; (b) aq. NH\textsubscript{3}, CuO, DMSO, 90 °C (sealed-tube), 16 h.
Aminochalcone 55 was accessed directly via a one-step base-catalyzed Claisen condensation\textsuperscript{196} of 4-aminoacetophenone (16) and benzaldehyde (57) (scheme 19).

\[
\text{Reagents and conditions: aq. 1 M NaOH, EtOH, r.t., 24 h.}
\]

\textbf{Scheme 19: Synthesis of 4'-aminochalcone (55).}

B) \textbf{Physicochemical evaluation of 4'-aminobenzophenone (54) and 4'-aminochalcone (55)}

The partition coefficient observed for aminobenzophenone 54 (log $P_{\text{exp.}} = 2.42$) was similar to that of PAVP (log $P_{\text{exp.}} = 2.48$) (table 11). As expected, aminochalcone 55 (log $P_{\text{exp.}} = 3.20$) was more lipophilic compared to aminobenzophenone 54, owing to the presence of the two additional carbons present in the $\alpha,\beta$-unsaturated olefin linker moiety of 55 (table 11). Broadly, as anticipated, the partition coefficients of aminophenones 54 (log $P_{\text{exp.}} = 2.42$) and 55 (log $P_{\text{exp.}} = 3.20$) demonstrated that the introduction of an aryl ring resulted in an overall increase in lipophilicity when compared to PAPP (log $P_{\text{exp.}} = 1.65$) (table 11). The observed ipso $^{13}$C shifts of aminobenzophenone 54 (-0.06 ppm) and aminochalcone 55 (+0.10 ppm) were very similar to that of PAPP, suggesting that the additional unsaturation present in these aryl analogues has a minimal effect on electronic contribution to the aryl amine ring (table 11). This result was in agreement with experimentally measured Hammett constants for the propionyl ($\sigma = +0.48$) and benzoyl ($\sigma = +0.43$) substituents attached to PAPP and aminobenzophenone 54, respectively.\textsuperscript{179}
Table 11: Electronic and lipophilic parameters of 4’-aminobenzophenone (54) and 4’-aminochalcone (55).

<table>
<thead>
<tr>
<th>Compound</th>
<th>NH$_2$ ipso $^{13}$C shift (ppm, relative to PAPP)</th>
<th>log $P_{\text{exp.}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP</td>
<td>0.00</td>
<td>1.65</td>
</tr>
<tr>
<td><img src="image" alt="Chemical Structure" /></td>
<td>-0.06</td>
<td>2.42</td>
</tr>
<tr>
<td>PAVP</td>
<td>-0.03</td>
<td>2.48</td>
</tr>
<tr>
<td><img src="image" alt="Chemical Structure" /></td>
<td>+0.10</td>
<td>3.20</td>
</tr>
</tbody>
</table>

C) *In vitro* evaluation of 4’-aminobenzophenone (54) and 4’-aminochalcone (55)

Despite exhibiting similar lipophilic profiles, aminobenzophenone 54 (log $P_{\text{exp.}} = 2.42$, MtHb = 39.1 ± 2.1%) displayed levels of *in vitro* MtHb induction considerably inferior to that of PAVP (log $P_{\text{exp.}} = 2.48$, MtHb = 62.5 ± 4.4%) (graph 18). Bearing more favourable lipophilic properties still, for *in vitro* MtHb induction, aminochalcone 55 (log $P_{\text{exp.}} = 3.20$, MtHb = 40.3 ± 3.6%) was likewise found to be less active than PAVP, displaying levels of activity comparable to that of aminobenzophenone 54 (graph 18). Both aminobenzophenone 54 and aminochalcone 55, however, exhibited greater *in vitro* MtHb induction than PAPP (16.3 ± 0.9%), albeit possibly a consequence of more favourable lipophilic profiles (graph 18). At this stage, aminochalcone 55 was considered a promising candidate for further elaboration.
3.2. Aryl 4’-aminophenones

It is known that the electronic nature of substituted anilines has a significant effect on the level of MtHb induced by these compounds.\textsuperscript{104} Based on the \textit{in vitro} MtHb induction data presented above (section 3.2.2.C), combined with the relative ease of synthetic accessibility, aminochalcone 55 was put forward as a lead structure for the synthesis of analogues designed to probe the role of electronics in MtHb induction. To facilitate this investigation, the electronic influence on the aryl amine moiety was modulated through the synthesis of aminochalcones 58-62 (table 12). Each aminochalcone analogue 58-62 consisted of an ‘electronically distinct’ substituent at the 4’-position on the styrene ring, designed to allow subtle and remote manipulation of the electronic effect through the α,β-unsaturated olefin linker (table 12). Based on a Topliss approach,\textsuperscript{197} ring substituents were carefully selected with the aim of representing a broad range of Hammett constants, with consideration given to their corresponding steric and lipophilic parameters. It is important to note that the aryl aminophenone analogues proposed from here on in will be discussed, at this stage, solely as pharmacological tools, with a main focus on gathering further information with respect to the relationship between electronics and \textit{in vitro} MtHb
toxicity; with the further intention of addressing potential pharmacokinetic concerns at a later stage.

Table 12: 4’-Aminochalcones 55 and 58-62.

<table>
<thead>
<tr>
<th></th>
<th>Hammett Constant ($\sigma$) of R substituent</th>
</tr>
</thead>
<tbody>
<tr>
<td>58 R = NO$_2$</td>
<td>+0.78</td>
</tr>
<tr>
<td>59 R = Cl</td>
<td>+0.23</td>
</tr>
<tr>
<td>55 R = H</td>
<td>+0.00</td>
</tr>
<tr>
<td>60 R = Me</td>
<td>-0.17</td>
</tr>
<tr>
<td>61 R = OMe</td>
<td>-0.27</td>
</tr>
<tr>
<td>62 R = NMe$_2$</td>
<td>-0.83</td>
</tr>
</tbody>
</table>

A regioisomer of aminochalcone 55, aminochalcone 63 was also prepared and evaluated in parallel to aminochalcones 58-62, in order to investigate whether aminochalcone 63 could likewise provide a platform for further exploration into the role that electronics play in MtHb induction (figure 29).

Figure 29: 4’-Aminochalcone (55) and 4''-aminochalcone (63).

**A) Synthesis of 4’-aminochalcones 58-62 and 4''-aminochalcone (63)**

Aminochalcones 58-62 were accessed in variable yield via the previously described base-catalyzed Claisen condensation,$^{196}$ using 4-aminoacetophenone (16) and the appropriate benzaldehyde 64-68 (scheme 20).
Aminochalcone 63 was obtained in 59% yield over two steps, with the first step again employing a base-catalyzed Claisen condensation reaction, using 4-nitrobenzaldehyde (69) and acetophenone (7) (scheme 21). With nitrochalcone 70 in hand, the desired aminochalcone 63 was accessed by reduction using tin(II) chloride (scheme 21).

Reagents and conditions: (a) aq. 1 M NaOH, EtOH, r.t., 24 h (to give 70); (b) 70, SnCl₂, EtOH, reflux, 1 h.

Scheme 21: Synthesis of 4''-aminochalcone 63.

B) Physicochemical evaluation of 4'-aminochalcones 58-62 and 4''-aminochalcone (63)

As a result of adjusting the electronic contribution through the α,β-unsaturated olefin linker, the ipso ¹³C shifts of aminochalcones 58-62 were dependent on the electronic...
nature of the substituent on the styrene ring, falling in the range of $+0.60 \rightarrow -0.39$ ppm (relative to PAPP) (table 13).

**Table 13:** Electronic and lipophilic parameters of 4'-aminochalcones 58-62 and 4''-aminochalcone 63.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\text{NH}_2 \text{ ipso }^{13}\text{C shift}$ (ppm, relative to PAPP)</th>
<th>$\log P_{\text{exp.}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP</td>
<td>0.00</td>
<td>1.65</td>
</tr>
<tr>
<td>PAVP</td>
<td>-0.03</td>
<td>2.48</td>
</tr>
<tr>
<td>58 $R = \text{NO}_2$</td>
<td>+0.60</td>
<td>3.37</td>
</tr>
<tr>
<td>59 $R = \text{Cl}$</td>
<td>+0.24</td>
<td>3.69</td>
</tr>
<tr>
<td>55 $R = \text{H}$</td>
<td>+0.10</td>
<td>3.20</td>
</tr>
<tr>
<td>60 $R = \text{Me}$</td>
<td>+0.05</td>
<td>3.54</td>
</tr>
<tr>
<td>61 $R = \text{OMe}$</td>
<td>-0.01</td>
<td>3.26</td>
</tr>
<tr>
<td>62 $R = \text{NMe}_2$</td>
<td>-0.39</td>
<td>3.47</td>
</tr>
<tr>
<td>63</td>
<td>-1.88</td>
<td>3.18</td>
</tr>
</tbody>
</table>

Evaluation of the partition coefficients by RP-HPLC established that aminochalones 58-62 were slightly more lipophilic than their parent aminochalcone 55 ($\log P_{\text{exp.}} = 3.20$), however, the ‘lipophilic range’ for this series of compounds was limited to only ca. 0.5 log $P$ units ($\text{55 }\log P_{\text{exp.}} = 3.20 \rightarrow \text{59 }\log P_{\text{exp.}} = 3.69$) (table 13). With this parameter now having been largely standardized, it was anticipated that this series of compounds could provide some insight into how MtHb induction is influenced by electronics.
Aminochalcone 63 displayed a considerably altered ipso $^{13}$C shift (-1.88 ppm) compared to aminochalcone 55 (+0.10), suggesting that inversion of the $\alpha,\beta$-unsaturated ketone linker presented a less electron-withdrawing ring substituent (table 13). In terms of their lipophilic properties, however, aminochalcone 55 (log $P_{\text{exp.}} = 3.20$) and regioisomer aminochalcone 63 (log $P_{\text{exp.}} = 3.18$) were found to be very similar (table 13).

C) In vitro evaluation of $4'$-aminochalcones 58-62 and $4''$-aminochalcone (63)

Aminochalcone 55 (R = H, MtHb = 29.6 ± 1.4%) demonstrated the greatest in vitro MtHb induction within this series, with subtle electronic adjustments in either direction resulting in a loss in MtHb activity (graph 19). Electron-withdrawing substituents (59 R = Cl, 24.6 ± 3.1% and 58 R = NO$_2$, 20.7 ± 2.5%) generally appeared to be better tolerated than electron-donating substituents (60 R = Me, 16.0 ± 0.6%, 61 R = OMe, 10.6 ± 2.1% and 62 R = NMe$_2$, 3.0 ± 0.9%) (graph 19). Despite the electronics appearing to be the dominant factor affecting MtHb within this series, the observed trend could also be influenced by the presumed unfavourable steric bulk introduced to the styrene ring of aminochalcones 58-62. With regards to the chalcone family of MtHb inducers, consideration also needs to be given to the effect of ring substitution upon $s$-cis/$s$-trans conformational preference, with electronics once again playing a significant role.
Aminochalcone 63 exhibited inferior in vitro MtHb induction (10.2 ± 2.2%) compared to its regioisomer 55 (29.6 ± 1.4%) (graph 20). The considerably altered ipso $^{13}$C shift observed for aminochalcone 63 (-1.88 ppm, table 13) versus aminochalcone 55 (+0.10 ppm, table 13), suggested that inversion of the α,β-unsaturated olefin linker presented an aniline ring with greater electron-richness, leading to less optimal electronics, and consequently diminished MtHb inducing properties (graph 21). This hypothesis is somewhat supported by the Hammett constant reported for this substituent ($\sigma = +0.25$)\textsuperscript{179}. Given that aminochalcone 63 provides our first example of an aniline ring directly bearing a substituent other than a ketone, a second explanation for the observed fall in MtHb activity for this compound could relate to, from a structural perspective, the importance of the positioning of the ketone moiety within the linker.
3. Design, synthesis and bioevaluation of PAPP-related analogues

3.2. Aryl 4'-aminophenones

Graph 20: *In vitro* MtHb induction vs. log $P_{exp}$ of 4'-aminochalcone (55) and 4''-aminochalcone (63).

Graph 21: *In vitro* MtHb induction vs. ipso $^{13}$C shift (ppm, relative to PAPP) of 4'-aminochalcone (55) and 4''-aminochalcone (63).

Ultimately, neither aminochalcones 58-62 nor regioisomer aminochalcone 63 exhibited greater *in vitro* MtHb induction compared to aminochalcone 55 (29.6 ± 1.4%) or PAVP (58.1 ± 1.5%), hence these compounds were not further investigated (graph 19, 20 and 21).
3. Design, synthesis and bioevaluation of PAPP-related analogues

3.2. Aryl 4’-aminophenones

3.2.3. 4’-Aminochalcone (55) and 4’-aminodihydrochalcone (71)

In parallel to studies on aminochalcones 58-62, attention focused on the preparation and evaluation of aminodihydrochalcone 71, in order to investigate the importance of the \( \alpha, \beta \)-unsaturated olefin linker of 55 with regards to its influence on MtHb induction (figure 30).

![Figure 30: 4’-Aminochalcone (55) and 4’-aminodihydrochalcone (71).](image)

**Figure 30:** 4’-Aminochalcone (55) and 4’-aminodihydrochalcone (71).

**A) Synthesis of 4’-aminodihydrochalcone (71)**

Aminodihydrochalcone 71 was prepared using a similar procedure to that previously described for alkyl chain aminophenones 27-30. Specifically, a one-step Ru(II)-catalyzed \( \alpha \)-alkylation, using 4-aminoacetophenone (16) and benzaldehyde (57), in the presence of dichlorotris(triphenylphosphine)ruthenium(II) and potassium hydroxide at elevated temperature, was employed (scheme 22).

![Scheme 22: Synthesis of 4’-aminodihydrochalcone (71).](image)

**Scheme 22:** Synthesis of 4’-aminodihydrochalcone (71).

**Reagents and conditions:** RuCl\(_3\)(PPh\(_3\))\(_3\), KOH, 1,4-dioxane, 90\(^\circ\)C (sealed-tube), 42 h.

**B) Physicochemical evaluation of 4’-aminodihydrochalcone (71)**

Despite the absence of the \( \alpha, \beta \)-unsaturated olefin linker in aminodihydrochalcone 71, the electronic and lipophilic properties of this compound remained largely unchanged (log
3. Design, synthesis and bioevaluation of PAPP-related analogues 3.2. Aryl 4’-aminophenones

$P_{\text{exp.}} = 3.07$, ipso $^{13}\text{C}$ shift = +0.15) when compared to aminochalcone 55 (log $P_{\text{exp.}}$ = 3.20, ipso $^{13}\text{C}$ shift = +0.10) (table 14). With these parameters now largely standardized, it was postulated that aminodihydrochalcone 71 could potentially provide some insight into the significance of the $\alpha,\beta$-unsaturated olefin linker (from a structural perspective).

Table 14: Electronic and lipophilic parameters of 4’-aminodihydrochalcone (71).

<table>
<thead>
<tr>
<th>Compound</th>
<th>NH$_2$ ipso $^{13}\text{C}$ shift (ppm, relative to PAPP)</th>
<th>log $P_{\text{exp.}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP</td>
<td>0.00</td>
<td>1.65</td>
</tr>
<tr>
<td>PAVP</td>
<td>-0.03</td>
<td>2.48</td>
</tr>
<tr>
<td><img src="https://example.com/chemistry.png" alt="" /></td>
<td>+0.10</td>
<td>3.20</td>
</tr>
<tr>
<td><img src="https://example.com/chemistry.png" alt="" /></td>
<td>+0.15</td>
<td>3.07</td>
</tr>
</tbody>
</table>

C) In vitro evaluation of 4’-aminodihydrochalcone (71) compared to 4’-aminochalcone (55)

Aminodihydrochalcone 71 (46.3 \(\pm\) 1.6%) exhibited a level of in vitro MtHb induction comparable to that of PAVP (42.0 \(\pm\) 1.6%), demonstrating that removal of the $\alpha,\beta$-unsaturated olefin linker moiety results in a significant increase in MtHb potency (graph 22). Given that both the partition coefficient and electronic properties of aminodihydrochalcone 71 remained largely unchanged upon saturation of the olefinic bond, it is hypothesized that an increase in conformational flexibility may be responsible for the observed increase in MtHb induction within this series. Moreover, this result once again raised the subject of a potential side-chain binding interaction, and an opportunity
to probe further the structural requirements for enhanced MtHb induction within the aryl aminophenone series. As a consequence, aminodihydrochalcone 71 was seen as an attractive scaffold for further elaboration.

**Graph 22:** *In vitro* MtHb induction of 4'-aminochalcone (55) and 4'-aminodihydrochalcone (71).

### 3.2.4. 4'-Aminodihydrochalcone (71) and homologues 54, 72 and 73

As a result of the improved MtHb induction elicited by aminodihydrochalcone 71, further investigation into this structural sub-class was achieved through the synthesis, physicochemical and *in vitro* evaluation of aminodihydrochalcone 71 homologues, 54 (n = 0), 72 (n = 1) and 73 (n = 3) (figure 31). Preparation of the aforementioned homologous series of compounds was proposed to enable the probing of the existence and/or location of a putative hydrophobic binding pocket involved in the aforementioned biochemical processes which may, for compounds of this type, play a role in MtHb induction.
3. Design, synthesis and bioevaluation of PAPP-related analogues

3.2. Aryl 4'-aminophenones

![Chemical structure](image)

**Figure 31:** 4'-Aminodihydrochalcone (71) (n = 2) and homologues 54 (4'-aminobenzophenone, n = 0), 72 (n = 1) and 73 (n = 3), probing a putative hydrophobic binding pocket.

**A) Synthesis of 4'-aminodihydrochalcone (71) and homologues 54, 72 and 73**

Aminobenzophenone 54 and aminodihydrochalcone 71 were prepared as described previously in section 3.2.1.A and 3.2.3.A, respectively. Amino-2-phenylacetophenone 72 was accessed by a similar route to that used to prepare PABP and PACP, namely via Friedel-Crafts acylation\(^{169}\) and subsequent amination,\(^{171}\) affording 72 in 21% yield over two steps (scheme 23).

\[
\begin{align*}
\text{Reagents and conditions:} & \quad (a) \text{ PhCH}_2\text{COCl, AlCl}_3, \text{ r.t., 3-6 h; (b) aq. NH}_3, \text{ CuO, DMSO, 90 °C (sealed-tube), 16 h.} \\
\text{Scheme 23:} & \quad \text{Synthesis of 2-phenyl-4'-amino-acetophenone (72).}
\end{align*}
\]

Amino-4-phenylbutyrophenone 73 was prepared by a similar route to that used to prepare alkyl chain aminophenones 27-30,\(^{175}\) namely via a one-step Ru(II)-catalyzed \(\alpha\)-alkylation of 4-aminoacetophenone (16) with phenylethanol (75) in the presence of dichlorotris(triphenylphosphine)ruthenium(II) and potassium hydroxide, at elevated temperature (scheme 24).
3. Design, synthesis and bioevaluation of PAPP-related analogues

3.2. Aryl 4'-aminophenones

Reagents and conditions: RuCl2(PPh3)3, KOH, 1,4-dioxane, 90°C (sealed-tube), 42 h.

Scheme 24: Synthesis of 4'-amino-4-phenylbutyrophenone (73).

B) Physicochemical evaluation of 4'-aminodihydrochalcone (71) and homologues 54, 72 and 73

In general, aminodihydrochalcone homologues 54 and 71-73 exhibited a similar trend to that observed for earlier homologous series, with regards to the stepwise increase in lipophilicity observed upon insertion of each methylene unit (ca. 0.4 log \( P \) units per carbon) (table 15).

Table 15: Electronic and lipophilic parameters of 4'-aminodihydrochalcone (71) homologues 54, 72 and 73.

<table>
<thead>
<tr>
<th></th>
<th>NH(_2) ipso (^{13})C shift (ppm, relative to PAPP)</th>
<th>( \log P_{\text{exp.}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP</td>
<td>0.00</td>
<td>1.65</td>
</tr>
<tr>
<td>54 ( n = 0 )</td>
<td>-0.06</td>
<td>2.42</td>
</tr>
<tr>
<td>PAVP</td>
<td>-0.03</td>
<td>2.48</td>
</tr>
<tr>
<td>72 ( n = 1 )</td>
<td>+0.24</td>
<td>2.58</td>
</tr>
<tr>
<td>71 ( n = 2 )</td>
<td>+0.15</td>
<td>3.07</td>
</tr>
<tr>
<td>73 ( n = 3 )</td>
<td>-0.05</td>
<td>3.37</td>
</tr>
</tbody>
</table>
Contrary to the general trend, however, amino-2-phenylacetophenone 72 (n = 1) exhibited a level of lipophilicity (log $P_{\text{exp.}} = 2.58$) only marginally (= 0.16 log $P$ units) greater than that of aminobenzophenone 54 (n = 0, log $P_{\text{exp.}} = 2.42$) (table 15). Upon consultation of the literature, it became apparent that this observation was in agreement with that of Hansch et al.,200 who demonstrated that, while the general trend exhibited an increase in $\pi$ by ca. +0.50 per methylene unit, the hydrophobic ($\pi$) constants for phenyl and benzyl substituents were, counterintuitively, very similar ($\pi(\text{Ph}) = +1.96$, $\pi(\text{Bzl}) = +2.01$). The \textit{ipso} $^{13}$C shifts for the aminodihydrochalcone homologues 54 and 71-73 (-0.06 $\rightarrow$ +0.24 ppm) were similar to that of the parent compound, thus suggesting that electronics would be less of a contributing factor within this series of compounds (table 15).

C) \textit{In vitro} evaluation of 4'-aminodihydrochalcone (71) and homologues 54, 72 and 73

As a general trend, aminodihydrochalcone homologues 54 and 71-73 demonstrated that \textit{in vitro} MtHb induction was again predominantly influenced by the partition coefficient of each compound, with the near ‘optimally lipophilic’ homologue 73 (n = 3, log $P_{\text{exp.}} = 3.37$) exhibiting the greatest activity within the series (62.4 ± 0.4%) (graph 23). Given the similarity in both the lipophilic and electronic properties of aminobenzophenone 54 (n = 0, log $P_{\text{exp.}} = 2.42$) and amino-2-phenylacetophenone 72 (n = 1, log $P_{\text{exp.}} = 2.58$), it was expected that these homologues would display similar MtHb induction profiles (table 15). On the contrary, amino-2-phenylacetophenone 72 (n = 1, 47.0 ± 1.9%) displayed levels of \textit{in vitro} MtHb induction considerably greater than that of aminobenzophenone 54 (n = 0, 38.0 ± 3.6%), suggesting that free rotation of the aryl ring is beneficial to activity (graph 23). As a result of the dependency of \textit{in vitro} MtHb induction on lipophilicity, it remained unclear as to whether a hydrophobic binding pocket indeed existed, and if so, where it was located relative to the aryl amine ring. In light of this, aminodihydrochalcone 71 was further elaborated.
3. Design, synthesis and bioevaluation of PAPP-related analogues

3.2. Aryl 4'-aminophenones

Graph 23: *In vitro* MtHb induction of 4'-aminodihydrochalcone (71) and homologues 54, 72 and 73.

3.2.5. Heterocyclic 4'-aminodihydrochalcone analogues 76-78 and cyclohexyl aminodihydrochalcone analogue 79

In the structure-activity development of non-steroid anti-inflammatory agents, it has been demonstrated that phenyl rings can be successfully replaced by heterocyclic moieties such as furans, thiophenes, and pyridines. In light of this, aminodihydrochalcone 71 was proposed as an attractive structural platform to investigate the effect of replacing the phenyl group with various heterocyclic rings, in an effort to further probe the nature of any potential side-chain binding interactions involved in MtHb induction (figure 32). In addition to heterocyclic aminodihydrochalcone analogues 76-78, the synthesis, physicochemical and *in vitro* evaluation of non-planar cyclohexyl aminodihydrochalcone analogue 79, was undertaken, in an effort to determine whether
aromaticity, through possible \( \pi \)-stacking interactions, was a feature for the proposed hydrophobic binding (figure 32).

![Figure 32](image)

**Figure 32:** 4'-Aminodihydrochalcone (71), and heterocyclic analogues 76-78 and cyclohexyl analogue 79.

A) Synthesis of heterocyclic 4'-aminodihydrochalcone analogues 76-78 and cyclohexyl 4'-aminodihydrochalcone analogue 79

Heterocyclic aminodihydrochalcone analogues 76-78 and cyclohexyl aminodihydrochalcone analogue 79 were synthesized in variable yield using a similar route to that used to prepare aminodihydrochalcone 71. Specifically, a one-step Ru(II)-catalyzed \( \alpha \)-alkylation\(^{199} \) of 4-aminoacetophenone (16) and the corresponding aldehydes 80-83 in the presence of dichlorotris(triphenylphosphine)ruthenium(II) and potassium hydroxide at elevated temperature, was used (scheme 25).

![Scheme 25](image)

**Scheme 25:** Synthesis of heterocyclic 4'-dihydroaminochalcone analogues 76-78 and cyclohexyl 4'-aminodihydrochalcone analogue 79.

Reagents and conditions: RuCl\(_3\)(PPh\(_3\))\(_3\), KOH, 1,4-dioxane, 90°C (sealed-tube), 42 h.
B) Physicochemical evaluation of heterocyclic 4'-aminodihydrochalcone analogues 76-78 and cyclohexyl 4'-aminodihydrochalcone analogue 79

In general, the partition coefficients of heterocyclic aminodihydrochalcone analogues 76-78 were largely influenced by the lipophilic contribution of each phenyl ring replacement. Replacement of the phenyl ring with polar heterocycles, such as pyridyl and furanyl groups as found in 76 and 77, respectively, resulted in an overall decrease in lipophilicity (76 log $P_{\text{exp.}} = 1.84$, 77 log $P_{\text{exp.}} = 2.58$) compared to the parent aminodihydrochalcone 71 (log $P_{\text{exp.}} = 3.07$) (table 16).

<table>
<thead>
<tr>
<th>NH$_2$ ipso $^{13}$C shift (ppm, relative to PAPP)</th>
<th>log $P_{\text{exp.}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP</td>
<td>0.00</td>
</tr>
<tr>
<td>76 R = 2''-pyridyl</td>
<td>+0.06</td>
</tr>
<tr>
<td>PAVP</td>
<td>-0.03</td>
</tr>
<tr>
<td>77 R = 2''-furanyl</td>
<td>+0.34</td>
</tr>
<tr>
<td>78 R = 2''-thienyl</td>
<td>+0.16</td>
</tr>
<tr>
<td>71 R = phenyl</td>
<td>+0.15</td>
</tr>
<tr>
<td>79 R = cyclohexyl</td>
<td>-0.01</td>
</tr>
</tbody>
</table>

Replacement of the phenyl group with a thienyl moiety led only to a subtle decrease in lipophilicity (71 R = phenyl, log $P_{\text{exp.}} = 3.07$, 78 R = 2-thienyl, log $P_{\text{exp.}} = 2.92$), while substitution of the phenyl group with the cyclohexyl ring increased the lipophilicity considerably (79 R = cyclohexyl, log $P_{\text{exp.}} = 3.81$) (table 16). Across the series of aminodihydrochalcone analogues 76-79, the ipso $^{13}$C shifts (-0.01 → +0.16 ppm) were
found to be similar to that of PAPP and aminodihydrochalcone 71 (+0.16 ppm), with the exception of furanyl aminodihydrochalcone analogue 77, which exhibited a slight difference in electronic effect (+0.34 ppm). These findings implied that the electronic parameter would be unlikely to play a leading role in the observed in vitro MtHb induction within this series of compounds, again focusing on compound lipophilicity and structure (table 16).

C) In vitro evaluation of heterocyclic 4'-aminodihydrochalcone analogues 76-78 and cyclohexyl 4'-aminodihydrochalcone analogue 79

All heterocyclic phenyl ring replacements (76 R = 2''-pyridyl, MtHb = 34.5 ± 1.1%, 77 R = 2''-furanyl, MtHb = 50.6 ± 1.1%, 78 R = 2''-thienyl, MtHb = 58.2 ± 1.3%) explored within this series resulted in decreased in vitro MtHb induction compared to that displayed by the parent compound, aminodihydrochalcone 71 (63.9 ± 0.6%) (graph 24). These results suggested that the heterocyclic replacements for the phenyl ring were not beneficial for the induction of MtHb in vitro.

Cyclohexyl aminodihydrochalcone analogue 79 was evaluated next to investigate whether planarity (π-stacking) favourably contributed to the putative hydrophobic binding within this series. Physicochemical evaluation of 79 revealed a rare example of an analogue exhibiting a lipophilic profile (log $P_{\text{exp.}}$ = 3.81) greater than that previously established for optimal in vitro MtHb induction (log $P_{\text{ca.}}$ 3 - 3.5), within an earlier series (section 3.1.1.F) (table 16). With this in mind, combined with an assumption that the hypothesized hydrophobic binding pocket could potentially favour π-stacking interactions,203 cyclohexyl aminodihydrochalcone analogue 79 could be expected to exhibit inferior MtHb induction compared to its planar counterparts 77 and 78. Contrary to this however, 79 exhibited a level of in vitro MtHb activity (56.9 ± 0.3%) comparable to that observed for heterocyclic aminodihydrochalcone analogues 77 (50.6 ± 1.1%) and 78 (58.2 ± 1.3%) (graph 24). This result suggested that π-stacking interactions, through the planarity of the above phenyl ring equivalents, did not contribute to any putative hydrophobic bonding interactions involved in MtHb induction.
Graph 24: In vitro MtHb induction of heterocyclic 4'-aminodihydrochalcone analogues 76-78 and cyclohexyl 4'-aminodihydrochalcone analogue 79.

Given that none of the aminodihydrochalcone analogues 76-79 exhibited enhanced in vitro MtHb induction compared to aminodihydrochalcone 71 or PAVP, this series of compounds was not investigated further.
3.2.6. Ring-fused aryl 4’-aminophenones 84-87

As described earlier, the manipulation of the electronic properties of 4’-aminochalcones 58-62, in an effort to enhance MtHb induction through the remote attachment of various electron-withdrawing and electron-donating groups, produced variable results (section 3.2.2.). Using previously established MtHb inducers aminochalcone 55 and aminodihydrochalcone 71 as structural platforms, attention next turned to an alternative strategy in the examination of the role of the electronic effect on MtHb induction, through the introduction of a ring-fused electron-donating group in the case of aminoindanone 84 and aminoindanone 85 (cf. \( \sigma_{\text{meta}} \) (Me) = -0.07), or an electron-withdrawing group in the case of aminoaurone 86 and aminobenzofuranone 87 (cf. \( \sigma_{\text{meta}} \) (OMe) = +0.12), meta to the aryl amine (figure 33). It was envisaged that this approach would bring about a subtle change in the electronic nature of the aryl amine whilst to some extent conserving the desirable chalcone-like structure.

![Chemical structures](image)

**Figure 33:** *In silico* log P predictions for 4’-aminochalcone (55), 4’-aminodihydrochalcone (71) and ring-fused aryl 4’-aminophenones; 2-benzylidene-5-aminoindanone (84) and 2-benzyl-5-aminoindanone (85), 6-aminoaurone (86) and 2-benzyl-6-aminobenzofuranone (87).

Based on *in silico* log P predictions, it was also anticipated that the ring-fused aryl aminophenones 84-87 would possess the additional physicochemical advantage of reduced log P values, which were postulated to potentially confer improved bioavailability.
A) Attempted synthesis of 2-benzylidene-5-aminooindanone (84) and 2-benzyl-5-aminooindanone (85)

It was envisaged that aminooindanones 84 and 85 could be accessed from the hydrolysis of their corresponding acetamides, 88 and 89, which in turn could be obtained from a coupling reaction involving common acetamidoindanone intermediate 90 (scheme 26).

Scheme 26: Synthetic strategy towards ring-fused aryl 4'-aminophenones, 2-benzylidene-5-aminooindanone (84) and 2-benzyl-5-aminooindanone (85).

More specifically, acetamidoindanone 88 could in turn be prepared via condensation of acetamidoindanone 90 with benzaldehyde (57), and acetamidoindanone 85 accessed using the previously described α-alkylation of acetamidoindanone 90 and benzyl alcohol (91) (scheme 26).
Synthesis of the key acetamidoindanone intermediate 90 proceeds via cinnamic acid 92, itself the Knoevenagel condensation\textsuperscript{206} product of 3-nitrobenzaldehyde (93) and malonic acid (scheme 26).

The Knoevenagel condensation\textsuperscript{206} of 3-nitrobenzaldehyde (93) proceeded smoothly to afford cinnamic acid 92 in near quantitative yield (scheme 28), however subsequent hydrogenation of 92 at elevated pressure (40 psi) to access aminodihydrocinnamic acid 94 proved problematic, with hydrogenation affording a complex and inseparable mixture of partially reduced products 94-96 (scheme 27). Additionally, the desired carboxylic acid 94, being zwitterionic in nature, proved difficult to handle with respect to both its isolation and characterization (scheme 27).

\begin{align*}
\text{Reagents and conditions:} & \quad \text{(a) H}_2 (40 \text{ psi}), 10 \text{ mol\% Pd/C, r.t., 3 h.} \\
\text{Scheme 27: Attempted synthesis of aminodihydrocinnamic acid 94.}
\end{align*}

Faced with these complications, attention turned to protecting cinnamic acid 92 as its methyl ester 99 prior to reduction (scheme 28).\textsuperscript{207} Palladium-catalyzed hydrogenation\textsuperscript{208} of methyl ester 99 at elevated pressure led to the exclusive isolation of dihydrocinnamate 100 in near quantitative yield (scheme 28). In order to avoid formation of a zwitterionic intermediate in the subsequent step, dihydrocinnamate 100 was acetylated\textsuperscript{208} prior to
saponification, affording dihydrocinnamic acid 101 in 71% yield over two steps (scheme 28).

Reagents and conditions: (a) CH\(_2\)(COOH)\(_2\), piperidine, pyridine, reflux, 18 h; (b) H\(_2\)SO\(_4\), MeOH, reflux, 24 h; (c) H\(_2\) (60 psi), 10 mol% Pd/C, r.t., 18 h; (d) AcCl, NEt\(_3\), C\(_6\)H\(_6\), reflux, 1 h; (e) aq. 1 M NaOH, reflux, 1 h; (f) i) DMF, (COCl)\(_2\), r.t., 2 h; ii) acid chloride 102, AlCl\(_3\), CH\(_2\)Cl\(_2\), r.t., 4 h; (g) aq. 1 M HCl, MeOH, reflux, 1 h.

Scheme 28: Synthesis of 5-aminooxindole (104).

Treatment of dihydrocinnamic acid 101 with oxalyl chloride in the presence of a catalytic quantity of dimethylformamide afforded the required acid chloride 102, which was used directly without further purification (scheme 28). The limiting step of the synthesis was the intramolecular Friedel-Crafts acylation of acid chloride 102 to access acetamidooxindole 103. Unexpectedly, the predominant acylation product from this reaction was found to be ortho regioisomer 103a, however the para regioisomer 103b could be conveniently isolated following purification by flash chromatography (scheme 28). The considerable difference in RF values for the two regioisomers 103a and 103b, considering their structural similarity, was due to the internal hydrogen bonding in ortho isomer 103a, which resulted in a lower affinity of 103a for the polar stationary phase (SiO\(_2\)) during chromatographic separation (figure 34).
As a consequence of the synthetic complications associated with accessing intermediate acetamidoindanone \(103\), and the subsequent failure to generate sufficient material to proceed as originally intended, it was decided to postpone the key coupling steps towards aminooindanones \(84\) and \(85\), and instead hydrolyze\(^{210}\) intermediate \(103\) to aminoindanone \(104\), which would now be the revised subject of physicochemical and \textit{in vitro} MtHb evaluation (scheme \(28\)) (section \(3.2.6.C\) and \(3.2.6.D\)).

\section*{B) Synthesis of 6-aminoaurone (86)}

Efforts towards the preparation of aminoaurone \(86\) initially focused on the synthesis of key bromobenzofuranone intermediate \(105\), which in turn was postulated to undergo a previously described copper(I)-catalyzed amination to the desired aminobenzofuranone \(109\) (scheme \(29\)). Firstly, alkylation\(^{211}\) of the sodium salt of 3-bromophenol \((106)\) with bromoacetic acid afforded phenoxyacetic acid \(107\) in 51\% yield (scheme \(29\)). Unfortunately, subsequent acid-catalyzed cyclization\(^{211}\) of phenoxyacetic acid \(107\) led only to the degradation of starting materials (scheme \(29\)). This problem was circumnavigated via the preparation of phenol \(108\), itself accessed by a boron(III)
chloride mediated Hoesch reaction\textsuperscript{212} of 3-bromophenol (106) with chloroacetonitrile. Subsequent base-catalyzed cyclization\textsuperscript{212} of phenol 108 afforded bromobenzofuranone 105 in 7\% yield over two steps (scheme 29). Disappointingly, copper(I)-catalyzed amination of bromobenzofuranone 105 resulted in a complex mixture, with the by-products possibly emanating from benzofuranone ring opening, however these were not isolated (scheme 29).

\begin{center}
\textit{Reagents and conditions:} (a) (i) \text{NaH}, \text{DMF}, 0 °C, 0.5 h; (ii) \text{BrCH}_2\text{COOH}, \text{DMF}, \text{r.t.}, 24 h; (b) (i) \text{ClCH}_2\text{CN}, \text{AlCl}_3, \text{BCl}_3, \text{CH}_2\text{Cl}_2, 0 °C \rightarrow \text{r.t.}, 24 h; (ii) \text{H}_2\text{O}/\text{H}^+; (c) \text{PPA}, 75 °C, 3 h; (d) \text{NEt}_3, \text{CH}_3\text{CN}, \text{r.t.}, 0.5 h; (e) \text{aq. NH}_3, \text{CuO, DMSO, 90 °C (sealed-tube)}, 16 h.
\end{center}

\begin{center}
\textbf{Scheme 29: Attempted synthesis of 6-aminourone (86).}
\end{center}

Attention next focused on accessing aminourone 86 via late-stage hydrolysis of acetamidoaurone 110, thereby avoiding the aforementioned high pressure amination reaction. Starting with 3-acetamidophenol (111), attempted acylation under similar conditions to those described previously,\textsuperscript{212} failed due to solubility problems, however, methylation\textsuperscript{213} of 3-acetamidophenol (111) with subsequent Friedel-Crafts acylation\textsuperscript{213} using chloroacetyl chloride, afforded the desired the intermediate 112 in 4\% yield over two steps (scheme 30). Subsequent base-catalyzed cyclization\textsuperscript{212} of 112 afforded acetamidobenzofuranone 113, which in turn underwent an acid-catalyzed condensation\textsuperscript{214}.
with benzaldehyde (57) to afford acetamidoaurone 110 in 83% yield (scheme 30). Finally, acid-hydrolysis furnished the desired aminoaurone 86 as a mixture of E/Z isomers, which were deemed inseparable due to isomerization taking place during the chromatographic purification step (scheme 30).

For similar reasons to that discussed above for aminoindanones 84 and 85, the synthesis of aminobenzofuranone 87 was postponed until physicochemical and in vitro MtHb evaluation of aminoaurone 86 was performed.

\[\text{Reagents and conditions:} \ (a) \ CICH_2CN, \ AlCl_3, \ BCl_3, \ CH_2Cl_2, \ 0 \ ^\circ C \to r.t., \ 24 \ h; \ (b) \ MeI, \ K_2CO_3, \ DMF, \ r.t., \ 18 \ h; \ (c) \ CICOCH_2Cl, \ AlCl_3, \ (CH_2)Cl_2, \ 0 \ ^\circ C \to r.t., \ 1 \ h, \ 75 \ ^\circ C, \ 3 \ h; \ (d) \ NEt_3, \ CH_3CN, \ r.t., \ 1.5 \ h; \ (e) \ Benzaldehyde \ (57), \ aq. \ 10 \ M \ HCl, \ AcOH, \ r.t., \ 3 \ h; \ (f) \ aq. \ 1 \ M \ HCl, \ MeOH, \ reflux, \ 1 \ h.\]

\textbf{Scheme 30:} Synthesis of 6-aminoaurone (86).

\textbf{C) Physicochemical evaluation of 5-aminoindanone (104) and 6-aminoaurone (86)}

In order to examine the value of the ring fusion incorporated into chalcone-like structures 55 and 71, aminoindanone 104, given its greater structural resemblance, was compared directly to PAPP, and aminoaurone 86 compared to aminochalcone 55 (table 17).
Table 17: Electronic and lipophilic parameters of 5-aminoindanone (104) and 6-aminoaurone (86).

<table>
<thead>
<tr>
<th>Compound</th>
<th>NH$_2$ ipso $^{13}$C shift (ppm, relative to PAPP)</th>
<th>log P$_{exp.}$</th>
</tr>
</thead>
<tbody>
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<td>PAAP</td>
<td>+0.11</td>
<td>1.34</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="PAAP" /></td>
<td></td>
</tr>
<tr>
<td>PAPP</td>
<td>0.00</td>
<td>1.65</td>
</tr>
<tr>
<td>PAVP</td>
<td>-0.03</td>
<td>2.48</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="PAVP" /></td>
<td></td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="6-aminoaurone" /></td>
<td></td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="5-aminoaurone" /></td>
<td></td>
</tr>
</tbody>
</table>

Unexpectedly, the lipophilicity of aminoindanone 104 (log P$_{exp.}$ = 1.43) was found to be closer to that of PAAP (log P$_{exp.}$ = 1.34) than that of PAPP (log P$_{exp.}$ = 1.65) (table 17). This observation was postulated to be the result of decreased surface area in the case of aminoindanone 104, brought about by the presence of ring fusion.\textsuperscript{215} Again, counterintuitively, aminoindanone 104 displayed an ipso $^{13}$C shift considerably downfield (+1.84 ppm) compared to that of PAPP, suggesting that the strain caused by ring fusion plays a significant role in contributing to the electronics of the aryl amine (table 17). This result was in agreement with studies by Frimer \textit{et al.},\textsuperscript{216} who demonstrated that, for unsubstituted indanone (115), the strain induced by the fused ring results in a
considerable deshielding and a consequential downfield $^{13}$C shift, for the C-4 carbon (table 18). This is the same carbon of the indanone structure that is located ipso to that of the NH$_2$ group in aminooindanone 104 (table 18).

Table 18: Effect of indanone ring strain on $^{13}$C NMR shift and electronic effects.$^{216}$

<table>
<thead>
<tr>
<th>Frimer’s work$^{216}$</th>
<th>Current work</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsubstituted</td>
<td>NH$_2$ ipso $^{13}$C shift (ppm)</td>
</tr>
<tr>
<td>$^{13}$C-4 shift (ppm)</td>
<td>Amino-substituted</td>
</tr>
<tr>
<td>133.06</td>
<td>151.06 (C-4)</td>
</tr>
<tr>
<td>131.48</td>
<td>152.79 (C-5)</td>
</tr>
</tbody>
</table>

Interestingly, Frimer demonstrated that, as would be anticipated, the $^{13}$C shift of the C-4 carbon in acetophenone (7) (133.06 ppm) decreased when the C-2 position of this compound was substituted with a weakly electron-donating methyl group (cf. $\sigma_{\text{meta}}$ (Me) = -0.07) (116 $^{13}$C shift of C-4 = 131.48 ppm) (table 18). With the only structural difference between 2-methylacetophenone (116) and indanone (115) being ring fusion, this observation further suggests that ring strain is responsible for inducing a dominant effect on the observed ipso $^{13}$C shift, and hence the global electronics of the molecule.

For aminoaurone 86, it was assumed that the presence of an additional hydrogen bond acceptor would increase the overall polarity of the compound. Contrary to expectation, aminoaurone 86 exhibited a partition coefficient ($\log P_{\exp.} = 3.15$) similar to that of
aminochalcone 55 (log $P_{\text{exp.}} = 3.20$), suggesting that the hydrogen bond acceptor was in some way shielded (table 17).

As expected, the ipso $^{13}$C shift of aminoaurone 86 was downfield (+4.30 ppm) compared with that of aminochalcone 55 (+0.10 ppm), however it was not anticipated that the difference between the two shifts would be so great (table 17). Similar to that described for aminoindanone 104, this result suggests that, while the electronegative oxygen atom would contribute some degree of electron-withdrawing inductive effect, the ring fusion present in aminoaurone 86 would also appear to be a major contributing factor towards the global electronics of the molecule.

**D) In vitro evaluation of 5-aminoindanone (104) and 6-aminoaurone (86)**

Due to the unanticipated polarity of aminoindanone 104 (log $P_{\text{exp.}} = 1.43$), PAAP (log $P_{\text{exp.}} = 1.34$) was concurrently evaluated for comparative in vitro MtHb induction (graph 25 and 26). Aminoindanone 104 demonstrated inferior MtHb induction ($8.8 \pm 0.4\%$) compared to that of PAAP ($22.0 \pm 1.7\%$), suggesting that ring fusion and the ensuing unfavourable electronics was responsible for suboptimal in vitro MtHb induction (graph 25 and 26). Moreover, the introduction of substituents meta to the aryl amine may not be structurally tolerated with respect to MtHb inducers of this sub-structural class.

Despite demonstrating favourable lipophilic properties for in vitro MtHb induction, aminoaurone 86 (log $P_{\text{exp.}} = 3.15$) exhibited near baseline levels of MtHb activity ($2.9 \pm 0.2\%$), comparing poorly to both aminodihydrochalcone 71 ($81.2 \pm 1.5\%$) and aminochalcone 55 ($70.1 \pm 1.1\%$) (graph 25 and 26). The vastly inferior in vitro MtHb activity observed for this compound may again be a result of the considerable detrimental change in electronics brought about through the introduction of the ring-fused electron-withdrawing substituent. It is important to note that the MtHb induction exhibited by the positive controls (PAPP and PAVP) in this particular in vitro assay were unusually high as a result of the necessity to use higher concentrations of compounds 104 and 86 to elevate their in vitro MtHb activity from the baseline (graph 25 and 26).
3. Design, synthesis and bioevaluation of PAPP-related analogues

3.2. Aryl 4'-aminophenones

Upon consideration of the inferior *in vitro* MtHb induction observed for both aminoindanone 104 and aminoaurone 86, in combination with difficulties encountered in their respective syntheses, these compounds were no longer considered a good platform for further optimization. Efforts towards the syntheses of aminoindanones 84 and 85, and aminobenzofuranone 87 were therefore discontinued.

3.3. *In vivo* studies (1)
At this juncture, two candidates, PAHP and aminodihydrochalcone 71, were nominated for *in vivo* evaluation in rats, alongside known *in vivo* MtHb inducer, PAVP, as a positive control (figure 35).

![PAVP, PAHP and 4'-aminodihydrochalcone (71)](image)

**Figure 35:** Candidates (PAVP, PAHP and 4'-aminodihydrochalcone (71)) selected for *in vivo* evaluation.

PAHP was selected on the basis that it demonstrated the greatest *in vitro* MtHb induction within the alkyl aminophenone series (section 3.1.1.F). Likewise, aminodihydrochalcone 71 exhibited comparable *in vitro* MtHb toxicity to PAVP (section 3.2.3.C), and while not being the most potent MtHb inducer within its series of homologues, aminodihydrochalcone 71 was the most amenable to the synthetic scale up required for *in vivo* evaluation.

The main objective for this experiment was to explore the aforementioned discrepancy between the level of lipophilicity required for optimal *in vitro* MtHb activity, and that required for *in vivo* oral toxicity (section 3.1.1.). While both PAHP and aminodihydrochalcone 71 exhibited promising *in vitro* MtHb induction profiles, possibly as a result of enhanced uptake into the erythrocyte, it remained unknown whether these compounds possessed a favourable pharmacokinetic profile for *in vivo* MtHb toxicity.

The *in vivo* experiments were conducted as described in section 7.3.6. The results obtained (table 19) demonstrated that PAVP was the most toxic MtHb inducer *in vivo*, while both PAHP and aminodihydrochalcone 71 were demonstrated to be considerably less toxic.

Approximately 15 mins after dosing with PAHP and aminodihydrochalcone 71, rats exhibited symptoms characteristic of sub-lethal MtHb induction (blue paws, tail and nose, lethargy and ataxia). These symptoms, did however, ultimately eventuate to the death of
one of the test species for both PAHP and 71. For rats dosed with PAVP, symptoms of MtHb induction were again evident, leading to lethality in all cases (table 19).

<table>
<thead>
<tr>
<th>Table 19: In vivo results for PAVP, PAHP and 4'-aminodihydrochalcone (71).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compound</strong></td>
</tr>
<tr>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td><img src="image" alt="PAVP" /></td>
</tr>
<tr>
<td><img src="image" alt="PAHP" /></td>
</tr>
<tr>
<td><img src="image" alt="71" /></td>
</tr>
</tbody>
</table>


These results were in accordance with the in vivo LD_{50} data reported by Pan et al., and provided support for the notion that the optimal lipophilicity required for in vivo oral toxicity is lower than that required for in vitro MtHb activity. One explanation for the inferior in vivo MtHb toxicity observed for PAHP and aminodihydrochalcone 71 may be a result of elevated absorption and trapping of these compounds in the fatty tissues of the rat, which consequently may reduce their delivery to the target red blood cells. In light of this data, an emphasis on the design and synthesis of MtHb inducers with lipophilic profiles tailored to be comparable to that of PAVP was considered critical to the attainment of a superior in vivo MtHb toxicant.

3.4. 4'-Aminophenone isosteres
Having previously explored the influence of the group attached to the ketone on MtHb toxicity through a series of PAPP-like analogues, attention next turned to investigating the role of the ketone itself. Considering the aforementioned biochemical nature of MtHb induction, it was hypothesized that the electron-withdrawing nature of the ketone moiety of PAPP was central to the observed toxicity. In order to probe this notion further, a number of isosteric replacements for the ketone were investigated.

### 3.4.1. PAPP O-methyl ketoxime 117

Isosterism, a concept first proposed by Langmuir in 1919, describes the replacement of an atom or group of atoms in a molecule by other atoms that confer a comparable electronic and steric arrangement. A common isosteric replacement for the carbonyl group is the ketoxime moiety. An in silico log P prediction suggested that the O-methyl ketoxime 117 (log P_{exp.} = 2.59 ± 0.43) of PAPP would have a similar lipophilic profile to PAVP (log P_{exp.} = 2.36 ± 0.23) (figure 36). In light of this observation, the synthesis, physicochemical and in vitro evaluation of ketoxime 117 was undertaken (figure 36).

**Figure 36:** In silico log P predictions for PAPP, PAPP O-methyl ketoxime 117 and PAVP.

A) Synthesis of PAPP O-methyl ketoxime 117
Treatment of PAPP with \(O\)-methyl hydroxylamine hydrochloride and sodium carbonate at elevated temperature\(^{220}\) directly afforded PAPP \(O\)-methyl ketoxime 117 in 70\% yield, exclusively as the \(E\) isomer (scheme 31).

\[
\text{Reagents and conditions: NH}_2\text{OMe.HCl, Na}_2\text{CO}_3, \text{EtOH, H}_2\text{O, reflux, 36 h.}
\]

**Scheme 31:** Synthesis of PAPP \(O\)-methyl ketoxime 117.

### B) Physicochemical evaluation of PAPP \(O\)-methyl ketoxime 117

As expected, the partition coefficient of ketoxime 117 (log \(P_{\text{exp.}} = 2.30\)) was similar to that of PAVP (log \(P_{\text{exp.}} = 2.48\)) (table 20). The \(ipso\) \(^{13}\text{C}\) shift of 117, however, was considerably upfield (-3.68 ppm) from that of the parent ketone, suggesting that the \(O\)-methyl ketoxime moiety is significantly less electron-withdrawing than the ketone (table 20).

### Table 20: Electronic and lipophilic parameters of PAPP \(O\)-methyl ketoxime 117.

<table>
<thead>
<tr>
<th>Compound</th>
<th>(\text{NH}_2) (^{13}\text{C}) shift (ppm, relative to PAPP)</th>
<th>log (P_{\text{exp.}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP</td>
<td>0.00</td>
<td>1.65</td>
</tr>
<tr>
<td>117</td>
<td>-3.68</td>
<td>2.30</td>
</tr>
<tr>
<td>PAVP</td>
<td>-0.03</td>
<td>2.48</td>
</tr>
</tbody>
</table>

The observed
difference in electronics between these two groups was in agreement with the most structurally related Hammett substituent constants for each moiety (table 21).\textsuperscript{179}

**Table 21:** Hammett substituent constants for the propionyl and $O$-methyl aldoxime substituents.

<table>
<thead>
<tr>
<th>Substituent</th>
<th>Hammett constant ($\sigma_{\text{para}}$)\textsuperscript{179}</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{\begin{array}{c} \text{|} \ \text{|} \ \text{|} \ \text{|} \end{array}}$</td>
<td>+0.48</td>
</tr>
<tr>
<td>$\text{\begin{array}{c} \text{|} \ \text{|} \end{array}}$</td>
<td>+0.30</td>
</tr>
</tbody>
</table>
C) In vitro evaluation of PAPP O-methyl ketoxime 117

Despite exhibiting a favourable lipophilic profile (log $P_{\text{exp.}} = 2.30$), ketoxime 117 displayed considerably inferior in vitro MtHb induction ($7.2 \pm 0.9\%$) compared to both PAPP ($29.2 \pm 3.9\%$) and PAVP ($63.1 \pm 0.9\%$) (graph 27 and 28), suggesting that the electronic balance of the aryl amine ring was ‘sub-optimal’ in this particular example.

**Graph 27:** In vitro MtHb induction vs. log $P_{\text{exp.}}$ of PAPP O-methyl ketoxime 117.

**Graph 28:** In vitro MtHb induction vs. ipso $^{13}$C shift of PAPP O-methyl ketoxime 11.
3.4.2. Chloro-oximes 118 and 119, and cyano-oximes 120 and 121

The particularly low level of in vitro MtHb induction exhibited by ketoxime 117 was postulated to be a consequence of the weak electron-withdrawing nature of the oxime moiety (σ = +0.30) compared to that of the ketone in PAPP (σ = +0.48) (table 21). In a study using O-alkyl ketoxime moieties as ester surrogates, Bromidge et al. demonstrated that the pKa of azabicyclic muscarinic agonists could be modified through the introduction of an electron-withdrawing group into the O-alkyl ketoxime function. As described in section 1.3.3., the electronic contribution of a particular substituent is derived from its influence on the pKa of the overall molecule, thus these two physicochemical properties are interdependent. In light of this, it was postulated that the introduction of an electron-withdrawing group into the O-alkyl ketoxime function in our system could result in a favourable increase in the electron-deficiency of the aryl amine ring. Moreover, from a synthetic perspective, a favourable lipophilic profile could be conveniently built into each analogue through the incorporation of an appropriate alkyl chain via the oxygen of the ketoxime group (figure 37). Based on this design rationale, with guidance from in silico log P predictions, the synthesis, physicochemical and in vitro evaluation of chloro-oximes 118 and 119 and cyano-oximes 120 and 121 were investigated (figure 37).204

Figure 37: In silico log P predictions for PAPP O-methyl ketoxime 117, chloro-oximes 118 and 119, and cyano-oximes 120 and 121.204

A) Synthesis of chloro-oxime 118 and cyano-oximes 120 and 121

Synthesis of chloro-oxime 118 commenced with the condensation of 4-nitrobenzaldehyde (69) and hydroxylamine hydrochloride to afford unsubstituted
aldoxime 122 in 90% yield (scheme 32). Alkylation$^{223}$ of 122 with methyl iodide led to O-methyl ketoxime 123 in 59% yield (scheme 32). Treatment of 123 with $N$-chlorosuccinimide$^{222}$ afforded chloro-oxime 124 in 39% yield, with concomitant reduction$^{224}$ using iron and hydrochloric acid furnishing the desired chloro-oxime 118 in 35% yield, exclusively as the Z isomer (scheme 32). For reasons discussed in section 3.4.2.B, the synthesis of chloro-oxime 119 was not pursued.

\[
\text{Reagents and conditions: (a) NH}_2\text{OH.HCl, NaOH, H}_2\text{O, EtOH, r.t., 3 h; (b) i) NaH, DMF, r.t., 0.25 h; ii) MeI, DMF, r.t., 0.5 h; (c) NCS, DMF, 0 °C → r.t., 48 h; (d) Fe, aq. 10 M HCl, EtOH, H}_2\text{O, 70 °C, 3 h.}
\]

**Scheme 32: Synthesis of chloro-oxime 118.**

The preparation of cyano-oximes 120 and 121 began with the chlorination of aldoxime 122, as previously described$^{222}$ (scheme 33). The cyano group was installed through treatment of 125 with sodium cyanide in the presence of triethylamine,$^{224}$ affording 126 in 19% yield (scheme 33). Alkylation$^{224}$ of hydroximoyl cyanide 126 using methyl iodide or ethyl iodide in the presence of tetra-$n$-butylammonium bromide and sodium hydroxide at elevated temperature afforded 127 and 128 in 66% and 29% yield, respectively (scheme 33). Ultimately, nitro cyano-oximes 127 and 128 were reduced as described previously$^{224}$ to access the desired amino cyano-oximes 120 and 121 in 32% and 38% yield, respectively, exclusively as their Z isomers (scheme 33).
Reagents and conditions: (a) NCS, DMF, 0 °C → r.t., 24 h; (b) NaCN, NEt₃, iPrOH, DCE, H₂O, 0 °C, 5 h; (c) RI, TBAB, NaOH, THF, H₂O, 70 °C, 3 h; (d) Fe, aq. 10 M HCl, EtOH, H₂O, 70 °C, 3 h.

Scheme 33: Synthesis of cyano-oximes 120 and 121.

B) Physicochemical evaluation of chloro-oxime 118 and cyano-oximes 120 and 121

Contrary to expectation, chloro-oxime 118 was found to be considerably more lipophilic (log $P_{\text{exp.}} = 2.42$) than the in silico prediction suggested (log $P_{\text{calc.}} = 1.56 \pm 0.41$), with the level of lipophilicity being comparable to that of PAPP O-methyl ketoxime 117 (log $P_{\text{exp.}} = 2.30$) and PAVP (log $P_{\text{exp.}} = 2.48$) (table 22). Based on the observation that chloro-oxime 118 (R = Me) appeared to display ‘optimal’ levels of lipophilicity for MtHb induction, the synthesis, physicochemical and in vitro evaluation of chloro-oxime 119 (R = Et) was no longer pursued. Again, based on in silico log $P$ calculations, cyano-oxime 121 was predicted to possess a relatively low partition coefficient (log $P_{\text{calc.}}$ ca. 1.8), largely due to the polarity of the nitrile (figure 37). RP-HPLC evaluation, however, established that the lipophilicity of cyano-oxime 121 was significantly greater (log $P_{\text{exp.}} = 2.91$) than anticipated (table 22). Based on this result, the shorter chain O-methyl ether cyano-oxime 120 was also prepared and subjected to physicochemical evaluation. This compound also exhibited lipophilic properties (120, log $P_{\text{exp.}} = 2.44$) similar to that of PAVP (log $P_{\text{exp.}} = 2.48$), and thus was considered appropriate for comparison to PAVP with regards to its in vitro MtHb inducing properties (table 22).
Table 22: Electronic and lipophilic parameters of chloro-oxime 118 and cyano-oximes 120 and 121.

<table>
<thead>
<tr>
<th></th>
<th>NH$_2$ ipso $^{13}$C shift (ppm, relative to PAPP)</th>
<th>log $P_{exp}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP</td>
<td>0.00</td>
<td>1.65</td>
</tr>
<tr>
<td>117 R = Me X = Et</td>
<td>-3.68</td>
<td>2.30</td>
</tr>
<tr>
<td>118 R = Me X = Cl</td>
<td>-2.29</td>
<td>2.42</td>
</tr>
<tr>
<td>120 R = Me X = CN</td>
<td>-1.69</td>
<td>2.44</td>
</tr>
<tr>
<td>PAVP</td>
<td>-0.03</td>
<td>2.48</td>
</tr>
<tr>
<td>121 R = Et X = CN</td>
<td>-1.84</td>
<td>2.91</td>
</tr>
</tbody>
</table>

As presumed, the electronic trend within this series of compounds (ketoxime 117, chloro-oxime 118 and cyano-oxime 120) was of increasing ipso $^{13}$C shift (117 (X = Et) = -3.68 ppm, 118 (X = Cl) = -2.29 ppm and 120 (X = CN) = -1.69 ppm) relative to the increasing electron-withdrawing potential of the ketoxime derivative (table 22). In light of RP-HPLC evaluation, it was considered that the direct comparison of the lipophilically similar PAPP O-methyl ketoxime 117 (log $P_{exp.}$ = 2.30), chloro-oxime 118 (log $P_{exp.}$ = 2.42) and cyano-oxime 120 (log $P_{exp.}$ = 2.44) would provide the greatest insight into the role of electronics on MtHb induction within this series (table 22).

C) *In vitro* evaluation of chloro-oxime 118 and cyano-oximes 120 and 121

Contrary to expectation, ketoxime 117 (6.4 ± 0.8%), chloro-oxime 118 (8.4 ± 0.9%) and cyano-oxime 120 (8.2 ± 1.9%) exhibited similar levels of activity, thus disappointingly providing no clear relationship between the electronic parameter and MtHb induction within this series (graph 29 and 30).

In summary, these results would suggest that the derivatized ketoxime moieties evaluated were insufficiently electron-withdrawing to achieve an optimal electronic contribution.
the aryl amine ring, including the ketoxime analogue possessing the strongest electron-withdrawing group (120, X = CN), exhibiting an ipso $^{13}$C shift (-1.69 ppm) somewhat upfield from that of PAPP (table 22). Upon consideration of the aforementioned structural requirements (section 3.1. and 3.2.), it should be noted that the derivatized ketoxime moiety may not be as well tolerated as the ketone, potentially providing another explanation for the inferior in vitro MtHb activity observed within this series.

Graph 29: In vitro MtHb induction vs. log $P_{exp}$ of chloro-oxime 118 and cyano-oximes 120 and 121.

Graph 30: In vitro MtHb induction vs. ipso $^{13}$C shift (ppm, relative to PAPP) of chloro-oxime 118 and cyano-oximes 120 and 121.
As a result of the inferior MtHb induction exhibited by chloro-oxime 118 and cyano-oximes 120 and 121, this series of compounds was not considered for further elaboration.

### 3.4.3. 4'-Aminophenyl alkyl sulfoxide 129, sulfone 130, and sulfonamides 131 and 132, and dapsone

Due to a lack of success in efforts to improve the electronic contribution of the ketoxime moiety through ketoxime analogues 118, 120 and 121 (table 22), attention next turned to the use of alternative carbonyl isosteric replacements that possessed greater electron-withdrawing properties, namely sulfur-based moieties such as sulfoxides and sulfones (table 23).

**Table 23:** Aldoxime, sulfoxide, sulfonamide and sulfone substituents as isosteric replacements for the propionyl moiety of PAPP, and their corresponding Hammett $\sigma$ constants.

<table>
<thead>
<tr>
<th>Substituent</th>
<th>Hammett constant ($\sigma_{\text{para}}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{CH}_2$</td>
<td>+0.48</td>
</tr>
<tr>
<td>$\text{N}^\text{O}$</td>
<td>+0.30</td>
</tr>
<tr>
<td>$\text{H}$</td>
<td>+0.49</td>
</tr>
<tr>
<td>$\text{SO}$</td>
<td>+0.65</td>
</tr>
<tr>
<td>$\text{SO}_2$</td>
<td>+0.72</td>
</tr>
</tbody>
</table>

Replacement of the ketone with isosteric substituents possessing larger Hammett constants ($\sigma_{\text{para}} = +0.49 \rightarrow +0.72$) was postulated to further probe the role of the ketone.
group of PAPP on MtHb toxicity, specifically how its electron-withdrawing properties might contribute to its influence (table 23). A traditional carboxylate isostere, the sulfonamide group, was also explored due to its similar electron-withdrawing properties ($\sigma_{\text{para}} = +0.65$) to the sulfoxide and sulfonamide substituents (table 23). With guidance from in silico log $P$ predictions and consideration given to synthetic accessibility, aminophenyl alkyl sulfoxide 129, sulfone 130, and sulfonamide 131 were proposed, with each compound incorporating an alkyl chain tailored to achieve a lipophilic profile comparable to that of PAVP (most potent in vivo MtHb inducer) (figure 38).

![Chemical structures](image)

**Figure 38:** In silico log $P$ predictions of PAVP, 4'-aminophenyl alkyl sulfoxide 129, sulfone 130, sulfonamide 131 and dapsone.

It was considered that this series of sulfur-based isosteres presented a good opportunity to concurrently examine the potency of a structurally-related aryl amine, dapsone, a compound which is known to induce MtHb, in our own in-house in vitro MtHb assay (figure 38).
A) Synthesis of 4'-aminophenyl alkyl sulfoxide 129, sulfone 130, and sulfonamides 131 and 132, and dapsone

Aminophenyl alkyl sulfoxide 129 and aminophenyl alkyl sulfone 130 were prepared from the common nitrophenyl alkyl sulfide intermediate 133, which in turn was obtained by thiolation of 4-chloronitrobenzene (134), using n-pentanethiol in the presence of potassium hydroxide at elevated temperature (scheme 34). Reduction of nitrophenyl alkyl sulfide 133 with tin(II) chloride at elevated temperature afforded aminophenyl alkyl sulfide 135, which was subsequently subjected to mild oxidation conditions using hydrogen peroxide to afford the desired aminophenyl alkyl sulfoxide 129 in low yield over two steps (scheme 34).

Reagents and conditions: (a) CH₃(CH₂)₄SH, KOH, DMF, 100 °C, 6 h; (b) SnCl₂, EtOH, 70 °C, 5 h; (c) 135, H₂O₂, H₂O, 70 °C, 1 h; (d) m-CPBA, CH₂Cl₂, 0 °C → r.t., 4 h; (e) 136, SnCl₂, EtOH, reflux, 4 h.

Scheme 34: Synthesis of 4'-aminophenyl alkyl sulfoxide 129 and 4'-aminophenyl alkyl sulfone 130.

Oxidation of nitrophenyl alkyl sulfide 133 using m-chloroperoxybenzoic acid (m-CPBA) afforded nitrophenyl alkyl sulfone 136. Subsequent selective nitro group
3. Design, synthesis and bioevaluation of PAPP-related analogues  3.4. 4'-Aminophenone isosteres

reduction$^{227}$ of 136 using tin(II) chloride at elevated temperature furnished aminophenyl alkyl sulfonyl chloride 130, again in low yield, over two steps (scheme 34).

The first step towards the synthesis of aminophenyl alkyl sulfonyl chloride 131 involved diazotization$^{230}$ of 4-nitroaniline (137), with treatment of the diazonium salt with thionyl chloride to yield sulfonyl chloride 138 (scheme 35). Subsequent amination$^{231}$ of 138 with butylamine afforded nitrophenyl alkyl sulfonyl chloride 139 in 68% yield over two steps (scheme 35). Reduction$^{232}$ of the nitro group of 139 using iron and ammonium chloride at elevated temperature furnished the desired aminophenyl alkyl sulfonyl chloride 131 in 38% yield (scheme 35).

![Scheme 35: Synthesis of 4'-aminophenyl alkyl sulfonyl chloride 131 and N-methyl 4'-aminophenyl alkyl sulfonyl chloride 132.](image)

Reagents and conditions: (a) i) NaNO$_2$/H$_2$O, HCl/H$_2$O, 0 °C, 0.25 h; ii) SOCl$_2$/H$_2$O, CuCl, 0 °C → r.t., 1.25 h; (b) 138, CH$_3$(CH$_2$)$_3$NH$_2$, THF, r.t., 0.5 h; (c) NaH, MeI, 0 °C → reflux, 18 h; (d) Fe, NH$_4$Cl, MeOH, H$_2$O, r.t. → 70 °C, 2.5 h; (e) (CH$_3$O)$_2$CHNMe$_2$, 90 °C, 18 h.

For reasons discussed in section 3.4.3.C, N-methyl aminophenyl alkyl sulfonyl chloride 132 was also synthesized. Initial attempts$^{233}$ to prepare N-methyl aminophenyl alkyl sulfonyl chloride 132 from sulfonyl chloride 131 using dimethylformamide dimethyl acetal failed, affording only mixed alkylation products (scheme 35). Ultimately, alkylation$^{234}$ of nitrophenyl alkyl sulfonyl chloride 139 using sodium hydride and methyl iodide afforded N-methyl nitrophenyl alkyl sulfonyl chloride 140 in 82% yield, which underwent reduction of the nitro group as described previously$^{232}$ to afford aminophenyl alkyl N-methyl sulfonyl chloride 132 in 86% yield (scheme 35). Dapsone was obtained from commercial sources.$^{235}$

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3. Design, synthesis and bioevaluation of PAPP-related analogues

3.4. 4'-Aminophenone isosteres

B) Physicochemical evaluation of 4'-aminophenyl sulfoxide 129, sulfone 130, and sulfonamides 131 and 132, and dapsone

The partition coefficient of aminophenyl alkyl sulfonamide 131 (log $P_{\text{exp.}} = 2.05$) fell between that of PAPP (log $P_{\text{exp.}} = 1.65$) and PAVP (log $P_{\text{exp.}} = 2.48$) (table 24). Interestingly, the $ipso$ $^{13}$C shift of sulfonamide 131 (-0.53 ppm) was similar to that of PAPP, suggesting that the sulfonamide group is only slightly less electron-withdrawing than the ketone (table 24).

For reasons described in section 3.4.3.C, $N$-methyl aminophenyl alkyl sulfonamide 132 was also prepared and subjected to physicochemical evaluation. Sulfonamide 132 exhibited an elevation in lipophilicity (log $P_{\text{exp.}} = 2.73$), compared to its mono-substituted counterpart 131 (log $P_{\text{exp.}} = 2.05$), greater than the $ca. 0.4 \log P$ units generally observed upon the incorporation of a single methylene unit (table 24). This observation was postulated to be due to the removal of the N-H hydrogen bond donor site in 131 upon methylation, which may have significantly contributed to the overall polarity of sulfonamide 131.

Despite possessing the same alkyl chain length, aminophenyl alkyl sulfoxide 129 unexpectedly exhibited greater polarity (log $P_{\text{exp.}} = 2.26$) than aminophenyl alkyl sulfone 130 (log $P_{\text{exp.}} = 2.53$), for reasons which cannot be explained (table 24). Again, contrary to expectation, the $ipso$ $^{13}$C shift of aminophenyl alkyl sulfone 130 was considerably closer (+0.34 ppm) to PAPP than the $ipso$ $^{13}$C shift of sulfoxide 129 (-1.59 ppm) was to PAPP (table 24). This observation suggested that the electron-withdrawing nature of the sulfone substituent (rather than the sulfoxide group, as anticipated) more closely resembled that of the parent ketone.

Dapsone exhibited a partition coefficient (log $P_{\text{exp.}} = 1.68$) similar to that of PAPP (log $P_{\text{exp.}} = 1.65$) (table 24). Additionally, the $ipso$ $^{13}$C shift of dapsone (-0.60 ppm) was comparable to that of PAPP, suggesting that its sulfonylanilino substituent possesses electron-withdrawing properties most closely resembling that of sulfonamide 131 (-0.53 ppm) (table 24).
**Table 24**: Electronic and lipophilic parameters of 4'-aminophenyl alkyl sulfoxide 129, sulfone 130, and sulfonamides 131 and 132, and dapsone.

<table>
<thead>
<tr>
<th></th>
<th>NH$_2$ ipso $^{13}$C shift (ppm, relative to PAPP)</th>
<th>log $P_{exp.}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP</td>
<td>0.00</td>
<td>1.65</td>
</tr>
<tr>
<td>Dapsone</td>
<td>-0.60</td>
<td>1.68</td>
</tr>
<tr>
<td>131</td>
<td>-0.53</td>
<td>2.05</td>
</tr>
<tr>
<td>129</td>
<td>-1.59</td>
<td>2.26</td>
</tr>
<tr>
<td>PAVP</td>
<td>-0.03</td>
<td>2.48</td>
</tr>
<tr>
<td>130</td>
<td>+0.34</td>
<td>2.53</td>
</tr>
<tr>
<td>132</td>
<td>-0.66</td>
<td>2.73</td>
</tr>
</tbody>
</table>

In summary, the trend in ipso $^{13}$C shifts of aminophenone isosteres, sulfoxide 129, alkyl sulfonamide 132 and sulfone 130, were found to correlate to their established literature electronic properties (i.e. the greater the Hammett constant for the substituent, the greater the observed ipso $^{13}$C shift) (graph 31). When comparing the ipso $^{13}$C shifts of 129, 132 and 130 to PAVP, however, all appeared further upfield (lower ppm value) than expected, with respect to their Hammett constants (graph 31).
Graph 31: Ipso $^{13}$C shifts of the ‘lipophilically equivalent’ PAVP, 4'-aminophenyl alkyl sulfoxide 129, N-methyl 4'-aminophenyl alkyl sulfonamide 132, 4'-aminophenyl alkyl sulfone 130, and their corresponding Hammett substituent constants.$^{179}$

C) \textit{In vitro} evaluation of 4'-aminophenyl alkyl sulfoxide 129, sulfone 130, and sulfonamides 131 and 132, and dapsone

As largely anticipated, sulfoxide 129, possessing the most unfavourable electronics (\textit{ipso} $^{13}$C shift = -1.59 ppm) within this series, exhibited suboptimal \textit{in vitro} MtHb induction (13.2 ± 0.8\%) (graph 32 and 33). Contrary to expectation, however, sulfonamide 131, a compound which appeared to possess an electronic contribution similar to PAPP (\textit{ipso} $^{13}$C shift = -0.53 ppm), exhibited the poorest \textit{in vitro} MtHb induction within this series (5.1 ± 0.4\%) (graph 32 and 33). To date, only hydrogen bond accepting aryl amine ring substituents (e.g. ketones, ketoximes etc.) have been explored within this family of aminophenone-like compounds. The low level of activity observed for sulfonamide 131, despite its favourable electronics, was loosely attributed to the presence of the N-H hydrogen bond donor of the ketone isostere being detrimental to MtHb induction. To further investigate this hypothesis, efforts subsequently focused on the synthesis, physicochemical and \textit{in vitro} evaluation of the
3. Design, synthesis and bioevaluation of PAPP-related analogues 3.4. 4'-Aminophenone isosteres

N-methyl aminophenyl alkyl sulfonamide 132, in an effort to eliminate the hydrogen bond donor site through N-methylation (table 24). Gratifyingly, N-methylation of sulfonamide 131 resulted in an increase in activity (132, 22.0 ± 0.5%), however, upon consideration of the physicochemical properties of sulfonamide 132 (log $P_{\text{exp.}} = 2.73$, table 24), it remained unclear as to whether the observed increase in in vitro MtHb induction was due to the elimination of the hydrogen bond donor site, or a consequence of its more favourable lipophilic profile (graph 32 and 33).

Sulfone 130, exhibiting the most favourable electronics ($ipso^{13}$C shift = +0.34 ppm), demonstrated the greatest in vitro MtHb induction within this series (33.5 ± 2.9%) (graph 32 and 33).

Graph 32: In vitro MtHb induction vs. log $P_{\text{exp.}}$ of 4'-aminophenyl alkyl sulfoxide 129, sulfone 130, and sulfonamides 131 and 132, and dapsone.

Graph 33: In vitro MtHb induction vs. $ipso^{13}$C shift (ppm, relative to PAPP) of 4'-aminophenyl alkyl sulfoxide 129, sulfone 130, and sulfonamides 131 and 132, and dapsone.
In general, the level of MtHb induction exhibited by the lipophilically similar sulfur-based isosteres; sulfoxide 129, sulfonamide 132 and sulfone 130 followed a pattern based largely on the electronic nature (based on ipso $^{13}$C shifts rather than Hammett constants) of their respective substituents (graph 31, 32 and 33).

In comparison to the ‘lipophically equivalent’ PAPP (log $P_{\text{exp.}} = 1.65$, MtHb = 22.9 $\pm$ 0.3%), dapsone exhibited inferior in vitro MtHb induction (log $P_{\text{exp.}} = 1.68$, 7.4 $\pm$ 0.6%) (graph 32 and 33). Based on this result, dapsone was not considered an appropriate platform for further elaboration.

Despite displaying ‘near optimal’ electronic (ipso $^{13}$C shift = +0.34 ppm) and lipophilic properties (log $P_{\text{exp.}} = 2.53$), and exhibiting the greatest activity within this series (33.5 $\pm$ 2.9%), the in vitro MtHb induction of sulfone 130 remained somewhat behind that of the lead compound, PAVP (66.5 $\pm$ 3.0%) (graph 32 and 33). One explanation for this and the general relative inferiority of other sulfur-based isosteres such as the sulfoxide and sulfonamide replacements, as in 129 and 132, respectively, may relate to the ‘specific’ electronic contribution of these ketone isosteres.

It is known that the Hammett $\sigma$ constant is composed of two key parts, a ‘field’ ($F$) component (or inductive) and a ‘resonance’ ($R$) component. The inductive effect is dependent on the electronegativity of the atoms, bond order, charge and the position of the atoms within a group, while the resonance effect is derived from the delocalization of the electrons in a $\pi$-system.

On the surface, evaluation of the Hammett constants ($\sigma$) for the propionyl (+0.48) and sulfoxide (+0.49) substituents of PAPP and sulfoxide 129, respectively, suggests these substituents to be relatively similar with respect to their electronic nature (table 25).
Table 25: $\sigma_{para}, F$ and $R$ parameters for the propionyl, sulfoxide, sulfonamide and sulfone substituents.

<table>
<thead>
<tr>
<th>Substituent</th>
<th>$\sigma_{para}$</th>
<th>$F$</th>
<th>$R$</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image.png" alt="Propionyl" /></td>
<td>+0.48</td>
<td>+0.34</td>
<td>+0.14</td>
</tr>
<tr>
<td><img src="image.png" alt="Sulfoxide" /></td>
<td>+0.49</td>
<td>+0.52</td>
<td>-0.03</td>
</tr>
<tr>
<td><img src="image.png" alt="Sulfonamide" /></td>
<td>+0.65</td>
<td>+0.44</td>
<td>+0.21</td>
</tr>
<tr>
<td><img src="image.png" alt="Sulfone" /></td>
<td>+0.72</td>
<td>+0.53</td>
<td>+0.19</td>
</tr>
</tbody>
</table>

Upon closer inspection of each substituents corresponding $F$ and $R$ parameters, however, it was observed that the resonance ($R$) parameter afforded a larger contribution to the overall $\sigma$ constant of that found in PAPP ($R = +0.14$) compared with that found in sulfoxide 129 ($R = -0.03$) (table 25). This contrasting electronic composition of sulfoxide 129 may influence one of the two critical factors ($N$-hydroxylation and the coupled redox reaction) involved the mechanism of MtHb toxicity. It could be further postulated that a specific ratio of field and resonance parameters for the $\sigma$ constant of the substituent is required for strong MtHb induction (table 25).

Sulfonamide 132 and sulfone 130 appear to possess electronic properties more comparable to that of PAPP, perhaps offering some explanation as to why these compounds exhibited greater *in vitro* MtHb induction than sulfoxide 129 (table 25). It would appear that, for sulfonamide 132 and sulfone 130, their respective electronic composition is not a limiting factor with respect to their MtHb induction. Given that there may be structural requirements for MtHb induction, some consideration needs be given to the tetrahedral geometry of sulfonamide 132 and sulfone 130 (versus that of the planar ketone present in PAPP), in that they may not be tolerated structurally with respect to the processes relevant to MtHb induction.
The specific objectives for the design, synthesis and bioevaluation of benzocaine-related analogues were:

A) A comparison of the physicochemical properties and \textit{in vitro} activity of known MtHb inducers, PAPP and benzocaine, with the aim of evaluating benzocaine as a potential lead compound for the design of MtHb inducers (figure 39).

\textbf{Figure 39:} Structure PAPP and benzocaine.

B) To explore the viability of replacing the metabolically unstable ethyl ester functionality of benzocaine with a number of heterocyclic ester bioisosteres, with the aim of attaining a more potent \textit{in vivo} MtHb inducer.

- This was envisaged to be achieved through the synthesis and bioevaluation of a number of series of oxadiazoles and thiadiazoles (figure 40).

\textbf{Figure 40:} Benzocaine and heterocyclic benzocaine bioisosteres.
4.1. PAPP and benzocaine

In 1901, benzocaine (141), or ethyl para-aminobenzoate, was pharmacologically tested by Kober-Rostock® and found to possess remarkable anaesthetic properties. By 1904, benzocaine was on the market under the trade name Anesthesin. Still widely used today in clinical practice as a topical anaesthetic in the form of creams, ointments and sprays, benzocaine has been observed, as a side effect, to induce MtHb in patients in some cases leading to potentially lethal levels. While of concern in the field of medicine, benzocaine-induced MtHb is of considerable interest to the current research. Structurally, benzocaine bears a close resemblance to PAPP, differing only slightly in the nature of the aniline substituent (figure 41).

![Figure 41: Structure PAPP and benzocaine (141).](image)

Consequently, a number of studies have been conducted focusing on a comparison of their MtHb inducing properties. Guertler et al. demonstrated that sheep, dosed either intranasally with benzocaine, or intravenously with PAPP, developed similar levels of MtHb induction. Further to these studies, Coleman et al. demonstrated that benzocaine was a more potent in vitro (rat liver systems) MtHb inducer than PAPP (table 26).
Table 26: NAPDH-dependent (rat liver systems) MtHb induction exhibited by PAPP and benzocaine.\textsuperscript{246}

<table>
<thead>
<tr>
<th>Compound</th>
<th>MtHb(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>w/o NADPH</td>
</tr>
<tr>
<td>PAPP</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Benzocaine</td>
<td>1.2 ± 0.1</td>
</tr>
</tbody>
</table>

Considering their structural similarities, in combination with the apparent NAPDH-dependency of both PAPP and benzocaine for MtHb induction\textsuperscript{246}, it is generally accepted that these compounds induce their toxic effect by analogous biochemical pathways\textsuperscript{245}. Having earlier established the role of electronics with respect to MtHb induction, it was postulated that the similar mode of action of PAPP and benzocaine may largely be a consequence of the similar electronic contributions of their ketone and ester functionalities, respectively. By comparing the Hammett $\sigma$ substituent constants of these two functionalities, it can be demonstrated that the ketone and ester groups are very similar with regards to their electron-withdrawing nature (table 27).\textsuperscript{179}

Table 27: Hammett substituent constants ($\sigma$) of ketone and ester functionalities present in PAPP and benzocaine, respectively.\textsuperscript{179}

<table>
<thead>
<tr>
<th>Substituent</th>
<th>Hammett constant ($\sigma$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[\text{\rotatebox{90}{$\text{O}$}}\text{\rotatebox{90}{$\text{O}$}}]</td>
<td>+0.48</td>
</tr>
<tr>
<td>[\text{\rotatebox{90}{$\text{O}$}}\text{\rotatebox{90}{$\text{O}$}}]</td>
<td>+0.45</td>
</tr>
</tbody>
</table>

Based on this similarity, it was hypothesized that both these functionalities offered a favourable electronic contribution for the biochemical processes critical to MtHb induction (section 1.3.3.).
In order to investigate this notion further, the synthesis, physicochemical and *in vitro* evaluation of benzocaine was conducted for comparison to PAPP.

**A) Synthesis of benzocaine**

Benzocaine was prepared through the acid-catalyzed esterification of *para*-aminobenzoic acid (PABA) (142) with ethanol, in the presence of concentrated sulfuric acid, in 35% yield (scheme 36).

Benzocaine exhibited a partition coefficient (log $P_{exp.} = 2.04$) greater than that of PAPP (log $P_{exp.} = 1.65$) (table 28). This trend appeared to be in general agreement with lipophilic ($\pi$) substituent constants reported by Hansch\textsuperscript{200} for the closely related methyl ketone ($\pi = -0.55$) and methyl ester ($\pi = -0.01$) substituents. As expected, the *ipso* $^{13}$C shift of benzocaine (-0.30 ppm) was similar to that of PAPP, further supporting the notion that the electronic contribution provided by the ketone and ester functionalities of PAPP and benzocaine, respectively, closely resemble each other (table 28).
4. Design, synthesis and bioevaluation of benzocaine-related analogues

4.1. PAPP and benzocaine

Table 28: Electronic and lipophilic parameters of PAPP and benzocaine.

<table>
<thead>
<tr>
<th>Compound</th>
<th>NH$_2$ $ipso$ $^{13}$C shift (ppm, relative to PAPP)</th>
<th>log $P_{exp.}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP</td>
<td>0.00</td>
<td>1.65</td>
</tr>
<tr>
<td>Benzocaine</td>
<td>-0.30</td>
<td>2.04</td>
</tr>
</tbody>
</table>

C) In vitro evaluation of PAPP and benzocaine

Based on physicochemical evaluation, it was postulated that benzocaine would elicit a similar or possibly elevated level of in vitro MtHb induction compared to PAPP. Contrary to expectation, at a standard compound concentration of 10µM, PAPP induced typical levels of MtHb (15.5 ± 0.8%), whereas benzocaine exhibited merely baseline levels of activity (graph 34). In an effort to elevate the level of MtHb induction exhibited by benzocaine, the assay was repeated at a compound concentration of 100µM. Consequently, benzocaine demonstrated some MtHb induction (24.4 ± 1.5%), however it remained largely inferior compared to PAPP (35.8 ± 1.8%) (graph 34).

Graph 34: In vitro MtHb induction of PAPP and benzocaine.

The observed difference between the levels of in vitro MtHb induction exhibited by PAPP and benzocaine was postulated to be the consequence of the latter’s susceptibility to hydrolysis. The hydrolytic process, assumed to be catalyzed by plasma esterases present in the blood, leads to the formation of para-aminobenzoic acid (PABA) (scheme 37). While the acid
functionality of PABA possesses an electronic contribution ($\sigma = +0.45$) closely resembling that of the ester, its high polarity, a consequence of ionization at physiological pH, severely compromises the passive diffusion of this compound through the red blood cell membrane. In light of this, it would be unlikely that any MtHb induction would occur post-hydrolysis of benzocaine.\textsuperscript{248}

Scheme 37: Hydrolysis of benzocaine, catalyzed by plasma esterases.

To investigate whether hydrolysis was indeed occurring during the \textit{in vitro} MtHb assay (and thus responsible for benzocaine’s low level of MtHb induction), bis(4-nitrophenyl) phosphate (BNPP) (143), a reversible esterase inhibitor, was employed to inhibit the perceived hydrolytic action of the plasma esterases (figure 42).

Figure 42: Bis(4-nitrophenyl) phosphate (143) (BNPP), a reversible esterase inhibitor.

\textit{In vitro} MtHb induction was measured at a range of concentrations of both benzocaine and BNPP, with the results demonstrating that an increase in MtHb induction correlated with an increase in the concentration of BNPP (graph 35). This result suggested that, to a certain extent, hydrolysis was indeed hindering the \textit{in vitro} MtHb induction by benzocaine.
4. Design, synthesis and bioevaluation of benzocaine-related analogues

4.1. PAPP and benzocaine

Graph 35: Effect of BNPP esterase inhibitor on MtHb induction at different concentrations of benzocaine (1μM, 10μM and 100μM).

It is important to note that PABA, the cleavage product of benzocaine hydrolysis, was likewise assayed for *in vitro* MtHb induction, with standard incubation times displaying baseline levels of MtHb induction at three different concentrations (1μM, 10μM and 100μM). This observation further supported the hypothesis that hydrolytic breakdown was responsible for the inferior *in vitro* MtHb induction observed for benzocaine.

### 4.2. Bioisosterism

In 1951, Friedman\textsuperscript{249} introduced a concept as an extension of Langmuir’s\textsuperscript{218} isosterism, namely ‘bioisosterism’, describing bioisosteric compounds as those that ‘fit the broadest definition of isosteres and have the same type of biological activity.’ This definition received rapid acceptance and is still commonly referred to today,\textsuperscript{250} serving as a research tool of paramount importance to the field of medicinal chemistry. More recently, Thornber\textsuperscript{251} proposed a more loose and flexible definition of bioisosteres, describing them as ‘groups or molecules that have chemical and physical similarities producing broadly similar biological effects.’

In parallel to the design, synthesis, physicochemical and *in vitro* evaluation of PAPP-related analogues, efforts focused on strategies for circumnavigating the susceptibility of benzocaine to
hydrolysis, while endeavouring to retain its favourable physicochemical properties for MtHb induction. This was postulated to be achieved through the design, synthesis, physicochemical and in vitro evaluation of heterocyclic bioisosteres as hydrolytically stable substitutes for benzocaine.

**4.3. Benzocaine bioisosteres and related heterocycles (1)**

**4.3.1. 3-Substituted-1,2,4-oxadiazoles 144-149**

Investigation into non-classical bioisosteres for the ester functionality revealed the 1,2,4-oxadiazole moiety as the most frequently employed surrogate in the literature.\(^{250,252,253}\) In light of this, with guidance from *in silico* log *P* predictions, a number of 3-substituted-1,2,4-oxadiazoles 144-149 bearing various alkyl chains and aryl groups were prepared for physicochemical and in vitro evaluation, in order to explore various structure-activity relationships of this new structural sub-class (figure 43).

![Figure 43: In silico log P predictions for benzocaine bioisosteres, 3-substituted-1,2,4-oxadiazoles 144-149.\(^{204}\)](image)

**A) Synthesis of 3-substituted-1,2,4-oxadiazoles 144-149**

3-Substituted-1,2,4-oxadiazoles 144-149 were accessed conveniently in three steps, via amidoximes 150-155,\(^{254}\) themselves the products of the reaction between the corresponding nitriles and hydroxylamine hydrochloride (scheme 38). Subsequent one-pot base-catalyzed acylation and dehydration\(^{254}\) of 150-155 with 4-nitrobenzoyl chloride (156) allowed conversion through to oxadiazoles 157-162 in variable yield (2-18%) over two steps (scheme 38).
Compounds 157-162 were then reduced, adopting a procedure established for the selective reduction of nitro-heterocycles, using sodium sulfide in aqueous 1,4-dioxane to afford the desired oxadiazoles 144-149 in moderate yield (47-87%) (scheme 38).

![Reagents and conditions: (a) NH₂OH.HCl, NaOH, EtOH, H₂O, reflux, 36 h; (b) 4-nitrobenzoyl chloride (156), pyridine, reflux, 0.5 h; (c) Na₂S.9H₂O, H₂O, 1,4-dioxane, 80 °C, 1 h.](image)

**Scheme 38:** Synthesis of 3-substituted-1,2,4-oxadiazoles 144-149.

### B) Physicochemical evaluation of 3-substituted-1,2,4-oxadiazoles 144-149

The partition coefficients of oxadiazoles 144-149 were largely influenced by the nature of the R group at the 3-position, with manipulation at this position within this series of compounds providing a broad range of lipophilic profiles (log $P_{\text{exp.}} = 1.89-3.48$) (table 29). Contrary to expectation, oxadiazole 149 (R = Bzl, log $P_{\text{exp.}} = 3.24$) exhibited a slightly lower lipophilicity than that of oxadiazole 148 (R = Ph, log $P_{\text{exp.}} = 3.48$), despite possessing an additional methylene unit (table 29). This result appears to be in agreement with the lipophilic relationship between aminodihydrochalcone 71 homologues, amino-2-phenylacetophenone 72 and aminobenzophenone 54, and Hansch’s hydrophobic constants for phenyl and benzyl substituents, as described earlier (section 3.2.4.B).

Oxadiazoles 144-149 demonstrated an ipso $^{13}$C shift (-0.39 ppm $\rightarrow$ -0.46 ppm) closely resembling that of benzocaine (-0.30 ppm), suggesting that the 1,2,4-oxadiazole moiety provides an electronic contribution similar to that of the ester group present in benzocaine (table...
4. Design, synthesis and bioevaluation of benzocaine-related analogues

4.3. Benzocaine bioisosteres

This data upheld the notion that the 1,2,4-oxadiazole moiety constitutes an excellent non-classical ester bioisostere.

Table 29: Electronic and lipophilic parameters of 3-substituted-1,2,4-oxadiazoles 144-149.

<table>
<thead>
<tr>
<th></th>
<th>NH$_2$ ipso $^{13}$C shift (ppm, relative to PAPP)</th>
<th>log $P_{\text{exp.}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP</td>
<td>0.00</td>
<td>1.65</td>
</tr>
<tr>
<td>144 R = Me</td>
<td>-0.42</td>
<td>1.89</td>
</tr>
<tr>
<td>Benzocaine</td>
<td>-0.30</td>
<td>2.04</td>
</tr>
<tr>
<td>145 R = Et</td>
<td>-0.41</td>
<td>2.22</td>
</tr>
<tr>
<td>PAVP</td>
<td>-0.03</td>
<td>2.48</td>
</tr>
<tr>
<td>147 R = iPr</td>
<td>-0.42</td>
<td>2.57</td>
</tr>
<tr>
<td>146 R = nPr</td>
<td>-0.46</td>
<td>2.61</td>
</tr>
<tr>
<td>149 R = Bzl</td>
<td>-0.39</td>
<td>3.24</td>
</tr>
<tr>
<td>148 R = Ph</td>
<td>-0.39</td>
<td>3.48</td>
</tr>
</tbody>
</table>

**C) In vitro evaluation of 3-substituted-1,2,4-oxadiazoles 144-149**

Owing to concerns over the hydrolytic stability of benzocaine during *in vitro* MtHb appraisal, PAPP and PAVP were once again employed as positive controls. Gratifyingly, a high level of *in vitro* MtHb induction was exhibited by oxadiazoles 144-149 (graph 36). This promising result was attributed to the favourable electronic contribution of the 1,2,4-oxadiazole substituent, thus proving a worthy surrogate for the ethyl ester substituent of benzocaine. In general, the oxadiazoles 144-147 (R = alkyl) exhibited a similar trend compared to alkyl aminophenones 1 and 16-19, whereby increasing the length of the alkyl chain at the 3-position (and thus increasing the lipophilicity of the molecule, i.e. 144 R = Me; log $P_{\text{exp.}}$ = 1.89, 145 R = Et; log
4. Design, synthesis and bioevaluation of benzocaine-related analogues

4.3. Benzocaine bioisosteres

$P_{\text{exp.}} = 2.22$, 146 $R = nPr$, log $P_{\text{exp.}} = 2.61$), led to enhanced in vitro MtHb activity within this series (144 $R = Me$, MtHb = 38.7 ± 1.3%, 145 $R = Et$, MtHb = 42.7 ± 0.6%, 146 $R = nPr$, MtHb = 47.9 ± 1.0%) (graph 36).

\[
\begin{align*}
\text{Graph 36: In vitro MtHb induction for 3-substituted-1,2,4-oxadiazoles 144-149.}
\end{align*}
\]

Based on the potency demonstrated by oxadiazoles 144-147 ($R = alkyl$), it was anticipated that oxadiazoles 149 ($R = Bzl$, log $P_{\text{exp.}} = 3.24$) and 148 ($R = Ph$, log $P_{\text{exp.}} = 3.48$), possessing favourable lipophilic properties for in vitro MtHb induction, would exhibit similarly high levels of activity, akin to that observed for aryl aminophenones, amino-2-phenylacetophenone 72 and aminobenzophenone 54 (section 3.2.4.C). Contrary to expectation, a considerable decrease in MtHb induction was observed for oxadiazoles 149 (35.4 ± 1.1%) and 148 (6.9 ± 1.2%), compared to their alkyl counterparts 144-147 (graph 36). Also worth noting was the significantly greater MtHb induction observed for oxadiazole 149 compared to its lipophilically similar oxadiazole 148 (table 29, graph 36), which provided further support to the previously
described hypothesis (section 3.2.3.C) that conformational flexibility may play a role in the putative hydrophobic binding involved in MtHb induction. Not only did oxadiazole 146 (R = nPr) exhibit the greatest MtHb induction within the series (47.9 ± 1.0%), this compound also marginally outperformed the lead alkyl aminophenone, PAVP (45.3 ± 1.8%) (graph 36). With this promising result in hand, further investigation into the strategy of employing bioisosteric alternatives for benzocaine was pursued.

### 4.3.2. 5-Substituted-1,3,4-oxadiazoles 163-169

Although employed traditionally as an amide bioisostere, the 1,3,4-oxadiazole moiety was considered worthy of investigation on the grounds that it may possess favourably comparable global electronics to that of the 1,2,4-oxadiazole. As a result of the exceptional MtHb induction demonstrated by 3-substituted-1,2,4-oxadiazoles 144-149, with guidance from in silico log P predictions, attention turned to the synthesis, physicochemical and in vitro evaluation of the regioisomeric 5-substituted-1,3,4-oxadiazoles 163-169 (figure 44).

![Figure 44: In silico log P predictions for 5-substituted-1,3,4-oxadiazoles 163-169.](image)

**A) Synthesis of 5-substituted-1,3,4-oxadiazoles 163-169**

Synthesis of 5-substituted-1,3,4-oxadiazoles 163-169, through the reaction of 4-nitrobenzoyl chloride (156) with methanol, proceeded via the in situ formation of the corresponding methyl ester 170 (scheme 39). Subsequent addition of hydrazine hydrate under heating afforded common hydrazide intermediate 171 in 55% yield, which in turn was acylated with the corresponding acid chlorides to form N-acylhydrazides 172-178 in variable yield (10-87%) (scheme 39). Subsequent cyclization in the presence of phosphorus oxychloride at elevated
temperature afforded oxadiazoles 179-185 (scheme 39). Ultimately, sodium sulfide reduction, as described previously, afforded target oxadiazoles 163-169 in variable yield (9-98%) (scheme 39).

\[ 
\begin{align*}
\text{Reagents and conditions:} & \text{ (a) (i) MeOH, r.t., 0.5 h (to give 170); (ii) 170, N}_2\text{H}_4\cdot\text{H}_2\text{O, MeOH, reflux, 0.5 h; (b) RCOCl, DMA, 0 °C, 6 h; (c) POCl}_3, 80 °C, 20-48 h; (d) Na}_2\text{S.9H}_2\text{O, H}_2\text{O, 1,4-dioxane, 80 °C, 1 h.} \\
\text{Scheme 39: Synthesis of 5-substituted-1,3,4-oxadiazoles 163-169.}
\end{align*}
\]

**B) Physicochemical evaluation of 5-substituted-1,3,4-oxadiazoles 163-169**

As expected, the lipophilicity of oxadiazoles 163-169 increased in a stepwise trend, largely influenced by the lipophilic nature of the R group at the 5-position. Manipulation at this position provided a series of compounds possessing a broad range of partition coefficients (log \( P_{\text{exp.}} = 1.63 - 2.91 \)) (table 30). Initial lipophilic measurement of oxadiazole 163 (R = Me, log \( P_{\text{exp.}} = 1.63 \)) suggested that this structural sub-class was inherently more polar than its
1,2,4-oxadiazole counterparts, hence the alkyl chain incorporated at the 5-position of the 1,3,4-oxadiazole was extended (167 $R = nBu$, log $P_{\text{exp.}} = 2.66$) to achieve a level of lipophilicity comparable to that of PAVP (log $P_{\text{exp.}} = 2.48$) and the potent 1,2,4-oxadiazole 146 ($R = nPr$, log $P_{\text{exp.}} = 2.61$) (table 30).

Table 30: Electronic and lipophilic parameters of 5-substituted-1,3,4-oxadiazoles 163-169.

<table>
<thead>
<tr>
<th></th>
<th>$\text{NH}_2$ ipso $^{13}$C shift (ppm, relative to PAPP)</th>
<th>log $P_{\text{exp.}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>163 $R = \text{Me}$</td>
<td>-1.45</td>
<td>1.63</td>
</tr>
<tr>
<td>PAPP</td>
<td>0.00</td>
<td>1.65</td>
</tr>
<tr>
<td>164 $R = \text{Et}$</td>
<td>-1.50</td>
<td>1.92</td>
</tr>
<tr>
<td>Benzocaine</td>
<td>-0.30</td>
<td>2.04</td>
</tr>
<tr>
<td>166 $R = i\text{Pr}$</td>
<td>-1.46</td>
<td>2.23</td>
</tr>
<tr>
<td>165 $R = n\text{Pr}$</td>
<td>-1.46</td>
<td>2.27</td>
</tr>
<tr>
<td>PAVP</td>
<td>-0.03</td>
<td>2.48</td>
</tr>
<tr>
<td>146 ($R = n\text{Pr}$, 1,2,4-)</td>
<td>-0.46</td>
<td>2.61</td>
</tr>
<tr>
<td>167 $R = n\text{Bu}$</td>
<td>-1.49</td>
<td>2.66</td>
</tr>
<tr>
<td>169 $R = \text{Bzl}$</td>
<td>-1.28</td>
<td>2.82</td>
</tr>
<tr>
<td>168 $R = \text{Ph}$</td>
<td>-1.37</td>
<td>2.91</td>
</tr>
</tbody>
</table>

Providing further confirmation of the counterintuitive trend observed for 1,2,4-oxadiazoles 149 ($R = \text{Bzl}$) and 148 ($R = \text{Ph}$), 1,3,4-oxadiazole 168 ($R = \text{Bzl}$, log $P_{\text{exp.}} = 2.82$) also exhibited a slightly lower lipophilicity than that of 1,3,4-oxadiazole 168 ($R = \text{Ph}$, log $P_{\text{exp.}} = 2.91$) (table 30), despite the only structural difference being a single methylene unit. 1,3,4-Oxadiazoles 163-169 exhibited a range of ipso $^{13}$C shifts (-1.28 ppm $\rightarrow$ -1.50 ppm), upfield from that of benzocaine (-0.30 ppm), 1,2,4-oxadiazoles 144-149 (-0.39 ppm $\rightarrow$ -0.46 ppm) and PAPP (table
29 and 30). This result suggests that the 1,3,4-oxadiazoles 163-169 were in general less electron-withdrawing than their corresponding 1,2,4-oxadiazole regioisomers 144-149.

C) *In vitro* evaluation of 5-substituted-1,3,4-oxadiazoles 163-169

For comparative purposes, the leading heterocycle, 1,2,4-oxadiazole 146 (R = nPr), was examined concurrently. Oxadiazole 165 (R = nPr, log \( P_{\exp} \) = 2.27, MtHb% = 52.4 ± 0.9%) and oxadiazole 167 (R = nBu, log \( P_{\exp} \) = 2.66, MtHb% = 52.8 ± 0.7%) exhibited the greatest *in vitro* MtHb induction within this series, possibly due to the favourable lipophilic profiles possessed by these compounds (table 30, graph 37). The *in vitro* MtHb induction exhibited by PAVP (61.9 ± 1.9%) and 1,2,4-oxadiazole 146 (R = nPr, 61.0 ± 3.6%), however, was superior (graph 37). One explanation for this observation may be that the weaker electron-withdrawing nature of the 1,3,4-oxadiazole substituent was responsible for a presumed sub-optimal electronic contribution and consequently reduced MtHb induction.

![Graph 37: In vitro MtHb induction for 5-substituted-1,3,4-oxadiazoles 163-169.](image-url)
In a comparison of the lipophilically similar oxadiazole 165 (R = nPr, log $P_{exp} = 2.27$, MtHb = 52.4 ± 0.9%) and oxadiazole 166 (R = iPr, log $P_{exp} = 2.23$, MtHb = 45.6 ± 1.3%), it was revealed that branching of the alkyl chain attached to the oxadiazole ring was not beneficial for in vitro MtHb induction (graph 37). In contrast to the in vitro MtHb induction observed for 1,2,4-oxadiazoles 148 (R = Ph, 6.9 ± 1.2%) and 149 (R = Bzl, 35.4 ± 1.1%), the aryl groups present in 1,3,4-oxadiazoles 168 (R = Ph, 20.8 ± 2.7%) and 169 (R = Bzl, 42.7 ± 3.4%), appeared to be structurally tolerated to a greater extent with regards to MtHb induction within this series (graph 36 and 37). Akin to that observed for 1,2,4-oxadiazoles 148 and 149, 1,3,4-oxadiazole 169 (R = Bzl, log $P_{exp} = 2.82$) exhibited considerably greater MtHb activity than the lipophilically similar 1,3,4-oxadiazole 168 (R = Ph, log $P_{exp} = 2.91$) (table 30, graph 37), adding further support towards the previously proposed requirement (section 3.2.3.C) for conformational flexibility with respect to the putative hydrophobic binding pocket.

At this stage, it was considered pertinent to confirm that the 1,2,4- and 1,3,4-oxadiazole ring structures were indeed resistant to the hydrolytic processes that such compounds were designed to overcome. In order to confirm this, benzocaine, 1,2,4-oxadiazole 146 (R = nPr) and 1,3,4-oxadiazole 165 (R = nPr) were each assayed for in vitro MtHb induction in the presence of a range of BNPP concentrations, the same esterase inhibitor as employed previously during stability studies on benzocaine (section 4.2.C).

As expected, the levels of MtHb induced by benzocaine increased in relation to an increase in the concentration of BNPP (graph 38). This observation was again assumed to be the result of a decrease in the extent of hydrolysis taking place as the concentration of esterase inhibitor increased. In contrast, the levels of MtHb induced by oxadiazoles 146 and 165 remained relatively high across the selected concentrations of BNPP (graph 38), suggesting that the hydrolysis responsible for precluding the in vitro MtHb induction of benzocaine was not a factor in the MtHb induced by oxadiazoles 146 and 165. This result supported the notion that, at least in vitro, the oxadiazole ring acts as a hydrolytically stable replacement for the ester group, while retaining the favourable lipophilic and electronic characteristics required for optimal MtHb induction.
Graph 38: Effect of BNPP (esterase inhibitor) on \textit{in vitro} MtHb induction by benzocaine, 3-propyl-1,2,4-oxadiazole \textbf{146} and 5-propyl-1,3,4-oxadiazole \textbf{165}. 
4.3.3. 5-Alkyl-1,2,4-oxadiazoles 186-189

Again, based on the high MtHb induction exhibited by 3-substituted-1,2,4-oxadiazoles 144-149, attention focused in parallel on a second regioisomeric structural sub-class, namely 5-alkyl-1,2,4-oxadiazoles 186-189. It was anticipated that oxadiazoles 186-189 may not only possess similar electronics to that of the potent 3-substituted-1,2,4-oxadiazoles 144-147, but may also confer a different metabolic profile in vivo. This structural sub-class was considered to add diversity to the emerging library of bioisosteric MtHb inducers, and eliminate further risk in developing a novel in vivo MtHb inducer. With guidance from in silico log P predictions, a series of oxadiazoles 186-189 (R = alkyl) were synthesized for physicochemical and in vitro evaluation (figure 45).204 It is important to note that, at this juncture, the number of compounds investigated within this series was refined. As a result of the inferior in vitro MtHb induction exhibited by 1,2,4- and 1,3,4- oxadiazoles 148 and 168 (R = Ph), and 149 and 169 (R = Bzl), and 146 and 166 (R = iPr), the corresponding 5-substituted-1,2,4-oxadiazoles were not pursued.

![Figure 45: In silico log P predictions for 5-alkyl-1,2,4-oxadiazoles 186-189](image)

A) Synthesis of 5-alkyl-1,2,4-oxadiazoles 186-189

Preparation of 5-alkyl-1,2,4-oxadiazoles 186-189 centred around the synthesis of key benzamidoxime intermediate 190 (scheme 40). Dehydration257 of 4-nitrobenzaldehyde (69) using hydroxylamine hydrochloride in the presence of dimethyl sulfoxide, allowed conversion to 4-nitrobenzonitrile (191) in 94% yield (scheme 40). Further treatment of 191 with hydroxylamine hydrochloride as previously described,254 afforded common benzamidoxime intermediate 190, albeit in low yield (scheme 40). Sufficient material was raised in order to effect a one-pot base-catalyzed acylation and dehydration254 in the presence of the corresponding acid chlorides to afford oxadiazoles 192-195 in moderate yields (21-56%) (scheme 40). Subsequent reduction of 192-195 as previously described255 afforded desired
oxadiazoles 186-189 in a range of yields (42-86%) (scheme 40). Acknowledgements go to Thorsten Beck for the synthesis of oxadiazoles 187 (R = Et), 188 (R = nPr) and 189 (R = nBu).258

Reagents and conditions: (a) NH2OH.HCl, DMSO, r.t. → 100 °C, 3 h; (b) NH2OH.HCl, NaOH, EtOH, H2O, reflux, 36 h; (c) RCOCl, pyridine, reflux, 0.5 h; (c) Na2S.9H2O, H2O, 1,4-dioxane, 80 °C, 1 h.

Scheme 40: Synthesis of 5-alkyl-1,2,4-oxadiazoles 186-189.

B) Physicochemical evaluation of 5-alkyl-1,2,4-oxadiazoles 186-189

The lipophilicity of oxadiazoles 186-189 increased in a stepwise fashion influenced by each extension of the alkyl chain at the 5-position, in a manner similar to that of 3-alkyl-1,2,4-oxadiazoles 144-146, with oxadiazole 188 (R = nPr, log Pexp. = 2.54) possessing a degree of lipophilicity most closely resembling that of PAVP (log Pexp. = 2.48) (table 31). Oxadiazoles 186-189 exhibited a range of ipso 13C shifts (-1.80 ppm → -1.87 ppm), considerably upfield from that of benzocaine (-0.30 ppm) and the regioisomeric 3-substituted-1,2,4-oxadiazoles 144-149 (-0.39 ppm → -0.46 ppm), suggesting that the 5-alkyl-1,2,4-oxadiazole substituent was less electron-withdrawing than that of
3-alkyl-1,2,4-oxadiazoles 144-149 (table 29 and 31). The 5-alkyl-1,2,4-oxadiazole substituent, however, appeared to be only slightly less electron-withdrawing (ipso $^{13}$C shift = -1.80 ppm → -1.87 ppm) than that of regioisomeric 5-alkyl-1,3,4-oxadiazoles 163-169 (-1.28 ppm → -1.50 ppm) (table 30 and 31).

Table 31: Electronic and lipophilic parameters of 5-alkyl-1,2,4-oxadiazoles 186-189.

<table>
<thead>
<tr>
<th>NH$_2$ ipso $^{13}$C shift (ppm, relative to PAPP)</th>
<th>log $P_{exp.}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP</td>
<td>0.00</td>
</tr>
<tr>
<td>186 R = Me</td>
<td>-1.80</td>
</tr>
<tr>
<td>Benzocaine</td>
<td>-0.30</td>
</tr>
<tr>
<td>187 R = Et</td>
<td>-1.88</td>
</tr>
<tr>
<td>PAVP</td>
<td>-0.03</td>
</tr>
<tr>
<td>188 R = nPr</td>
<td>-1.84</td>
</tr>
<tr>
<td>146 (R = nPr, 1,2,4-)</td>
<td>-0.46</td>
</tr>
<tr>
<td>189 R = nBu</td>
<td>1.87</td>
</tr>
</tbody>
</table>

C) **In vitro** evaluation of 5-alkyl-1,2,4-oxadiazoles 186-189

In general, the oxadiazoles 186-189 exhibited a similar trend to that of 3-alkyl-1,2,4-oxadiazoles 144-146, whereby an increase in the lipophilicity correlated to greater observed **in vitro** MtHb activity within this series (186 R = Me, log $P_{exp.}$ = 1.71, MtHb = 47.6 ± 2.7%, 187 R = Et, log $P_{exp.}$ = 2.13, MtHb = 56.2 ± 4.9%, 188 R = nPr, log $P_{exp.}$ = 2.54, MtHb = 65.4 ± 1.7%) (table 31, graph 38). Contrary to the observed trend, 5-butyl-1,2,4-oxadiazole 189 (R = nBu, log $P_{exp.}$ = 3.01, MtHb% = 62.4 ± 1.8%), despite possessing a favourable lipophilic profile for **in vitro** MtHb induction, exhibited a slightly inferior MtHb activity compared to its homologue, oxadiazole 188 (R = nPr) (graph 38).
4. Design, synthesis and bioevaluation of benzocaine-related analogues

4.3. Benzocaine bioisosteres (1)

Graph 38: *In vitro* MtHb induction for 5-alkyl-1,2,4-oxadiazoles 186-189.

The leading heterocycle, 3-propyl-1,2,4-oxadiazole 146, was once again examined concurrently for comparative purposes. Ultimately, the *in vitro* MtHb induction exhibited by oxadiazoles 186-189 was marginally inferior to both that of the lead oxadiazole 146 (68.1 ± 3.4%) and PAVP (73.2 ± 1.6%) (graph 38). Akin to 5-substituted-1,3,4-oxadiazoles 163-169, the lower levels of *in vitro* MtHb induction displayed by oxadiazoles 186-189 is most likely attributed to the sub-optimal electronic contribution of the 5-alkyl-1,2,4-oxadiazole substituent. Nevertheless, this series of compounds provided another potent structural sub-class to complement the growing catalogue of benzocaine bioisosteres available for selection for *in vivo* evaluation.
4. Design, synthesis and bioevaluation of benzocaine-related analogues

4.3. Benzocaine bioisosteres (1)

4.3.4. 5-Alkyl-1,3,4-thiadiazoles 196 and 197, and 3-ethyl-1,2,4-thiadiazole 198

In a further elaboration of 5-substituted-1,3,4-oxadiazoles 163-169 and 3-substituted-1,2,4-oxadiazoles 144-149, attention turned to potential modifications of the oxadiazole moiety. In structure-activity relationship studies exploring the replacement of the oxygen atom in the oxadiazole ring with sulfur, it has been demonstrated that an increase in potency can be achieved in the corresponding thiadiazole. The near isoelectronic replacement of the oxygen atom with sulfur in our oxadiazole ring system was postulated to allow a subtle alteration of the global electronic contribution towards the aryl amine ring, while in parallel exploring the tolerance of such heterocyclic modifications upon MtHb induction. In addition, the sulfur-oxygen exchange was considered to be of interest with respect to further complementing the emerging suite of benzocaine bioisosteres, acting to facilitate the reduction of risk in the development and identification of novel in vivo MtHb inducers. With guidance from experimental physicochemical and in vitro MtHb induction data obtained from previous oxadiazoles 165, 167 and 145, attention now focused on the preparation and evaluation of 5-alkyl-1,3,4-thiadiazoles 196 and 197, and 3-ethyl-1,2,4-thiadiazole 198, with each analogue incorporating an alkyl chain substituent chosen to mirror the lipophilic profile of PAVP (figure 46).

Figure 46: 5-Propyl-1,3,4-oxadiazole 165, 5-butyl-1,3,4-oxadiazole 167 and 3-ethyl-1,2,4-oxadiazole 145, and their corresponding thio homologues; 5-alkyl-1,3,4-thiadiazoles 196 and 197, and 3-ethyl-1,2,4-thiadiazole 198.
A) Synthesis of 5-alkyl-1,3,4-thiadiazoles 196 and 197

5-Alkyl-1,3,4-thiadiazoles 196 and 197 were conveniently accessed in two steps. Previously synthesized\textsuperscript{256} N-acylhydrazides 174 and 176 underwent a one-pot thiolation and condensation\textsuperscript{261} using Lawesson’s reagent (LR) at elevated temperature to afford 1,3,4-thiadiazoles 199 (R = nPr) and 200 (R = nBu), in 56% and 70% yields, respectively (scheme 41). Subsequent reduction as previously described\textsuperscript{255} afforded target 1,3,4-thiadiazoles 196 (R = nPr) and 197 (R = nBu) in 46% and 56% yields, respectively (scheme 41).

![](image)

Reagents and conditions: (a) LR, PhMe, reflux, 5 h; (b) Na₂S.9H₂O, H₂O, 1,4-dioxane, 80 °C, 1 h.

Scheme 41: Synthesis of 5-alkyl-1,3,4-thiadiazoles 196 and 197.

B) Synthesis of 3-ethyl-1,2,4-thiadiazole 198

Synthesis of 1,2,4-thiadiazole 198 (R = Et) employed a convergent approach, with the key steps consisting of the union of dimethylacetal 201 and thiobenzamide 202, with concomitant cyclization of carbothioamide 203 to form 1,2,4-thiadiazole 204 (R = Et) (scheme 43).

Synthesis of dimethylacetal 201 began with the acylation\textsuperscript{262} of dimethylamine hydrochloride (205), using propionic acid in the presence of triethylamine, to afford N,N-dimethylpropanamide (206), which in turn was treated with dimethyl sulfate\textsuperscript{263} under heating to form dimethylpropanamide dimethyl sulfate complex (207) (scheme 42). Subsequent treatment\textsuperscript{263} of 207 with freshly prepared sodium methoxide allowed access to target dimethylacetal 201 in 10% yield over three steps (scheme 42).
4. Design, synthesis and bioevaluation of benzocaine-related analogues

4.3. Benzocaine bioisosteres (1)

**Reagents and conditions:** (a) CH$_3$CH$_2$COOH, NEt$_3$, C$_6$H$_6$, r.t., 10 h; (b) Me$_2$SO$_4$, 80 °C, 5 h; (c) i) Na, MeOH, r.t., 0.5 h; ii) then 207, NaOMe, MeOH, r.t., 20 h.

**Scheme 42:** Synthesis of dimethylacetal 201.

4-Nitrobenzoyl chloride (156) was treated with aqueous ammonia to afford 4-nitrobenzamide (208) in near quantitative yield.$^{264}$ Thiobenzamide 202 was accessed in 58% yield via thiolation$^{265}$ of 4-nitrobenzamide (208), using Lawesson’s reagent at elevated temperature (scheme 43). Union$^{266}$ of thiobenzamide 202 with dimethyl acetal 201 proceeded smoothly to afford carbothioamide 203 in 92% yield (scheme 43).

**Reagents and conditions:** (a) aq. NH$_3$, r.t., 0.5 h; (b) LR, THF, reflux, 5 h; (c) 201, r.t., 1 h; (d) HSA, pyridine, EtOH, MeOH, r.t., 1 h; (e) Na$_2$S,9H$_2$O, H$_2$O, 1,4-dioxane, 80 °C, 1 h.

**Scheme 43:** Synthesis of 3-ethyl-1,2,4-thiadiazole 198.
Subsequent cyclization of 203 in the presence of aminating agent hydroxylamine-O-sulfonic acid (HSA) afforded 1,2,4-thiadiazole 204 (R = Et) in 70% yield (scheme 43). Ultimately, reduction as previously described furnished the desired 1,2,4-thiadazole 198 (R = Et) in 69% yield (scheme 43).

C) Physicochemical evaluation of 5-alkyl-1,3,4-thiadiazoles 196 and 197

A comparison of 1,3,4-thiadiazoles 196 (R = nPr, log \( P_{\text{exp.}} \) = 2.22) and 197 (R = nBu, log \( P_{\text{exp.}} \) = 2.61) to their corresponding oxadiazoles 165 (R = nPr, log \( P_{\text{exp.}} \) = 2.53) and 167 (R = nBu, log \( P_{\text{exp.}} \) = 2.92) demonstrated that the oxygen-sulfur exchange led to a subtle increase in the lipophilicity of the overall molecule (table 32).

Table 32: Electronic and lipophilic parameters of 5-alkyl-1,3,4-thiadiazoles 196 and 197.

<table>
<thead>
<tr>
<th></th>
<th>NH(_2) ipso (^{13})C shift (ppm, relative to PAPP)</th>
<th>log ( P_{\text{exp.}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP</td>
<td>0.00</td>
<td>1.65</td>
</tr>
<tr>
<td>Benzocaine</td>
<td>-0.30</td>
<td>2.04</td>
</tr>
<tr>
<td>165 R = nPr X = O</td>
<td>-1.46</td>
<td>2.22</td>
</tr>
<tr>
<td>PAVP</td>
<td>-0.03</td>
<td>2.48</td>
</tr>
<tr>
<td>196 R = nPr X = S</td>
<td>-1.89</td>
<td>2.53</td>
</tr>
<tr>
<td>167 R = nBu X = O</td>
<td>-1.49</td>
<td>2.61</td>
</tr>
<tr>
<td>146 (R = nPr, 1,2,4-)</td>
<td>-0.46</td>
<td>2.61</td>
</tr>
<tr>
<td>197 R = nBu X = S</td>
<td>-1.98</td>
<td>2.92</td>
</tr>
</tbody>
</table>

Comparison of the ipso \(^{13}\)C shifts of 1,3,4-thiadiazoles 196 (R = nPr, = -1.89 ppm) and 197 (R = nBu = -1.98 ppm) to their corresponding 1,3,4-oxadiazoles 165 (R = nPr, = -1.89 ppm) and 167...
(R = nBu, = -1.98 ppm), suggested that the oxygen-sulfur exchange resulted in a subtle decrease in the electron-withdrawing nature of the heterocyclic substituent (table 32).

**D) Physicochemical evaluation of 3-ethyl-1,2,4-thiadiazole 198**

The leading heterocycle, 3-propyl-1,2,4-oxadiazole 146, was included for comparative purposes. In a similar trend to 1,3,4-thiadiazoles 196 (R = nPr) and 197 (R = nBu), the oxygen-sulfur exchange within this series led to 1,2,4-thiadiazole 198 (R = Et, log $P_{exp.} = 2.39$) exhibiting a slightly elevated lipophilic profile compared to that of the corresponding 1,2,4-oxadiazole 145 (R = Et, log $P_{exp.} = 2.22$) (table 33).

**Table 33: Electronic and lipophilic parameters of 3-ethyl-1,2,4-thiadiazole 198.**

<table>
<thead>
<tr>
<th></th>
<th>NH$_2$ ipso $^{13}$C shift (ppm, relative to PAPP)</th>
<th>log $P_{exp.}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP</td>
<td>0.00</td>
<td>1.65</td>
</tr>
<tr>
<td>Benzocaine</td>
<td>-0.30</td>
<td>2.04</td>
</tr>
<tr>
<td>145 R = Et X = O</td>
<td>-0.39</td>
<td>2.22</td>
</tr>
<tr>
<td>198 R = Et X = S</td>
<td>-1.11</td>
<td>2.39</td>
</tr>
<tr>
<td>PAVP</td>
<td>-0.03</td>
<td>2.48</td>
</tr>
<tr>
<td>146 R = nPr X = O</td>
<td>-0.46</td>
<td>2.61</td>
</tr>
</tbody>
</table>

Likewise, the ipso $^{13}$C shift of 1,2,4-thiadiazole 198 (R = Et, -1.11 ppm) was found to be upfield from that of corresponding 1,2,4-oxadiazole 145 (R = Et, -0.39 ppm), adding further support towards the notion that the oxygen-sulfur exchange led to a subtle decrease in the electron-withdrawing nature of the heterocyclic ring (table 33).
E) In vitro evaluation of 5-alkyl-1,3,4-thiadiazoles 196 and 197

Based on the observation that the replacement of the oxygen atom of the oxadiazole ring with sulfur appeared to effect only a subtle change on the physicochemical properties (table 32), the in vitro MtHb induction of 1,3,4-thiadiazoles 196 (R = nPr) and 197 (R = nBu), and 1,2,4-thiadiazole 198 (R = Et) were examined concurrently against their most structurally related oxadiazole counterparts (in terms of alkyl chain length, and their closest oxadiazole analogues with respect to lipophilic profiles). The leading heterocycle, 3-propyl-1,2,4-oxadiazole 146, was also examined for comparative purposes.

Graph 39: In vitro MtHb induction vs. log $P_{\text{exp}}$ of 5-alkyl-1,3,4-thiadiazoles 196 and 197.

Graph 40: In vitro MtHb induction vs. ipso $^{13}$C shift (ppm, relative to PAPP) of 5-alkyl-1,3,4-thiadiazoles 196 and 197.

The in vitro MtHb activity of 1,3,4-thiadiazole 196 (R = nPr, 32.7 ± 1.2%) compared to its alkyl chain length equivalent, 1,3,4-oxadiazole 165 (R = nPr, 45.1 ± 4.2%), and the lipophilically equivalent 1,3,4-oxadiazole 167 (R = nBu, 50.5 ± 1.4%), suggested that the oxygen-sulfur
exchange within the heterocyclic ring was not beneficial for enhanced MtHb induction within this series (graph 39 and 40). While the observed decrease in *in vitro* MtHb activity for 1,3,4-thiadiazoles 196 (R = nPr) and 197 (R = nBu) may be assumed to be a consequence of the slightly reduced electron-withdrawing capability of the 1,3,4-thiadiazole moiety (table 32, graph 40), another explanation may be a slight intolerance of the considerably larger sulfur atom within the heterocyclic system. While 1,3,4-thiadiazole 197 (R = nBu, log $P_{exp.} = 2.92$, MtHb% = 49.2 ± 1.1%) exhibited relatively high *in vitro* MtHb activity, this was assumed to be primarily a consequence of its favourable lipophilic profile for *in vitro* MtHb induction (table 32, graph 39).

**F) In vitro evaluation of 3-ethyl-1,2,4-thiadiazole 198**

Comparison of the *in vitro* MtHb activity of 1,2,4-thiadiazole 198 (R = Et, 52.3 ± 1.3%) to its alkyl chain length equivalent, 1,2,4-oxadiazole 145 (R = Et, 55.7 ± 4.8%), suggested a similar trend to that of 1,3,4-thiadiazoles 196 (R = nPr) and 197 (R = nBu), whereby the oxygen-sulfur exchange appeared to be slightly detrimental for *in vitro* MtHb induction for this structural sub-class (graph 41 and 42). A similar explanation as described for the 1,3,4-thiadiazoles is offered for the lower levels *in vitro* MtHb induction observed for 1,2,4-thiadiazole 198 (R = Et) compared to its 1,2,4-oxadiazole counterparts 145 (R = Et) and 146 (R = nPr) (section 4.3.4.E).
Despite the slight loss in *in vitro* MtHb activity observed for the thio derivatives of oxadiazoles 165, 167 and 145, 1,3,4-thiadiazoles 196 (R = nPr) and 197 (R = nBu), and 1,2,4-thiadiazole 198 (R = Et) were considered valuable additions to the library of benzocaine bioisosteres, due to their potential to exhibit an improved pharmacokinetic profile with regards to *in vivo* MtHb toxicity.
4.3.5. Unsuccessful attempts to effect derivatization of 1,3,4-oxadiazoles and 1,2,4-oxadiazoles

At this stage, 3-substituted-1,2,4-oxadiazoles 144-149, 5-substituted-1,3,4-oxadiazoles 163-169 and 5-alkyl-1,2,4-oxadiazoles 186-189 had, to varying degrees, demonstrated some promising in vitro MtHb activity. Combining the knowledge gained from these results with the understanding that electronics play a significant role in MtHb induction, it was postulated that subtle alterations to the electronic contribution provided by the oxadiazole moiety could result in improved MtHb induction. This approach has been met with some success in other biological studies employing 1,2,4-oxadiazole and 1,3,4-oxadiazole systems.\(^{253,267,268}\) In light of this literature precedence, further derivatization within these structural sub-classes was explored, namely through attempts to install electronic-withdrawing or electron-donating groups at the 2-position of 1,3,4-oxadiazoles, and at the 3- or 5-position of 1,2,4-oxadiazoles (figure 47).

![Figure 47](image)

**Figure 47**: Various proposed derivatizations of 3-substituted-1,2,4-oxadiazoles 209 and 210 and 5-substituted-1,3,4-oxadiazoles 211, 212 and 213, 5-substituted-1,2,4-oxadiazole 214.

A) 2-Trifluoromethyl-1,3,4-oxadiazole 213 and 3- or 5-trifluoromethyl-1,2,4-oxadiazoles 209 and 214

Employing the acylation procedure as previously described\(^ {256}\) for the synthesis of 1,3,4-oxadiazoles 163-169, conversion of hydrazide 171 to N-trifluoroacetyl hydrazide 215 proceeded smoothly in 78% yield (scheme 44). Subsequent phosphorus oxychloride induced cyclization\(^ {256}\) converted 215 to 1,3,4-oxadiazole 216 (R = CF\(_3\)) in 66% yield (scheme 44). On
this occasion, however, sodium sulfide reduction of 216 as previously described\textsuperscript{255} gave rise to a complex mixture of ring-opened products, with no trace of desired 1,3,4-oxadiazole 213 (R = CF\textsubscript{3}) (scheme 44). Likewise, palladium-catalyzed hydrogenation\textsuperscript{269} failed to afford the desired 1,3,4-oxadiazole 213 (R = CF\textsubscript{3}) (scheme 44).

\[ \text{Reagents and conditions: (a) (CF}_3\text{CO})_2\text{O, DMA, 0 °C, 6 h; (b) POCl}_3, 80 °C, 20 h; (c) Na}_2\text{S.9H}_2\text{O, H}_2\text{O, 1,4-dioxane, 80 °C, 1 h; (d) H}_2 (1 \text{ atm.}), 10 \text{ mol\% Pd/C, EtOAc, r.t., 18 h.} \]

\textbf{Scheme 44: Attempted synthesis of 2-trifluoromethyl-1,3,4-oxadiazole 213.}

Upon closer investigation into preparation of 3-trifluoromethyl-1,2,4-oxadiazole 209, it was considered impractical to employ the one-pot base-catalyzed acylation and dehydration procedure previously described for the preparation of 1,2,4-oxadiazoles\textsuperscript{144-149,254} as this method would require the use of the relatively expensive\textsuperscript{270} trifluoromethylacetonitrile (217).

Instead, attention turned to accessing 3-trifluoromethyl-1,2,4-oxadiazole 209 through an irreversible base-catalyzed ring-degenerate rearrangement of 5-trifluoromethyl-1,2,4-oxadiazole 218 to 3-trifluoromethyl-1,2,4-oxadiazole 219, using hydroxylamine hydrochloride in the presence of potassium tert-butoxide (scheme 45)\textsuperscript{271} In preparation for this key step, 4-nitrobenzamidoxime (190) was successfully cyclized\textsuperscript{254} in the presence of trifluoroacetic anhydride at elevated temperature to afford 5-trifluoromethyl-1,2,4-oxadiazole 218 in 90% yield (scheme 46).
Mindful of previous difficulties in accessing 1,3,4-oxadiazole 213 (R = CF$_3$), attention focused on the reduction$^{255}$ of 5-trifluoromethyl-1,2,4-oxadiazole 218 prior to addressing the base-catalyzed ring-degenerate rearrangement step. Disappointingly, reduction conditions as previously described$^{255}$ were again unsuccessful in affording desired 3-trifluoromethyl-1,2,4-oxadiazole 214, with the products not being isolated but again appearing to be the result of 1,2,4-oxadiazole ring opening (scheme 46). This observation was in agreement with kinetic and stability studies of Yu et al.$^{272}$ who reported that the high electronegativity of the trifluoromethyl group increases the lability of the 1,2,4-oxadiazole ring. As a result, base-catalyzed ring-degenerate rearrangement of 218 was not pursued, under the assumption that the subsequent reduction of 3-trifluoro-1,2,4-oxadiazole 219 would in all likelihood not be fruitful (scheme 46).
4. Design, synthesis and bioevaluation of benzocaine-related analogues

4.3. Benzocaine bioisosteres (1)

Reagents and conditions: (a) (CF₃CO)₂O, PhMe, reflux, 0.5 h; (b) Na₂S·9H₂O, H₂O, 1,4-dioxane, 80 °C, 1 h.

Scheme 46: Attempted syntheses of 3- and 5-trifluoromethyl 1,2,4-oxadiazoles 209 and 214, respectively.

Exploration into alternative procedures for the reduction of the nitro group in the presence of the trifluoromethyl substituent (the use of reducing agents such as zinc,²⁷³ iron,²⁷⁴ hydrazine hydrate,²⁷⁵ tin(II) chloride²⁷⁶) were not pursued due to reasons discussed in sections 4.4-4.6.

B) 2-Methoxy-1,3,4-oxadiazole 212

With regards to the proposed introduction of an alkoxy substituent into the oxadiazole ring, although marginally less potent in vitro MtHb inducers, the 1,3,4-oxadiazole structural sub-class was preferred over the corresponding 1,2,4-oxadiazoles on synthetic grounds. Initial efforts towards 1,3,4-oxadiazole 212 (R = OMe) followed a similar route to that established for 1,3,4-oxadiazoles 163-169.²⁵⁶ Thus, beginning with the acylation of hydrazide 171 using methyl chloroformate, N-carboxyhydrazide 223 was successfully prepared in 79% yield (scheme 47). Subsequent phosphorus oxychloride induced cyclization²⁵⁶ of 223, however, proved problematic, with only starting material observed after 24 h, and significant degradation
resulting from use of extended reaction times at elevated temperature (scheme 47). Rigo et al.\cite{277} demonstrated that a mixture of phosphorus pentoxide (\(\text{P}_2\text{O}_5\)) and methanesulfonic acid (MSA) (1:10, w/w) exhibited superior results over conventional dehydration agents such as polyphosphoric acid and phosphorus oxychloride, in effecting the cyclization of \(\text{N}\)-acylhydrazides to 1,3,4-oxadiazoles. Disappointingly, application of Rigo’s conditions\cite{277} to \(\text{N}\)-carboxylhydrazide 223 failed to afford the desired 1,3,4-oxadiazole 224 (\(\text{R} = \text{OMe}\)), with only decomposition of the starting material being observed (scheme 47).

Reagents and conditions: (a) \text{MeOCOCl}, \text{DMA}, 0 °C, 6 h; (b) \text{POCl}_3, reflux, 24-72 h; (c) \text{P}_2\text{O}_5/\text{MSA}, 75 °C, 4 h.

Scheme 47: Attempted synthesis of 2-methoxy-1,3,4-oxadiazole 212 (1).

Faced with a lack of success in accessing 1,3,4-oxadiazole 212 (\(\text{R} = \text{OMe}\)) via the dehydration route,\cite{256} attention next turned to a strategy involving an oxidative cyclization\cite{278} as the key step in forming the 1,3,4-oxadiazole ring. Firstly, 4-nitrobenzaldehyde (69) was reacted with methyl carbazate in the presence of acetic acid\cite{279} to give \(\text{N}\)-carboxyhydrazone 225 in 11% yield, which in turn was subjected to an oxidative cyclization\cite{278} in the presence of the hypervalent iodine oxidant bis(trifluoroacetoxyl)iodobenzene (BTI) (scheme 48). Interestingly, this reaction formed predominantly \(\text{N}\)-carboxyhydrazide 223 and none of the desired 1,3,4-oxadiazole 224 (\(\text{R} = \text{OMe}\)) (scheme 48). An alternative oxidant, lead(IV) acetate, was also employed in a further
attempt to promote oxidative cyclization of 225, however this procedure also failed to access the desired 1,3,4-oxadiazole 224 (R = OMe) (scheme 48).

Reagents and conditions: (a) H$_3$COCONHNH$_2$, AcOH, MeOH, r.t., 0.5 h; (b) BTI, CHCl$_3$, r.t., 0.5 h; (c) Pb(OAc)$_4$, C$_6$H$_6$, reflux, 0.25 h.

Scheme 48: Attempted synthesis of 2-methoxy-1,3,4-oxadiazole 212 (2).

C) 2-Bromo-1,3,4-oxadiazole 211 and 3-bromo-1,2,4-oxadiazole 210

In parallel to efforts towards the synthesis of 1,3,4-oxadiazole 212 (R = OMe), attention also focused on the preparation of 2-halo-1,3,4-oxadiazoles (scheme 51). Zarudnitskii et al. demonstrated that various 2-substituted-1,3,4-oxadiazoles could be accessed from 2-trimethylsilyl-1,3,4-oxadiazole 226 (scheme 49). In light of this, attention next focused on the preparation of the common 2-trimethylsilyl-1,3,4-oxadiazole intermediate 227 (scheme 51).
Scheme 49: 2-Substituted-1,3,4-oxadiazoles potentially available from 2-trimethylsilyl-1,3,4-oxadiazole 226.281

Efforts towards the preparation of key intermediate 227 started with the cyclization of hydrazide 171 to form 5H-1,3,4-oxadiazole 228 (scheme 50). Employment of the acylation procedure as previously described256 using ethyl orthoformate as a one carbon acid chloride equivalent failed to afford the desired 5H-1,3,4-oxadiazole 228 (scheme 50). Upon consultation of the literature,282 it became apparent that 1,3,4-oxadiazole ring formation employing orthoesters may require the presence of an acid catalyst. Gratifyingly, silica sulfonic acid282 (SiO2-OSO3H) acted to catalyze 1,3,4-oxadiazole ring formation of hydrazide 171 with triethyl orthoformate, affording oxadiazole 228 in 11% yield (scheme 50). Moreover, silica sulfonic acid was conveniently prepared from readily available starting materials silica and chlorosulfonic acid (scheme 50).283 With oxadiazole 228 now in hand, silylation284 using bromotrimethylsilane in the presence of triethylamine was employed to access common intermediate 227 (scheme 50). Unfortunately, this reaction led only to the recovery of starting materials.
Reagents and conditions: (a) CH(OEt)_3, DMA, 0 °C, 6 h; (b) i) SiO_2-OH, ClSO_3H, r.t., 0.5 h (to give SiO_2-OSO_3H) ii) then 171, SiO_2-OSO_3H, CH(OEt)_3, MeOH, r.t., 0.25 h; (c) Me_3SiBr, NEt_3, pyridine/PhMe, r.t., 24 h; (d) i) ZnCl_2, (tmp)MgCl·LiCl, THF, r.t., 15 h (to give (tmp)_2Zn·2MgCl_2·2LiCl (0.5 M in THF)) 229; (ii) then 228, (tmp)_2Zn·2MgCl_2·2LiCl (0.5 M in THF) 229, r.t., 0.3 h; (iii) I_2, THF, 0 °C → r.t., 0.5 h.

Scheme 50: Attempted synthesis of 2-bromo-1,3,4-oxadiazole 211.

In order to circumnavigate this problem, an alternative strategy employing an ortho-metalation reaction was explored. Wunderlich et al.^{285} showed that the complex base (2,2,6,6-tetramethylpiperidide)zinc-magnesium chloride–lithium chloride ((tmp)_2Zn·2MgCl_2·2LiCl) 229 exhibits a high reactivity to effect zinication of sensitive heterocycles such as 2-phenyl-1,3,4-oxadiazole (230) (scheme 51). *Ortho*-metalated species 231 can then be quenched with an electrophile such as iodine to afford 2-ido-1,3,4-oxadiazole 232 (scheme 51).
4. Design, synthesis and bioevaluation of benzocaine-related analogues  4.3. Benzocaine bioisosteres (1)

Reagents and conditions: (a) ZnCl₂, (tmp)MgCl₂·LiCl, THF, r.t., 15 h; (b) (tmp)₂Zn·2MgCl₂·2LiCl (0.5 M in THF) (229), r.t., 0.3 h; (c) I₂, THF, 0 °C → r.t., 0.5 h.

Scheme 51: Alternative ortho-metalation strategy towards the synthesis of 2-halo-1,3,4-oxadiazoles.

It was envisaged that this procedure could be extended to the preparation of 1,3,4-oxadiazole 211 (R = Br), by ortho-metalation of 5H-1,3,4-oxadiazole 228, followed by quenching with bromine as the electrophile (scheme 50).

Complex zinc base (tmp)₂Zn·2MgCl₂·2LiCl 229 was prepared as described from zinc(II) chloride and titrated prior to use, with a concentration of 0.5 M in THF obtained. 5H-1,3,4-Oxadiazole 228 was then subjected to zincation conditions, followed by the addition of bromine to quench the ortho-metalated species in an effort to access 1,3,4-oxadiazole 234 (R = Br) (scheme 50). Unfortunately, the reaction mostly returned unreacted starting material 228, suggesting that the initial ortho-metalation step was unsuccessful.

In parallel to the work focusing on 1,3,4-oxadiazole 211 (R = Br), synthetic studies towards 3-halo-1,2,4-oxadiazoles were also explored (scheme 52). The synthetic strategy centred on a key 1,3-dipolar cycloaddition between dibromonitrile oxide (235) (formed in situ upon the
addition of base to dibromoformaldoxime (236) and 4-nitrobenzonitrile (191) to form 1,2,4-oxadiazole 237 (R = Br) (scheme 52).

Condensation\textsuperscript{287} of glyoxylic acid hydrate (238) using hydroxylamine hydrochloride allowed access to oximinoacetic acid (239), which in turn was subsequently brominated\textsuperscript{288} to afford dibromoformaldoxime intermediate (236) in 22% yield over two steps (scheme 52). With key precursor 236 now in hand, 1,3-dipolar cycloaddition\textsuperscript{286} with 4-nitrobenzonitrile (191) was attempted in the presence of sodium hydrogen carbonate at elevated temperature, in an effort to access 1,2,4-oxadiazole 237 (R = Br) (scheme 52). Unfortunately, however, only unreacted starting materials could be recovered.

\textit{Reagents and conditions:} (a) NH\textsubscript{2}OH.HCl, H\textsubscript{2}O, r.t., 18 h; (b) Br\textsubscript{2}, H\textsubscript{2}O, r.t., 1.25 h; (c) 4-Nitrobenzonitrile (191), NaHCO\textsubscript{3}, PhMe, 90 °C, 3 h.

\textbf{Scheme 52: Attempted synthesis of 3-bromo-1,2,4-oxadiazole 210.}

In light of \textit{in vivo} studies conducted in parallel to the synthetic programme, efforts towards the synthesis of derivatized oxadiazoles 209-214 were discontinued for reasons discussed in detail in section 4.4.-4.6.
4. Design, synthesis and bioevaluation of benzocaine-related analogues  

4.3. Benzocaine bioisosteres (1)

4.3.6. 4-Ethyl-1,2,5-oxadiazole 240 and 3-ethoxy-1,2,5-oxadiazole 241

In studies investigating hydrolytically stable bioisosteric replacements for the ester functionality, the 1,2,5-oxadiazole moiety has been successfully employed as a regioisomeric alternative to the 1,2,4-oxadiazoles. As a result of the MtHb potency exhibited by various 1,2,4- and 1,3,4-oxadiazoles, with some consideration towards in silico log P predictions, attention next focused on the synthesis, physicochemical and in vitro evaluation of 4-ethyl-1,2,5-oxadiazole 240, with the objective of further exploring oxadiazole regioisomers as potent MtHb inducers (figure 48).

With the knowledge that electronics play a significant role in MtHb induction, it was postulated that subtle alterations to the electronic contribution of 1,2,5-oxadiazole substituent could result in enhanced MtHb induction. In an attempt to probe the global electronic contribution of the 1,2,5-oxadiazole moiety, synthesis, physicochemical and in vitro evaluation of 3-ethoxy-1,2,5-oxadiazole 241, possessing an inductively electron-withdrawing group at the 3-position of the 1,2,5-oxadiazole ring, was also conducted (figure 48). It was envisaged that 1,2,5-oxadiazoles 240 (R = Et) and 241 (R = OEt) could potentially confer a different metabolic profile in vivo, adding further diversity to the established library of benzocaine bioisosteres.

![Figure 48: In silico log P predictions for 4-ethyl-1,2,5-oxadiazole 240 and 4-ethoxy-1,2,5-oxadiazole 241.](image)

**Figure 48:** In silico log P predictions for 4-ethyl-1,2,5-oxadiazole 240 and 4-ethoxy-1,2,5-oxadiazole 241. 204

A) Synthesis of 4-ethyl-1,2,5-oxadiazole 240

Initial efforts towards the synthesis of oxadiazole 240 (R = Et) focused on accessing key 1,2-diketone intermediate 242 (scheme 53), which would undergo condensation to form glyoxime 243, that would then undergo cyclization to form oxadiazole 244 (R = Et).
Subsequent reduction\textsuperscript{255} of 244 would then allow access to the desired oxadiazole 240 (R = Et) (scheme 53).

\[
\begin{align*}
\text{Reagents and conditions:} & \quad (a) (\text{CH}_3\text{CH}_2\text{CO})_2\text{O}, \text{CoCl}_2, \text{MeCN}, \text{r.t.}, 24 \text{ h}; (b) \text{Br}_2, \text{CH}_3\text{Cl}_2, \text{H}_2\text{O}, \text{r.t.}, 1.5 \text{ h}; \quad (c) \text{NH}_4\text{NO}_3, \text{Cu(OAc)}_2, \text{AcOH}, \text{H}_2\text{O}, \text{reflux}, 18 \text{ h}; (d) \text{CuSO}_4\cdot5\text{H}_2\text{O}, \text{pyridine}, \text{H}_2\text{O}, \text{reflux} 18 \text{ h}; (e) i) \text{CH}_3\text{CH}_2\text{CH}_2\text{PPh}_3^-, n\text{BuLi}, \text{THF}, -20 \text{ °C}, 0.75 \text{ h}; (ii) then 69, \text{THF}, -20 \text{ °C} \rightarrow \text{r.t.}, 2 \text{ h}; (f) \text{IBX}, \text{NBS}, \text{DMSO}, 65 \text{ °C}, 18 \text{ h}; \\
\text{Scheme 53: Attempted synthesis of 4-ethyl-1,2,5-oxadiazole 240.}
\end{align*}
\]

Ahmad \textit{et al.}\textsuperscript{290} reported that asymmetric 1,2-diketones could conveniently be accessed through a cobalt(II) chloride-catalyzed coupling of acetic anhydride with various aldehydes. It was anticipated that this procedure could be extended to the present system for the union of propionic anhydride with 4-nitrobenzaldehyde (69), to allow access to the desired 1,2-diketone 242. Disappointingly, however, in our hands, the only product isolated from the reaction was the by-product $\alpha$-hydroxyketone 245 (scheme 53). Consequently, a number of methods to promote the oxidation of the $\alpha$-hydroxy group of 245 to form target 1,2-diketone 242 were
explored (scheme 53). Oxidation using bromine,\textsuperscript{291} catalytic oxidation by cupric salts,\textsuperscript{292} and the use of copper(II) sulfate as a modified Fehling’s solution\textsuperscript{293} all failed to facilitate conversion of \( \alpha \)-hydroxyketone 245 to the desired 1,2-diketone 242, in all cases only cleavage of 245 to 4-nitrobenzaldehyde resulted (69) (scheme 53).

In an effort to access 1,2-diketone 242 via a different route, 4-nitrobenzaldehyde (69) was successfully treated\textsuperscript{294} with the Wittig salt propyltriphenylphosphonium iodide to form olefin 246 in 54% yield, as a mixture of \( E \) and \( Z \) isomers (scheme 53). Moorthy \textit{et al.}\textsuperscript{295} demonstrated that \textit{para}-nitro substituted olefins subjected to 2-iodoxybenzoic acid (IBX) in the presence of \( N \)-bromosuccinimide (NBS), form \( \alpha \)-bromoketones which concomitantly undergo further oxidation to form 1,2-diketones. It was postulated that this approach could be extended to our system, however, treatment of olefin 246 with IBX and NBS afforded a mixture of the two \( \alpha \)-bromoketones, and despite using an extended period of heating overnight, further conversion to 1,2-diketone 242 could not be achieved (scheme 53).

Due to the lack of success towards the synthesis of oxadiazole 240 (\( R = \text{Et} \)) via 1,2-diketone intermediate 242, attention now turned to an alternative approach, again proceeding through olefin 246 to form \( N \)-oxide-oxadiazole 247 (\( R = \text{Et} \)) (scheme 54). Despite modifications to the number of equivalents, temperature, solvent and rate of addition of sodium nitrite to olefin 246, the reaction in all cases yielded a complex mixture of pseudonitrosites 248 and 249, and their respective \( \alpha \)-nitro-oxime tautomers 250 and 251, themselves intermediates towards \( N \)-oxide-oxadiazole 247 (\( R = \text{Et} \)) (scheme 54). These intermediates were deemed inseparable by chromatography.
4. Design, synthesis and bioevaluation of benzocaine-related analogues  
4.3. Benzocaine bioisosteres (1)

**Scheme 54:** Synthesis of 4-ethyl-1,2,5-oxadiazole 240.

Gratifyingly, it was found that this complex mixture of intermediates 248-251 could be directly converted into N-oxide-oxadiazole 247 (R = Et)\(^\text{296}\) in 45% yield over two steps, using chlorosulfonic acid in dimethylformamide (scheme 54). Ultimately, the N-oxide and the nitro moieties of 247 were reduced concomitantly in the presence of zinc and an excess of ammonium formate,\(^\text{297}\) furnishing the desired oxadiazole 240 (R = Et) in a single step in 16% yield (scheme 54).

**B) Synthesis of 3-ethoxy-1,2,5-oxadiazole 241**

Synthesis of oxadiazole 241 (R = OEt) proceeded via hydroximoyl cyanide 126, which was prepared in three steps as described previously,\(^\text{224}\) in section 4.3.1.A. Treatment\(^\text{260}\) of 126 with hydroxylamine hydrochloride in the presence of sodium acetate afforded \(\alpha\)-oximidoacetoxime
252 in 49% yield (scheme 55). Unfortunately, subsequent treatment\textsuperscript{260} of 252 with phosphorus pentachloride at elevated temperature afforded a complex mixture of products (scheme 55). Base-catalyzed cyclization\textsuperscript{298} in the presence of urea likewise failed to provide access to 253 (scheme 55). Gratifyingly, treatment of 252 with sodium acetate at elevated temperature for five days afforded the target oxadiazole 253 (R = NH\textsubscript{2}) in 44% yield (scheme 55).

\textit{Reagents and conditions:} (a) NH\textsubscript{2}OH.HCl, NaOAc, EtOH, reflux, 6 h; (b) PCl\textsubscript{5}, Et\textsubscript{2}O, reflux, 6 h; (c) (NH\textsubscript{2})\textsubscript{2}CO, aq. 10 M NaOH, reflux, 3 h; (d) NaOAc, EtOH, reflux, 120 h; (e) i) NaNO\textsubscript{2}/H\textsubscript{2}O, AcOH/aq. 10 M HCl/H\textsubscript{2}O, 0 °C, 0. 5 h; ii) CuCl, aq. 10 M HCl, 0 °C → r.t., 0.5 h; (f) i) Na, EtOH, r.t., 0.5 h (ii) then 255, NaOEt, EtOH, r.t., 2 h; (g) Na\textsubscript{2}S.9H\textsubscript{2}O, H\textsubscript{2}O, 1,4-dioxane, 80 °C, 1 h.

\textbf{Scheme 55:} Synthesis of 3-ethoxy-1,2,5-oxadiazole 241.

Diazotization\textsuperscript{260} of 253 to access oxadiazole 254 (R = Cl) at first proved problematic, however, it was discovered that the order of addition was critical to the formation of the diazonium salt intermediate and consequently formation of product 254. Gratifyingly, a modified diazotization procedure\textsuperscript{299} employing the correct order of addition afforded the desired oxadiazole 254 (R = Cl) in 22% yield (scheme 55). Subsequent treatment\textsuperscript{260} of 254 with freshly prepared sodium ethoxide afforded oxadiazole 255 (R = OEt), which, due to its instability towards chromatographic conditions as observed in preliminary experiments, was immediately subjected
to the established selective nitro-heterocycle reduction procedure,\textsuperscript{255} to furnish the target oxadiazole 241 (R = OEt) in 55% yield over two steps (scheme 55).

C) Physicochemical evaluation of 4-ethyl-1,2,5-oxadiazole 240 and 3-ethoxy-1,2,5-oxadiazole 241

Physicochemical evaluation demonstrated oxadiazole 240 (R = Et, \( \log P_{\text{exp.}} = 2.46 \)) to be lipophilically similar to PAVP (\( \log P_{\text{exp.}} = 2.48 \)) (table 34). Contrary to expectation, oxadiazole 241 (R = OEt, \( \log P_{\text{exp.}} = 2.79 \)) was observed to be more lipophilic than oxadiazole 240 (R = Et) (table 34).

Table 34: Electronic and lipophilic parameters of 4-ethyl-1,2,5-oxadiazole 240 and 3-ethoxy-1,2,5-oxadiazole 241.

<table>
<thead>
<tr>
<th></th>
<th>( \text{NH}_2 ) ipso ( ^{13}\text{C} ) shift (ppm, relative to PAPP)</th>
<th>( \log P_{\text{exp.}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP</td>
<td>0.00</td>
<td>1.65</td>
</tr>
<tr>
<td>Benzocaine</td>
<td>-0.30</td>
<td>2.04</td>
</tr>
<tr>
<td>240 R = Et</td>
<td>-2.60</td>
<td>2.46</td>
</tr>
<tr>
<td>PAVP</td>
<td>-0.03</td>
<td>2.48</td>
</tr>
<tr>
<td>146 (R = nPr, 1,2,4-)</td>
<td>-0.46</td>
<td>2.61</td>
</tr>
<tr>
<td>241 R = OEt</td>
<td>-2.40</td>
<td>2.79</td>
</tr>
</tbody>
</table>

The ipso \( ^{13}\text{C} \) shifts of both oxadiazole 240 (R = Et, -2.60 ppm) and oxadiazole 241 (R = OEt, -2.40 ppm) were demonstrated to be considerably further upfield than that of PAPP (table 34). This result provided some evidence towards the hypothesis that the 1,2,5-oxadiazole ring is less electron-withdrawing than its 1,2,4-counterpart. Attachment of the electronegative oxygen atom at the 3-position of the 1,2,5-oxadiazole ring resulted only in a subtle increase (+0.20 ppm
between the ipso $^{13}$C shifts of 240 and 241) in the electron-withdrawing nature of the 1,2,5-oxadiazole substituent (table 34).

D) *In vitro* evaluation of 4-ethyl-1,2,5-oxadiazole 240 and 3-ethoxy-1,2,5-oxadiazole 241

Contrary to expectation, oxadiazole 241 (R = OEt, MtHb% = 25.7 ± 1.9%) exhibited inferior *in vitro* MtHb induction compared to oxadiazole 240 (R = Et, MtHb% = 38.1 ± 4.3%) (graph 43 and 44). Considering that attachment of an alkoxyl group at the 3-position of the 1,2,5-oxadiazole ring did not significantly alter its electronic contribution, this result suggests that there may be an intolerance to the positioning of an electronegative atom adjacent to the oxadiazole ring.

**Graph 43:** *In vitro* MtHb induction vs. log $P_{exp}$ of 4-ethyl-1,2,5-oxadiazole 240 and 3-ethoxy-1,2,5-oxadiazole 241.

**Graph 44:** *In vitro* MtHb induction vs. ipso $^{13}$C shift (ppm, relative to PAPP) of 4-ethyl-1,2,5-oxadiazole 240 and 3-ethoxy-1,2,5-oxadiazole 241.
The leading heterocycle, 3-propyl-1,2,4-oxadiazole 146, was once again examined concurrently for comparative purposes. In general, despite being ‘lipophilically optimized’, oxadiazole 240 (R = Et) and oxadiazole 241 (R = OEt) displayed inferior *in vitro* MtHb induction compared to both PAVP (59.1 ± 5.6%) and the leading heterocycle, 3-propyl-1,2,4-oxadiazole 146 (55.9 ± 0.4%) (graph 43 and 44). This observation was attributed to the suboptimal electronic contribution of the 1,2,5-oxadiazole ring. Based on this data, in combination with their demanding syntheses, further investigation into the 1,2,5-oxadiazole structural sub-class of benzocaine bioisostere series was not pursued.
4.4. In vivo studies (2)

Having now evaluated a number of oxadiazole and thiadiazole structures for MtHb induction in vitro, attention turned to the appraisal of selected candidates for toxicity in vivo, to examine whether the leading heterocyclic-based benzocaine bioisosteres possessed sufficient MtHb potency to induce death in the test species. Two benzocaine bioisosteres, 3-propyl-1,2,4-oxadiazole 146 and 5-propyl-1,3,4-oxadiazole 165, were nominated (figure 49).

![Figure 49: PAVP, and benzocaine bioisosteres, 3-propyl-1,2,4-oxadiazole 146 and 5-propyl-1,3,4-oxadiazole 165, selected for in vivo evaluation.](image)

1,2,4-Oxadiazole 146 (R = nPr) was chosen on the basis that it had exhibited the greatest in vitro MtHb induction within the benzocaine bioisosteres evaluated thus far. 1,3,4-Oxadiazole 165 (R = nPr) was also selected on the basis of its potent in vitro MtHb induction profile, however an additional basis for its selection was its possession of a different heterocyclic arrangement to that of oxadiazole 146. The different regioisomers of the oxadiazole ring were anticipated to be of significance with regards to in vivo evaluation, due to potential differences that might arise with regards to their respective metabolic profiles, and consequently the MtHb toxicities of oxadiazoles 146 and 165 in the test species. Oxadiazoles 146 and 165 were compared to PAVP, the leading in vivo MtHb inducer, which was again to act as a positive control, using the standard in vivo experimental procedure as described in section 7.3.6. Disappointingly, at doses of 120 mg/kg, both oxadiazole 146 and 165 failed to elicit a lethal endpoint (table 35). Shortly after dosing with oxadiazoles 146 and 165, rats exhibited symptoms characteristic of sub-lethal MtHb induction (blue paws, tail and nose, lethargy), however these symptoms were short lived (ca. 45 mins), with all rats making a full recovery. Conversely, the
symptoms observed (blue paws, tail and nose, lethargy, ataxia, prone) for rats dosed with PAVP continued to become more pronounced, leading to death in all cases (table 35).

**Table 35:** *In vivo* results for 3-propyl-1,2,4-oxadiazole 146 and 5-propyl-1,3,4-oxadiazole 165.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Result (No. of Kills)</th>
<th>Symptoms(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="PAVP" /></td>
<td>60</td>
<td>4/4</td>
<td>✓ ✓ ✓ ✓</td>
</tr>
<tr>
<td><img src="image" alt="146" /></td>
<td>120</td>
<td>4/4</td>
<td>✓ ✓ × ×</td>
</tr>
<tr>
<td><img src="image" alt="165" /></td>
<td>120</td>
<td>0/4</td>
<td>✓ ✓ × ×</td>
</tr>
</tbody>
</table>

\(^a\)B = Blue paws, tail and nose. L = lethargy. A = ataxia. P = prone.

Combined with the knowledge that these compounds exhibited potent *in vitro* MtHb induction, the observations from the above *in vivo* experiment led to the hypothesis that both oxadiazoles 146 and 165 were in fact the subject of presumed metabolism, thus rendering these compounds inactive as MtHb inducers shortly after administration.
4.5. Stability studies on 3-propyl-1,2,4-oxadiazole 146 and 5-propyl-1,3,4-oxadiazole 165

Based on data emanating from the aforementioned in vivo studies, attention next focused on determining the cause for the low levels in vivo MtHb toxicity exhibited by oxadiazoles 146 and 165. A number of stability experiments in various biological mediums, measuring the loss of parent compound, and identification of metabolite formation, were postulated to provide an explanation for the aforementioned in vivo observations.

Firstly, appraisal of the aqueous stability of oxadiazoles 146 and 165 in phosphate buffer (pH 7.4) was performed, in order to determine whether oxadiazoles 146 and 165 were susceptible to chemical hydrolysis at physiological pH (table 36). The hydrolytic stability assay was performed as described in section 7.3.2., with PAVP included as a negative control, using RP-HPLC methods as described in section 7.1.3. The results displayed a negligible loss of parent compound for PAVP, oxadiazoles 146 and 165, with no new peaks being identified, suggesting these compounds to be stable in aqueous solution at physiological pH (table 36).

Subsequently, the potential for chemical degradation of oxadiazoles 146 and 165 in the acidic environment of the stomach was investigated, through subjection of the compounds of interest to a simulated gastric fluid (SGF) assay. The SGF simulates the concentrations of acid and salt present in the stomach, thus it was postulated that measurement of the stability in this medium would facilitate an investigation into whether the stomach is implicated in the breakdown of these compounds when dosed orally. Post-incubation, however, RP-HPLC evaluation confirmed negligible loss of parent compound for PAVP, oxadiazoles 146 and 165, and again no identification of degradation products, demonstrating that these compounds were stable when subjected to low pH (table 36).

The serum component of the blood in rats is known to possess a large expression of carboxyesterases, thus attention next turned to examining the susceptibility of oxadiazoles 146 and 165 to the hydrolytic action of enzymes present in this particular biological medium. PAVP, oxadiazoles 146 and 165 were incubated with rat serum (80%, diluted with PBS) as described in section 7.3.3., with RP-HPLC evaluation of these compounds post-incubation showing minimal loss of parent compound without formation of metabolites, suggesting that
oxadiazoles 146 and 165 were not vulnerable to metabolic breakdown catalyzed by carboxyesterases present in the serum (table 36).

Table 36: Stability of 3-propyl-1,2,4-oxadiazole 146, 5-propyl-1,3,4-oxadiazole 165, PAVP and benzocaine in various biological mediums.

<table>
<thead>
<tr>
<th>Compound</th>
<th>% PARENT COMPOUND REMAINING(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hydrolytic stability(^b)</td>
</tr>
<tr>
<td>146</td>
<td>&gt;99</td>
</tr>
<tr>
<td>165</td>
<td>&gt;99</td>
</tr>
<tr>
<td>PAVP</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Benzocaine</td>
<td>&gt;99</td>
</tr>
</tbody>
</table>

\(^a\)Various assay conditions and RP-HPLC measurement as described in sections 7.1. and 7.3, respectively.
\(^b\)Phosphate buffer (PB) (0.1 M, pH 7.4). \(^c\)Simulated gastric fluid (SGF) (0.03 M NaCl, 0.1 M HCl, pH 1.2). \(^d\)80% Rat serum (in PB)\(^e\)4 mg/ml of S9 (in PB). \(^f\)43% PABA metabolite identified. \(^g\)93% PABA metabolite identified.

Despite demonstrating that oxadiazoles 146 and 165 were resistant to the hydrolytic action of the enzymes present in the serum, it is known that the S9 fraction of the rat liver possesses a ‘powerhouse’ of various hydrolases that could also be implicated in the metabolic breakdown of these compounds \textit{in vivo}.\(^{302}\) PAVP, oxadiazoles 146 and 165 were subject to incubation with S9 as described in section 7.3.4., with RP-HPLC evaluation of these also demonstrating almost no
loss of parent compound and no metabolite formation, suggesting that oxadiazoles 146 and 165 are likewise stable to the hydrolases present in the S9 fraction of the liver. In order to confirm that the hydrolases of the rat serum and S9 fraction were in fact active, the stability of benzocaine was appraised as a positive control in parallel to PAVP and oxadiazoles 146 and 165. RP-HPLC evaluation of benzocaine post-incubation verified that both the rat serum and S9 fraction employed in these experiments indeed possessed considerable levels of hydrolytic activity, with each biological medium effecting ca. 50% and 100% loss of parent compound, with ca. 40% and 90% concomitant formation of the hydrolytic product PABA, respectively (table 36).

Due to the lack of success in identifying a pathway for the putative metabolism of oxadiazoles 146 and 165 through evaluation in various biological mediums, attention turned to the microsomal fraction of the rat liver as the potential source for metabolic inactivation. A literature search afforded a number of examples of microsomal CYP-catalyzed 1,2,4-oxadiazole ring opening, primarily by means of a reductive or oxidative cleavage process, with the former being more prominent. Lan et al. reported that the 1,2,4-oxadiazole ring undergoes a reductive ring opening through an NADPH-dependent reductive N-O bond cleavage, forming amidine, amide and carboxylic acid metabolites. On the other hand, an unusual cleavage of the 1,2,4-oxadiazole ring has been reported by Yabuki et al., whereby hydroxylation of a methylene group attached to the 3-position of the oxadiazole ring resulted in a spontaneous rearrangement and formation of a novel nitrile metabolite in rats. A similar in vitro hydroxylation and ring cleavage of a 3-methyl-1,2,4-oxadiazole moiety has also been reported by Bateman et al.

Conversely, reported biotransformations of the 1,3,4-oxadiazole ring are rare, suggesting this regioisomer to be generally more metabolically stable than its 1,2,4- counterpart. Maciolek et al. reported that the 1,3,4-oxadiazole moiety, however, exhibits some susceptibility to oxidative ring opening, catalyzed by the CYP1A2 isozyme expressed largely in rat liver microsomes.

In our hands, attempts to identify various routes for the metabolic breakdown for oxadiazoles 146 and 165 through incubation with microsomes proved problematic, as methods for extraction proved unreliable. Moreover, incubation of oxadiazoles 146 and 165 with the microsomal
fraction created difficulties in determining the formation of new metabolites, as the microsomes likewise facilitated the formation of the aryl hydroxylamine active metabolite, itself unstable and prone to undergoing redox-mediated side reactions with its oxidation product, the aryl nitroso derivative, to produce a number of dimeric (azoxy andazo) metabolites.\textsuperscript{312} At this stage, further efforts towards the identification of the metabolic pathway responsible for the detoxification of oxadiazoles \textbf{146} and \textbf{165} was considered beyond the scope of this project. Instead, attention turned to alternative synthetic strategies to overcome the metabolic shortcomings of oxadiazoles \textbf{146} and \textbf{165}. 
4.6. Benzocaine bioisosteres and related heterocycles (2)

As a result of the presumed *in vivo* instability observed for 3-propyl-1,2,4-oxadiazole 146 and 5-propyl-1,3,4-oxadiazole 165, further efforts towards the synthesis of 1,2,4-oxadiazoles 209 and 210-214 and 1,3,4-oxadiazoles 211-213 (with derivatization at the 3- or 5-position) were discontinued. Moreover, as a general consequence of the inconclusive stability studies on oxadiazoles 146 and 165, further *in vivo* evaluation within the oxadiazole family was not pursued. Instead, attention turned to the design and synthesis of more structurally diverse heterocycles, postulated to offer greater stability *in vivo*, while retaining favourable lipophilic and electronic parameters for MtHb induction.

4.6.1. 2-Propytetrazole 256 and 5-propyltetrazole 257

One structurally diverse bioisosteric alternative for the 1,2,4-oxadiazole moiety which has received attention in the literature is the tetrazole moiety. Traditionally employed as a heterocyclic alternative to the carboxylic acid functionality, the tetrazole moiety is able to retain acidic character, and afford greater potency, bioavailability selectivity and stability. Significant to the current research was the capacity of tetrazoles to resist oxidative metabolism, a pathway reported to be prevalent in the deactivation of 1,2,4-oxadiazoles. In light of this, replacement of the oxadiazole ring with a tetrazole was postulated to potentially confer a superior metabolic profile for MtHb inducers.

Also of importance to this study were the electronic properties of the tetrazole, and also that of its parent function, the carboxylic acid group. Interestingly, the acid substituent ($\sigma = +0.45$) possesses an identical Hammett constant to that of the ethyl ester ($\sigma = +0.45$), suggesting that esterification of the acid has a negligible impact on electronic contribution (table 37). Moreover, unsubstituted 5H-tetrazole ($\sigma = +0.50$) and 2H-tetrazole ($\sigma = +0.56$) were shown to exhibit Hammett constants comparable not only to the acid functionality which they are designed to mimic, but also to the propionyl (+0.48) and ethyl ester (+0.45) substituents of PAPP and benzocaine, respectively (table 37).
Table 37: Hammett constants for propionyl, ethyl ester, carboxylic acid, 5-substituted-tetrazole and 1-substituted-tetrazole substituents.\textsuperscript{179}

<table>
<thead>
<tr>
<th>Substituent</th>
<th>Hammett constant ($\sigma_{\text{para}}$)\textsuperscript{179}</th>
</tr>
</thead>
</table>
| \[ \begin{array}{c}
\text{CHO} \\
\text{O} \\
\text{O} \\
\text{\text{N} = \text{N} = \text{N} = \text{N}}
\end{array} \] | \[ +0.48 \] |
| \[ \begin{array}{c}
\text{\text{N} = \text{N} = \text{N} = \text{N}}
\end{array} \] | \[ +0.50 \] |

In light of this data, it was postulated that, with regards to electronics, the alkylation of 5\textit{H}-tetrazole and 1\textit{H}-tetrazole substituents was potentially analogous to the alkylation of a carboxylic acid in the formation of an ester, thus resulting in the removal of acidic character with little impact to the electronic nature of the heterocycle.

With guidance from \textit{in silico} log $P$ predictions, attention focused on the preparation and evaluation of 2-propytetrazole \textit{256} and 5-propyltetrazole \textit{257}, each possessing an alkyl chain substituent designed to mirror the lipophilic profile of PAVP (figure 50).\textsuperscript{204}

![Figure 50: In silico log $P$ predictions for benzocaine and its potentially more stable bioisosteres, 2-propytetrazole 256 and 5-propyltetrazole 257.\textsuperscript{204}](image-url)
A) Syntheses of 2-propyltetrazole 256 and 5-propyltetrazole 257

2-Propyltetrazole 256 was prepared in three steps, beginning with the [2+3] fusion or ‘click’ reaction \(^{319}\) of 4-nitrobenzonitrile (191) with sodium azide in the presence of ammonium chloride, which proceeded smoothly to afford \(1H\)-tetrazole 258 in 93% yield (scheme 56). In turn, \(1H\)-tetrazole 258 was alkylated \(^{320}\) with propyl iodide to access substituted tetrazole 259 45% yield. Finally, reduction of 259 as previously described \(^{255}\) furnished the desired target 2-propyltetrazole 256 in 93% yield (scheme 56).

\[
\begin{array}{c}
\text{191} \xrightarrow{(a)} \text{256} (93\%) \xrightarrow{(b)} \text{258} (93\%) \xrightarrow{(c)} \text{259} (45\%) \xrightarrow{(d)} \text{260} (93\%)
\end{array}
\]

Reagents and conditions: (a) NaN₃, NH₄Cl, DMF, reflux, 12 h; (b) NaH, nPrI, DMF, 0 °C → 5 °C, 1.5 h; (c) Na₂S.9H₂O, H₂O, 1,4-dioxane, 80 °C, 1 h.

Scheme 56: Synthesis of 2-propyltetrazole 256.

5-Propyltetrazole 257 was prepared similarly over three steps, commencing with acylation \(^{321}\) of 4-nitroaniline (137) with butyric anhydride, in the presence of triethylamine, to access amide 260 in 80% yield, followed by treatment with phosphorus oxychloride and sodium azide, \(^{321}\) at elevated temperature, to afford 5-propyltetrazole 261 in 17% yield (scheme 57). Reduction of 261 as previously described \(^{255}\) afforded target 5-propyltetrazole 257 in 52% yield (scheme 57).

\[
\begin{array}{c}
\text{137} \xrightarrow{(a)} \text{260} (88\%) \xrightarrow{(b)} \text{261} (17\%) \xrightarrow{(c)} \text{257} (52\%)
\end{array}
\]

Reagents and conditions: (a) (CH₃CH₂CH₂CO)₂O, NEt₃, 1,4-dioxane, r.t., 1 h; (b) NaN₃, POCl₃, MeCN, reflux 10 h; (c) Na₂S.9H₂O, H₂O, 1,4-dioxane, 80 °C, 1 h.

Scheme 57: Synthesis of 5-propyltetrazole 257.
B) Physicochemical evaluation of 2-propyltetrazole 256 and 5-propyltetrazole 257

The partition coefficient of 5-propyltetrazole 257 (log $P_{\text{exp.}} = 1.84$) fell between the values of PAPP (log $P_{\text{exp.}} = 1.65$) and benzocaine (log $P_{\text{exp.}} = 2.04$), while that of 2-propyltetrazole 257 (log $P = 2.29$) was found to sit between benzocaine (log $P_{\text{exp.}} = 2.04$) and PAVP (log $P_{\text{exp.}} = 2.48$) (Table 38).

Table 38: Electronic and lipophilic parameters of 2-propyltetrazole 256 and 5-alkyltetrazole 257.

<table>
<thead>
<tr>
<th>Compound</th>
<th>NH$_2$ ipso $^{13}$C shift (ppm, relative to PAPP)</th>
<th>log $P_{\text{exp.}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP</td>
<td>0.00</td>
<td>1.65</td>
</tr>
<tr>
<td><img src="image" alt="Structure 256" /></td>
<td>-2.60</td>
<td>2.29</td>
</tr>
<tr>
<td>Benzocaine</td>
<td>-0.30</td>
<td>2.04</td>
</tr>
<tr>
<td>PAVP</td>
<td>-0.03</td>
<td>2.48</td>
</tr>
<tr>
<td><img src="image" alt="Structure 146" /></td>
<td>-0.46</td>
<td>2.61</td>
</tr>
</tbody>
</table>

Contrary to expectation, 2-propyltetrazole 256 (-2.60 ppm) and 5-propyltetrazole 257 (-2.41 ppm) each exhibited an ipso $^{13}$C shift that was considerably upfield compared to PAPP (0.00...
4. Design, synthesis and bioevaluation of benzocaine-related analogues

4.6. Benzocaine bioisosteres (2)

ppm) (table 38). These results suggested that the electron-withdrawing nature of the tetrazole substituent was significantly less than that of the acid, ethyl ester and propionyl substituents.

C) *In vitro* evaluation of 2-propyltetrazole 256 and 5-propyltetrazole 257

Although being lower than expected (relative to *in silico* log $P$ predictions), the partition coefficients of tetrazoles 257 (log $P_{exp.} = 1.84$) and 256 (log $P_{exp.} = 2.29$) demonstrated that these compounds were sufficiently lipophilic for evaluation of their *in vitro* MtHb properties compared to PAPP, PAVP and our most potent *in vitro* heterocycle to date, oxadiazole 146 (table 38).

Graph 45: *In vitro* MtHb induction vs. log $P$ of 2-propyltetrazole 256 and 5-propyltetrazole 257.

Graph 46: *In vitro* MtHb induction vs. ipso $^{13}$C shift (ppm, relative to PAPP) of 2-propyltetrazole 256 and 5-propyltetrazole 257.

Despite being only marginally less lipophilic than PAVP (log $P_{exp.} = 2.48$, MtHb = 72.3 ± 1.6%), to which it was most comparable, 2-propyltetrazole 256 (log $P_{exp.} = 2.29$, MtHb = 47.4 ±
3.3%) was found to be significantly less active than the former (graph 45). More pronounced still, and contrary to expectation, 5-propyltetrazole 257 (log $P_{\text{exp}} = 1.85$, 9.4 ± 0.5%) demonstrated considerably inferior in vitro MtHb induction when compared to PAPP (log $P_{\text{exp}} = 1.65$, 22.5 ± 3.2%), despite possessing a more favourable lipophilic profile with regards to in vitro MtHb induction (graph 45). Generally, the in vitro MtHb induction of tetrazoles 256 and 257, in light of their physicochemical evaluation, suggests that the electronic contribution of the tetrazole moiety was suboptimal for the critical biochemical processes involved in MtHb induction (table 38, graph 46). Despite their similar electronic contributions, 2-propyltetrazole 256 ($ipso$ $^{13}$C shift = -2.60 ppm, MtHb = 47.4 ± 3.3%) and 5-propyltetrazole 257 ($ipso$ $^{13}$C shift = -2.41 ppm, MtHb = 9.4 ± 0.5%) exhibited marked differences in in vitro MtHb induction (graph 45 and 46). In terms of structural acceptance, one explanation for this could be an intolerance of the positioning of the alkyl chain as in 5-propyltetrazole 257. With regards to electronics, a second hypothesis for the apparent suboptimal contribution of the tetrazole substituent could be a consequence of alkylation of the tetrazole substituent (as in tetrazoles 256 and 257). This structural modification may indeed not lead to the negligible changes in electronic properties observed for the alkylation of a carboxylic acid, as originally hypothesized. A further explanation for the suboptimal electronic contribution of the tetrazole substituent may relate to that offered for the poor activity observed for aminophenyl alkyl sulfoxide 129, sulfonamide 132 and sulfone 130 (section 3.4.3.3). Akin to substituents found in these sulfur-based isosteres, the $5\text{H}$-tetrazole ($\sigma = +0.50$) and $2\text{H}$-tetrazole ($\sigma = +0.56$) substituents, on the surface, appear to possess a similar electronic composition to that of propionyl ($\sigma = +0.48$) and ethyl ester ($\sigma = +0.45$) moieties, however closer inspection of their corresponding field (F) and resonance (R) parameters reveals marked differences (table 39).
Table 39: $\sigma_{\text{para}}, F$ and $R$ parameters for propionyl, ethyl ester, $5H$-tetrazole and $2H$-tetrazole substituents.

<table>
<thead>
<tr>
<th>Substituent</th>
<th>$\sigma_{\text{para}}$</th>
<th>$F$</th>
<th>$R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{CH}_3$</td>
<td>+0.48</td>
<td>+0.34</td>
<td>+0.14</td>
</tr>
<tr>
<td>$\text{O}$</td>
<td>+0.45</td>
<td>+0.34</td>
<td>+0.11</td>
</tr>
<tr>
<td>$\text{OH}$</td>
<td>+0.45</td>
<td>+0.34</td>
<td>+0.11</td>
</tr>
<tr>
<td>$\text{N}=\text{N}$</td>
<td>+0.56</td>
<td>+0.65</td>
<td>-0.09</td>
</tr>
<tr>
<td>$\text{N}=\text{N}$</td>
<td>+0.50</td>
<td>+0.52</td>
<td>-0.02</td>
</tr>
</tbody>
</table>

This hypothesis, however, is limited, due to literature Hammett constants only being available for the $2H$- and $5H$- unsubstituted tetrazole substituents,\textsuperscript{179} with no experimental evidence available to suggest that the Hammett constants for the alkylated substituents (as in tetrazoles 256 and 257) would be similar.

Despite physicochemical evaluation demonstrating that tetrazoles 256 and 257 were not strictly ‘lipophilically optimized’, these compounds exhibited levels of \textit{in vitro} MtHb induction considerably lower than that of PAVP (72.3 ± 1.6%) and our most potent \textit{in vitro} heterocycle, oxadiazole 146 (68.1 ± 3.4%) (graph 45 and 46). Although tetrazoles 256 and 257 may potentially confer greater metabolic stability than oxadiazoles 146 and 165 \textit{in vivo}, and thus perhaps a superior pharmacokinetic profile, the levels of \textit{in vitro} MtHb induction displayed by tetrazoles 256 and 257 were considered insufficient to warrant further investigation into this structural sub-class.
4.6.2. 6-Membered heterocycles; 4-pyridine 262 and pyrimidines 263-265, pyrazine 266 and 3-pyridazine 267

Other structurally diverse bioisosteric alternatives employed as a replacement for the 1,2,4-oxadiazole moiety include 6-membered heteroaromatic diazine rings such as pyrimidines, pyrazine and pyridazine. Of interest to the current research were, again, the electronic properties of these heterocycles, as each of these groups exhibited a Hammett constant comparable to that of the desired propionyl and ethyl ester substituents of PAPP and benzocaine, respectively (table 40).

Table 40: Hammett constants for propionyl, ethyl ester, 4-pyridinyl and heteroaromatic diazine substituents.

<table>
<thead>
<tr>
<th>Substituent</th>
<th>Hammett constant ( (\sigma_{\text{para}}) )</th>
<th>Substituent</th>
<th>Hammett constant ( (\sigma_{\text{para}}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>+0.48</td>
<td><img src="image" alt="4-Pyridine" /></td>
<td>+0.48</td>
<td></td>
</tr>
<tr>
<td>+0.45</td>
<td><img src="image" alt="Pyrimidine" /></td>
<td>+0.53</td>
<td></td>
</tr>
<tr>
<td>+0.39</td>
<td><img src="image" alt="Pyrazine" /></td>
<td>+0.63</td>
<td></td>
</tr>
<tr>
<td>+0.44</td>
<td><img src="image" alt="Pyridazine" /></td>
<td>n/a</td>
<td></td>
</tr>
</tbody>
</table>

Upon examination of the Hammett constants of related heterocycles, it was found that the 4-pyridyl substituent also possessed electronic properties similar to that of the propionyl and ethyl ester substituents of PAPP and benzocaine, respectively (table 40). Despite not possessing an experimentally measured Hammett constant, the pyrazinyl substituent was
considered worthy of further consideration, on the basis that its electrostatic mapping has been reported to be similar to that of the 1,2,4-oxadiazole ring. In light of these findings, a series of 6-membered heterocycles, consisting of 4-pyridine 262, pyrimidines 263-265, pyrazine 266 and 3-pyridazine 267 were prepared for physicochemical and in vitro evaluation (figure 51).

![Figure 51: 4-Pyridine 262, and heteroaromatic diazines 263-267.](image)

**A) Synthesis of 4-pyridine 262 and pyrimidines 263-265, pyrazine 266 and 3-pyridazine 267**

Initial efforts focused on a Suzuki cross-coupling approach to access heterocycles 262-267. Fundamentally, it was preferred that the aryl amine moiety constituted the boronate coupling partner, and the heterocyclic component provided the aryl halide. It was envisioned that a large batch of the common aryl amine boronic acid 268 or boronate ester 269 could be prepared for use in Suzuki cross-coupling reactions with a range of heterocyclic partners, rather than preparing a unique boronic acid or ester for each reaction. In light of this approach, the synthesis of boronic acid 268 and of boronate ester 269 were explored. 4-Bromoaniline (270) was treated with pinacolborane in the presence of bis(triphenylphosphine)palladium(II) chloride (20 mol%) and triethylamine, at elevated temperature; however, only unreacted starting material 270 was recovered (scheme 58). Increased catalytic loading generally resulted in decomposition of starting material 270. Hypothesizing that bromide 270 may not have been
sufficiently reactive, a modified procedure\(^3\) was employed using 4-iodoaniline (271), adopting similar conditions to that previously described\(^3\) (scheme 58). Despite further investigation into different catalyst loadings and adherence to strict de-gassing procedures, only unreacted starting material 271 was recovered.

\[
\begin{align*}
270 & \quad X = \text{Br} \\
271 & \quad X = \text{I}
\end{align*}
\]

\[
\text{Reagents and conditions:} \quad \begin{align*}
(a) \text{ pinacolborane, PdCl}_2(\text{PPh}_3)_2, \text{ NEt}_3, 1,4\text{-dioxane, 80 °C, 5 h (X = Br)); (b) pinacolborane, PdCl}_2(\text{PPh}_3)_2, \text{ NEt}_3, \text{ PhMe, 80 °C, 3 h (X = I).}
\end{align*}
\]

\textbf{Scheme 58: Attempted syntheses of boronate ester 269.}

Due to the failure of the original cross-coupling strategy, efforts now turned to employing the heterocyclic component as the boronic acid partner in the Suzuki cross-coupling. Treatment\(^3\) of 4-bromopyridine hydrochloride (272) with \(n\)-butyllithium and triisopropyl borate (\(\text{B(OiPr)}_3\)) at low temperature resulted in no product formation (scheme 59). Upon further consultation of the literature, it was postulated that the order of addition\(^3\) of the reagents was critical to the quenching of the organolithiate intermediate with the triisopropyl borate species. To test this hypothesis, a similar procedure\(^3\) utilizing a different order of addition of the reagents was conducted, however this revised procedure again failed to allow access to boronic acid 268 (scheme 59).
4. Design, synthesis and bioevaluation of benzocaine-related analogues

4.6. Benzocaine bioisosteres (2)

**Reagents and conditions:** (a) nBuLi, then 272, B(OiPr)_3, THF, PhMe, -60 °C → -15 °C, 0.75 h; (b) B(OiPr)_3, nBuLi, then 272, THF, PhMe, -40 °C → -20 °C, 0.5 h; (c) i) aq. 1 M NaOH, Et_2O, 0 °C, (to give 4-bromopyridine (272) as 0.23 M solution in Et_2O), or NEt_3, r.t., 24 h (to give 4-bromopyridine (272)); ii) nBuLi, then 272, B(OiPr)_3, Et_2O, -78 °C → 20 °C, 1 h.

**Scheme 59: Attempted syntheses of boronic acid 268.**

4-Bromopyridine (272) is only commercially available as its hydrochloride salt, due to its inherent instability as a free base. The procedures followed previously necessitated *in situ* liberation of 4-bromopyridine (272) through the addition of an excess of *n*-butyllithium to the hydrochloride salt. In a final effort, conversion of 4-bromopyridine hydrochloride (272) to its free base was carried out prior to its introduction to the reaction mixture, via either extraction into ether following neutralization with aqueous sodium hydroxide, or by stirring in triethylamine overnight with removal of the resultant triethylamine salt by filtration (scheme 59). Despite these modifications to the original procedure, efforts to form boronic acid 268 remained unsuccessful.

As a consequence of the shortcomings of the Suzuki-cross coupling strategy, it was next decided to investigate whether Stille cross-coupling methods could facilitate access to desired heterocycles 262-267. Bearing in mind the aforementioned instability associated with 4-bromopyridine (272), 2-chloropyrimidine (274) was instead employed as the model compound for exploring Stille cross-coupling methods. Stannane precursor 274 was treated with tributyltin(III) hydride in the presence of preformed LDA, however no product could be isolated from the reaction mixture (scheme 60).
Reagents and conditions: (a) (i) iPrNH, nBuLi, THF, -10 °C, 0.1 h (to give LDA); (ii) LDA, SnBu3H, 0 °C → -78 °C, 0.3 h, then 274, THF, -78 °C, 1 h.

Scheme 60: Attempted synthesis of stannane 275.

Ultimately, heterocycles 262-267 were prepared by the original Suzuki-cross coupling strategy, employing commercially available 4-acetamidophenylboronic acid pinacol ester (276) as the boronate coupling partner. Treatment\(^{330}\) of 276 with the corresponding aryl halides 274 and 277-279 in the presence of bis(triphenylphosphine)palladium(II) chloride and sodium bicarbonate under microwave irradiation afforded heterocycles 280-283 in moderate yield (47%-80%) (scheme 61). Subsequent acid-hydrolysis of acetamides 280-283, as previously described,\(^{210}\) proceeded smoothly to furnish target heterocycles 262, 263, 265 and 266 in good yield (78-96%) (scheme 61).
Reagents and conditions: (a) PdCl$_2$(PPh$_3$)$_2$, NaHCO$_3$, PhMe, EtOH, H$_2$O, 120 °C, MW, 50W, 1 h; (b) aq. 1 M HCl, MeOH, reflux, 1 h.

Scheme 61: Synthesis of 4-pyridine 262, 2-pyrimidine 263, 5-pyrimidine 265 and pyrazine 266.

In the pursuit of 4-pyrimidine 264, via the established method as described above,$^{210,330}$ a procedure for the synthesis of aryl halide 284 was required, owing to it being commercially unavailable. Efforts to synthesize aryl halide cross-coupling precursor 284 from 4-(3H)-pyrimidinone (285), in the presence of phosphorus oxychloride at elevated
temperature,\textsuperscript{331} proved problematic, as the reaction resulted in either unreacted starting material \textsuperscript{285}, or a decomposition of \textsuperscript{285} following extended reaction times (scheme \textsuperscript{62}). Initially, it was postulated that \textsuperscript{284} was undergoing hydrolysis back to \textsuperscript{285} during the basic aqueous work up procedure, however, when a procedure with similar reaction conditions\textsuperscript{332} was adopted that involved simply cooling and collecting the resulting solid by filtration, it was still not possible to isolate the desired 4-chloropyrimidine (\textsuperscript{284}) (scheme \textsuperscript{62}). The inclusion of a catalytic amount of dimethylaniline\textsuperscript{333} in the reaction mixture also failed to facilitate the conversion of \textsuperscript{285} to 4-chloropyrimidine (\textsuperscript{284}) (scheme \textsuperscript{62}). The \textsuperscript{1}H NMR chemical shifts observed for the crude mixture isolated from this reaction did not match the chemical shifts reported for 4-chloropyrimidine (\textsuperscript{284}) in the literature,\textsuperscript{334,335} however, it was postulated that, due to the release of hydrogen chloride gas upon reaction of \textsuperscript{285} with phosphorus oxychloride, \textsuperscript{284} may exist as its hydrochloride salt (scheme \textsuperscript{62}). To the best of our knowledge, no literature NMR data was available for the hydrochloride salt of 4-chloropyrimidine (\textsuperscript{284}). The crude mixture isolated from the reaction was then taken through to the established Suzuki cross-coupling conditions,\textsuperscript{330} adding excess sodium bicarbonate in an attempt to liberate any \textsuperscript{284} if indeed present (scheme \textsuperscript{62}). Disappointingly, none of desired 4-pyrimidine \textsuperscript{286} was formed, suggesting the hydrochloride salt of 4-chloropyrimidine (\textsuperscript{284}) was not present in the mixture (scheme \textsuperscript{62}).

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {\textsuperscript{285}}; \node (b) at (2,0) {\textsuperscript{284}}; \node (c) at (4,0) {\textsuperscript{286}}; \node (d) at (0,-0.5) {\textsuperscript{284}}; \node (e) at (0,-1) {\textsuperscript{264}}; \node (f) at (2,0) {\textsuperscript{284}}; \node (g) at (2,-0.5) {\textsuperscript{284}}; \node (h) at (4,-0.5) {\textsuperscript{286}}; \node (i) at (4,-1) {\textsuperscript{286}}; \node (j) at (2,0) {\textsuperscript{284}}; \node (k) at (2,-0.5) {\textsuperscript{284}}; \node (l) at (4,-0.5) {\textsuperscript{286}}; \node (m) at (4,-1) {\textsuperscript{286}};
\draw[->,thick] (a) -- (b) node[midway,above] {\Large (a) or (b)};
\draw[->,thick] (b) -- (c) node[midway,above] {\Large (c)};
\draw[->,thick] (e) -- (f) node[midway,above] {\Large (c)};
\end{tikzpicture}
\end{center}

\textit{Reagents and conditions:} (a) POCl\textsubscript{3}, 100 °C, 0.5-1 h; (b) POCl\textsubscript{3}, PhNMe\textsubscript{2}, reflux, 2 h; (c) 4-acetamidophenylboronic acid pinacol ester (\textsuperscript{276}), PdCl\textsubscript{2}(PPh\textsubscript{3})\textsubscript{2}, NaHCO\textsubscript{3}, PhMe, EtOH, H\textsubscript{2}O, 120 °C, MW, 50W, 1 h.

\textit{Scheme 62:} Attempted synthesis of 4-pyrimidine \textsuperscript{264} (1).
Owing to the lack of success in attempts to form the required 4-chloropyrimidine (284) via chlorination methods,\textsuperscript{331,332,333} an alternative approach was pursued whereby the desired 4-pyrimidyl ring structure could be formed via a one-step reaction between aldehyde 69 and formamidine acetate (287), thus by-passing the need to form 4-chloropyrimidine (284) as an intermediate (scheme 63). In order to investigate this potential route, triethyl orthoformate (288) was treated\textsuperscript{336} with acetic acid and a stream of ammonia gas, with heating, to afford the desired formamidine acetate (287) in 46% yield (scheme 63). 4-Nitrobenzaldehyde (69) was then treated with an excess of formamidine acetate (287) at elevated temperature,\textsuperscript{337} however after two days, only unreacted starting material was recovered (scheme 63).

\begin{align*}
\text{CH(OEt)}_3 & \xrightarrow{(a)} \text{OAc} \quad \text{H}_2\text{N} \quad \text{H} \quad \xrightarrow{(b)} \text{O}_2\text{N} \quad \text{H}_2\text{N} \\
288 & \quad 287 (46\%) & \quad 289 & \quad 264
\end{align*}

\textit{Reagents and conditions:} (a) AcOH, NH\textsubscript{3}, H\textsubscript{2}O, 100 °C, 0.3 h; (b) 4-nitrobenzaldehyde (69), nPrOH, 100 °C, 48 h.

\textbf{Scheme 63:} Attempted synthesis of 4-pyrimidine 264 (2).

Returning to the earlier strategy to prepare the desired 4-pyrimidine 264 via cross-coupling methods, an alternative route was to proceed using 4,6-dichloropyrimidine (290) as the aryl halide coupling partner (scheme 64). Gratifyingly, Suzuki cross coupling\textsuperscript{330} of 290 in the presence of an excess of boronic acid pinacol ester (276) formed the desired mono-coupled product 291, which itself was subsequently reduced using zinc and aqueous ammonia at elevated temperature to afford acetamide 292 in 15% yield over two steps (scheme 64). Hydrolysis of 292 as previously described\textsuperscript{210} furnished the desired 4-pyrimidine 264 in 93% yield (scheme 64).
4. Design, synthesis and bioevaluation of benzocaine-related analogues  

4.6. Benzocaine bioisosteres (2)

Reagents and conditions: (a) PdCl$_2$(PPh$_3$)$_2$, NaHCO$_3$, PhMe, EtOH, H$_2$O, 120 °C, MW, 50W, 1 h; (b) Zn, aq. NH$_3$, THF, reflux, 5 h; (c) aq. 1 M HCl, MeOH, reflux, 1 h; (d) NH$_4^+$COO$, 10$ mol% Pd/C, MeOH, reflux, 18 h.

Scheme 64: Synthesis of 4-pyrimidine 264 and 3-pyridazine 267.

A similar strategy was also applied to the synthesis of 3-pyridazine 267. Subjection of 3,6-dichloropyridazine (293) to Suzuki cross-coupling conditions, again using an excess of pinacol ester 276, afforded 3-pyridazine 294 in 25% yield (scheme 64). Unfortunately, reduction of 294 as described previously formed an unknown by-product, however, pleasingly, it was discovered that acetamide 295 could be accessed through treatment of 294 with ammonium formate in the presence of palladium-on-carbon (scheme 64). Subsequent acid-hydrolysis of 295 as previously described afforded target 3-pyridazine 267 in 15% yield over two steps (scheme 64).
B) Physicochemical evaluation of 4-pyridine 262 and pyrimidines 263-265, pyrazine 266 and 3-pyridazine 267

In general, heteroaromatic diazines 263-267 (log $P_{\text{exp.}} = 1.54$-1.70) exhibited similar lipophilic profiles to that of PAPP (log $P_{\text{exp.}} = 1.65$) (table 41). A very broad peak was observed for the RP-HPLC evaluation of 4-pyridine 262 (log $P_{\text{exp.}} = 1.47$), and as a consequence, this partition coefficient was considered unreliable (table 41).

Table 41: Electronic and lipophilic parameters of 4-pyridine 262 and pyrimidines 263-265, pyrazine 266 and 3-pyridazine 267.

<table>
<thead>
<tr>
<th>Compound</th>
<th>NH$_2$ ipso $^{13}$C shift (ppm, relative to PAPP)</th>
<th>log $P_{\text{exp.}}$</th>
<th>Compound</th>
<th>NH$_2$ ipso $^{13}$C shift (ppm, relative to PAPP)</th>
<th>log $P_{\text{exp.}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAAP</td>
<td>+0.11</td>
<td>1.34</td>
<td>263</td>
<td>-1.90</td>
<td>1.67</td>
</tr>
<tr>
<td>262</td>
<td>-3.22</td>
<td>1.47$^a$</td>
<td>266</td>
<td>-2.66</td>
<td>1.68</td>
</tr>
<tr>
<td>264</td>
<td>-2.49</td>
<td>1.54</td>
<td>265</td>
<td>-3.54</td>
<td>1.58</td>
</tr>
<tr>
<td>267</td>
<td>-2.49</td>
<td>1.54</td>
<td>264</td>
<td>-1.45</td>
<td>1.70</td>
</tr>
<tr>
<td>265</td>
<td>-3.54</td>
<td>1.58</td>
<td>Benzocaine</td>
<td>-0.30</td>
<td>2.04</td>
</tr>
<tr>
<td>PAPP</td>
<td>0.00</td>
<td>1.65</td>
<td>PAVP</td>
<td>-0.03</td>
<td>2.48</td>
</tr>
</tbody>
</table>

$^a$Broad peak observed in RP-HPLC log $P$ evaluation.
In summary, heterocycles 262-267 exhibited a broad range of ipso $^{13}$C shifts (-1.45 → -3.54 ppm), and thus a large array of electronic properties (table 41). In a similar fashion to the physicochemical evaluation of aminophenone sulfur-based isosteres 129, 132 and 130 (section 3.4.3.C), the ipso $^{13}$C shifts of the heterocycles 262-265 and 267, based on their electronic properties, followed the expected trend in relation to each other (i.e. the greater the Hammett constant for the substituent, the greater the observed ipso $^{13}$C shift) (graph 47). When comparing the ipso $^{13}$C shifts of compounds 262-265 and 267 to PAPP, however, these values also appeared to be all significantly upfield (lower ppm value) from that expected, with respect to their Hammett constants (graph 47). \(^{179}\)

**Graph 47: Ipso $^{13}$C shifts of the lipophilically equivalent PAPP, 4-pyrimidyl 262, 2-pyrimidyl 263, 3-pyridazinyl 267, 4-pyridyl 262 and 5-pyrimidyl 265, and their corresponding Hammett substituent constants.** \(^{179}\)

**C) In vitro evaluation of 4-pyridine 262 and pyrimidines 263-265, pyrazine 266 and 3-pyridazine 267**

Due to the polarity of heterocycles 262-267 (log $P_{\text{exp.}}$ = 1.47-1.70), these compounds were evaluated for in vitro MtHb induction compared to the lipophilically similar PAPP (log $P_{\text{exp.}}$ = 1.65) and the slightly more polar PAAP (log $P_{\text{exp.}}$ = 1.34).
Physicochemical evaluation demonstrated that 4-pyrimidine 264 (*ipso* $^{13}$C shift = -1.45 ppm) was, in this series, the most similar to PAPP with respect to electronics (table 41). Based on this observation, it was anticipated that the 4-pyrimidine 264 would display the greatest *in vitro* MtHb activity within this series, however, contrary to expectation, regioisomer 2-pyrimidine 263 (15.7 ± 3.5%) exhibited the highest level of *in vitro* MtHb induction (graph 48 and 49). Although exhibiting an *ipso* $^{13}$C shift that appeared to represent a suboptimal electronic contribution, the 2-pyrimidyl substituent has been reported to possess a very similar Hammett constant ($\sigma$ = +0.53) to that of the ketone group ($\sigma$ = +0.50) (table 41).¹⁷⁹ These findings would suggest that, within this series, the *ipso* $^{13}$C shift was not a reliable indicator of favourable electronics for MtHb induction. A second explanation for this unexpected result could be that the positioning of the heteroatoms within the ring was of significance with regards to MtHb induction.

Although lipophilically similar, the remaining heterocycles exhibited considerably inferior *in vitro* MtHb induction (264 $R=4$-pyrimidyl, MtHb = 3.9 ± 0.5%, 267 $R=3$-pyridazinyl, MtHb = 0 ± 0.0%, 266 $R=4$-pyrazinyl, MtHb = 4.7 ± 4.0%, 262 $R=4$-pyridyl, MtHb = 5.5 ± 0.7%, 265 $R=5$-pyrimidyl, MtHb = 0.0 ± 0.0%) not only when compared to PAPP (MtHb = 24.3 ±

**Graph 48:** *In vitro* MtHb induction vs. log $P$ of 4-pyridine 262 and pyrimidines 263-265, pyrazine 266 and 3-pyridazine 267.

**Graph 49:** *In vitro* MtHb induction vs. *ipso* $^{13}$C shift (ppm, relative to PAPP) of 4-pyridine 262 and pyrimidines 263-265, pyrazine 266 and 3-pyridazine 267.
Based on these results, these structural sub-classes were not considered for further elaboration.

Although no clear relationship could be identified with regards to electronic properties and MtHb induction within this series, one explanation for the inferior in vitro activity of heterocycles 262-267 may be similar to that described for sulfur-based isosteres 129, 132 and 130 (section 3.4.3.C), and tetrazole based heterocycles 256 and 257 (section 4.6.1.C). In a similar fashion to these aforementioned compounds, the heteroaromatic substituents of heterocycles 262-267 appeared to possess a range of Hammett constants ($\sigma = +0.39 \rightarrow +0.63$) comparable to that of desired propionyl ($\sigma = +0.48$) and ethyl ester ($\sigma = +0.45$) substituents. Further examination of their corresponding field (F) and resonance (R) parameters, however, reveals that the Hammett constants of heterocycles 262-267 possess a markedly greater contribution from the resonance (R) parameter than that of desired propionyl and ethyl ester substituents (table 42). These findings, suggest that, again, a very fine balance of field (F) and resonance (R) parameters may be required for the biochemical processes critical to MtHb induction.

**Table 42: $\sigma_{para}$, F and R parameters for propionyl, ethyl ester, and various 6-membered heterocyclic substituents.**

<table>
<thead>
<tr>
<th>Substituent</th>
<th>$\sigma_{para}$</th>
<th>F</th>
<th>R</th>
<th>Substituent</th>
<th>$\sigma_{para}$</th>
<th>F</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Propionyl" /></td>
<td>+0.48</td>
<td>+0.34</td>
<td>+0.14</td>
<td><img src="image" alt="Ethyl ester" /></td>
<td>+0.48</td>
<td>+0.21</td>
<td>+0.27</td>
</tr>
<tr>
<td><img src="image" alt="Heterocycle" /></td>
<td>+0.45</td>
<td>+0.34</td>
<td>+0.11</td>
<td><img src="image" alt="Heterocycle" /></td>
<td>+0.53</td>
<td>+0.13</td>
<td>+0.40</td>
</tr>
<tr>
<td><img src="image" alt="Heterocycle" /></td>
<td>+0.39</td>
<td>+0.25</td>
<td>+0.14</td>
<td><img src="image" alt="Heterocycle" /></td>
<td>+0.63</td>
<td>+0.18</td>
<td>+0.45</td>
</tr>
<tr>
<td><img src="image" alt="Heterocycle" /></td>
<td>+0.44</td>
<td>+0.21</td>
<td>+0.23</td>
<td><img src="image" alt="Heterocycle" /></td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>
5. DESIGN, SYNTHESIS AND BIOEVALUATION OF 4'-AMINOPHENONE KETOXIME PRODRUGS

The specific objective for the design, synthesis and bioevaluation of aminophenone ketoxime prodrugs was:

- To investigate the potential of replacing the ketone with a ketoxime promoiety, for the purpose of accessing compounds which possess improved bioavailability and ultimately greater \textit{in vivo} MtHb toxicity. It was envisaged that this could be achieved through the synthesis and bioevaluation of a series of ketoxime prodrugs (figure 52).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure52.png}
\caption{The ketoxime moiety as a potential prodrug strategy for carbonyl group of PAVP.}
\end{figure}
5.1. The prodrug concept

Drug development utilizing in vitro screening systems has led to the discovery of numerous potent and selective drug candidates. However, the bioavailability of these molecules screened exclusively using in vitro assays can be low. The presence of certain functional groups in a molecule can result in poor absorption, undesirable distribution, or early metabolism, all of which can lead to a short biological half-life. One strategy to overcome these drawbacks often involves the design of a ‘prodrug’. First introduced by Albert in 1958, a prodrug describes ‘any compound that undergoes biotransformation prior to exhibiting its pharmacological effects.’ A more developed definition was proposed by Harper in 1959, who defined the prodrug strategy as ‘the chemical modification of a biologically active compound to form a new compound that, upon in vivo enzymatic attack, will liberate the parent compound.’ The purpose of the ‘prodrug’ approach within the current research was to ultimately improve the bioavailability of the parent compound (figure 53).

![Figure 53: Prodrug concept.](image)

5.2. Pharmacokinetic shortcomings of PAVP and related aminophenones

There are a number of reports in the literature which suggest that aminophenones (such as those found in section 3.1.1.), depending on their route of administration, experience poor bioavailability. Rockwood et al. demonstrated that PAHP and PAOP exhibited considerably inferior in vivo MtHb induction when administered to mice intramuscularly (i.m.),
compared to when dosed intraperitoneally (i.p.), offering ‘drug sequestration’ as an explanation for the observed discrepancy. Bright and Marrs\textsuperscript{345} reported that in dogs, PAPP was a more effective MtHb inducer than PACP and PAHP via oral administration, possibly due to faster absorption in the gut of the former. Based on pharmacodynamic and pharmacokinetic models, Marino \textit{et al.}\textsuperscript{346} performed pharmacokinetic calculations (EC\textsubscript{50}’s) that were in agreement with the studies of Bright and Marrs,\textsuperscript{345} and further hypothesized that the discrepancy between oral and intravenous routes was due to oral first-pass inactivation. More pertinent to the our research, Bright and Marrs\textsuperscript{87} also demonstrated, in rats, that there was a significant discrepancy between the oral and intravenous doses of PAPP required to achieve similar levels of \textit{in vivo} MtHb induction.

In summary, there are a number of studies that suggest that aminophenones such as PAVP, PACP and PAHP suffer from poor absorption, and that potentially these compounds are susceptible to some degree of first-pass metabolism. It was postulated that these pharmacokinetic shortcomings affect the level of \textit{in vivo} MtHb induction observed for these compounds. In light of this, attention turned to PAVP and other known potent \textit{in vitro} aminophenones (e.g. PAHP or aminodihydrochalcone\textsuperscript{71}) as structural platforms for the design, synthesis, physicochemical and \textit{in vitro} evaluation of prodrugs, which were hypothesized to confer potentially, improved bioavailability and thus enhanced \textit{in vivo} MtHb toxicity.

\textit{5.3. Ketoxime prodrugs 296-300 of PAVP}

Upon consultation of the literature, it was postulated that the carbonyl group of PAVP was a potentially viable site for prodrug derivatization.\textsuperscript{250} Replacement of the ketone with a ketoxime promoiety is a prodrug approach that has experienced success, particularly in the site-specific delivery of anti-glaucoma agents.\textsuperscript{347-349} More importantly, there are a number of studies\textsuperscript{350-355} that suggest that the ketoxime function is an attractive promoiety for biotransformation back to the corresponding ketone in the liver, via hydrolysis catalyzed by microsomal cytochrome P450 enzymes (scheme 65). The cytochrome P450 enzymes that catalyze these reactions have been demonstrated to be relatively non-specific with respect to the oxime substrate, with hydrolysis of the amidoximes, guanidoximes, aldoximes and ketoximes being reported.\textsuperscript{356}
Although *in vivo* hydrolysis of ketoxime moieties to their corresponding ketones is generally accepted, some concerns have been raised with respect to the effectiveness of its application in prodrug strategies. In opposition to the aforementioned studies, other reports suggest that the dominant metabolic pathway of ketoximes in the rat is reduction through to the corresponding hydroxylamine, as opposed to hydrolysis. Moreover, a further study demonstrates that the ketoxime-metabolizing activity of rat liver is not as high as in other mammals.

Despite a lack of consensus in the literature, the ketoxime prodrug strategy was considered advantageous on the basis that it provided a handle for a broad range of functionalizations. Polar groups can be attached to the ketoxime moiety to improve water solubility and absorption in the small intestine, or peptides can be incorporated in order to target specific transport mechanisms. Due to variable enzyme expression across species, an additional advantage of the ketoxime prodrug approach could include greater species selectivity.

In light of these potential benefits, the synthesis and evaluation of a series of ketoxime prodrugs was undertaken, with the objective to not only improve bioavailability, and consequently the toxicity of PAVP *in vivo*, but to extend this approach to the more lipophilic and perhaps more intrinsically potent aminophenones (PACP and PAHP) (scheme 65).

![Scheme 65: Ketoxime prodrugs and their bioactivation to PAVP.](image)

PAVP ketoxime 296 (R = H) and PAVP *O*-methyl ketoxime 297 (R = Me) were prepared and evaluated in order to examine whether the ketoxime moiety, in its simplest structural forms, is indeed susceptible to the metabolic processes required to release sufficient quantities of PAVP...
in vivo (scheme 65). It is known that a pH gradient exists in the progression of the gastrointestinal tract (stomach pH 2 → small intestine 6.8). Basic prodrugs O-dimethylaminoethyl ketoxime 298 and O-morpholinoethyl ketoxime 299 were prepared and evaluated with the aim of investigating whether manipulation of the pKa of these prodrugs could potentially confer greater solubility along this pH gradient and thus greater absorption within the small intestine (scheme 65). O-Carboxymethyl ketoxime 300 was prepared and evaluated on the basis that the acid functionality has been reported to provide assistance in the intramolecular liberation of the parent ketone (PAVP) (scheme 65).

**A) Synthesis of ketoxime prodrugs 296-300**

Ketoximes 296 and 297 were prepared in 89% and 25% yields, respectively, as a mixture of E and Z isomers, via the condensation of PAVP with the appropriate hydroxylamine hydrochloride, in the presence of sodium carbonate at elevated temperature (scheme 66).

![Scheme 66: Synthesis of PAVP ketoxime prodrugs 296 and 297.](image)

**Reagents and conditions:** NH₂OR.HCl, Na₂CO₃, EtOH, H₂O, reflux, 36-96 h.

The alkylation of previously synthesized ketoxime 296 using 2-dimethylaminoethyl chloride hydrochloride (301) and potassium tert-butoxide at elevated temperature directly afforded O-dimethylaminoethyl ketoxime 298 in 23% yield, exclusively as the E isomer (scheme 67). Unfortunately, treatment of ketoxime 296 with 4-(2-chloroethyl)morpholine hydrochloride (302) and potassium tert-butoxide failed to afford the desired ketoxime 299, instead giving the dialkylated by-product 303 (scheme 67). Changing the conditions for the
alkylation procedure to sodium hydride in ethanol\textsuperscript{360} also met with failure (scheme 67). In parallel to these studies, attempts to alkylate\textsuperscript{361} PAVP ketoxime 296 with ethyl bromoacetate (304) in the presence of sodium ethoxide, followed by saponification,\textsuperscript{208} to access ketoxime 300, were likewise unsuccessful, instead affording alkylated by-product 305 (scheme 67).

\begin{align*}
\text{Reagents and conditions:} & \ (a) \ \text{tBuOK, RCl (301 or 302), THF, reflux, 2 h, } (R = (\text{CH}_2)_2\text{NMe}_2 \text{ or } (\text{CH}_2)_2\text{N(}\text{CH}_2\text{)_4O}) \ (b) \ NaH, \ RCl (301 \text{ or } 302), \ \text{EtOH, reflux, 24 h, } (R = (\text{CH}_2)_2\text{NMe}_2 \text{ or } (\text{CH}_2)_2\text{N(}\text{CH}_2\text{)_4O}) \ (c) \ (i) \ \text{BrCH}_2\text{COOMe 304, NaOEt, EtOH, r.t., 48 h; (ii) aq. 1 M NaOH, reflux, 1 h,}
\end{align*}
\[(R = \text{CH}_2\text{COOH}).

\textbf{Scheme 67:} Synthesis of PAVP $O$-dimethylaminoethyl ketoxime 298 and attempted syntheses of PAVP $O$-morpholinoethyl ketoxime 299 and PAVP $O$-carboxymethyl ketoxime 300.

Faced with the lack of selectivity in the attempted alkylation of ketoxime 296, an alternative approach towards target ketoximes 299 and 300 was adopted, similar to that used for the synthesis of ketoxime 297. This new approach initially required the synthesis of the appropriate hydroxylamines 306 and 307 (scheme 68).
The successful synthesis of ketoximes 299 and 300 commenced with the conversion of either acetone (308) or benzophenone (309) to their corresponding ketoximes 310 and 311 using hydroxylamine hydrochloride, in the presence of sodium carbonate and pyridine, respectively (scheme 68). Alkylation of acetone oxime (310) with bromoacetic acid in the presence of sodium hydroxide allowed access to acetone carboxymethoxime (312) in 31% yield (scheme 68).

**Reagents and conditions:**
(a) NH₂OH.HCl, Na₂CO₃, H₂O, r.t., 16 h (R = Me); (b) NH₂OH.HCl, pyridine, MeOH, r.t., 18 h (R = Ph); (c) BrCH₂COOH, aq. 2 M NaOH, 0 °C → r.t., 3 h (R = Me); (d) (i) HO(CH₂)₃N(CH₂)₄O 313, SOCl₂, CHCl₃, reflux, 4 h (to give Cl(CH₂)₃N(CH₂)₄O 302); (ii) then 311, KOH, Cl(CH₂)₃N(CH₂)₄O 302, DMSO, r.t., 18 h (R = Ph); (e) aq. 6 M HCl, reflux, 2 h; (f) PAVP, pyridine, MeOH, reflux, 3 h, (R = CH₂COOH); (g) PAVP, K₂CO₃, EtOH, reflux, 4 h (R = (CH₂)₃N(CH₂)₄O).

**Scheme 68:** Synthesis of PAVP O-morpholinoethyl ketoxime 299 and PAVP O-carboxymethyl ketoxime 300.
Likewise, treatment\textsuperscript{365} of benzophenone oxime (311) with 4-(2-chloroethyl)morpholine hydrochloride (302), prepared itself from 2-morpholinoethanol (313), afforded benzophenone O-morpholinoethyl ketoxime 314 in 44\% yield (scheme 68). Acid-hydrolysis of 312\textsuperscript{364} and 314\textsuperscript{365} allowed access to hydroxylamines 307 and 306 in 23\% and 81\% yields, respectively (scheme 68). Subsequent condensation of hydroxylamines 307\textsuperscript{366} and 306\textsuperscript{367} with PAVP afforded the target ketoximes 300 and 299 in 83\% and 57\% yields, respectively, exclusively as their $E$ isomers (scheme 68).

B) \textit{In vitro} and physicochemical evaluation of ketoxime prodrugs 296-300

The earlier established systematic approach adopted for the previously described ‘direct-acting’ MtHb inducers (e.g. synthesis $\rightarrow$ physicochemical evaluation $\rightarrow$ \textit{in vitro} MtHb assay; section 1.3.4.) was not considered appropriate for the evaluation of ‘indirect-acting’ ketoxime prodrugs 296-300. Instead, the first objective was to examine these compounds for \textit{in vitro} MtHb induction. The rationale behind commencing with the \textit{in vitro} evaluation of ketoxime prodrugs 296-300 was to investigate whether these compounds exhibited any intrinsic \textit{in vitro} MtHb activity. If ketoxime prodrugs 296-300 were found to be inactive \textit{in vitro}, then any symptoms observed during \textit{in vivo} evaluation representative of MtHb induction could be attributed to the metabolic release of PAVP \textit{in vivo}, in turn providing support for the ketoxime prodrug concept within this structural sub-class.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{graph50.png}
\caption{\textit{In vitro} MtHb induction for ketoxime prodrugs 296-300.}
\end{figure}
In general, ketoxime prodrugs \textit{296-300} exhibited a range of low \textit{in vitro} MtHb activities (7.2 - 14.7\%) similar to that of structurally-related PAPP O-methyl ketoxime \textit{117} (7.2 \pm 0.9\%, section \textit{3.4.1.C})(graph \textit{50}).

Given that the cleavage process could be catalyzed by microsomal cytochrome P450 enzymes,\textsuperscript{354} it was unclear (with the exception of ketoxime \textit{300}, MtHb(\%) = 0) whether the observed \textit{in vitro} MtHb activity for these compounds was a consequence of their hydrolysis and subsequent release of the potent MtHb inducer, PAVP, or due to their intrinsic \textit{in vitro} MtHb activity.

At this stage, it was postulated that physicochemical evaluation may be of assistance in elucidating the origin of the \textit{in vitro} MtHb activity observed for ketoxime prodrugs \textit{296-300} (table \textit{43}). As with PAPP O-methyl ketoxime \textit{117} (-3.68 ppm), the \textit{ipso} $^{13}$C shifts of ketoxime prodrugs \textit{296-300} were considerably downfield (-3.60 $\rightarrow$ -3.65 ppm) from that of PAPP and PAVP (-0.03 ppm), verifying that the ketoxime moiety present within this series is significantly less electron-withdrawing than the parent ketone (table \textit{43}). In light of this, and data obtained from earlier studies evaluating a broad range of electronically distinct analogues, it was assumed that ketoxime prodrugs \textit{296-300}, due to their poor electronic properties (-3.60 $\rightarrow$ -3.65 ppm), would themselves be unlikely to be responsible for the observed \textit{in vitro} MtHb induction (table \textit{43}).

The partition coefficients of ketoximes \textit{296} (log $P_{\text{exp.}}$ = 2.50) and \textit{297} (log $P_{\text{exp.}}$ = 3.23) however, demonstrated these compounds to possess a level of lipophilicity that would be considered favourable for \textit{in vitro} MtHb induction (table \textit{43}), thus this parameter may have played a role in contributing to any inherent \textit{in vitro} MtHb activity if indeed present.

Unfortunately, the partition coefficients of ketoxime prodrugs \textit{298}, \textit{299} and \textit{300} could not be determined accurately, due to their acid or base character inducing broadening of their RP-HPLC peaks. It is therefore unknown whether the lipophilicity of these compounds influenced their observed \textit{in vitro} MtHb induction.

In summary, ketoximes \textit{296-300} demonstrated low levels of MtHb induction, however it was not possible to attribute this observation to hydrolysis and the subsequent release of PAVP, or to the intrinsic MtHb inducing properties of these compounds.
5. Design, synthesis and bioevaluation of PAVP ketoxime prodrugs

5.3. Ketoxime prodrugs of PAVP

Table 43: Electronic and lipophilic parameters of ketoxime prodrugs 296-300.

<table>
<thead>
<tr>
<th></th>
<th>NH$_2$ ipso $^{13}$C shift (ppm, relative to PAPP)</th>
<th>log $P_{exp.}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP</td>
<td>0.00</td>
<td>1.65</td>
</tr>
<tr>
<td>298 $R = CH_2CH_2N(CH_3)_2$</td>
<td>-3.65</td>
<td>1.20-1.70$^b$</td>
</tr>
<tr>
<td>299 $R = CH_2CH_2N(CH_2)_4O$</td>
<td>-3.60</td>
<td>1.84-2.41$^b$</td>
</tr>
<tr>
<td>PAVP</td>
<td>-0.03</td>
<td>2.48</td>
</tr>
<tr>
<td>296 $R = H$</td>
<td>-3.62</td>
<td>2.50</td>
</tr>
<tr>
<td>297 $R = Me$</td>
<td>-3.64</td>
<td>3.23</td>
</tr>
<tr>
<td>300 $R = CH_2COOH$</td>
<td>+0.01$^a$</td>
<td>n/a</td>
</tr>
</tbody>
</table>

$^a$ NH$_2$ ipso $^{13}$C shift measure using d$_6$-DMSO as a solvent. $^b$log $P_{exp.}$ values reported as a range due to acid or base character producing very broad HPLC peaks.

C) Stability studies for ketoxime prodrugs 296-300

As a result of inconclusive findings with respect to the origins of the observed in vitro MtHb activity for ketoxime prodrugs 296-300 through physicochemical evaluation, attention turned to the examination of the stability of these compounds in a selection of biological media. This experiment was conducted in order to learn more about the metabolic profile of the ketoxime moiety, substituted or unsubstituted, and to ultimately probe its susceptibility to hydrolysis.

The metabolic profile of ketoximes 296 and 297 were examined in three biological systems (SGF, rat serum and S9 fraction of the rat liver) as described in section 7.3. (table 44). In the SGF stability assay, low levels of hydrolysis of ketoxime 296 to PAVP (ca. 7%) were observed, suggesting that this compound is slightly susceptible to chemical hydrolysis under acidic conditions. Ketoxime 297, however, appeared to be stable within this simulated gastric environment (table 44).
Table 44: Stability of PAVP ketoxime 296, PAVP O-methyl ketoxime 297, PAVP and benzocaine in various biological mediums.

<table>
<thead>
<tr>
<th>Compound</th>
<th>% PARENT COMPOUND REMAINING&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Gastric fluid stability&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Serum stability&lt;sup&gt;c&lt;/sup&gt;</th>
<th>S9 stability&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>296 R = H</td>
<td>93&lt;sup&gt;e&lt;/sup&gt;</td>
<td>&gt;99</td>
<td>&gt;99</td>
<td></td>
</tr>
<tr>
<td>297 R = Me</td>
<td>96</td>
<td>&gt;99</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>Benzocaine</td>
<td>95</td>
<td>52&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0&lt;sup&gt;g&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>PAVP</td>
<td>96</td>
<td>98</td>
<td>&gt;99</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Various assay conditions and RP-HPLC measurement as described in sections 7.1. and 7.3, respectively.

<sup>b</sup>Simulated gastric fluid (SGF) (0.03 M NaCl, 0.1 M HCl, pH 1.2).<sup>c</sup>80% Rat serum (in PB).<sup>d</sup>4 mg/ml of S9 (in PB).<sup>e</sup>7% PAVP metabolite identified. <sup>f</sup>43% PABA metabolite identified. <sup>g</sup>93% PABA metabolite identified.

When ketoximes 296 and 297 were subjected to incubation with rat serum or rat liver S9 fraction, negligible loss of parent prodrug was observed, and no formation of PAVP could be identified (table 44). In order to verify the respective hydrolytic activities of the aforementioned biological mediums, benzocaine was, once again, employed as a positive control. RP-HPLC evaluation of benzocaine post-incubation with both rat serum and liver S9 fractions confirmed their activity, with each biological medium effecting considerable loss of benzocaine, with formation of the corresponding hydrolytic product (PABA) (table 44).

Despite not being able to demonstrate conversion of these compounds to PAVP by various hydrolytic systems, the ketoxime prodrug strategy was deemed worthy of further consideration on the basis that the largest extent of hydrolysis of linkages of this type have been reported to occur in the liver, through catalysis by powerful CYP enzymes. Due to potential problems associated with the identification of metabolites post-incubation with microsomes (as discussed in section 4.5.), the subjection of ketoxime prodrugs 296-300 to microsomes in vitro was not considered practical. Instead, attention turned to the in vivo comparison of ketoxime prodrugs 296-300 to PAVP, as it was postulated that results from such an experiment would ultimately confirm the viability of the ketoxime prodrug approach. Further to this rationale, examination of
the metabolic profiles of ketoxime prodrugs 298, 299 and 300 were postponed until their *in vivo* MtHb toxicity profiles were examined.

**5.4. In vivo studies (3)**

Symptoms characteristic of MtHb induction were observed (blue paws, tail and nose, lethargy, ataxia and prone) for rats dosed with ketoxime 297, with these symptoms leading to death in one of two of the test species examined (table 45). This compound exhibited the greatest MtHb toxicity within the prodrug series, however it was unclear as to whether the activity of ketoxime 297 was due to its inherent MtHb inducing capacity, or a result of its superior bioavailability and subsequent liberation of PAVP (table 45).

Likewise, ketoximes 296, 298 and 299 were observed to induce symptoms characteristic of *in vivo* MtHb toxicity (blue paws, tail and nose, lethargy, ataxia and prone) in one of the test species dosed, however these symptoms did not lead to a lethal endpoint (table 45). Similarly, the observed toxicity for ketoximes 296, 298 and 299 could not strictly be attributed to the cleavage of the prodrug *in vivo*, as both these compounds were shown to exhibit a degree of *in vitro* MtHb induction. Ketoxime 300 induced less pronounced symptoms of *in vivo* MtHb toxicity (blue paws, tail and nose, lethargy and ataxia) (table 45). Considering the absence of any observable *in vitro* MtHb activity for this compound, the *in vivo* data suggested that ketoxime 300 was indeed undergoing hydrolytic cleavage to liberate PAVP, consequently inducing MtHb *in vivo*, potentially through assistance of the aforementioned intramolecular release mechanism. The degree to which this process was occurring, however, was insufficient for this compound to prove lethal (table 45).

In general, ketoxime prodrugs 296-300 appeared to induce symptoms that suggested some degree of *in vivo* MtHb toxicity, however the toxicity observed was considerably less than that of PAVP, which, when dosed at 120 mg/kg, led to a lethal endpoint in all cases (table 45). On this basis, the ketoxime prodrug strategy was deemed to be unsuccessful, hence further investigation into these compounds was not pursued.
5. Design, synthesis and bioevaluation of PAVP ketoxime prodrugs  

5.4. *In vivo* studies (3)

Table 45: *In vivo* results for ketoxime prodrugs 296-300.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Result (No. of Kills)</th>
<th>Symptoms$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>B</td>
</tr>
<tr>
<td>$\text{H}_2\text{N}\text{PAVP}$</td>
<td>60</td>
<td>1/3</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>3/3</td>
<td>✓</td>
</tr>
<tr>
<td>$\text{H}_2\text{N}\text{N}^+\text{CH}$</td>
<td>120</td>
<td>0/2</td>
<td>✓</td>
</tr>
<tr>
<td>296</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{H}_2\text{N}$</td>
<td>120</td>
<td>1/2</td>
<td>✓</td>
</tr>
<tr>
<td>297</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{H}_2\text{N}$</td>
<td>120</td>
<td>0/2</td>
<td>✓</td>
</tr>
<tr>
<td>298</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{H}_2\text{N}$</td>
<td>120</td>
<td>0/2</td>
<td>✓</td>
</tr>
<tr>
<td>299</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{H}_2\text{N}$</td>
<td>120</td>
<td>0/2</td>
<td>✓</td>
</tr>
<tr>
<td>300</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6. CONCLUSIONS AND FUTURE WORK

Part 1

Using PAPP as a starting point, three series of alkyl aminophenones (figure 54) were prepared and evaluated for their physicochemical properties and in vitro MtHb induction, primarily to investigate the significance of the lipophilicity parameter with respect to activity. From these studies, it was discovered that the relationship between lipophilicity (modulated by alkyl chain length) and MtHb induction was parabolic, with an optimal lipophilicity for in vitro MtHb induction sitting in the range of log $P = 3-3.5$ units, as demonstrated by PAHP (log $P_{\text{exp.}} = 3.49$). Further to this observation, extensive studies suggested that there were certain structural requirements for in vitro activity, with both branched (31-37) and cyclic (46-49) alkyl chains appearing to be detrimental to MtHb induction.

![Figure 54: Linear, branched and cyclic alkyl 4'-aminophenones (section 3.1.).](image)

Similarly, five different series of aryl aminophenones (figure 55-57) were likewise prepared and evaluated, with the primary aim of investigating the apparent link between the radioprotective and MtHb inducing properties of PAPP. Known radioprotective aryl analogues of PAPP, namely benzophenone 54 and aminochalcone 55, were found to exhibit levels of in vitro activity comparable to that of the parent compound, thus the aforementioned relationship was considered beneficial in the design of further aryl-based analogues. In order to examine the influence of the electronic parameter on MtHb
induction, a series of aminochalcones 58-62 were investigated. From this study, the
results suggested that electronics play a significant role in MtHb induction, with the
highest levels of activity observed for aminochalcone 55 (R = H). In an extension of this
work, it was shown that saturation of the double bond present in aminochalcone 55 led to
enhanced MtHb activity, as demonstrated by aminodihydrochalcone 71. This result was
postulated to be consequential of an increase in conformational flexibility, therefore
further corroborating the role of structure in MtHb induction.

![Figure 55: Aryl 4'-aminophenone analogues (section 3.2.).](image)

Evaluation of a series of homologous analogues of aminodihydrochalcone 71, namely 54,
72 and 73, revealed *in vitro* MtHb induction data that was largely influenced by the
lipophilicity parameter, while replacing the phenyl ring for heterocyclic substituents (as
found in 76-78) generally resulted in a marginal loss in activity.

![Figure 56: 4'-Aminodihydrochalcone analogues (section 3.2.).](image)

In contrast, ring-fused derivatives of 55 and 71, namely aminoaurone 86 and
aminooindanone 104, also revealed a severe loss of MtHb activity, postulated to be the
result of either unfavourable electronics or intolerance to *meta*-substitution of the aryl
amine ring.
From the aforementioned series, three candidates, PAVP, PAHP and aminodihydrochalcone 71 exhibited the most promising in vitro MtHb induction, and thus were nominated for in vivo evaluation in rats (figure 58). Ultimately, PAVP exhibited the greatest toxicity within this series. In contrast, PAHP and aminodihydrochalcone 71, were both only found to induce symptoms characteristic of sub-lethal MtHb induction (blue paws, tail and nose, lethargy and ataxia), hence these compounds were confirmed to be inferior in vivo toxicants. These observations were postulated to be a consequence of these compounds exhibiting an inferior pharmacokinetic profile for MtHb induction.

Subsequent to this, in an effort to probe the role of the ketone in MtHb induction, two series of aminophenone isosteres (figure 59) were investigated. Ketoxime 117 displayed inferior MtHb activity compared to that of PAPP, an observation postulated to be a consequence of its suboptimal electronics. Increasing the electron-withdrawing nature of the ketoxime moiety, through the synthesis of chloro- and cyano-oximes 118 and 120, respectively, failed to improve the in vitro MtHb activity within this series. Sulfur-based ketone isosteres (sulfoxide 129, sulfone 130 and sulfonamides 131 and 132), possessing
greater electron-withdrawing nature than their ketoxime counterparts, generally exhibited superior activity, with aminophenyl alkyl sulfone 130 demonstrating the greatest \textit{in vitro} MtHb induction within this series. However, it remained inferior to the lead compound, PAVP. Although these ketone isosteres appeared to possess similar electronic profiles to that of the parent ketone, closer examination of the Hammett constants of these substituents suggested that a particular ratio of the field and resonance parameters may be required for the biochemical processes involved in MtHb induction.

\textbf{Figure 59:} 4’-Aminophenone isosteres 117, 118 and 120, and 129-132 (section 3.4.).
6. Conclusions and future work

**Part 2**

In an endeavour to circumvent the well documented susceptibility of benzocaine to \textit{in vivo} hydrolysis, attention focused on the preparation of five series of benzocaine bioisosteres, employing heterocyclic replacements such as oxadiazoles and thiadiazoles (figure 60).

**Figure 60:** Benzocaine bioisosteres (1), oxadiazoles and thiadiazoles (section 4.3.).

From these series, two candidates, 3-propyl-1,2,4-oxadiazole 146 and 5-propyl-1,3,4-oxadiazole 165, exhibited potent \textit{in vitro} MtHb activity, and thus were nominated for \textit{in vivo} evaluation in rats (figure 61).

**Figure 61:** Benzocaine bioisosteres, 3-propyl-1,2,4-oxadiazole 146 and 5-propyl-1,3,4-oxadiazole 165, selected for \textit{in vivo} evaluation (2) (section 4.4.).

Disappointingly, neither of these compounds exhibited \textit{in vivo} MtHb toxicity anywhere close to that observed for the lead compound, PAVP. This result was postulated to be a
6. Conclusions and future work

Consequence of extensive metabolism of oxadiazoles 146 and 165 in vivo. Disappointingly, the source of this metabolic deactivation could not be identified.

In light of these results, attention subsequently focused on the synthesis and bioevaluation of structurally diverse heterocycles, such as tetrazoles 256 and 257 (figure 62), and a series of diazines 262-267 (figure 63). These compounds were postulated to offer greater stability in vivo, however, due to their poor in vitro MtHb activity, these compounds were not investigated further. The failure of these compounds was again assumed to be the result of the suboptimal electronic contribution of the corresponding heterocyclic substituent.

**Figure 62:** Benzocaine bioisosteres (2), tetrazoles 256 and 257 (section 4.6.).

**Figure 63:** Benzocaine bioisosteres (2), diazines 262-267 (section 4.6.).
Finally, a prodrug approach was employed, in an effort to access compounds with improved bioavailability and consequently greater *in vivo* MtHb toxicity. This was explored through the synthesis and evaluation of a select series of ketoximes of PAVP (figure 64). Unfortunately, when evaluated *in vivo*, the toxicity observed for the ketoxime prodrugs was considerably less than that of PAVP, with these compounds only revealing symptoms characteristic of sub-lethal MtHb induction (blue paws, tail and nose, lethargy and ataxia). It remained unclear as to whether the source of the observed toxicity could be attributed to either the release of PAVP, or the intrinsic MtHb inducing properties of ketoxime prodrugs 296-300.

**Figure 64:** Ketoxime prodrugs 296-300 (section 5.3.).
Future work

Subsequent to this research, bait trials were conducted by our industrial collaborators on the lead toxicant, PAVP (table 46).\textsuperscript{368} Broadly, the PAVP bait employed in this study was not well received, and consequently, none of the rats consumed a dose sufficient to effect a lethal endpoint. Interestingly, the results demonstrated that a lower consumption of the bait ($0.18 \pm 0.13$ g) occurred when a higher percentage of PAVP (4\%) was used (table 46).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Mean amount eaten (g) (of 10 g total)</th>
<th>Symptoms observed</th>
<th>Result (No. of kills)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2% PAVP</td>
<td>$0.76 \pm 0.41$</td>
<td>7/7</td>
<td>0/7</td>
</tr>
<tr>
<td>4% PAVP</td>
<td>$0.18 \pm 0.13$</td>
<td>1/7</td>
<td>0/7</td>
</tr>
</tbody>
</table>

This observation was postulated to be the consequence of a known evolutionary trait developed by rats, relating to how they sample food, in particular novel food. The feeding habits of rats can result in an aversion to acute toxins (such as PAVP), as they cautiously feed so as not to ingest a potentially toxic dose at the first encounter.\textsuperscript{369,370} A rapid onset of MtHb induction within the pest species post toxicant consumption is one explanation for their apparent ‘bait-shyness’.

In light of this study, future work could involve further prodrug studies on PAVP, with an emphasis on improving palatability. Rats are known to masticate (or chew) their food thoroughly before swallowing, therefore, during this period, some degree of sublingual uptake of the toxicant may occur, resulting in the aforementioned bait-shyness. It was postulated that a delay in the release of PAVP, post ingestion, could help circumvent the apparent poor palatability and allow greater consumption of the toxicant by the pest species. One strategy postulated to achieve this involves the protection of the parent ketone of PAVP as a ketal functionality. A ketal prodrug of PAVP is predicted to lack the MtHb inducing properties of the parent toxicant, due to the significantly lower
6. Conclusions and future work

electron-withdrawing nature of the ketal group compared to the ketone. Consequently, it was hypothesized that this would allow this structural sub-class of compounds to be consumed to a greater extent by the pest species. Once ingested, the ketal prodrug is susceptible to chemical hydrolysis under acidic conditions, which would allow liberation of PAVP within the stomach (pH ca. 1.2).\(^371\) The rate of release of the parent toxicant can thus be conveniently manipulated through modification of the ketal group (scheme 69).\(^367,368,372,373\)

![Scheme 69: The hydrolysis of ketal and oxazolidine prodrugs to PAVP.](image)

Alternatively, the point of release of PAVP could be delayed to a further point along the pH gradient of the GI tract, through the preparation and evaluation of oxazolidine prodrugs of PAVP. Likewise predicted to lack the MtHb inducing properties of the parent toxicant, these compounds have been demonstrated to be relatively stable at low pH (pH ca. 1.2), however they are susceptible to chemical hydrolysis at elevated pH values, as found further along the GI tract (small intestine, pH ca. 6.5).\(^117\) The extent to which this type of hydrolysis can occur can also be modulated through derivatization of the oxazolidine group (scheme 69).\(^374-376\)

In summary, it is proposed that the masking of the parent ketone with a ketal or oxazolidine group may facilitate enhanced palatability through the elimination of undesired premature MtHb induction, resulting in decreased bait-shyness and greater potential for lethal consumption of the toxic bait.
6. Conclusions and future work
7. EXPERIMENTAL
7. Experimental

7.1. General details – chemistry

7.1.1. Reaction conditions

All non-aqueous reactions were carried out in oven dried glassware under dry nitrogen unless otherwise stated. Tetrahydrofuran was distilled from sodium wire and dichloromethane was dried over calcium hydride. All dried solvents were distilled before use. All other commercially supplied liquid reagents were used without further purification unless otherwise stated and solids were dried under high vacuum before use. Flash chromatography was carried out using silica gel (Riedel-de Haën, particle size 40-63 μm) with the desired eluent. Thin layer chromatography (thin layer chromatography) was performed using silica coated aluminium plates (60, F254; Merck) and compounds were identified using staining with ninhydrin, potassium permanganate or iodine on silica.

7.1.2. RP-HPLC assay for purity and log P determination

Purity and log P values for all pharmacologically test compounds were assigned using RP-HPLC [Dionex Ultimate 3000 System using a Phenomenex Gemini C18-Si column (100 Å, 50 mm × 2.0 mm; 5 μm)] – eluted using a gradient of 99:1% A/B to 0:100% A/B over 60 min at 0.5 mL/min; where solvent A was water and solvent B was acetonitrile; with detection at 254 nm. Solvents for RP-HPLC were purchased as HPLC grade and used without further purification.

7.1.3. RP-HPLC assay for stability appraisal

Selected compounds analyzed for their stability in various biological mediums were assigned using RP-HPLC [Dionex Ultimate 3000 System using a Phenomenex Gemini C18-Si column (100 Å, 150 mm × 4.6 mm; 5 μm)] – eluted using a gradient of 99:1% A/B to 5:95% A/B over 15 min at 1 mL/min; where solvent A was water (0.1% TFA) and solvent B was acetonitrile (0.1% TFA); with detection at 254 nm. Solvents for RP-HPLC were purchased as HPLC grade and used without further purification.
7.1.4. Physical and spectroscopic characterization

Melting point determinations were performed on an Electrom thermal® and X-4 Melting Point Apparatus With Microscope® and are reported in degrees Celsius (°C) and are uncorrected.

Infrared spectra were recorded using a Perkin-Elmer Spectrum 1000 series Fourier Transform IR spectrometer as a thin film between sodium chloride plates where the absorption maxima are expressed in wavenumbers (ν, cm⁻¹).

NMR spectra were recorded on either a Bruker DRX300 spectrometer (300 MHz for ¹H nuclei and at 75 MHz for ¹³C nuclei); or a Bruker DRX400 spectrometer (400 MHz for ¹H nuclei and at 100 MHz for ¹³C nuclei). All chemical shifts are reported in parts per million (ppm) relative to CDCl₃, d₆-DMSO or CD₃OD. The ¹H NMR data is reported in the following order: chemical shift (δ, ppm), relative integral, multiplicity (s, singlet; d, doublet; dd, doublet of doublets; dt, doublet of triplets; t, triplet; q, quartet; m, multiplet), coupling constant(s) (J in Hz), signal assignment. The ¹³C NMR data is reported in the following format: chemical shift (δ, ppm), degree of hybridization, signal assignment.

High resolution mass spectra were recorded on a VG-70SE spectrometer using electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) methods. Prominent fragments are quoted in the form a(b) where a is the mass to charge ratio and b is the percentage abundance relative to the base peak (100%).
7.2. Synthesis

7.2.1. Alkyl 4'-aminophenones

7.2.1.1. Linear alkyl chain 4'-aminophenones 17-19 and 27-30

4'-Aminobutyrophenone (PABP) (17)[377]

Compound 17 was prepared by a two-step procedure similar to that of Dohner and co-workers[169] and Lang and co-workers.[171] In the first step, to a stirring solution of bromobenzene (5.00 g, 31.80 mmol) and anhydrous aluminum chloride (6.00 g, 45.00 mmol) at room temperature was added dropwise butyric anhydride (3.30 g, 20.86 mmol). Once the evolution of hydrogen chloride ceased, the mixture was poured onto ice water (200 mL), extracted with dichloromethane (3 × 100 mL), dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo to afford crude 4'-bromobutyrophenone (22) (2.99 g), which was used without further purification. In the second step, a suspension of crude 4'-bromobutyrophenone (22) (2.99 g), cuprous oxide (0.47 g, 3.29 mmol) and aqueous ammonia (2 mL, 15 M) in dimethyl sulfoxide (2 mL) was heated in a sealed-tube at 90 °C for 16 h. The reaction was allowed to cool, diluted with ethyl acetate (50 mL), washed with water (3 × 50 mL), the separated aqueous layer further extracted with ethyl acetate (3 × 50 mL) and the combined organic phases dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate, 2:1) afforded 17 as a light brown solid (0.83 g, 5.09 mmol, 16% over two steps); $R_f = 0.15$ (hexane:ethyl acetate, 2:1); $^1$H NMR (300 MHz; CDCl$_3$) $\delta$H 0.99 (3H, t, $J = 9.0$ Hz, CH$_2$CH$_2$CH$_3$), 1.40 (2H, m, CH$_2$CH$_2$CH$_3$), 2.84 (2H, t, $J = 7.5$ Hz, CH$_2$CH$_2$CH$_3$), 4.08 (2H, s, NH$_2$), 6.64 (2H, d, $J = 8.6$ Hz, H-3', H-5') and 7.81 (2H, d, $J = 8.6$ Hz, H-2', H-6'); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 14.0 (CH$_3$), 18.2 (CH$_2$), 39.9 (CH$_2$), 113.7 (CH), 127.7 (C), 130.5 (CH), 150.9 (C) and 198.9
7. Experimental

7.2. Synthesis

(C); \textbf{MS} (ESI, 70 eV) \textit{m/z} 164 (M$^+$, 100%); Found (M$^+$, 164.1062), C$_{10}$H$_{14}$NO requires 164.1070.

**4'-Aminovalerophenone (PAVP) (18)**

\[
\text{H}_2\text{N-}
\]

Compound 18 was prepared by a procedure similar to that of Brown and co-workers. 4'-Acetamidovalerophenone (26) (17.00 g, 77.52 mmol) and hydrochloric acid (100 mL, 4 M) in ethanol (100 mL) was heated under reflux for 2.5 h. The solution was allowed to cool, quenched with sat. ammonium hydroxide (200 mL), extracted with dichloromethane (3 × 200 mL), dried over anhydrous magnesium sulfate, filtered and the solvent removed \textit{in vacuo}. Purification by flash chromatography (hexane:ethyl acetate, 4:1) afforded 18 as a light brown solid (13.05 g, 73.65 mmol, 95%). \textit{Rf} = 0.25 (hexane:ethyl acetate, 4:1); mp 70-75 °C [lit.\textsuperscript{378} mp 71-72 °C]; $^1\text{H NMR}$ (300 MHz; CDCl$_3$) \textit{$\delta$} 0.94 (3H, t, \textit{J} = 7.5 Hz, CH$_2$CH$_2$CH$_2$C$_3$), 1.40 (2H, m, CH$_2$CH$_2$CH$_2$C$_3$), 1.69 (2H, m, CH$_2$CH$_2$CH$_2$C$_3$), 2.86 (2H, t, \textit{J} = 7.5 Hz, CH$_2$CH$_2$CH$_2$CH$_3$), 4.14 (2H, s, NH$_2$), 4.64 (2H, d, \textit{J} = 8.8 Hz, H-3', H-5') and 7.81 (2H, d, \textit{J} = 8.8 Hz, H-2', H-6'); $^{13}\text{C NMR}$ (75 MHz, CDCl$_3$) \textit{$\delta$}C: 13.9 (CH$_3$), 22.6 (CH$_2$), 27.0 (CH$_2$), 37.7 (CH$_2$), 113.7 (CH), 127.8 (C), 130.5 (CH), 150.9 (C) and 198.9 (C); \textbf{MS} (ESI, 70 eV) \textit{m/z} 178 (M$^+$, 100%); Found (M$^+$, 178.1239), C$_{11}$H$_{16}$NO requires 178.1226.

**4'-Aminocaprophenone (PACP) (19)**

\[
\text{H}_2\text{N-}
\]

A similar procedure\textsuperscript{169,171} to that previously described for the preparation of 17 was followed using caproyl chloride (2.00 g, 14.86 mmol), bromobenzene (7.00 g, 44.57 mmol) and anhydrous aluminum chloride (2.00 g, 14.86 mmol) in step 1, and crude
4'-bromocaprophenone (23) (3.15 g), cuprous oxide (0.44 g, 3.08 mmol) and aqueous ammonia (8.80 mL, 15 M) in dimethyl sulfoxide (4 mL) in step 2. Purification by flash chromatography (hexane:ethyl acetate, 4:1) afforded 19 as a light brown solid (0.86 g, 4.47 mmol, 31% over two steps). \( R_f = 0.27 \) (hexane:ethyl acetate, 2:1); \( ^1H\text{ NMR} \) (400 MHz; CDCl\(_3\)) \( \delta \) 0.90 (3H, t, \( J = 7.2 \) Hz, CH\(_2\)CH\(_2\)(CH\(_2\)_2CH\(_3\)), 1.33-1.37 (4H, m, CH\(_2\)CH\(_2\)(CH\(_2\)_2CH\(_3\)), 1.70 (2H, m, CH\(_2\)CH\(_2\)(CH\(_2\)_2CH\(_3\)), 2.85 (2H, t, \( J = 7.6 \) Hz, CH\(_2\)CH\(_2\)(CH\(_2\)_3CH\(_3\)), 4.10 (2H, s, NH\(_2\)), 6.64 (2H, d, \( J = 8.6 \) Hz, H-3', H-5') and 7.81 (2H, d, \( J = 8.6 \) Hz, H-2', H-6'); \( ^13C\text{ NMR} \) (100 MHz, CDCl\(_3\)) \( \delta \)C 14.0 (CH\(_3\)), 22.5 (CH\(_2\)), 24.6 (CH\(_2\)), 31.7 (CH\(_2\)), 38.0 (CH\(_2\)), 113.7 (CH), 127.8 (C), 130.5 (CH), 150.9 (C) and 198.9 (C); \( \text{MS} \) (ESI, 70 eV) \( m/z \) 192 (M\(^+\), 100%); Found (M\(^+\), 192.1381), C\(_{12}\)H\(_{18}\)NO requires 192.1383.

4'-Aminoheptanoylphenone (PAHP) (27)

\[
\text{H}_2\text{N} \quad \text{O} \\
\text{CH}_2\text{CH}_2\text{CH}_3
\]

Compound 27 was prepared by a procedure similar to that of Cho and co-workers.\(^{175}\) A suspension of 4-aminoacetophenone (16) (2.12 g, 15.70 mmol), \( n \)-pentanol (1.39 g, 15.70 mmol), potassium hydroxide (0.88 g, 15.70 mmol) and dichlorotris(triphenylphosphine)ruthenium(II) (0.30 g, 0.31 mmol) in 1,4-dioxane (45 mL) was heated in a sealed-tube at 90 °C for 42 h. The mixture was allowed to cool and the solvent removed in vacuo. The residue was taken up in ethyl acetate (5 mL), filtered through a short plug of silica (ethyl acetate) and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate, 4:1) afforded 27 as a light brown solid (0.55 g, 0.98 mmol, 17%). \( R_f = 0.25 \) (hexane:ethyl acetate, 2:1); mp 85-90 °C [ lit.\(^{378}\) mp 83-84 °C]; \( ^1H\text{ NMR} \) (400 MHz; CDCl\(_3\)) \( \delta \) 0.88 (3H, t, \( J = 8.8 \) Hz, CH\(_2\)CH\(_2\)(CH\(_2\)_2CH\(_3\)), 1.29-1.38 (6H, m, CH\(_2\)CH\(_2\)(CH\(_2\)_2CH\(_3\)), 1.70 (2H, m, CH\(_2\)CH\(_2\)(CH\(_2\)_2CH\(_3\)), 2.85 (2H, m, CH\(_2\)CH\(_2\)(CH\(_2\)_2CH\(_3\)), 4.11 (2H, s, NH\(_2\)), 6.63 (2H, d, \( J = 8.8 \) Hz, H-3', H-5') and 7.81 (2H, d, \( J = 8.8 \) Hz, H-2', H-6').
7. Experimental

7.2. Synthesis

J = 8.6 Hz, H-3', H-5') and 7.81 (2H, d, J = 8.6 Hz, H-2', H-6'); 13C NMR (100 MHz, CDCl3) δc 14.0 (CH3), 22.5 (CH2), 24.8 (CH2), 29.2 (CH2), 31.7 (CH2), 38.0 (CH2), 113.8 (CH), 127.8 (C), 130.5 (CH), 150.8 (C) and 198.9 (C); MS (ESI, 70 eV) m/z 206 (M+, 100%); Found (M+, 206.1541), C13H20NO requires 206.1539.

4'-Aminooctanoylphenone (PAOP) (28)

A similar procedure to that previously described for the preparation of 27 was followed using 4-aminoacetophenone (16) (0.25 g, 1.85 mmol), n-hexanol (0.19 g, 1.85 mmol), potassium hydroxide (0.10 g, 1.85 mmol), and dichlorotris(triphenylphosphine)ruthenium(II) (35 mg, 0.04 mmol) in 1,4-dioxane (5 mL). Purification by flash chromatography (hexane:ethyl acetate, 2:1) afforded 28 as a light brown solid (38 mg, 0.17 mmol, 9%). Rf = 0.40 (hexane:ethyl acetate, 2:1); mp 102-104 °C [lit. mp 105-105.5 °C]; 1H NMR (400 MHz; CDCl3) δh 0.88 (3H, t, J = 8.8 Hz, CH2CH2(CH2)4CH3), 1.26-1.35 (8H, m, CH2CH2(CH2)4CH3), 1.70 (2H, m, CH2CH2(CH2)4CH3), 2.85 (2H, t, J = 8.8 Hz, CH2CH2(CH2)4CH3), 4.08 (2H, s, NH2), 6.64 (2H, d, J = 8.6 Hz, H-3', H-5') and 7.81 (2H, d, J = 8.6 Hz, H-2', H-6'); 13C NMR (100 MHz, CDCl3) δc 14.1 (CH3), 22.6 (CH2), 24.9 (CH2), 29.2 (CH2), 29.5 (CH2), 31.7 (CH2), 38.0 (CH2), 113.8 (CH), 127.8 (C), 130.5 (CH), 150.8 (C) and 199.0 (C); MS (ESI, 70 eV) m/z 220 (M+, 100%); Found (M+, 220.1685), C14H22NO requires 220.1696.
4'-Aminodecanoylphenone (PADP) (29)\(^{380}\)

A similar procedure\(^{175}\) to that previously described for the preparation of 27 was followed using 4-aminoacetophenone (16) (0.25 g, 1.85 mmol), \(n\)-octanol (0.29 mL, 1.85 mmol), potassium hydroxide (0.10 g, 1.85 mmol) and dichlorotris(triphenylphosphine)ruthenium(II) (35 mg, 0.04 mmol) in 1,4-dioxane (5 mL). Purification by flash chromatography (hexane:ethyl acetate, 2:1) afforded 29 as a light brown solid (50 mg, 0.20 mmol, 11\%). \(R_f = 0.42\) (hexane:ethyl acetate, 2:1); mp 97-102 °C [ lit.\(^{380}\) mp 96 °C]; \(^1\)H NMR (400 MHz; CDCl\(_3\)) \(\delta_H\) 0.88 (3H, t, \(J = 6.8\) Hz, CH\(_2\)CH\(_2\)(CH\(_2\))\(_6\)CH\(_3\)), 1.26-1.36 (12H, m, CH\(_2\)CH\(_2\)(CH\(_2\))\(_6\)CH\(_3\)), 1.70 (2H, m, CH\(_2\)CH\(_2\)(CH\(_2\))\(_6\)CH\(_3\)), 2.85 (2H, t, \(J = 7.5\) Hz, CH\(_2\)CH\(_2\)(CH\(_2\))\(_6\)CH\(_3\)), 4.13 (2H, s, NH\(_2\)), 6.64 (2H, d, \(J = 8.6\) Hz, H-3', H-5') and 7.81 (2H, d, \(J = 8.6\) Hz, H-2', H-6'); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta_C\) 14.1 (CH\(_3\)), 22.6 (CH\(_2\)), 24.9 (CH\(_2\)), 29.3 (CH\(_2\)), 29.5 (3 × CH\(_2\)), 31.8 (CH\(_2\)), 38.0 (CH\(_2\)), 113.7 (CH), 127.7 (C), 130.5 (CH), 150.8 (C) and 199.0 (C); MS (ESI, 70 eV) \(m/z\) 248 (M\(^+\), 100\%); Found (M\(^+\), 248.1997), C\(_{16}\)H\(_{26}\)NO requires 248.2009.

4'-Aminododecanoylphenone (PADOP) (30)\(^{381}\)

A similar procedure\(^{175}\) to that previously described for the preparation of 27 was followed using 4-aminoacetophenone (16) (0.25 g, 1.85 mmol), \(n\)-decanol (0.35 mL, 1.85 mmol), potassium hydroxide (0.10 g, 1.85 mmol) and dichlorotris(triphenylphosphine)ruthenium(II) (35 mg, 0.04 mmol) in 1,4-dioxane (5 mL). Purification by flash chromatography (hexane:ethyl acetate, 2:1) afforded 30 as a
7. Experimental

7.2. Synthesis

light brown solid (46 mg, 0.17 mmol, 9%). \( R_f = 0.47 \) (hexane:ethyl acetate, 2:1); mp 100-105 °C [ lit.\(^{381} \) mp 102.8-103 °C]; \(^1\)H NMR (400 MHz; CDCl\(_3\)) \( \delta_H \) 0.88 (3H, t, J = 7.0 Hz, CH\(_2\)CH\(_2\)(CH\(_2\))\(_8\)CH\(_3\)), 1.26-1.37 (16H, m, CH\(_2\)CH\(_2\)(CH\(_2\))\(_8\)CH\(_3\)), 1.70 (2H, m, CH\(_2\)CH\(_2\)(CH\(_2\))\(_8\)CH\(_3\)), 2.85 (2H, t, J = 7.6 Hz, CH\(_2\)CH\(_2\)(CH\(_2\))\(_8\)CH\(_3\)), 4.10 (2H, s, NH\(_2\)), 6.64 (2H, d, J = 8.6 Hz, H-3', H-5') and 7.81 (2H, d, J = 8.6 Hz, H-2', H-6'); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta_C \) 14.1 (CH\(_3\)), 22.7 (CH\(_2\)), 24.9 (CH\(_2\)), 29.3 (CH\(_2\)), 29.6 (4 × CH\(_2\)), 31.9 (CH\(_2\)), 38.0 (CH\(_2\)), 113.7 (CH), 127.8 (C), 130.5 (CH), 150.9 (C) and 199.0 (C); MS (ESI, 70 eV) \( m/z \) 276 (M\(^+\), 100%); Found (M\(^+\), 276.2302), C\(_{18}\)H\(_{30}\)NO requires 276.2322.

7.2.1.2. Branched alkyl chain 4'-aminophenones 31-37

4'-Aminoisobutyrophenone (31)\(^{382} \)

\[ \begin{align*} &\text{A similar procedure}\(^{169,171} \) \text{ to that previously described for the preparation of 17 was} \\
&\text{followed using isobutyryl chloride (1.00 g, 9.38 mmol), bromobenzene (21) (4.42 g,} \\
&\text{28.14 mmol) and anhydrous aluminum chloride (0.82 g, 6.25 mmol) in step 1, and crude} \\
&\text{4'-bromoisobutyrophenone (38) (1.50 g), cuprous oxide (0.19 g, 1.32 mmol) and aqueous} \\
&\text{ammonia (0.90 mL, 15 M) in dimethyl sulfoxide (2 mL) in step 2. Purification by flash} \\
&\text{chromatography (hexane:ethyl acetate, 4:1) afforded 31 as a light brown solid (0.23 g,} \\
&\text{1.43 mmol, 15% over two steps).} \ R_f = 0.12 \ (\text{hexane:ethyl acetate, 4:1}); \\
&\(^1\)H NMR (300 MHz; CDCl\(_3\)) \( \delta_H \) 1.18 (6H, d, J = 6.9 Hz, CH(CH\(_3\))\(_2\)), 3.48 (1H, m, CH(CH\(_3\))\(_2\)), 4.09 (2H, s, NH\(_2\)), 6.65 (2H, d, J = 8.8 Hz, H-3', H-5') and 7.82 (2H, d, J = 8.8 Hz, H-2', H-6'); \\
&\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta_C \) 19.4 (CH\(_3\)), 34.5 (CH), 113.8 (CH), 126.6 (C), 130.7 (CH), 150.9 (C) and 202.8 (C); MS (ESI, 70 eV) \( m/z \) 164 (M\(^+\), 100%); Found (M\(^+\), 164.1071), C\(_{10}\)H\(_{14}\)NO requires 164.1070.} \]
4'-Amino-2-methylbutyrophenone (32)

A similar procedure\textsuperscript{169,171} to that previously described for the preparation of 17 was followed using 2-methylbutanoyl chloride (1.00 g, 8.29 mmol), bromobenzene (21) (3.91 g, 24.87 mmol) and anhydrous aluminum chloride (1.10 g, 8.29 mmol) in step 1, and crude 4'-bromo-2-methylbutyrophenone (39) (0.38 g), cuprous oxide (0.06 g, 0.40 mmol) and aqueous ammonia (1.5 mL, 15 M) in dimethyl sulfoxide (2 mL) in step 2. Purification by flash chromatography (hexane:ethyl acetate, 4:1) afforded 32 as a light brown solid (30 mg, 0.17 mmol, 2% over two steps). \( R_f = 0.10 \) (hexane:ethyl acetate, 4:1); mp 68-72 °C; \(^1\text{H NMR} \) (400 MHz; CDCl\textsubscript{3}) \( \delta \) 0.90 (3H, t, \( J = 7.6 \) Hz, CH\((\text{CH}_3)\text{CH}_2\text{CH}_3\)), 1.16 (3H, d, \( J = 6.8 \) Hz, CH\((\text{CH}_3)\text{CH}_2\text{CH}_3\)), 1.47 (1H, m, CH\((\text{CH}_3)\text{CH}_2\text{CH}_3\)), 1.81 (1H, m, CH\((\text{CH}_3)\text{CH}_2\text{CH}_3\)), 3.31 (1H, m, CH\((\text{CH}_3)\text{CH}_2\text{CH}_3\)), 4.07 (2H, s, NH\textsubscript{2}), 6.64 (2H, d, \( J = 8.8 \) Hz, H-3', H-5') and 7.83 (2H, d, \( J = 8.8 \) Hz, H-2', H-6'); \(^{13}\text{C NMR} \) (100 MHz, CDCl\textsubscript{3}) \( \delta \) 11.9 (CH\(_3\)), 17.1 (CH\(_3\)), 27.0 (CH\(_2\)), 41.4 (CH), 114.0 (CH), 127.5 (C), 130.6 (CH), 150.8 (C) and 202.7 (C); \( \text{IR} \) (\( \nu_{\text{max}}/\text{cm}^{-1} \)) 1226 (C-N), 1365, 1587 (N-H), 1738 (C=O), 2871, 2929, 2969, 3233 (N-H) and 3337; \( \text{MS} \) (ESI, 70 eV) \( m/z \) 178 (M\(^+\), 100%); Found (M\(^+\), 178.1226), C\(_{11}\)H\(_{16}\)NO requires 178.1226.

4'-Aminoisovalerophenone (33)

A similar procedure\textsuperscript{169,171} to that previously described for the preparation of 17 was followed using isovaleroyl chloride (1.00 g, 8.29 mmol), bromobenzene (21) (3.91 g,
24.87 mmol) and anhydrous aluminum chloride (1.10 g, 8.29 mmol) in step 1, and crude 4'-bromoisonovalerophenone (40) (1.60 g), cuprous oxide (0.23 g, 1.66 mmol) and aqueous ammonia (4.5 mL, 15 M) in dimethyl sulfoxide (2 mL) in step 2. Purification by flash chromatography (hexane:ethyl acetate, 9:1) afforded 33 as a light brown solid (0.72 g, 4.06 mmol, 49% over two steps). Rf = 0.08 (hexane:ethyl acetate, 9:1); mp 85-91 °C; 1H NMR (400 MHz; CDCl3) δH 0.98 (6H, d, J = 6.4 Hz, CH2CH(CH3)2), 2.26 (1H, m, CH2CH(CH3)2), 2.72 (2H, d, J = 6.8 Hz, CH2CH(CH3)2), 4.20 (2H, s, NH2), 6.64 (2H, d, J = 8.4 Hz, H-3′, H-5′) and 7.80 (2H, d, J = 8.4 Hz, H-2′, H-6′); 13C NMR (100 MHz, CDCl3) δC 22.8 (2 × CH3), 25.5 (CH), 46.9 (CH2), 113.6 (CH), 127.9 (C), 130.5 (CH), 151.0 (C) and 198.6 (C); IR (νmax/cm⁻¹) 813, 826, 1172, 1221 (C-N), 1364, 1583 (N-H), 1737 (C=O), 2867, 2949, 3221, 3334 (N-H) and 3414; MS (ESI, 70 eV) m/z 178 (M⁺, 100%); Found (M⁺, 178.1233), C11H16NO requires 178.1226.

4'-Bromopivalophenone (44)\textsuperscript{383}

![4'-Bromopivalophenone (44)](image)

Compound 44 was prepared by a procedure similar to that of Diaz-Valenzuela and co-workers.\textsuperscript{178} To a stirring suspension of copper bromide dimethyl sulfide complex (0.59 g, 2.87 mmol) in tetrahydrofuran (10 mL) at -78 °C, was added a solution of tert-butyllithium (1.70 mL, 2.87 mmol, 1.7 M in pentane) and the mixture stirred for 30 min. A solution of 4-bromobenzoyl chloride (45) (0.7 g, 3.19 mmol) in tetrahydrofuran (3 mL) was added dropwise at -78 °C, and mixture stirred at -78 °C for a further 4 h. The solution was quenched through the addition of sat. ammonium chloride (25 mL), extracted with ethyl acetate (3 × 25 mL), dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Purification by distillation at reduced pressure (bp 140-150 °C/15 Torr) afforded 44 as a colourless oil (0.30 g, 1.24 mmol, 43%). Rf = 0.60 (hexane:ethyl acetate, 19:1); 1H NMR (400 MHz; CDCl3) δH 1.34 (9H, s, C(CH3)3),
7. Experimental                              7.2. Synthesis

7.53-7.58 (2H, m, H-2', H-3', H-5', H-6'); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 27.9 (CH$_3$), 44.1 (C), 125.6 (C), 129.6 (CH), 131.2 (CH), 137.0 (C) and 207.7 (C).

4'-Aminopivalophenone (37)

A similar procedure$^{171}$ to that previously described in step 2 for the preparation of 17 was followed using 4'-bromopivalophenone (44) (0.30 g, 1.24 mmol), cuprous oxide (44 mg, 0.31 mmol) and aqueous ammonia (1.00 mL, 15 M) in dimethyl sulfoxide (2 mL). Purification by flash chromatography (hexane:ethyl acetate, 4:1) afforded 37 as a light brown solid (30 mg, 0.17 mmol, 14%). $R_f = 0.08$ (hexane:ethyl acetate, 4:1); mp 83-88 °C; $^1$H NMR (400 MHz; CDCl$_3$) $\delta$H 1.36 (9H, s, C(CH$_3$)$_3$), 4.10 (2H, s, NH$_2$), 6.61 (2H, d, $J = 8.8$ Hz, H-3', H-5') and 7.78 (2H, d, $J = 8.8$ Hz, H-2', H-6'); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 28.5 (CH$_3$), 43.5 (C), 113.4 (CH), 126.9 (C), 131.4 (CH), 149.8 (C) and 205.4 (C); IR (v$_{\text{max}}$/cm$^{-1}$) 1202, 1229 (C-N), 1366, 1594 (N-H), 1738 (C=O), 2971, 3016, 3231, 3347 (N-H) and 3437; MS (ESI, 70 eV) m/z 178 (M$^+$, 100%); Found (M$^+$, 178.1223), C$_{11}$H$_{16}$NO requires 178.1226.

4'-Amino-2-methylvalerophenone (34)

A similar procedure$^{169,171}$ to that previously described for the preparation of 17 was followed using 2-methylvaleroyl chloride (2.70 g, 20.06 mmol), bromobenzene (21) (9.46 g, 60.17 mmol) and anhydrous aluminum chloride (2.70 g, 20.06 mmol) in step 1, and crude 4'-bromo-2-methylvalerophenone (41) (5.40 g), cuprous oxide (0.76 g, 5.29 mmol) and aqueous ammonia (14.40 mL, 15 M) in dimethyl sulfoxide (4 mL) in step 2.
Purification by flash chromatography (hexane:ethyl acetate, 4:1) afforded 34 as a light brown solid (0.24 g, 1.23 mmol, 6% over two steps). \( R_f = 0.25 \) (hexane:ethyl acetate, 2:1); mp 61-66 °C; \(^1\)H NMR (400 MHz; CDCl\(_3\)) \( \delta_H \) 0.88 (3H, t, \( J = 6.4 \) Hz, CH(CH\(_3\))CH\(_2\)CH\(_2\)CH\(_3\)), 1.15 (3H, d, \( J = 6.8 \) Hz, CH(CH\(_3\))CH\(_2\)CH\(_2\)CH\(_3\)), 1.26-1.44 (3H, m, CH(CH\(_3\))CH\(_2\)CH\(_2\)CH\(_3\)), 1.75 (1H, m, CH(CH\(_3\))CH\(_2\)CH\(_2\)CH\(_3\)), 4.24 (2H, s, NH\(_2\)), 6.64 (2H, d, \( J = 9.0 \) Hz, H-3', H-5') and 7.83 (2H, d, \( J = 9.0 \) Hz, H-2', H-6'); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta_C \) 14.1 (CH\(_3\)), 17.5 (CH\(_3\)), 20.6 (CH\(_2\)), 36.2 (CH\(_2\)), 39.4 (CH), 113.7 (CH), 126.9 (C), 130.6 (CH) 151.1 (C) and 202.9 (C); \( \text{IR} (\nu_{\text{max/cm}^{-1}}) \) 776, 844, 971, 1149, 1235 (C-N) 1564, 1587 (N-H), 1654 (C=O), 2869, 2930, 3231, 3335 (N-H) and 3496; MS (ESI, 70 eV) \( m/z \) 178 (M\(^+\), 100%); Found (M\(^+\), 192.1384), C\(_{12}\)H\(_{18}\)NO requires 192.1383.

4'-Amino-3-methylvalerophenone (35)

A similar procedure\(^{169,171}\) to that previously described for the preparation of 17 was followed using 3-methylvaleroyl chloride (1.15 g, 8.54 mmol), bromobenzene (21) (2.69 g, 17.09 mmol) and anhydrous aluminum chloride (1.14 g, 8.54 mmol) in step 1, and crude 4'-bromo-3-methylvalerophenone (42) (2.15 g), cuprous oxide (0.30 g, 2.10 mmol) and aqueous ammonia (5.70 mL, 15 M) in dimethyl sulfoxide (2 mL) in step 2. Purification by flash chromatography (hexane:ethyl acetate, 2:1) afforded 35 as a light brown solid (0.13 g, 0.67 mmol, 8% over two steps). \( R_f = 0.28 \) (hexane:ethyl acetate, 2:1); mp 62-65 °C; \(^1\)H NMR (400 MHz; CDCl\(_3\)) \( \delta_H \) 0.89-0.94 (6H, m, CH\(_2\)CH(CH\(_3\))CH\(_2\)CH\(_3\)), 1.25 (1H, m, CH\(_2\)CH(CH\(_3\))CH\(_2\)CH\(_3\)), 1.40 (1H, m, CH\(_2\)CH(CH\(_3\))CH\(_2\)CH\(_3\)), 1.75 (1H, m, CH\(_2\)CH(CH\(_3\))CH\(_2\)CH\(_3\)), 2.63 (1H, m, CH\(_2\)CH(CH\(_3\))CH\(_2\)CH\(_3\)), 2.83 (1H, m, CH\(_2\)CH(CH\(_3\))CH\(_2\)CH\(_3\)), 4.31 (2H, s, NH\(_2\)), 6.63 (2H, d, \( J = 8.8 \) Hz, H-3', H-5') and 7.79 (2H, d, \( J = 8.8 \) Hz, H-2', H-6'); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta_C \) 11.3 (CH\(_3\)), 19.4 (CH\(_3\)), 29.6 (CH\(_2\)), 31.7 (CH), 44.9 (CH\(_2\)), 113.6
(CH), 127.6 (C), 130.5 (CH), 151.2 (C) and 198.9 (C); **IR (ν<sub>max/cm<sup>-1</sup></sub>)** 811, 830, 1172, 1286 (C-N), 1564, 1585 (N-H), 1655 (C=O), 2872, 2958, 3223, 3337 (N-H) and 3417; **MS (ESI, 70 eV)** m/z 192 (M<sup>+</sup>, 100%); Found (M<sup>+</sup>, 192.1388), **C<sub>12</sub>H<sub>18</sub>NO requires** 192.1383.

**4'-Aminoisocaprophenone (36)**

![4'-Aminoisocaprophenone (36)]

A similar procedure<sup>169,171</sup> to that previously described for the preparation of 17 was followed using isocaproyl chloride (1.15 g, 8.54 mmol), bromobenzene (21) (2.69 g, 17.09 mmol) and anhydrous aluminum chloride (1.14 g, 8.54 mmol) in step 1, and crude 4'-bromoisocaprophenone (43) (2.15 g), cuprous oxide (0.30 g, 2.10 mmol) and aqueous ammonia (5.70 mL, 15 M) in dimethyl sulfoxide (2 mL) in step 2. Purification by flash chromatography (hexane:ethyl acetate, 2:1) afforded 36 as a light brown solid (0.53 g, 2.78 mmol, 33% over two steps). **R<sub>f</sub> = 0.27** (hexane:ethyl acetate, 2:1); mp 81-85 °C; **<sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>)** δ<sub>H</sub> 0.93 (6H, d, J = 6.4 Hz, CH<sub>2</sub>CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 1.58-1.65 (3H, m, CH<sub>2</sub>CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 2.86 (2H, t, J = 7.7 Hz, CH<sub>2</sub>CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 4.11 (2H, s, NH<sub>2</sub>), 6.64 (2H, d, J = 8.4 Hz, H-3', H-5') and 7.81 (2H, d, J = 8.4 Hz, H-2', H-6'); **<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)** δ<sub>C</sub> 22.5 (CH<sub>3</sub>), 28.0 (CH), 33.8 (CH<sub>2</sub>), 36.0 (CH<sub>2</sub>), 113.8 (CH), 127.7 (C), 130.5 (CH), 150.8 (C) and 199.1 (C); **IR (ν<sub>max/cm<sup>-1</sup></sub>)** 776, 843, 1173, 1278 (C-N), 1564, 1587 (N-H), 1638 (C=O), 2868, 2952, 3226, 3331 (N-H) and 3395; **MS (ESI, 70 eV)** m/z 192 (M<sup>+</sup>, 100%); Found (M<sup>+</sup>, 192.1386), **C<sub>12</sub>H<sub>18</sub>NO requires** 192.1383.
7.2.1.3. Cyclic alkyl chain 4'-aminophenones 46-49

(4'-Aminophenyl)Cyclopropyl ketone (46)

A similar procedure\textsuperscript{169,171} to that previously described for the preparation of 17 was followed using cyclopropanecarbonyl chloride (1.00 g, 9.56 mmol), bromobenzene (21) (4.51 g, 28.70 mmol) and anhydrous aluminum chloride (1.27 g, 9.58 mmol) in step 1, and crude (4'-bromophenyl)cyclopropyl ketone (50) (50 mg), cuprous oxide (8 mg, 0.06 mmol) and aqueous ammonia (0.15 mL, 15 M) in dimethyl sulfoxide (1 mL) in step 2. Purification by flash chromatography (hexane:ethyl acetate, 4:1) afforded 46 as a light brown solid (21 mg, 0.11 mmol, 1% over two steps). \(R_f = 0.14\) (hexane:ethyl acetate, 4:1); mp 117-121 °C; \(^1\text{H NMR}\) (400 MHz; CDCl\textsubscript{3}) \(\delta_H\) 0.94 (2H, m, CH\(\text{CH}_2\text{CH}_2\)), 1.17 (2H, m, CH\(\text{CH}_2\text{CH}_2\)), 2.59 (1H, m, CH\(\text{CH}_2\text{CH}_2\)), 4.13 (2H, s, NH\(\text{CH}_2\)), 6.66 (2H, d, \(J = 8.8\) Hz, H-3', H-5') and 7.89 (2H, d, \(J = 8.8\) Hz, H-2', H-6'); \(^{13}\text{C NMR}\) (100 MHz, CDCl\textsubscript{3}) \(\delta_C\) 10.8 (CH\(_2\)), 16.2 (CH), 113.8 (CH), 128.5 (C), 130.4 (CH), 150.9 (C) and 198.5 (C); \(\text{IR}\) (\(\nu_{\text{max}}/\text{cm}^{-1}\)) 1217, 1230, 1376 (C-N), 1586 (N-H), 1738 (C=O), 2947, 2970, 3014, 3224, 3327 (N-H) and 3382; \(\text{MS}\) (ESI, 70 eV) \(m/z\) 162 (M\(^+\), 100%). Found (M\(^+\), 162.0912), C\(_{10}\)H\(_{12}\)NO requires 162.0913.
(4'-Aminophenyl)Cyclobutyl ketone (47)

A similar procedure\textsuperscript{169,171} to that previously described for the preparation of 17 was followed using cyclobutanecarbonyl chloride (1.00 g, 8.43 mmol), bromobenzene (21) (3.98 g, 25.29 mmol) and anhydrous aluminum chloride (1.12 g, 8.43 mmol) in step 1, and crude (4'-bromophenyl)cyclobutyl ketone (51) (1.25 g), cuprous oxide (0.18 g, 1.31 mmol) and aqueous ammonia (3.56 mL, 15 M) in dimethyl sulfoxide (2 mL) in step 2. Purification by flash chromatography (hexane:ethyl acetate, 4:1) afforded 47 as a light brown solid (0.10 g, 0.58 mmol, 7\% over two steps). $R_f$ = 0.14 (hexane:ethyl acetate, 4:1); mp 126-129 °C; $^1$H NMR (400 MHz; CDCl$_3$) $\delta_H$ 1.84-2.45 (6H, m, CH(CH$_2$)$_3$), 3.92 (1H, m, CH(CH$_2$)$_3$), 4.13 (2H, s, NH$_2$), 6.63 (2H, d, $J$ = 8.6 Hz, H-3', H-5') and 7.74 (2H, d, $J$ = 8.6 Hz, H-2', H-6'); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_C$ 18.2 (CH$_2$), 25.2 (CH$_2$), 41.7 (CH), 113.8 (CH), 126.2 (C), 130.7 (CH), 150.8 (C) and 199.4 (C); IR ($\nu_{\text{max}}$/cm$^{-1}$) 1218, 1249, 1355 (C-N), 1562 (N-H), 1739 (C =O), 2865, 2938, 2967, 3219, 3332 (N-H) and 3414; MS (ESI, 70 eV) $m/z$ 176 (M$^+$, 100\%). Found (M$^+$, 176.1074), C$_{11}$H$_{14}$NO requires 176.1070.

(4'-Aminophenyl)Cyclopentyl ketone (48)

A similar procedure\textsuperscript{169,171} to that previously described for the preparation of 17 was followed using cyclopentanecarbonyl chloride (1.00 g, 7.54 mmol), bromobenzene (21) (3.56 g, 22.60 mmol) and anhydrous aluminum chloride (1.00 g, 7.54 mmol) in step 1, and crude (4'-bromophenyl)cyclopentyl ketone (52) (0.54 g), cuprous oxide (0.15 g, 1.07
mmol) and aqueous ammonia (0.60 mL, 15 M) in dimethyl sulfoxide (2 mL) in step 2. Purification by flash chromatography (hexane:ethyl acetate, 4:1) afforded 48 as a light brown solid (0.27 g, 1.43 mmol, 67% over two steps). $R_f = 0.18$ (hexane:ethyl acetate, 4:1); mp 119-113 °C; $^1H$ NMR (400 MHz; CDCl$_3$) $\delta_H$ 1.65-1.91 (8H, m, CH(CH$_2$)$_4$), 3.63 (1H, m, CH(CH$_2$)$_4$), 4.07 (2H, s, NH$_2$), 6.65 (2H, d, $J = 8.6$ Hz, H-3', H-5') and 7.84 (2H, d, $J = 8.6$ Hz, H-2', H-6'); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_C$ 26.3 (CH$_2$), 30.2 (CH$_2$), 45.7 (CH), 113.8 (CH), 127.6 (C), 130.9 (CH), 150.7 (C) and 201.1 (C); IR ($\nu_{\text{max}}$/cm$^{-1}$) 1206, 1229, 1366 (C-N), 1570 (N-H), 1738 (C=O), 2868, 2947, 2970, 3016, 3253, 3341 (N-H) and 3458; MS (ESI, 70 eV) m/z 204 (M$^+$, 100%); Found (M$^+$, 204.1395), C$_{13}$H$_{18}$NO requires 204.1383.

(4'-Aminophenyl)Cyclohexyl ketone (49)

![Chemical Structure](image)

A similar procedure$^{169,171}$ to that previously described for the preparation of 17 was followed using cyclohexanecarbonyl chloride (1.00 g, 6.82 mmol), bromobenzene (21) (3.22 g, 20.46 mmol) and anhydrous aluminum chloride (0.91 g, 6.82 mmol) in step 1, and crude (4'-bromophenyl)cyclohexyl ketone (53) (0.38 g), cuprous oxide (41 mg, 0.28 mmol) and aqueous ammonia (1 mL, 15 M) in dimethyl sulfoxide (1 mL) in step 2. Purification by flash chromatography (hexane:ethyl acetate, 4:1) afforded 49 as a light brown solid (0.11 g, 0.54 mmol, 38% over two steps). $R_f = 0.16$ (hexane:ethyl acetate, 4:1); mp 77-82 °C; $^1H$ NMR (400 MHz; CDCl$_3$) $\delta_H$ 1.33-1.86 (10H, m, CH(CH$_2$)$_5$), 3.19 (1H, m, CH(CH$_2$)$_5$), 4.07 (2H, s, NH$_2$), 6.65 (2H, d, $J = 8.8$ Hz, H-3', H-5') and 7.81 (2H, d, $J = 8.8$ Hz, H-2', H-6'); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_C$ 26.0 (2 × CH$_2$), 29.6 (CH$_2$), 45.0 (CH), 113.8 (CH), 126.7 (C), 130.6 (CH), 150.8 (C) and 202.1 (C); IR ($\nu_{\text{max}}$/cm$^{-1}$) 1216, 1229, 1366 (C-N), 1587 (N-H), 1738 (C=O), 2850, 2930, 2970, 3016, 3230, 3339 (N-H) and 3458; MS (ESI, 70 eV) m/z 190 (M$^+$, 100%); Found (M$^+$, 190.1234), C$_{12}$H$_{16}$NO requires 190.1226.
7. Experimental

7.2. Synthesis

7.2.2. Aryl 4’-aminophenones

7.2.2.1. 4’-Aminobenzophenone (54), 4-aminochalcones (55) and (63)

4’-Aminobenzophenone (54)\(^{384}\)

\[
\text{H}_2\text{N}\begin{array}{c}
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\text{CH} \\
\end{array}
\]

Compound 54 was prepared by a two-step procedure similar to that of Jeon and co-workers,\(^ {195}\) and a similar procedure\(^ {171}\) to that previously described in step two for the preparation of 17. In the first step, to a stirring suspension of anhydrous aluminum chloride (0.43 g, 3.24 mmol) in benzene (3 mL) at room temperature was added dropwise over 10 mins 4-bromobenzoyl chloride (45) (0.50 g, 2.28 mmol), and the mixture heated under reflux for 36 h. The reaction was quenched with sat. sodium hydrogen carbonate (25 mL), extracted with dichloromethane (3 × 25 mL), dried over anhydrous magnesium sulfate, filtered and the solvent removed \textit{in vacuo} to afford crude 4’-bromobenzophenone (56) as a light brown solid (0.29 g), which was used without further purification. In the second step, a similar procedure\(^ {171}\) to that previously described in step two for the preparation of 17 was followed using crude 4’-bromobenzophenone (56) (0.29 g), cuprous oxide (40 mg, 0.28 mmol) and aqueous ammonia (0.75 mL, 15 M) in dimethyl sulfoxide (3 mL). Purification by flash chromatography (hexane:ethyl acetate, 4:1) afforded 54 as a light brown solid (0.10 g, 0.51 mmol, 22%). \(R_t = 0.20\) (hexane:ethyl acetate, 4:1); \(^1\text{H NMR} (400 \text{ MHz}; \text{CDCl}_3) \delta_\text{H} 4.15 (2\text{H}, \text{s, NH}_2), 6.67 (2\text{H}, \text{d, J} = 8.8 \text{ Hz, H-3’, H-5’}), 7.45 (2\text{H}, \text{td, J} = 6.8 \text{ Hz, J} = 0.8 \text{ Hz, H-3”, H-5”}), 7.51-7.56 (1\text{H}, \text{m, H-4”}) \text{ and } 7.70-7.74 (4\text{H}, \text{m, H-2’, H-6’, H-2”, H-6”}); \(^{13}\text{C NMR} (100 \text{ MHz}, \text{CDCl}_3) \delta_\text{C} 113.6 \text{ (CH), 127.4 (C), 128.1 (CH), 129.5 (CH), 131.4 (CH), 132.9 (CH), 138.8 (C), 150.9 (C) and 195.3 (C); MS (ESI, 70 eV) } m/z \text{ 198 (M+, 100%); Found (M+, 198.0925), C}_{13}\text{H}_{12}\text{NO requires 198.0913.}
*(E)-4′-Aminochalcone (55)*

Compound 55 was prepared by a procedure similar to that of Kubota and co-workers. To a stirring solution of 4-aminoacetophenone (16) (0.54 g, 4.00 mmol) and benzaldehyde (57) (0.43 g, 4.00 mmol) in ethanol (15 mL) at room temperature was added dropwise an aqueous solution of sodium hydroxide (10 mL, 1 M), and the mixture stirred at room temperature for 24 h. The resulting precipitate was collected by filtration, washed with ice-cold ethanol (10 mL), then water (10 mL), and dried *in vacuo*. Purification by flash chromatography (dichloromethane:diethyl ether, 49:1) afforded 55 as a bright yellow solid (0.55 g, 2.46 mmol, 61%). \( R_f = 0.15 \) (dichloromethane:diethyl ether, 49:1); \(^1\)H NMR (300 MHz; CDCl₃) \( \delta_H \) 4.15 (2H, s, NH₂), 6.70 (2H, d, \( J = 8.6 \) Hz, H-3′, H-5′), 7.38-7.43 (3H, m, H-3″, H-4″, H-5″), 7.54 (1H, d, \( J = 15.6 \) Hz, COCH=CH), 7.63 (2H, dd, \( J = 5.6 \) Hz, \( J = 2.0 \) Hz, H-2″, H-6″), 7.78 (1H, d, \( J = 15.6 \) Hz, COCH=CH) and 7.81 (2H, d, \( J = 8.6 \) Hz, H-2′, H-6′); \(^{13}\)C NMR (75 MHz, CDCl₃) \( \delta_C \) 113.9 (CH), 122.1 (CH), 128.3 (CH), 128.6 (C), 128.9 (CH), 130.1 (CH), 131.1 (CH), 135.3 (C), 143.1 (CH), 151.1 (C), 188.7 (C); MS (ESI, 70 eV) \( m/z \) 224 (M⁺, 100%); Found (M⁺, 224.1092), C₁₅H₁₄NO requires 224.1070.

*(E)-4″-Aminochalcone (63)*

Compound 63 was prepared by a two-step procedure, with the first step similar to that previously described for the preparation of 55, and the second step similar to that of Ono
and co-workers. In the first step, to a stirring solution of 4-nitrobenzaldehyde (69) (1.00 g, 6.62 mmol) and acetophenone (7) (0.80 g, 6.62 mmol) in ethanol (15 mL) at room temperature was added an aqueous solution of sodium hydroxide (10 mL, 1 M), and the mixture stirred at room temperature for 24 h. The resulting precipitate was collected by filtration, washed with ice-cold ethanol (10 mL), then water (10 mL), and dried in vacuo to afford (E)-4′-nitrochalcone (70) as an orange solid (1.50 g), which was used without further purification. In the second step, to a stirring suspension of crude (E)-4′-nitrochalcone (70) (1.50 g) in ethanol (25 mL) was added tin(II) chloride (5.62 g, 29.62 mmol), and the mixture heated under reflux for 1 h. The mixture was then allowed to cool, quenched with aqueous sodium hydroxide (25 mL, 1 M), extracted with ethyl acetate (3 × 25 mL), dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate, 4:1) afforded 63 as a yellow solid (0.87 g, 3.90 mmol, 59%). Rf = 0.30 (hexane:ethyl acetate, 2:1); 1H NMR (300 MHz; CDCl3) δH 4.00 (2H, s, NH2), 6.68 (2H, d, J = 8.4 Hz, H-3″, H-5″), 7.34 (1H, d, J = 15.6 Hz, CH=CHCO), 7.46-7.59 (5H, m, H-3′, H-4′, H-5′, H-2″, H-6″), 7.76 (1H, d, J = 15.6 Hz, CH=CHCO) and 8.00 (2H, d, J = 6.9 Hz, H-2′, H-6′); 13C NMR (75 MHz, CDCl3) δC 114.9 (CH), 118.0 (CH), 125.2 (C), 128.3 (CH), 128.4 (CH), 130.5 (CH), 132.3 (CH), 139.2 (C), 145.5 (CH), 149.1 (C) and 190.7 (C); MS (ESI, 70 eV) m/z 224 (M+, 100%); Found (M+, 224.1087), C15H14NO requires 224.1070.

7.2.2.2. 4′-Aminochalcones 58-62

(E)-4′-Amino-4″-nitrochalcone (58)

A similar procedure to that previously described for the preparation of 55 was followed using 4-aminoacetophenone (16) (1.00 g, 7.40 mmol), 4-nitrobenzaldehyde (64) (0.34 g,
2.22 mmol) and an aqueous solution of sodium hydroxide (5 mL, 1 M) in ethanol (7.5 mL). Purification by flash chromatography (dichloromethane:diethyl ether, 49:1) afforded 58 as a bright orange solid (0.22 g, 0.82 mmol, 44%). $R_f = 0.25$ (dichloromethane:diethyl ether, 49:1); $^1$H NMR (300 MHz; CDCl$_3$) $\delta_H$ 4.22 (2H, s, NH$_2$), 6.71 (2H, d, $J = 8.7$ Hz, H-3', H-5'), 7.63 (1H, d, $J = 15.9$ Hz, COCH=CH), 7.75-7.81 (3H, m, COCH=CH, H-2", H-6") and 8.26 (2H, d, $J = 8.7$ Hz, H-2', H-6') and 8.26 (2H, d, $J = 8.7$ Hz, H-3", H-5"); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_C$ 113.9 (CH), 124.2 (CH), 125.4 (CH), 127.9 (C), 129.8 (CH), 131.4 (CH), 139.9 (CH), 141.6 (C), 148.3 (C), 151.6 (C), and 187.0 (C); MS (ESI, 70 eV) $m/z$ 269 (M$^+$, 100%); Found (M$^+$, 269.0930), C$_{15}$H$_{13}$N$_2$O$_3$ requires 269.0921.

(E)-4'-Amino-4''-chlorochalcone (59)$^{186}$

A similar procedure$^{196}$ to that previously described for the preparation of 55 was followed using 4-aminoacetophenone (16) (1.00 g, 7.40 mmol), 4-chlorobenzaldehyde (65) (0.83 g, 5.92 mmol) and an aqueous solution of sodium hydroxide (10 mL, 1 M) in ethanol (15 mL). Purification by flash chromatography (dichloromethane:diethyl ether, 49:1) afforded 59 as a bright yellow solid (0.20 g, 0.78 mmol, 13%). $R_f = 0.25$ (dichloromethane:diethyl ether, 49:1); $^1$H NMR (300 MHz; CDCl$_3$) $\delta_H$ 4.18 (2H, s, NH$_2$), 6.69 (2H, d, $J = 9.0$ Hz, H-3', H-5'), 7.37 (2H, d, $J = 9.0$ Hz, H-3", H-5") and 7.92 (2H, d, $J = 9.0$ Hz, H-2', H-6'); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta_C$ 113.9 (CH), 122.5 (CH), 128.2 (CH), 128.4 (C), 129.1 (CH), 131.1 (CH), 133.8 (C), 135.9 (C), 141.6 (CH), 151.2 (C), 187.7 (C); MS (ESI, 70 eV) $m/z$ 258 (M$^+$, 100%); Found (M$^+$, 258.0700), C$_{15}$H$_{13}$ClNO requires 258.0721.
(E)-4'-Amino-4''-methylchalcone (60)

A similar procedure\textsuperscript{[196]} to that previously described for the preparation of 55 was followed using 4-aminoacetophenone (16) (1.00 g, 7.40 mmol), 4-methylbenzaldehyde (66) (1.07 g, 8.90 mmol) and an aqueous solution of sodium hydroxide (10 mL, 1 M) in ethanol (15 mL). Purification by flash chromatography (dichloromethane:diethyl ether, 49:1) afforded 60 as a bright yellow solid (0.51 g, 2.01 mmol, 29%). $R_f = 0.30$ (dichloromethane:diethyl ether, 49:1); \textsuperscript{1}H NMR (300 MHz; CDCl\textsubscript{3}) $\delta$H 2.37 (3H, s, CH$_3$), 4.20 (2H, s, NH$_2$), 6.68 (2H, d, $J = 8.5$ Hz, H-3', H-5'), 7.19 (2H, d, $J = 9.0$ Hz, H-3'', H-5''), 7.47-7.53 (3H, m, COCH=CH, H-2'', H-6''), 7.76 (1H, d, $J = 16.0$ Hz, COCH=CH) and 7.92 (2H, d, $J = 8.5$ Hz, H-2', H-6'); \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}) $\delta$C 21.4 (CH$_3$), 113.9 (CH), 121.0 (CH), 128.2 (CH), 128.5 (C), 129.5 (CH), 131.0 (CH), 132.5 (C), 140.5 (C), 143.1 (CH), 151.1 (C), 188.2 (C); MS (ESI, 70 eV) m/z 238 (M$^+$, 100%); Found (M$^+$, 238.1234), C$_{16}$H$_{16}$NO requires 238.1226.

(E)-4'-Amino-4''-methoxychalcone (61)

A similar procedure\textsuperscript{[196]} to that previously described for the preparation of 55 was followed using 4-aminoacetophenone (16) (1.00 g, 7.40 mmol), 4-methoxybenzaldehyde (67) (0.80 g, 5.92 mmol) and an aqueous solution of sodium hydroxide (10 mL, 1 M) in ethanol (15 mL). Purification by flash chromatography (dichloromethane:diethyl ether, 49:1) afforded 61 as a tan brown solid (46 mg, 0.18 mmol, 3%). $R_f = 0.22$ (dichloromethane:diethyl ether, 49:1); \textsuperscript{1}H NMR (300 MHz; CDCl\textsubscript{3}) $\delta$H 3.84 (3H, s,
CH₃), 4.15 (2H, s, NH₂), 6.69 (2H, d, J = 9.0 Hz, H-3', H-5'), 6.92 (2H, d, J = 9.0 Hz, H-3'', H-5''), 7.43 (1H, d, J = 15.6 Hz, COCH=CH), 7.58 (2H, d, J = 9.0 Hz, H-2'', H-6''), 7.75 (1H, d, J = 15.0 Hz, COCH=CH) and 7.92 (2H, d, J = 9.0 Hz, H-2', H-6'); ¹³C NMR (75 MHz, CDCl₃) δC 55.4 (CH₃), 113.9 (CH), 114.3 (CH), 119.7 (CH), 128.1 (C), 128.8 (C), 129.9 (CH), 130.9 (CH), 142.9 (CH), 150.9 (C), 161.3 (C) and 188.2 (C); MS (ESI, 70 eV) m/z 254 (M⁺, 100%); Found (M⁺, 254.1191), C₁₆H₁₆NO₂ requires 254.1176.

(E)-4'-Amino-4''-dimethylaminochalcone (62)

![Chemical Structure (E)-4'-Amino-4''-dimethylaminochalcone (62)](image)

A similar procedure to that previously described for the preparation of 55 was followed using 4-aminoacetophenone (16) (1.00 g, 7.40 mmol), 4-dimethylaminobenzaldehyde (68) (1.10 g, 7.40 mmol) and an aqueous solution of sodium hydroxide (10 mL, 1 M) in ethanol (15 mL). Purification by flash chromatography (dichloromethane:diethyl ether, 49:1) afforded 62 as an orange brown solid (0.32 g, 1.20 mmol, 16%). Rf = 0.20 (dichloromethane:diethyl ether, 49:1); ¹H NMR (400 MHz; CDCl₃) δH 3.03 (6H, s, CH₃), 4.11 (2H, s, NH₂), 6.67-6.71 (4H, m, H-3', H-5', H-3'', H-5''), 7.35 (1H, d, J = 15.6 Hz, COCH=CH), 7.53 (2H, d, J = 9.0 Hz, H-2'', H-6''), 7.76 (1H, d, J = 15.0 Hz, COCH=CH) and 7.92 (2H, d, J = 6.8 Hz, H-2', H-6'); ¹³C NMR (100 MHz, CDCl₃) δC 40.1 (CH₃), 113.9 (CH), 116.9 (CH), 123.1 (C), 129.4 (C), 130.0 (CH), 130.8 (CH), 144.1 (CH), 150.6 (C), 151.7 (C) and 188.4 (C); MS (ESI, 70 eV) m/z 267 (M⁺, 100%). Found (M⁺, 267.1509), C₁₆H₁₆NO₂ requires 267.1492.
7.2.2.3. 4'-Aminodihydrochalcone (71) and homologues 72 and 73

2-Phenyl-4'-aminoacetophenone (72)

A similar procedure\textsuperscript{169,171} to that previously described for the preparation of 17 was followed using phenylacetyl chloride (0.25 g, 1.59 mmol), bromobenzene (21) (0.50 g, 3.18 mmol) and anhydrous aluminum chloride (0.21 g, 1.59 mmol) in step 1, and crude 2-phenyl-4'-bromoacetophenone (74) (0.38 g), cuprous oxide (50 mg, 0.34 mmol) and aqueous ammonia (1 mL, 15 M) in dimethyl sulfoxide (2 mL) in step 2. Purification by flash chromatography (hexane:ethyl acetate, 9:1) afforded 72 as a light brown solid (71 mg, 0.34 mmol, 21%). $R_f = 0.31$ (hexane:ethyl acetate, 9:1); $^1$H NMR (400 MHz; CDCl\textsubscript{3}) $\delta$H 4.12 (2H, s, NH$_2$), 4.18 (2H, s, CH$_2$), 6.61 (2H, d, $J = 9.0$ Hz, H-3', H-5'), 7.21-7.32 (5H, m, ArH) and 7.85 (2H, d, $J = 9.0$ Hz, H-2', H-6'); $^{13}$C NMR (100 MHz, CDCl\textsubscript{3}) $\delta$C 44.9 (CH$_2$), 113.8 (CH), 126.6 (CH), 127.1 (C), 128.5 (CH), 129.4 (CH), 131.1 (CH), 135.4 (C), 151.2 (C) and 195.8 (C); MS (ESI, 70 eV) m/z 212 (M$^+$, 100%). Found (M$^+$, 212.1073), C$_{14}$H$_{14}$NO requires 212.1070.

4'-Aminodihydrochalcone (71)

Compound 71 was prepared by a procedure similar to that of Cho and co-workers.\textsuperscript{199} A suspension of 4-aminoacetophenone (16) (0.25 g, 1.85 mmol), benzaldehyde (57) (0.20 g, 1.85 mmol), potassium hydroxide (0.10 g, 1.85 mmol) and dichlorotris(triphenylphosphine)ruthenium(II) (36 mg, 0.04 mmol) in 1,4-dioxane (5 mL)
was heated in a sealed-tube at 90 °C for 42 h. The mixture was then allowed to cool, the solvent removed *in vacuo*, the residue taken up in ethyl acetate (5 mL) and filtered through a short plug of silica (ethyl acetate) and the solvent removed *in vacuo*. Purification by flash chromatography (hexane:ethyl acetate, 4:1) afforded 71 as a grey solid (0.15 g, 0.65 mmol, 35%). *R*<sub>f</sub> = 0.25 (hexane:ethyl acetate, 2:1); 1<sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>) δ<sub>H</sub> 3.05 (2H, t, J = 9.2 Hz, COCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 3.15 (2H, t, J = 8.8 Hz, COCH<sub>2</sub>CH<sub>2</sub>), 4.23 (2H, s, NH<sub>2</sub>), 6.56 (2H, d, J = 8.8 Hz, H-3', H-5'), 7.13-7.29 (5H, m, ArH) and 7.77 (2H, d, J = 8.8 Hz, H-2', H-6'); 13<sup>C</sup> NMR (100 MHz, CDCl<sub>3</sub>) δ<sub>C</sub> 30.4 (CH<sub>2</sub>), 39.5 (CH<sub>2</sub>), 113.5 (CH), 125.8 (CH), 126.9 (C), 128.2 (CH), 128.3 (CH), 130.3 (CH), 141.5 (C), 151.3 (C) and 197.5 (C); IR (ν<sub>max/cm</sub>-1) 698, 842, 1172, 1243 (C-N), 1367, 1588 (N-H), 2926, 2963, 3228, 3335 (N-H) and 3406; MS (ESI, 70 eV) *m/z* 226 (M<sup>+</sup>, 100%); Found (M<sup>+</sup>, 226.1242), C<sub>15</sub>H<sub>16</sub>NO requires 226.1226.

4'-Amino-4-phenylbutyrophenone (73)

A similar procedure<sup>175</sup> to that previously described for the preparation of 27 was followed using 4-aminoacetophenone (16) (0.25 g, 1.85 mmol), 2-phenylethanol (75) (0.23 g, 1.85 mmol), potassium hydroxide (0.10 g, 1.85 mmol) and dichlorotris(triphenylphosphine)ruthenium(II) (35 mg, 0.04 mmol) in 1,4-dioxane (5 mL). Purification by flash chromatography (hexane:ethyl acetate, 4:1) afforded 73 as a light brown solid (0.11 g, 0.50 mmol, 27%). *R*<sub>f</sub> = 0.40 (hexane:ethyl acetate, 2:1); mp 128-132 °C; 1<sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>) δ<sub>H</sub> 2.05 (2H, m, COCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub>), 2.70 (2H, t, J = 7.6 Hz, COCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.88 (2H, t, J = 7.6 Hz, COCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 4.11 (2H, s, NH<sub>2</sub>), 6.62 (2H, d, J = 8.8 Hz, H-3', H-5'), 7.17-7.30 (5H, m, ArH) and 7.77 (2H, d, J = 8.8 Hz, H-2', H-6'); 13<sup>C</sup> NMR (100 MHz, CDCl<sub>3</sub>) δ<sub>C</sub> 26.1 (CH<sub>2</sub>), 35.3 (CH<sub>2</sub>), 37.1 (CH<sub>2</sub>), 113.7 (CH), 125.8 (CH), 127.7 (C), 128.3 (CH), 128.5 (CH), 130.5 (CH), 141.9 (C), 150.9 (C) and 198.4 (C); IR (ν<sub>max/cm</sub>-1) 697, 821, 980, 1171, 1268 (C-N), 1565, 1587 (N-
H), 1653 (C=O), 2947, 3031, 3332 (N-H) and 3395; MS (ESI, 70 eV) m/z 240 (M+, 100%). Found (M+, 240.1381), C_{16}H_{16}NO requires 240.1383.

7.2.2.4. Heterocyclic 4'-aminodihydrochalcone analogues 76-78 and cyclohexyl aminodihydrochalcone analogue 79

4'-Amino-3-(pyridin-2''-yl)propiophenone (76)

A similar procedure\textsuperscript{199} to that previously described for the preparation of 71 was followed using 4-aminoacetophenone (16) (0.50 g, 3.70 mmol), pyridine-2-carboxaldehyde (80) (0.35 mL, 3.70 mmol), potassium hydroxide (0.25 g, 4.44 mmol) and dichlorotris(triphenylphosphine)ruthenium(II) (71 mg, 0.07 mmol) in 1,4-dioxane (10 mL). Purification by flash chromatography (hexane:ethyl acetate, 1:1) afforded 76 as a light brown solid (31 mg, 0.14 mmol, 4%). R\textsubscript{f} = 0.05 (hexane:ethyl acetate, 2:1); mp 123-128 °C; \textsuperscript{1}H NMR (400 MHz; CDCl\textsubscript{3}) δ\textsubscript{H} 3.21 (2H, t, J = 7.5 Hz, COCH\textsubscript{2}C\textsubscript{H}\textsubscript{2}), 3.40 (2H, t, J = 7.5 Hz, COC\textsubscript{H}\textsubscript{2}CH\textsubscript{2}), 4.14 (2H, s, NH\textsubscript{2}), 6.63 (2H, d, J = 8.8 Hz, H-3', H-5'), 7.08-7.27 (2H, m, H-3'', H-5''), 7.58 (1H, td, , J = 7.6 Hz, J = 1.6 Hz, H-4''), 7.84 (2H, d, J = 8.8 Hz, H-2', H-6') and 8.52 (1H, d, , J = 4.4 Hz, H-6''); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) δ\textsubscript{C} 26.8 (CH\textsubscript{2}), 37.3 (CH\textsubscript{2}), 113.7 (CH), 121.1 (CH), 123.4 (CH), 127.4 (C), 130.5 (CH), 136.3 (CH), 149.2 (CH), 151.3 (C) 161.1 (C) and 197.4 (C); IR (ν\textsubscript{max}/cm\textsuperscript{-1}) 751, 833, 980, 1169, 1321 (C-N), 1562, 1590 (N-H), 1653 (C=O), 2851, 2916, 3203, 3329 (N-H) and 3442; MS (ESI, 70 eV) m/z 249 (MNa\textsuperscript{+}, 100%). Found (M\textsuperscript{+}, 227.1173), C\textsubscript{14}H\textsubscript{12}N\textsubscript{2}O requires 227.1179.
4'-Amino-3-(furan-2''-yl)propiophenone (77)

A similar procedure\textsuperscript{199} to that previously described for the preparation of 71 was followed using 4-aminoacetophenone (16) (0.50 g, 3.70 mmol), furan-2-carboxaldehyde (81) (0.31 mL, 3.70 mmol), potassium hydroxide (0.25 g, 4.44 mmol) and dichlorotris(triphenylphosphine)ruthenium(II) (71 mg, 0.07 mmol) in 1,4-dioxane (10 mL). Purification by flash chromatography (hexane:ethyl acetate, 2:1) afforded 77 as a light brown solid (0.12 g, 0.54 mmol, 15%). $R_f = 0.20$ (hexane:ethyl acetate, 2:1); mp 134-137 °C; $^1$H NMR (400 MHz; CDCl$_3$) $\delta$ 3.06 (2H, t, $J = 7.2$ Hz, COCH$_2$C$_6$H$_2$), 3.32 (2H, t, $J = 7.2$ Hz, COC$_6$H$_4$CH$_2$), 4.15 (2H, s, NH$_2$), 6.03 (1H, m, H-3''), 6.27 (1H, dd, $J = 3.0$ Hz, $J = 1.6$ Hz, H-4''), 6.64 (2H, d, $J = 8.8$ Hz, H-3', H-5'), 7.30 (1H, m, H-5'') and 7.83 (2H, d, $J = 8.8$ Hz, H-2', H-6'); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ C 22.3 (CH$_2$), 36.2 (CH$_2$), 105.1 (CH), 110.2 (CH), 113.7 (CH), 127.3 (C), 130.5 (CH), 141.0 (CH), 151.1 (C), 155.2 (C) and 196.8 (C); IR (\nu$_{\text{max}}$/cm$^{-1}$) 736, 835, 1147 (C-O), 1277 (C-N), 1367, 1590 (N-H), 1655 (C=O), 2904, 2982, 3221, 3331 (N-H) and 3443; MS (ESI, 70 eV) m/z 216 (M$^+$, 100%). Found (M$^+$, 216.1013), C$_{13}$H$_{14}$NO$_2$ requires 216.1019.

4'-Amino-3-(thiophen-2''-yl)propiophenone (78)

A similar procedure\textsuperscript{199} to that previously described for the preparation of 71 was followed using 4-aminoacetophenone (16) (0.50 g, 3.70 mmol), thiophene-2-carboxaldehyde (82) (0.44 mL, 4.07 mmol), potassium hydroxide (0.25 g, 4.44 mmol) and dichlorotris(triphenylphosphine)ruthenium(II) (71 mg, 0.07 mmol) in 1,4-dioxane (10 mL). Purification by flash chromatography (hexane:ethyl acetate, 2:1) afforded 78 as a light brown solid (0.10 g, 0.45 mmol, 16%). $R_f = 0.25$ (hexane:ethyl acetate, 2:1); mp 180-182 °C; $^1$H NMR (400 MHz; CDCl$_3$) $\delta$ 3.06 (2H, t, $J = 7.4$ Hz, COCH$_2$C$_6$H$_2$), 3.34 (2H, t, $J = 7.4$ Hz, COC$_6$H$_4$CH$_2$), 4.15 (2H, s, NH$_2$), 6.03 (1H, m, H-3''), 6.27 (1H, dd, $J = 3.0$ Hz, $J = 1.6$ Hz, H-4''), 6.64 (2H, d, $J = 8.8$ Hz, H-3', H-5'), 7.30 (1H, m, H-5'') and 7.83 (2H, d, $J = 8.8$ Hz, H-2', H-6'); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ C 25.2 (CH$_2$), 36.2 (CH$_2$), 105.1 (CH), 110.2 (CH), 113.7 (CH), 127.3 (C), 130.5 (CH), 141.0 (CH), 151.1 (C), 155.2 (C) and 196.8 (C); IR (\nu$_{\text{max}}$/cm$^{-1}$) 736, 835, 1147 (C-O), 1277 (C-N), 1367, 1590 (N-H), 1655 (C=O), 2904, 2982, 3221, 3331 (N-H) and 3443; MS (ESI, 70 eV) m/z 216 (M$^+$, 100%). Found (M$^+$, 216.1013), C$_{13}$H$_{14}$NO$_2$ requires 216.1019.
mL). Purification by flash chromatography (hexane:ethyl acetate, 4:1) afforded 78 as a light brown solid (0.34 g, 1.47 mmol, 40%). \( R_f = 0.25 \) (hexane:ethyl acetate, 2:1); \(^1\text{H NMR} \) (400 MHz; CDCl\(_3\)) \( \delta_H \) 3.26 (4H, s, COCH\(_2\)CH\(_2\)), 4.12 (2H, s, NH\(_2\)), 6.64 (2H, d, \( J = 8.6 \) Hz, H-3', H-5'), 6.85 (1H, d, \( J = 3.6 \) Hz, H-3'\(^\prime\)), 6.91 (1H, m, H-4'\(^\prime\)), 7.11 (1H, dd, \( J = 4.8 \) Hz, \( J = 1.0 \) Hz, H-5'\(^\prime\)) and 7.82 (2H, d, \( J = 8.6 \) Hz, H-2', H-6'); \(^13\text{C NMR} \) (100 MHz, CDCl\(_3\)) \( \delta_C \) 24.5 (CH\(_2\)), 39.8 (CH\(_2\)), 113.8 (CH), 123.2 (CH), 124.5 (CH), 126.8 (CH), 127.4 (C), 130.5 (CH), 144.3 (C), 151.1 (C) and 196.7 (C); \(^\text{IR} \) (\( \nu_{\text{max}}/\text{cm}^{-1} \)) 698, 831, 977, 1170 (C-O), 1216, 1287 (C-N), 1561, 1586 (N-H), 1629, 2917, 3220, 3332 (N-H) and 3431; \(^\text{MS} \) (ESI, 70 eV) \( m/z \) 232 (M\(^+\), 100%). Found (M\(^+\), 232.0781), C\(_{13}\)H\(_{14}\)NOS requires 232.0791.

\textbf{4'-Amino-3-cyclohexylpropiophenone (79)}

A similar procedure\(^{199}\) to that previously described for the preparation of 71 was followed using 4-aminoacetophenone (16) (0.25 g, 1.85 mmol), cyclohexane carboxaldehyde (83) (0.23 mL, 2.04 mmol), potassium hydroxide (0.10 g, 1.85 mmol) and dichlorotris(triphenylphosphine)ruthenium(II) (35 mg, 0.04 mmol) in 1,4-dioxane (5 mL). Purification by flash chromatography (hexane:ethyl acetate, 2:1) afforded 79 as a light brown solid (80 mg, 0.35 mmol, 19%). \( R_f = 0.40 \) (hexane:ethyl acetate, 1:1); mp 74-79 °C; \(^1\text{H NMR} \) (400 MHz; CDCl\(_3\)) \( \delta_H \) 0.91-1.76 (13H, m, COCH\(_2\)CH\(_2\)(CH(CH\(_2\))\(_3\))), 2.86 (2H, t, \( J = 8.0 \) Hz, COCH\(_2\)CH\(_2\)), 4.17 (2H, s, NH\(_2\)), 6.64 (2H, d, \( J = 8.6 \) Hz, H-3', H-5') and 7.81 (2H, d, \( J = 8.6 \) Hz, H-2', H-6'); \(^13\text{C NMR} \) (100 MHz, CDCl\(_3\)) \( \delta_C \) 26.2 (CH\(_2\)), 26.5 (CH\(_2\)), 32.3 (CH), 33.2 (CH\(_2\)), 35.5 (C H\(_2\)), 37.5 (CH\(_2\)), 113.7 (CH), 127.5 (C), 130.5 (CH), 150.9 (C) and 199.3 (C); \(^\text{IR} \) (\( \nu_{\text{max}}/\text{cm}^{-1} \)) 787, 835, 1168, 1279 (C-N), 1319, 1586 (N-H), 1625, 1652 (C=O), 2849, 2916, 3211, 3350 (N-H) and 3463; \(^\text{MS} \) (ESI, 70 eV) \( m/z \) 232 (M\(^+\), 100%). Found (M\(^+\), 232.1686), C\(_{15}\)H\(_{22}\)NO requires 232.1696.
7.2.2.5. Ring-fused aryl 4'-aminophenones 104 and 86

A) 5-aminooindanone (104)

(E)-3’-Nitrocinnamic acid (92)\textsuperscript{389}

\[ \text{O}_2\text{N} \]
\[ \text{O} \]
\[ \text{OH} \]

Compound 92 was prepared by a procedure similar to that of Dipal and co-workers.\textsuperscript{206} To a stirring solution of 3-nitrobenzaldehyde (93) (10.00 g, 66.17 mmol) in pyridine (34 mL) at room temperature was added dropwise malonic acid (12.42 g, 119.00 mmol), followed by piperidine (0.56 mL, 5.67 mmol), and the mixture heated under reflux for 18 h. The mixture was allowed to cool and quenched through the addition of hydrochloric acid (200 mL, 2 M). The mixture was stirred for a further 15 min and the resulting precipitate collected by filtration, washed with water, and dried in vacuo to afford 92 as a pale orange solid (10.00 g), which was used without further purification. $R_f = 0.25$ (hexane:ethyl acetate, 1:1); $^1\text{H NMR}$ (400 MHz; CDCl$_3$) $\delta_H$ 6.58 (1H, d, $J = 16.0$ Hz, CH=C=HCO), 7.72-7.78 (2H, m, CH=CHCO, H-5’), 8.21-8.26 (2H, m, H-4’, H-6’), 8.55 (1H, s, H-2’) and 12.67 (1H, s, OH); $^{13}\text{C NMR}$ (100 MHz, CDCl$_3$) $\delta_C$ 123.3 (CH), 123.8 (CH), 123.4 (CH), 131.3 (CH), 135.0 (CH), 137.1 (C), 142.5 (CH), 149.3 (C) and 168.1 (C).

(E)-Methyl-3’-nitrocinnamate (99)\textsuperscript{390}

\[ \text{O}_2\text{N} \]
\[ \text{O} \]

Compound 99 was prepared by a procedure similar to that of Stover and co-workers.\textsuperscript{207} To a stirring solution of (E)-3’-nitrocinnamic acid (99) (10 g) in methanol (50 mL) at
7. Experimental

7.2. Synthesis

Room temperature was added dropwise sulfuric acid (5 mL, 18 M), and the mixture heated under reflux for 24 h. The reaction was allowed to cool and the solvent removed in vacuo. The residue was taken up in ethyl acetate (50 mL), washed with sat. sodium bicarbonate (100 mL), the separated aqueous layer further extracted with ethyl acetate (2 × 100 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate 2:1) afforded 99 as a white solid (10.25 g, 49.48 mmol, 75% over two steps). \( R_t = 0.50 \) (hexane:ethyl acetate, 1:1); \(^1\text{H NMR} \) (400 MHz; CDCl\(_3\) \( \delta_H \)) 3.84 (3H, s, CH\(_3\)), 6.57 (1H, d, \( J = 16.2 \) Hz, CH=CHCO), 7.60 (1H, t, \( J = 8.0 \) Hz, H-5'), 7.73 (1H, d, \( J = 16.2 \) Hz, CH=CHCO), 7.83 (1H, d, \( J = 7.8 \) Hz, H-6'), 8.23 (1H, dd, \( J = 8.4 \) Hz, \( J = 1.5 \) Hz, H-4') and 8.38 (1H, t, \( J = 1.5 \) Hz, H-2'); \(^{13}\text{C NMR} \) (100 MHz, CDCl\(_3\) \( \delta_C \)) 51.9 (CH\(_3\)), 120.9 (CH), 122.4 (CH), 124.5 (CH), 129.9 (CH), 133.6 (CH), 136.1 (C), 141.9 (CH), 148.7 (C) and 166.5 (C).

Methyl-3'-aminodihydrocinnamate (100)

\[ \text{H}_2\text{N} \quad \text{O} \quad \text{O} \quad \text{H} \]

Compound 100 was prepared by a procedure similar to that of Strawn and co-workers.\(^{391}\) To a solution of (E)-methyl-3'-nitrocinnamate (99) (10.50 g, 50.69 mmol) in ethanol (100 mL) at room temperature was added 10% palladium-on-carbon (0.54 g, 5.69 mmol), and the reaction stirred under an atmosphere of hydrogen (60 psi) at room temperature for 18 h. The reaction was filtered through celite and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate 2:1) afforded 100 as a white solid (9.00 g, 50.22 mmol, 99%). \( R_t = 0.35 \) (hexane:ethyl acetate, 1:1); mp 115-118 °C [lit.\(^{208}\) mp 116-118 °C]; \(^1\text{H NMR} \) (300 MHz; CDCl\(_3\) \( \delta_H \)) 2.59 (2H, t, \( J = 8.2 \) Hz, CH\(_2\)CH\(_2\)CO), 2.85 (2H, t, \( J = 8.2 \) Hz, CH\(_2\)CH\(_2\)CO), 3.62 (2H, s, NH\(_2\)), 3.66 (3H, s, CH\(_3\)), 6.50-6.57 (3H, m, H-2', H-4', H-6') and 7.06 (1H, m, H-5'); \(^{13}\text{C NMR} \) (75 MHz, CDCl\(_3\))
δ_C 30.8 (CH₂), 35.5 (CH₂), 51.5 (CH₃), 113.0 (CH), 114.9 (CH), 118.3 (CH), 129.3 (CH), 141.7 (C), 146.5 (C) and 173.3 (C).

3'-Acetamidocinnamic acid (101)²⁰⁸

Compound 101 was prepared by a two-step procedure, similar to that of Biggs and co-workers.²⁰⁸ In the first step, to a stirring solution of methyl-3'-aminodihydrocinnamate (100) (9.00 g, 50.22 mmol) and triethylamine (8.35 mL, 60.26 mol) in benzene (200 mL) at room temperature was added dropwise acetyl chloride (3.94 mL, 55.24 mmol), and the mixture heated under reflux for 1 h. The solution was allowed to cool and the resulting precipitate collected by filtration, washed with water (50 mL) and the solvent was removed in vacuo to afford crude methyl-3'-acetamidodihydrocinnamate (100a), as a white solid (11.00 g), which was used without further purification. In the second step, a solution of crude methyl-3'-acetamidodihydrocinnamate (100a) (11.00 g) in an aqueous solution of sodium hydroxide (170 mL, 1 M) was heated under reflux for 1 h. The reaction was quenched by pouring onto a mixture of hydrochloric acid (50 mL, 4 M) and ice (150 mL), and the resulting precipitate collected by filtration and dried in vacuo. Purification by recrystallization (ethanol) afforded 101 as a pale yellow solid (9.85 g, 47.52 mmol, 95% over two steps). R_f = 0.05 (hexane:ethyl acetate, 1:1); mp 161-165 °C [lit.²⁰⁸ mp 162 °C]; H NMR (400 MHz; CDCl₃) δ_H 2.03 (3H, s, CH₃), 2.51 (2H, t, J = 7.8 Hz, CH₂CH₂CO), 2.78 (2H, t, J = 7.8 Hz, CH₂CH₂CO), 6.89 (1H, d, J = 7.9 Hz, H-6'), 7.18 (1H, t, J = 7.9 Hz, H-5'), 7.42-7.44 (2H, m, H-2', H-4') and 9.94 (1H, s, NH); C NMR (100 MHz, CDCl₃) δ_C 24.9 (CH₃), 31.4 (CH₂), 36.2 (CH₂), 117.8 (CH), 119.8 (CH), 123.9 (CH), 129.5 (CH), 140.3 (C), 142.3 (C), 169.3 (C) and 174.6 (C).
Compound **103** was prepared by a two-step procedure similar to that of El-Feraly and co-workers\(^{230}\) and Biggs and co-workers.\(^{228}\) In the first step, to a stirring solution of 3'-acetamidocinnamic acid (**101**) (2.00 g, 9.65 mmol) in oxayl chloride (35 mL) at room temperature was added dropwise dimethylformamide (0.04 mL, 0.48 mmol), and the mixture stirred at room temperature for 2 h. The solvent was removed \textit{in vacuo} to afford crude 3'-acetamidocinnamoyl chloride (**102**) as a brown gum (2.60 g), which was used without further purification. In the second step, to a solution of crude 3'-acetamidocinnamoyl chloride (**102**) (2.60 g) in dichloromethane (30 mL) at room temperature was added anhydrous aluminum chloride (2.50 g, 18.73 mmol), and the mixture stirred at room temperature for 4 h. The reaction was poured onto ice (50 mL), extracted with dichloromethane (3 × 50 mL), dried over anhydrous sodium sulfate, filtered and the solvent removed \textit{in vacuo}. Purification by flash chromatography (hexane:ethyl acetate 1:2) afforded **103** as a white solid (90 mg, 0.48 mmol, 5% over two steps). \(R_t = 0.20\) (hexane:ethyl acetate, 1:2). \(^1\)H NMR (400 MHz; CDCl\(_3\)) \(\delta_H\) 2.14 (3H, s, CH\(_3\)), 2.61 (2H, m, CH\(_2\)CH\(_2\)CO), 3.09 (2H, t, \(J = 5.6\) Hz, CH\(_2\)CH\(_2\)CO), 7.53 (1H, dd, \(J = 7.8\) Hz, \(J = 2.0\) Hz, H-6), 7.61 (1H, d, \(J = 8.4\) Hz, H-7), 7.95 (1H, s, H-4) and 10.35 (1H, s, NH); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta_C\) 25.2 (CH\(_3\)), 26.4 (CH\(_2\)), 36.9 (CH\(_2\)), 116.3 (CH), 119.0 (CH), 124.8 (CH), 132.4 (C), 146.0 (C), 157.7 (C), 170.0 (C) and 205.5 (C).
5-Amino-1-indanone (104)²⁰⁸

![5-Amino-1-indanone (104)](image)

Compound 104 was prepared by a procedure similar to that of Detsi and co-workers.²¹⁰ A mixture of 5-acetamido-1-indanone (103) (26 mg, 0.14 mmol) in methanol (2.5 mL) and hydrochloric acid (2.5 mL, 2 M) was heated under reflux for 1 h. The reaction was quenched with an aqueous solution of sodium hydroxide (20 mL, 1 M), extracted with ethyl acetate (20 mL × 2), dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate 2:1) afforded 104 as an orange solid (10 mg, 0.07 mmol, 49%). \( R_f = 0.30 \) (hexane:ethyl acetate, 1:1); \(^1\)H NMR (400 MHz; CDCl₃) \( \delta_H \) 2.62 (2H, m, CH₂C₄H₂CO), 3.00 (2H, t, \( J = 6.0 \) Hz, CH₂CH₂CO), 4.23 (2H, s, NH₂), 6.59-6.61 (2H, m, H-4, H-7) and 7.58 (1H, dd, \( J = 8.0 \) Hz, \( J = 0.8 \) Hz, H-6); \(^1^C \)NMR (100 MHz, CDCl₃) \( \delta_C \) 25.2 (CH₃), 26.4 (CH₂), 36.9 (CH₂), 116.3 (CH), 119.0 (CH), 124.8 (CH), 132.4 (C), 146.0 (C), 157.7 (C), 170.0 (C) and 205.5 (C); MS (ESI, 70 eV) \( m/z \) 148 (M⁺, 100%); Found (M⁺, 148.0750), C₉H₁₀NO requires 148.0757.

B) 6-Aminoaurone (86)

3-Acetamidoanisole (114)³⁹²

![3-Acetamidoanisole (114)](image)

Compound 114 was prepared by a procedure similar to that of Teramoto and co-workers.²¹³ To a stirring suspension of 3-acetamidophenol (114) (4.50 g, 29.77 mmol) and potassium carbonate (6.17 g, 44.65 mmol) in dimethylformamide (25 mL) at 0 °C
was added dropwise methyl iodide (2.05 mL, 32.75 mmol), and the mixture stirred at room temperature for 18 h. The solution was diluted with water (100 mL), extracted with dichloromethane (3 × 100 mL), the combined organic phases washed with water (3 × 100 mL), dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate 1:2) afforded 114 as a pale brown solid (4.31 g, 26.46 mmol, 88%). \( R_f = 0.25 \) (hexane:ethyl acetate, 1:2); \( ^1H \text{NMR} \) (400 MHz; CDCl\(_3\)) \( \delta \) 2.15 (3H, s, NHCOC\(_3\)H), 3.77 (3H, s, OCH\(_3\)), 6.65 (1H, dd, \( J = 7.9 \) Hz, \( J = 1.3 \) Hz, H-6), 6.98 (1H, dd, \( J = 7.9 \) Hz, \( J = 1.3 \) Hz, H-4), 7.19 (2H, t, \( J = 7.9 \) Hz, H-5), 7.27 (1H, s, H-2) and 7.69 (1H, s, NHCOCH\(_3\)); \( ^13C \text{NMR} \) (100 MHz, CDCl\(_3\)) \( \delta \) C 24.5 (CH\(_3\)), 55.2 (CH\(_3\)), 105.7 (CH), 110.0 (CH), 112.0 (CH), 129.6 (CH), 139.2 (C), 160.1 (C) and 168.6 (C).

6-(2'-Chloroacetyl)-3-acetamidophenol (112)

\[
\begin{align*}
\text{Cl} & \quad \text{NH} \\
\text{O} & \quad \text{OH} \\
\text{O} & \quad \text{CO} 
\end{align*}
\]

Compound 112 was prepared by a procedure similar to that of Teramoto and co-workers.\(^{213}\) To a stirring suspension of anhydrous aluminum chloride (3.27 g, 24.52 mmol) in dichloroethane (50 mL) at 0 °C was added chloroacetyl chloride (1.07 mL, 13.48 mmol), and the mixture stirred at room temperature for 1 h. To the resulting mixture at room temperature was then added 3-acetamidoanisole (114) (2.00 g, 12.26 mmol), and the mixture heated at 70 °C for 3 h. The reaction was poured into ice-water (100 mL), extracted with dichloromethane (3 × 100 mL), dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate 1:1) afforded 112 as a white solid (0.13 g, 0.57 mmol, 5%). \( R_f = 0.15 \) (hexane:ethyl acetate, 1:1); mp 63-68 °C; \( ^1H \text{NMR} \) (400 MHz; CDCl\(_3\)) \( \delta \) \( H \) 2.11 (3H, s, CH\(_3\)), 4.98 (2H, s, CH\(_2\)), 7.02 (1H, dd, \( J = 9.0 \) Hz, \( J = 2.0 \) Hz, H-4), 7.47 (1H, d, \( J = 2.0 \) Hz, H-2), 7.75 (1H, d, \( J = 9.0 \) Hz, H-5), 10.21 (1H, s, NH) and 11.41 (1H, s, OH); \( ^13C \text{NMR} \) (100 MHz, CDCl\(_3\)) \( \delta \) C 25.0 (CH\(_3\)), 49.1 (CH\(_2\)), 106.8 (CH), 111.1 (CH), 115.4
(C), 132.1 (CH), 147.0 (C), 162.3 (C), 168.6 (C) and 193.7 (C); IR (νmax/cm−1) 716 (C-Cl), 841, 957, 1037, 1159 (C-O), 1350, 1446, 1525, 1594 (N-H), 1742 (C=O), 2850, 2918 and 3552 (O-H); MS (ESI, 70 eV) m/z 338 (100%).

6-Acetamido-3(2H)-benzofuranone (113)

![Chemical structure of 6-Acetamido-3(2H)-benzofuranone (113)]

Compound 113 was prepared by a procedure similar to that of Tsou and co-workers. A mixture of 6-(2'-chloroacetyl)-3-acetamidophenol (112) (120 mg, 0.53 mmol) and triethylamine (0.10 mL, 0.63 mmol) in acetonitrile (5 mL) was stirred at room temperature for 1 h. The solvent was removed in vacuo, with purification by flash chromatography (hexane:ethyl acetate 1:1) affording 113 as a white solid (50 mg, 0.26 mmol, 50%). Rf = 0.50 (hexane:ethyl acetate, 1:3). mp 193-198 °C; 1H NMR (400 MHz; CDCl3) δH 2.24 (3H, s, CH3), 4.64 (2H, s, CH2), 6.91 (1H, dd, J = 8.4 Hz, J = 1.6 Hz, H-5), 7.59 (1H, d, J = 8.4 Hz, H-4), 7.73 (1H, d, J = 1.6 Hz, H-7) and 7.73 (1H, s, NH); 13C NMR (100 MHz, CDCl3) δC 24.9 (CH3), 75.4 (CH2), 103.1 (CH), 113.5 (CH), 116.9 (C), 124.8 (CH), 146.5 (C), 168.5 (C), 175.5 (C) and 198.0 (C); IR (νmax/cm−1) 793, 881, 1011, 1125 (C-O), 1251 (C-N), 1277, 1323, 1434, 1497, 1546, 1597 (N-H), 1670, 1710 (C=O), 2851, 2936, 3135, 3228 and 3296 (N-H); MS (ESI, 70 eV) m/z 192 (M+, 100%); Found (M+, 192.0647), C10H10NO3 requires 192.0655.
Compound 110 was prepared by a procedure similar to that of Cheng and co-workers.\textsuperscript{214} To a stirring solution of 6-acetamido-3(2\textit{H})-benzofuranone (113) (10 mg, 0.05 mmol) and benzaldehyde (57) (0.01 mL, 0.06 mmol) in glacial acetic acid (0.5 mL) at room temperature was added hydrochloric acid (0.05 mL, 10 M), and the reaction stirred at room temperature for 3 h. The reaction was poured onto ice-water (10 mL) and the resulting precipitate collected by filtration, washed with cold ethanol (1 mL) and dried \textit{in vacuo}. Purification by flash chromatography (hexane:ethyl acetate, 2:1) afforded 110 exclusively as the (\textit{Z}) isomer (bright yellow solid; 12 mg, 0.04 mmol, 83\%). $R_f = 0.40$ (hexane:ethyl acetate, 1:1); mp 63-68 $^\circ$C; $^1$H NMR (400 MHz; CDCl$_3$) $\delta$H 2.26 (3H, s, CH$_3$), 6.85 (1H, s, CH), 6.95 (1H, dd, $J = 8.1$ Hz, $J = 2.0$ Hz, H-5), 7.40-7.50 (3H, m, ArH), 7.64 (1H, s, NH), 7.71 (1H, d, $J = 8.1$ Hz, H-4), 7.91 (2H, d, $J = 8.8$ Hz, ArH) and 8.07 (1H, s, H-7); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 25.0 (CH$_3$), 103.1 (CH), 112.7 (CH), 114.5 (CH), 117.3 (C), 125.4 (CH), 128.9 (CH), 129.9 (CH), 131.5 (CH), 132.3 (C), 145.7 (C), 147.6 (C), 167.6 (C), 168.5 (C) and 183.3 (C); IR ($\nu_{\text{max}}$/cm$^{-1}$) 830, 1041 (C-O), 1297 (C-N), 1318, 1518, 1604 (N-H), 1629 (C=O), 2234, 2851, 2922, 3217, 3326 (N-H) and 3413; MS (APCI, 70 eV) m/z 280 (M$^+$, 100\%). Found (M$^+$, 280.0972), C$_{17}$H$_{14}$NO$_3$ requires 280.0968.
(E/Z)-6-Aminoaurone (86)

A similar procedure\textsuperscript{210} to that previously described for the preparation 104 was followed using (Z)-6-acetamidoaurone (110) (140 mg, 0.50 mmol), methanol (1.5 mL) and hydrochloric acid (1.5 mL, 2 M). Purification by flash chromatography (dichloromethane:ethyl acetate 99:1) afforded 86 as a mixture of (E):(Z) isomers (1:5) (bright yellow solid; 42 mg, 0.18 mmol, 35%). \( R_t = 0.30 \) (hexane:ethyl acetate, 1:1); mp 220-225 °C; \( (Z) \) isomer; \( ^1\text{H} \text{NMR} \) (400 MHz; CDCl\(_3\)) \( \delta \text{H} \) 6.35-6.37 (2H, m, H-5, H-7), 6.68 (1H, s, CH), 7.26-7.38 (3H, m, ArH), 7.52 (1H, d, \( J = 8.0 \) Hz, H-4) and 7.80 (2H, d, \( J = 8.4 \) Hz, ArH); \( ^{13}\text{C} \text{NMR} \) (100 MHz, CDCl\(_3\)) \( \delta \text{C} \) 95.8 (CH), 110.7 (CH), 111.2 (CH), 112.5 (C), 126.5 (CH), 128.8 (CH), 129.2 (CH), 131.1 (CH), 132.8 (C), 148.4 (C), 155.3 (C), 168.9 (C) and 182.3 (C); \( (E) \) isomer; \( ^1\text{H} \text{NMR} \) (400 MHz; CDCl\(_3\)) \( \delta \text{H} \) 4.40 (2H, s, NH\(_2\)), 6.30 (1H, d, \( J = 2.0 \) Hz, H-7), 6.37 (1H, dd, \( J = 8.3 \) Hz, \( J = 1.9 \) Hz, H-5), 6.81 (1H, s, CH), 7.34-7.43 (3H, m, ArH), 7.56 (1H, d, \( J = 8.3 \) Hz, H-4) and 8.15 (2H, d, \( J = 8.8 \) Hz, ArH); \( ^{13}\text{C} \text{NMR} \) (100 MHz, CDCl\(_3\)) \( \delta \text{C} \) 95.1 (CH), 110.8 (CH), 114.6 (C), 126.5 (CH), 120.1 (CH), 128.3 (CH), 129.6 (CH), 130.8 (CH), 132.2 (C), 149.5 (C), 155.0 (C), 167.9 (C) and 182.3 (C); IR (v\text{max}/cm\(^{-1}\)) 769, 1129 (C-O), 1279 (C-N), 1462, 1582 (N-H), 1683 (C=O), 2850, 2920, 3193, 3318 (N-H) and 3476; MS (ESI, 70 eV) \( m/\varepsilon \) 238 (M\(^+\), 100%); Found (M\(^+\),238.0856), C\(_{15}\)H\(_{12}\)NO\(_2\) requires 238.0863.
7. Experimental

7.2. Synthesis

7.2.3. 4'-Aminophenone isosteres

7.2.3.1. 4'-Aminophenone ketoxime 117

*(E)-4'-Aminopropiophenone O-methyl ketoxime (117)*

![Image of compound 117]

Compound 117 was prepared by a procedure similar to that of Aakeröy and co-workers. To a stirring solution of 4'-aminopropiophenone (1) (1.00 g, 6.70 mmol) in ethanol (25 mL) at room temperature was added *N*-methyl hydroxylamine hydrochloride (0.56 g, 6.7 mmol) in water (10 mL), followed by sodium carbonate (0.36 g, 3.35 mmol) in water (10 mL), and the mixture heated under reflux for 36 h. The reaction was allowed to cool and the solvent removed *in vacuo*. The residue was taken up in ethyl acetate (100 mL), washed with water (100 mL), the separated aqueous layer further extracted with ethyl acetate (2 × 100 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo*. Purification by flash chromatography (hexane:ethyl acetate 4:1) afforded 117 exclusively as the *(E)* isomer (brown oil; 0.83 g, 4.62 mmol, 70%). *R* = 0.35 (hexane:ethyl acetate, 2:1); ¹H NMR (400 MHz; CDCl₃)  𝛿H 1.11 (3H, t, *J* = 7.6 Hz, CH₂CH₃), 2.68 (2H, q, *J* = 7.6 Hz, CH₂CH₃), 3.94 (3H, s, OC₆H₄), 6.65 (2H, d, *J* = 8.8 Hz, H-3', H-5') and 7.45 (2H, d, *J* = 8.8 Hz, H-2', H-6'); ¹³C NMR (100 MHz, CDCl₃)  𝛿C 11.3 (CH₃), 19.8 (CH₂), 61.6 (CH₃), 114.7 (CH), 125.7 (C), 127.4 (CH), 147.3 (C) and 159.7 (C); IR (νmax/cm⁻¹) 830, 871, 1044 (C-O), 1180, 1292 (C-N), 1517, 1595 (N-H), 1622 (C=C), 2937 and 3356 (N-H); MS (ESI, 70 eV) *m/z* 179 (M⁺, 100%). Found (M⁺, 179.1200), C₁₀H₁₅N₂O requires 179.1165.
7.2.3.2. Chloro-oxime 118 and cyano-oximes 120 and 121

**(E)-4-Nitrobenzaldehyde oxime (122)**

Compound **122** was prepared by a procedure similar to that of Sanders and co-workers. A mixture of 4-nitrobenzaldehyde (69) (3.00 g, 19.9 mmol), hydroxylamine hydrochloride (1.52 g, 21.8 mmol) and sodium hydroxide (0.87 g, 21.8 mmol) in water (50 mL) and ethanol (50 mL) was stirred at room temperature for 3 h. The solvent was removed *in vacuo* and the residue taken up in ethyl acetate (100 mL), washed with water (100 mL), the separated aqueous layer further extracted with ethyl acetate (2 × 100 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo*. Purification by flash chromatography (hexane:ethyl acetate 2:1) afforded **122** exclusively as the (E) isomer (white solid; 2.99 g, 18.0 mmol, 90%). $R_f = 0.25$ (hexane:ethyl acetate, 2:1); $^1$H NMR (400 MHz; CDCl$_3$) $\delta$H 7.75 (2H, d, $J = 8.8$ Hz, H-2, H-6), 8.21 (1H, s, N=CH) and 8.25 (2H, d, $J = 8.8$ Hz, H-3, H-5); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 123.7 (CH), 127.7 (CH), 138.1 (C), 148.4 (CH) and 148.5 (C).
(E)-4-Nitrobenzaldehyde O-methyl oxime (123)\textsuperscript{394}

\[
\text{O} \quad \text{N} \\
\text{O} \\
\text{O}_2\text{N}
\]

Compound 123 was prepared by a procedure similar to that of Mauleon and co-workers.\textsuperscript{223} To a stirring suspension of sodium hydride (0.43 g, 10.80 mmol, 60\% w/w dispersion in mineral oil) in dimethylformamide (25 mL) at room temperature was added (E)-4-nitrobenzaldehyde oxime (122) (1.5 g, 9.03 mmol), and the mixture stirred at room temperature for 10 min. To the resulting solution was added dropwise methyl iodide (0.62 mL, 9.93 mmol) in dimethylformamide (5 mL), and the mixture stirred at room temperature for a further 30 min. The solution was diluted with water (100 mL), extracted with ethyl acetate (3 × 100 mL), the combined organic phases washed with water (3 × 100 mL), dried over anhydrous sodium sulfate, filtered and the solvent removed \textit{in vacuo}. Purification by flash chromatography (hexane:ethyl acetate 49:1) afforded 123 exclusively as the (E) isomer (white solid; 1.05 g, 5.36 mmol, 59\%). \(R \text{\textsubscript{f}} = 0.50\) (hexane:ethyl acetate, 19:1); \(\text{\textsuperscript{1}H NMR}\) (400 MHz; CDCl\textsubscript{3}) \(\delta_{\text{H}}\) 4.04 (3H, s, CH\textsubscript{3}), 7.75 (2H, d, \(J = 9.0\) Hz, H-2, H-6), 8.11 (1H, s, N=CH) and 8.23 (2H, d, \(J = 9.0\) Hz, H-3, H-5); \(\text{\textsuperscript{13}C NMR}\) (100 MHz, CDCl\textsubscript{3}) \(\delta_{\text{C}}\) 62.6 (CH\textsubscript{3}), 124.0 (CH), 127.5 (CH), 138.3 (C), 146.2 (CH) and 148.3 (C).

(Z)-O-Methyl-4-nitrobenzohydroximoyl chloride (124)\textsuperscript{395}

\[
\text{O} \quad \text{N} \\
\text{O} \\
\text{Cl}
\]

Compound 124 was prepared by a procedure similar to that of Sanders and co-workers.\textsuperscript{222} To a stirring solution of (E)-4-nitrobenzaldehyde O-methyl oxime (123) (0.90 g, 4.59
mmol) in dimethylformamide (25 mL) at 0 °C was added portionwise 
N-chlorosuccinimide (6.13 g, 4.59 mmol), and the mixture stirred at room temperature for 
48 h. The solution was diluted with water (100 mL), extracted with ethyl acetate (3 × 100 
ml), the combined organic phases washed with water (3 × 100 mL), dried over 
anhydrous sodium sulfate, filtered and the solvent removed in vacuo. Purification by 
flash chromatography (hexane:ethyl acetate 49:1) afforded 124 exclusively as the (Z) 
isomer (white solid; 0.39 g, 1.80 mmol, 39%).  
\[ R_f = 0.45 \text{ (hexane:ethyl acetate, 19:1); } \]
\[ ^1H \text{ NMR (400 MHz; CDCl}_3\text{) } \delta_H 4.17 \text{ (3H, s, CH}_3\text{), 8.04 \text{ (2H, d, } J = 9.0 \text{ Hz, H}_2\text{-H}_6\text{) and} \]
8.25 (2H, d, \( J = 9.0 \text{ Hz, H}_3\text{-H}_5\text{); } \]
\[ ^13C \text{ NMR (100 MHz, CDCl}_3\text{) } \delta_C 63.7 \text{ (CH}_3\text{), 123.6} \text{ (CH), 127.8 (CH), 135.2 (C), 138.2 (C) and 148.8 (C).} \]

(Z)-O-Methyl-4-aminobenzohydroximoyl chloride (118)

\[ \begin{align*}
\text{H}_2\text{N} & \quad \text{O} \\
\text{Cl} & \\
\end{align*} \]

Compound 118 was prepared by a procedure similar to that of Sun and co-workers.\textsuperscript{224} To 
a stirring solution of (Z)-O-methyl-4-nitrobenzohydroximoyl chloride (124) (0.10 g, 0.46 
mmol) in ethanol (2.5 mL) at 70 °C was added water (1 mL), hydrochloric acid (0.10 mL 
10 M) and iron powder (90 mg, 1.62 mmol), and the mixture stirred at 70 °C for 3 h. The 
reaction was quenched with an aqueous solution of sodium hydroxide (20 mL, 1 M), 
extracted with ethyl acetate (2 × 20 mL), dried over anhydrous sodium sulfate, filtered 
and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl 
acetate 2:1) afforded 118 exclusively as the (Z) isomer (yellow solid; 30 mg, 0.16 mmol, 
35%).  
\[ R_f = 0.40 \text{ (hexane:ethyl acetate, 1:1); mp 55-60 °C; } \]
\[ ^1H \text{ NMR (400 MHz; CDCl}_3\text{) } \delta_H 3.90 \text{ (2H, s, NH}_2\text{), 4.05 \text{ (3H, s, CH}_3\text{), 6.64 \text{ (2H, d, } J = 8.8 \text{ Hz, H}_3\text{-H}_5\text{) and} } \]
7.64 (2H, d, \( J = 8.8 \text{ Hz, H}_2\text{-H}_6\text{); } \]
\[ ^13C \text{ NMR (100 MHz, CDCl}_3\text{) } \delta_C 62.8 \text{ (CH}_3\text{), 114.3 (CH), 122.4} \text{ (C), 128.7 (CH), 137.4 (C) and 148.7 (C); } \]
\[ \text{IR (v}_\text{max/cm}^{-1}) 825, 828 (C-Cl), 952, 1041, 1176 (C-O), 1298 (C-N), 1510, 1599 (N-H), 2853, 2942, 3208, 3359 (N-H) and 3463; \]
**7. Experimental**

**7.2. Synthesis**

**MS** (ESI, 70 eV) m/z 185 (M⁺, 100%). Found (M⁺, 185.0472), C₈H₁₀N₂O requires 185.0476.

**(Z)-4-Nitrobenzohydroximoyl chloride (125)**

![Chemical structure of (Z)-4-Nitrobenzohydroximoyl chloride](image)

A similar procedure to that previously described for the preparation of 124 was followed using *(E)-4-nitrobenzaldehyde oxime (122)* (1.20 g, 7.94 mmol) and N-chlorosuccinimide (5.30 g, 39.70 mmol) in dimethylformamide (25 mL). Purification by flash chromatography (hexane:ethyl acetate, 49:1) afforded 125 exclusively as the (Z) isomer (light brown solid; 1.34 g, 7.15 mmol, 90%). R<sub>f</sub> = 0.50 (hexane:ethyl acetate, 19:1); <sup>1</sup>H NMR (400 MHz; CDCl₃) δ<sub>H</sub> 8.04 (2H, d, J = 9.0 Hz, H-2, H-6), 8.24 (2H, d, J = 9.0 Hz, H-3, H-5) and 10.88 (1H, s, OH); <sup>13</sup>C NMR (100 MHz, CDCl₃) δ<sub>C</sub> 123.4 (CH), 127.7 (CH), 136.4 (C), 138.5 (C) and 148.5 (C).

**(Z)-4-Nitrobenzohydroximoyl cyanide (126)**

![Chemical structure of (Z)-4-Nitrobenzohydroximoyl cyanide](image)

Compound 126 was prepared by a procedure similar to that of Sun and co-workers. To a stirring solution of *(Z)-4-nitrobenzohydroximoyl chloride (125)* (1.00 g, 5.00 mmol) in isopropanol (6.70 mL) and dichloroethane (1.70 mL) at -10 °C was added dropwise a mixture of sodium cyanide (0.27 g, 5.50 mmol) and triethylamine (0.61 g, 6.00 mmol) in water (3.30 mL), and the mixture stirred at 0 °C for 5 h. The reaction was diluted with
ethyl acetate (50 mL), washed with sulfuric acid (50 mL, 1 M), then water (50 mL), the separated aqueous layer further extracted with ethyl acetate (3 × 50 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate 4:1) afforded 126 exclusively as the (Z) isomer (pale yellow solid; 0.18 g, 0.94 mmol, 19%). $R_f = 0.25$ (hexane:ethyl acetate, 4:1); $^1$H NMR (400 MHz; CDCl$_3$) $\delta$H 8.00 (2H, d, $J = 9.0$ Hz, H-2, H-6) and 8.32 (2H, d, $J = 9.0$ Hz, H-3, H-5); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 108.6 (C), 124.3 (CH), 127.2 (CH), 132.1 (C), 134.9 (C) and 149.3 (C).

(Z)-O-Methyl-4-nitrobenzohydroximoyl cyanide (127)

Compound 127 was prepared by a procedure similar to that of Sun and co-workers.$^{224}$ To a stirring solution of (Z)-4-nitrobenzohydroximoyl cyanide (126) (25 mg, 0.16 mmol), tetrabutylammonium bromide (8 mg, 0.02 mmol) and sodium hydroxide (10 mg, 0.23 mmol) in tetrahydrofuran (1 mL) and water (0.5 mL) at 70 °C was added dropwise methyl iodide (0.012 mL, 0.19 mmol) in tetrahydrofuran (0.5 mL), and the mixture stirred at 70 °C for 3 h. The solution was allowed to cool and the solvent removed in vacuo. The resulting residue was taken up in ethyl acetate (10 mL), washed with water (10 mL), the separated aqueous layer further extracted with ethyl acetate (3 × 10 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate 9:1) afforded 127 exclusively as the (Z) isomer (pale brown solid; 18 mg, 0.10 mmol, 66%). $R_f = 0.70$ (hexane:ethyl acetate, 1:1); mp 177-182 °C; $^1$H NMR (400 MHz; CDCl$_3$) $\delta$H 4.29 (3H, s, CH$_3$), 7.99 (2H, d, $J = 9.0$ Hz, H-2, H-6) and 8.31 (2H, d, $J = 9.0$ Hz, H-3, H-5); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 65.1 (CH$_3$), 108.8 (C), 124.2 (CH), 127.0 (CH), 129.9 (C), 134.9 (C) and 149.2 (C); $\text{IR (} v_{\text{max}}/\text{cm}^{-1})$ 833, 1075, 1375 (C-N), 1447,
1665 (N-H), 2223 (C≡N), 2854, 2926, 2960 and 3347 (N-H); MS (ESI, 70 eV) m/z 180 (100%).

(Z)-O-Ethyl-4-nitrobenzohydroximoyl cyanide (128)

A similar procedure to that previously described for the preparation of 127 was followed using (Z)-4-nitrobenzohydroximoyl cyanide (126) (80 mg, 0.50 mmol), tetrabutylammonium bromide (25 mg, 0.075 mmol), sodium hydroxide (30 mg, 0.75 mmol), tetrahydrofuran (2 mL) and water (1 mL), and ethyl iodide (0.05 mL, 0.60 mmol) in tetrahydrofuran (1 mL). Purification by flash chromatography (hexane:ethyl acetate 4:1) afforded 128 exclusively as the (Z) isomer (pale brown solid; 32 mg, 0.15 mmol, 29%). \( R_f = 0.65 \) (hexane:ethyl acetate, 2:1); mp 76-79 °C; \(^1\)H NMR (400 MHz; CDCl\(_3\)) \( \delta \) 1.46 (3H, t, \( J = 7.2 \) Hz, CH\(_2\)C\(_3\)H), 4.54 (2H, q, \( J = 7.2 \) Hz, CH\(_2\)CH\(_3\)), 7.99 (2H, d, \( J = 8.8 \) Hz, H-2, H-6) and 8.31 (2H, d, \( J = 8.8 \) Hz, H-3, H-5); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta \) C 14.5 (CH\(_3\)), 73.7 (CH\(_2\)), 109.0 (C), 124.2 (CH), 126.9 (CH), 129.5 (C), 135.2 (C) and 149.1 (C); IR (\( \nu_{max}/cm^{-1} \)) 690, 754, 853, 1024, 1040 (C-O), 1312 (C-N), 1342, 1516 (N-O), 1600, 2254 (C≡N), 2854, 2990 and 3110; MS (ESI, 70 eV) m/z 242 (MNa\(^+\), 100%). Found (MNa\(^+\), 242.0529), C\(_{10}\)H\(_9\)N\(_3\)NaO\(_3\) requires 242.0533.
(Z)-O-Methyl-4-aminobenzohydroximoyl cyanide (120)

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{N} \quad \text{O} \\
& \quad \text{CN}
\end{align*}
\]

A similar procedure\textsuperscript{224} to that previously described for the preparation of 118 was followed using (Z)-O-methyl-4-nitrobenzohydroximoyl cyanide (127) (15 mg, 0.09 mmol) in ethanol (2 mL) and water (0.5 mL), hydrochloric acid (0.10 mL, 10 M) and iron powder (17 mg, 0.30 mmol). Purification by flash chromatography (hexane:ethyl acetate 2:1) afforded 120 exclusively as the (Z) isomer (pale brown solid; 4 mg, 0.03 mmol, 32%). \( R_f = 0.50 \) (hexane:ethyl acetate, 1:1); mp 65-70 °C; \( ^{1} \text{H NMR} \) (400 MHz; CDCl\textsubscript{3}) \( \delta \text{H} 3.99 \) (2H, s, NH\textsubscript{2}), 4.13 (3H, s, CH\textsubscript{3}), 6.68 (2H, d, \( J = 8.8 \) Hz, H-3, H-5) and 7.59 (2H, d, \( J = 8.8 \) Hz, H-2, H-6); \( ^{13} \text{C NMR} \) (100 MHz, CDCl\textsubscript{3}) \( \delta \text{C} 63.7 \) (CH\textsubscript{3}), 109.9 (C), 114.8 (CH), 119.3 (C), 127.8 (CH), 131.9 (C) and 149.3 (C); IR (\( \nu_{\text{max}}/\text{cm}^{-1} \)) 825, 895, 1052, 1172 (C-O), 1305 (C-N), 2235 (C≡N), 2827, 2937, 3232, 3359 (N-H) and 3433; MS (ESI, 70 eV) \( m/z \) 176 (M\textsuperscript{+}, 100%). Found (M\textsuperscript{+}, 176.0808), C\textsubscript{9}H\textsubscript{10}N\textsubscript{3}O requires 176.0818.

(Z)-O-Ethyl-4-aminobenzohydroximoyl cyanide (121)

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{N} \quad \text{O} \\
& \quad \text{CN}
\end{align*}
\]

A similar procedure\textsuperscript{224} to that previously described for the preparation of 118 was followed using (Z)-O-ethyl-4-nitrobenzohydroximoyl cyanide (128) (26 mg, 0.14 mmol) in ethanol (2 mL) and water (0.5 mL), hydrochloric acid (0.10 mL, 10 M) and iron powder (27 mg, 0.48 mmol). Purification by flash chromatography (hexane:ethyl acetate 4:1) afforded 121 exclusively as the (Z) isomer (pale brown solid; 10 mg, 0.05 mmol,
7. Experimental

7.2. Synthesis

38%). \( R_f = 0.25 \) (hexane:ethyl acetate, 4:1); mp 59-63°C; \(^1\)H NMR (400 MHz; CDCl\(_3\)) \( \delta_H \)
1.39 (3H, t, \( J = 7.2 \) Hz, CH\(_2\)CH\(_3\)), 3.98 (2H, s, NH\(_2\)), 4.54 (2H, q, \( J = 7.2 \) Hz, CH\(_2\)CH\(_3\)),
6.68 (2H, d, \( J = 8.8 \) Hz, H-3, H-5) and 7.59 (2H, d, \( J = 8.8 \) Hz, H-2, H-6); \(^{13}\)C NMR
(100 MHz, CDCl\(_3\)) \( \delta_C \)
14.5 (CH\(_3\)), 72.0 (CH\(_2\)), 110.1 (C), 114.8 (CH), 119.6 (C), 127.7 (CH), 131.8 (C) and 149.1 (C); IR (\( \nu_{\text{max}}/\text{cm}^{-1} \)) 828, 969, 1001, 1139 (C-O), 1299 (C-N),
1509, 1602 (N-H), 2234 (C\(=\)N), 2896, 2979, 3215, 3326 (N-H) and 3419; MS (ESI, 70 eV) \( m/z \) 190 (M\(^+\), 100%); Found (M\(^+\), 190.0966), C\(_{10}\)H\(_{12}\)N\(_3\)O requires 190.0975.

7.2.3.3. 4’-Aminophenyl alkyl sulfoxide 129, sulfone 130, and sulfonamides 131 and 132

\( \text{O}_2\text{N} \)

\( \text{S} \)

4’-Nitrophenyl pentyl sulfide (133)

\[
\text{O}_2\text{N}\quad \text{S} \quad \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3
\]

Compound 133 was prepared by a procedure similar to that of Pescatori and co-workers.\(^{227}\) A mixture of 1-chloro-4-nitrobenzene (134) (3.97 g, 25.2 mmol),
1-pentanethiol (2.98 mL, 24.00 mmol) and potassium hydroxide (1.35 g, 24.00 mmol) in
dimethylformamide (10 mL) was heated at 100 °C for 6 h. The solution was diluted with
water (100 mL), extracted with dichloromethane (3 × 100 mL), the combined organic
phases washed with water (3 × 50 mL), dried over anhydrous sodium sulfate, filtered and
the solvent removed \textit{in vacuo}. Purification by flash chromatography (hexane:ethyl acetate
99:1) afforded 133 as a yellow oil (5.18 g, 23.00 mmol, 91%). \( R_f = 0.75 \) (hexane:ethyl acetate,
19:1); \(^1\)H NMR (300 MHz; CDCl\(_3\)) \( \delta_H \) 0.92 (3H, t, \( J = 7.2 \) Hz,
CH\(_2\)CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 1.30-1.51 (4H, m, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 1.73 (2H, m,
CH\(_2\)CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 3.01 (2H, t, \( J = 7.2 \) Hz, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)),
7.31 (2H, d, \( J = 9.0 \) Hz, H-2’, H-6’) and 8.11 (2H, d, \( J = 9.0 \) Hz, H-3’, H-5’); \(^{13}\)C NMR (75 MHz, CDCl\(_3\))
\( \delta_C \)
13.8 (CH\(_3\)), 22.1 (CH\(_2\)), 28.1 (CH\(_2\)), 30.9 (CH\(_2\)), 31.9 (CH\(_2\)), 123.8 (CH), 125.9 (CH),
144.8 (C) and 148.1 (C); IR (\( \nu_{\text{max}}/\text{cm}^{-1} \)) 741, 852, 1090, 1334 (C-N), 1508 (N-O), 1577,
1593, 2857, 2928 and 2956; MS (ESI, 70 eV) m/z 248 (MNa⁺, 100%); Found (MNa⁺, 248.0715), C₁₁H₁₅NNaO₂S requires 248.0713.

4'-Aminophenyl pentyl sulfide (135)²²⁸

Compound 135 was prepared by a procedure similar to that of Braunerová and co-workers.²²⁸ A mixture of 4'-nitrophenyl pentyl sulfide (133) and tin(II) chloride (0.93 g, 4.88 mmol) in ethanol (10 mL) was heated at 70 °C for 5 h. The residue was taken up in ethyl acetate (50 mL), washed with an aqueous solution of sodium hydroxide (50 mL, 1 M), the separated aqueous layer further extracted with ethyl acetate (2 × 50 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate 9:1) afforded 135 as a pale yellow solid (0.12 g, 0.63 mmol, 14%). Rᵣ = 0.40 (hexane:ethyl acetate, 2:1); mp 84-88 °C [ lit.²²⁸ mp 85.5-90 °C]; ¹H NMR (400 MHz; CDCl₃) δH 0.87 (3H, t, J = 7.4 Hz, CH₂CH₂CH₂CH₂CH₃), 1.25-1.40 (4H, m, CH₂CH₂CH₂CH₃), 1.69 (2H, m, CH₂CH₂CH₂CH₃), 2.78 (2H, t, J = 7.4 Hz, CH₂CH₂CH₂CH₃), 3.68 (2H, s, NH₂), 6.60 (2H, d, J = 8.6 Hz, H-3', H-5') and 7.22 (2H, d, J = 8.6 Hz, H-2', H-6'); ¹³C NMR (100 MHz, CDCl₃) δC 13.9 (CH₃), 22.2 (CH₂), 29.0 (CH₂), 30.8 (CH₂), 36.3 (CH₂), 115.5 (CH), 123.8 (C), 133.6 (CH) and 145.6 (C).
4'-Aminophenyl pentyl sulfoxide (129)

Compound 129 was prepared by a procedure similar to that of Shi and co-workers. To a stirring solution of 4'-aminophenyl pentyl sulfide (135) (118 mg, 0.60 mmol) at room temperature was added dropwise hydrogen peroxide (0.07 mL, 0.06 mmol, 30 wt% in water) and the reaction heated at 70 °C for 1 h. The reaction was allowed to cool, diluted with ethyl acetate (50 mL), washed with an aqueous solution of sodium hydroxide (50 mL, 1 M), the separated aqueous layer further extracted with ethyl acetate (2 × 50 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate 1:2) afforded 129 as a white solid (18 mg, 0.09 mmol, 14%). $R_f = 0.15$ (hexane:ethyl acetate, 1:2). mp 116-120 °C; $^1$H NMR (400 MHz; CDCl$_3$) $\delta$H 0.88 (3H, t, $J = 7.2$ Hz, CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 1.25-1.42 (4H, m, CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 1.64 (2H, m, CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 2.69 (1H, m, CH$_2$CH$_2$CH$_2$CH$_3$), 2.81 (1H, m, CH$_2$CH$_2$CH$_2$CH$_3$), 4.00 (2H, s, NH$_2$), 6.75 (2H, d, J = 8.6 Hz, H-3', H-5') and 7.42 (2H, d, J = 8.6 Hz, H-2', H-6'); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 13.8 (CH$_3$), 22.2 (CH$_2$), 22.2 (CH$_2$), 30.8 (CH$_2$), 57.4 (CH$_2$), 115.0 (CH), 126.2 (CH), 131.8 (C) and 149.4 (C); IR ($\nu$$_{max}$/cm$^{-1}$) 818, 1003, 1090, 1171, 1287 (C-N), 1496, 1589 (N-H), 1637, 2857, 2927, 2954, 3192, 3302, 3375 (N-H) and 3103; MS (ESI, 70 eV) $m/z$ 212 (M$^+$, 100%); Found (M$^+$, 212.1088), C$_{11}$H$_{18}$NOS requires 212.1104.
4'-Nitrophenyl pentyl sulfone (136)

Compound 136 was prepared by a procedure similar to that of Pescatori and co-workers. To a stirring solution of 4'-nitrophenyl pentyl sulfide (133) (1.00 g, 4.44 mmol) in dichloromethane (20 mL) at 0 °C was added dropwise a solution of 3-chloroperbenzoic acid (1.17 g, 5.33 mmol) in dichloromethane (30 mL), and the mixture stirred at room temperature for 4 h. The reaction was quenched with an aqueous solution of sodium hydrogen sulfite (25 mL, 1 M), then sat. sodium hydrogen carbonate (25 mL), extracted with dichloromethane (3 × 50 mL), dried over anhydrous sodium sulfate, filtered and the solvent removed \textit{in vacuo}. Purification by flash chromatography (hexane:ethyl acetate 9:1) afforded 136 as a pale yellow solid (0.38 g, 1.46 mmol, 33%). $R_f = 0.50$ (hexane:ethyl acetate, 1:1); mp 48-50 °C; $^1$H NMR (300 MHz; CDCl$_3$) $\delta$ $H$ 0.87 (3H, t, $J$ = 6.9 Hz, CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 1.25-1.43 (4H, m, CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 1.74 (2H, m, CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 3.17 (2H, m, CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 8.15 (2H, d, $J$ = 9.0 Hz, H-2', H-6'); $^1$C NMR (75 MHz, CDCl$_3$) $\delta$ $C$ 13.5 (CH$_3$), 21.9 (CH$_2$), 22.0 (CH$_2$), 30.1 (CH$_2$), 55.9 (CH$_2$), 124.3 (CH), 129.4 (CH), 144.7 (C) and 150.7 (C); IR ($v_{\text{max}}$/cm$^{-1}$) 737, 771, 863, 1085, 1146, 1293 (C-N), 1528 (N-O), 1607, 2878, 2929 and 3103; MS (ESI, 70 eV) $m/z$ 280 (MNa$^+$, 100%); Found (MNa$^+$, 280.0609), C$_{11}$H$_{15}$NNaO$_4$S requires 280.0614.

4'-Aminophenyl pentyl sulfone (130)$^{397}$

Compound 130 was prepared by a procedure similar to that of Pescatori and co-workers. A mixture of 4'-nitrophenyl pentyl sulfone (136) (0.38 g, 1.45 mmol) and
tin(II) chloride (0.41 g, 2.17 mmol) in ethanol (25 mL) was heated under reflux for 4 h. The solution was allowed to cool and the solvent removed in vacuo. The residue was taken up in ethyl acetate (50 mL), washed with an aqueous solution of sodium hydroxide (50 mL, 1 M), the separated aqueous layer further extracted with ethyl acetate (2 × 50 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate 2:1) afforded **130** as a white solid (68 mg, 0.30 mmol, 21%). $R_f = 0.20$ (hexane:ethyl acetate, 2:1); $^1$H NMR (400 MHz; CDCl$_3$) $\delta_H$ 0.86 (3H, t, $J = 7.0$ Hz, CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 1.23-1.35 (4H, m, CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 1.69 (2H, m, CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 3.02 (2H, m, CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 4.23 (2H, s, NH$_2$), 6.70 (2H, d, $J = 8.4$ Hz, H-3', H-5') and 7.64 (2H, d, $J = 8.4$ Hz, H-2', H-6'); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_C$ 13.7 (CH$_3$), 22.1 (CH$_2$), 22.6 (CH$_2$), 30.4 (CH$_2$), 56.7 (CH$_2$), 114.0 (CH), 127.2 (C), 130.1 (CH) and 151.3 (C); MS (ESI, 70 eV) $m/z$ 229 (M$^+$, 100%). Found (M$^+$, 228.1040), C$_{11}$H$_{18}$NO$_2$S requires 228.1053.

4-Nitrobenzenesulfonyl chloride (**138**) $^{398}$

![4-Nitrobenzenesulfonyl chloride](image)

Compound **138** was prepared by a procedure similar to that of Hogan and co-workers.$^{230}$ To water (13 mL) at 0 °C was added dropwise thionyl chloride (2.10 mL, 28.96 mmol) over 1 h, and the solution allowed to warm to room temperature over 18 h. To the resulting solution at room temperature was added cuprous chloride (50 mg, 0.29 mmol) and the mixture stirred for 15 min at -5 °C (solution A). To a solution of 4-nitroaniline (**137**) (1.00 g, 7.24 mmol) in hydrochloric acid (7.24 mL, 72.4 mmol, 10 M) at 0 °C was added dropwise over 5 min a solution of sodium nitrite (0.56 g, 8.11 mmol) in water (2 mL), and the resulting mixture stirred for 10 min at -5 °C. To stirring solution A at -5 °C was added dropwise over 30 min the above preformed diazonium salt, and the mixture stirred at 0 °C for 1.25 h. The resulting precipitate was collected by filtration, washed
with water (2 × 50 mL) and dried in vacuo. Purification by flash chromatography (hexane:ethyl acetate 4:1) afforded 138 as a pale yellow solid (1.60 g, 7.22 mmol, 99% yield.). \( R_f = 0.75 \) (hexane:ethyl acetate, 1:1); \(^1\text{H NMR} \) (400 MHz; CDCl\(_3\)) \( \delta_H \) 8.28 (2H, d, \( J = 8.2 \) Hz, H-2, H-6) and 8.50 (2H, d, \( J = 8.2 \) Hz, H-3, H-5); \(^{13}\text{C NMR} \) (100 MHz, CDCl\(_3\)) \( \delta_C \) 125.0 (CH), 128.5 (CH), 148.5 (C) and 151.3(C).

**N-Butyl-4'-nitrobenzenesulfonamide (139)**

![Image](image_url)

Compound 139 was prepared by a procedure similar to that of Lawrence and co-workers.\(^{231}\) To a stirring solution of 4-nitrobenzenesulfonyl chloride (138) (0.25 g, 1.13 mmol) in tetrahydrofuran (2.5 mL) at room temperature was added dropwise \( n \)-butylamine (0.34 mL, 3.38 mmol), and the mixture stirred at room temperature for 30 min. The reaction was quenched with hydrochloric acid (50 mL, 1 M) at 0 °C, extracted with ethyl acetate (3 × 50 mL), dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate 4:1) afforded 139 as a white solid (0.20 g, 0.77 mmol, 69%). \( R_f = 0.75 \) (hexane:ethyl acetate, 1:1); mp 79-83 °C [ lit.\(^{399}\) mp 80-81 °C]; \(^1\text{H NMR} \) (400 MHz; CDCl\(_3\)) \( \delta_H \) 0.87 (3H, t, \( J = 7.0 \) Hz, CH\(_2\)CH\(_2\)CH\(_2\)C\(_3\)H), 1.30 (2H, m, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 1.48 (2H, m, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 3.02 (2H, m, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 4.61 (1H, t, \( J = 6.0 \) Hz, NH), 8.06 (2H, d, \( J = 9.0 \) Hz, H-2’, H-6’) and 8.37 (2H, d, \( J = 9.0 \) Hz, H-3’, H-5’); \(^{13}\text{C NMR} \) (100 MHz, CDCl\(_3\)) \( \delta_C \) 13.5 (CH\(_3\)), 19.6 (CH\(_2\)), 31.7 (CH\(_2\)), 43.1 (CH\(_2\)), 124.4 (CH), 128.3 (CH), 146.0 (C) and 150.1 (C).
Compound 131 was prepared by a procedure similar to that of Habens and co-workers.\textsuperscript{232} To a stirring solution of N-butyl-4'-nitrobenzenesulfonamide (139) (0.14 g, 0.55 mmol) and iron powder (92 mg, 1.65 mmol) in methanol (3 mL) at room temperature was added ammonium chloride (0.15 g, 2.74 mmol) in water (3 mL), and the mixture heated at 70 °C for 2.5 h. The reaction was quenched through the addition of sat. sodium hydrogen carbonate (50 mL), extracted with ethyl acetate (3 × 50 mL), dried over anhydrous magnesium sulfate, filtered and the solvent removed \textit{in vacuo}. Purification by flash chromatography (hexane:ethyl acetate 4:1) afforded 131 as a white solid (78 mg, 0.34 mmol, 62%). $R_f = 0.20$ (hexane:ethyl acetate, 4:1); mp 96-100 °C [lit.\textsuperscript{400} mp 102 °C]; $^1$H NMR (400 MHz; CDCl$_3$) $\delta_H$ 0.85 (3H, t, $J = 7.2$ Hz, CH$_2$CH$_2$CH$_2$C$_3$), 1.28 (2H, m, CH$_2$CH$_2$CH$_2$CH$_3$), 1.43 (2H, m, CH$_2$CH$_2$CH$_2$CH$_3$), 2.90 (2H, m, CH$_2$CH$_2$CH$_2$CH$_3$), 4.14 (2H, s, NH$_2$), 4.37 (1H, t, $J = 6.0$ Hz, SO$_2$NH), 6.68 (2H, d, $J = 8.6$ Hz, H-3', H-5') and 7.63 (2H, d, $J = 8.6$ Hz, H-2', H-6'); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_C$ 13.5 (CH$_3$), 19.7 (CH$_2$), 31.5 (CH$_2$), 42.8 (CH$_2$), 114.0 (CH), 128.0 (C), 129.2 (CH) and 150.4 (C); MS (ESI, 70 eV) $m/z$ 229 (M$^+$, 100%). Found (M$^+$, 229.0995), C$_{10}$H$_{17}$N$_2$O$_2$S requires 229.1005.
Compound 140 was prepared by a procedure similar to that of Zhang and co-workers.\textsuperscript{234} To a stirring suspension of sodium hydride (6 mg, 0.14 mmol, 60\% w/w in mineral dispersion oil) in anhydrous tetrahydrofuran (3 mL) under nitrogen at 0 °C was added portionwise N-butyl-4'-nitrobenzenesulfonamide (139) (30 mg, 0.11 mmol), and the mixture stirred at room temperature for 1 h. To the solution at 0 °C was added dropwise methyl iodide (0.15 mL, 2.31 mmol), and the mixture heated under reflux for 18 h. The solution was allowed to cool, the solvent removed \textit{in vacuo}, the resulting residue was taken up in ethyl acetate (20 mL), washed with water (20 mL), the separated aqueous layer further extracted with ethyl acetate (2 × 20 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed \textit{in vacuo}. Purification by flash chromatography (hexane:ethyl acetate 9:1) afforded 140 as a white solid (26 mg, 0.10 mmol, 82\%). \(R_f = 0.25\) (hexane:ethyl acetate, 4:1); mp 84-88 °C; \(^1\)H NMR (300 MHz; CDCl\(_3\)) \(\delta_H\) 0.93 (3H, t, \(J = 7.0\) Hz, CH\(_2\)CH\(_2\)CH\(_2\)C\(_3\)), 1.34 (2H, m, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 1.54 (2H, m, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 2.79 (3H, s, NCH\(_3\)), 3.07 (2H, t, \(J = 7.0\) Hz, C\(_2\)H\(_2\)CH\(_2\)CH\(_3\)), 7.97 (2H, d, \(J = 8.8\) Hz, H-2', H-6') and 8.37 (2H, d, \(J = 8.8\) Hz, H-3', H-5'); \(^13\)C NMR (75 MHz, CDCl\(_3\)) \(\delta_C\) 13.6 (CH\(_3\)), 19.6 (CH\(_2\)), 29.6 (CH\(_2\)), 34.5 (CH\(_2\)), 49.9 (CH\(_3\)), 124.3 (CH), 128.4 (CH), 143.9 (C) and 150.0 (C); IR (\(\nu_{max}/\text{cm}^{-1}\)) 833, 975, 1092, 1152, 1299 (C-N), 1504 (N-O), 1600 (N-H), 2853, 2949, 3191; MS (ESI, 70 eV) \(m/z\) 295 (MNa\(^+\), 100\%); Found (MNa\(^+\), 295.0724), C\(_{11}\)H\(_{16}\)N\(_2\)NaO\(_4\)S requires 295.0723.
**N-Butyl-N-methyl-4′-aminobenzenesulfonamide (132)**

A similar procedure\(^{232}\) to that previously described for the preparation of 131 was followed using N-butyl-N-methyl-4′-nitrobenzenesulfonamide (140) (26 mg, 0.10 mmol) and iron powder (16 mg, 0.28 mmol) in methanol (1 mL), and ammonium chloride (25 mg, 0.48 mmol) in water (1 mL). Purification by flash chromatography (hexane:ethyl acetate 2:1) afforded 132 as a pale brown solid (20 mg, 0.08 mmol, 86%). \( R_f = 0.22 \) (hexane:ethyl acetate, 2:1); mp 71-74 °C; \(^1\)H NMR (400 MHz; CDCl\(_3\)) \( \delta \) 0.92 (3H, t, \( J = 7.3 \) Hz, CH\(_2\)CH\(_2\)CH\(_3\)), 1.34 (2H, m, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 1.50 (2H, m, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 2.67 (3H, s, NCH\(_3\)), 2.95 (2H, t, \( J = 7.3 \) Hz, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 4.11 (2H, s, NH\(_2\)), 6.69 (2H, d, \( J = 8.6 \) Hz, H-3′, H-5′) and 7.55 (2H, d, \( J = 8.6 \) Hz, H-2′, H-6′); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta \) 13.7 (CH\(_3\)), 19.7 (CH\(_2\)), 29.7 (CH\(_2\)), 34.6 (CH\(_2\)), 49.8 (CH\(_3\)), 114.0 (CH), 125.9 (C), 129.5 (CH) and 150.3 (C); IR (\( \nu_{\text{max}}/\text{cm}^{-1} \)) 743, 825, 921, 960, 1092, 1143, 1305 (C-N), 1504 (N-O), 1597 (N-H), 1642, 2812, 2861, 2957, 3256, 3370 (N-H) and 3462; MS (ESI, 70 eV) \( m/z \) 243 (M\(^+\), 100%). Found (M\(^+\), 243.1157), C\(_{11}\)H\(_{19}\)N\(_2\)O\(_2\)S requires 243.1162.
7.2.4. Benzocaine bioisosteres and related heterocycles

7.2.4.1. Benzocaine (141)

Compound 141 was prepared by a procedure similar to that of Lindén and co-workers. To a stirring solution of 4-aminobenzoic acid (142) (0.68 g, 4.97 mmol) in ethanol (5 mL) at room temperature was added dropwise sulfuric acid (0.5 mL, 18 M), and the mixture stirred overnight. The reaction was quenched through the addition of aqueous sodium hydroxide (50 mL, 2 M), extracted with dichloromethane (3 × 50 mL), dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate, 4:1) afforded 141 as a light brown solid (0.29 g, 1.76 mmol, 35%). $R_f = 0.25$ (hexane:ethyl acetate, 2:1); $^1H$ NMR (300 MHz; CDCl$_3$) $\delta$ 1.36 (3H, t, $J = 7.2$ Hz, OCH$_2$C$_6$H$_5$), 4.05 (2H, s, NH$_2$), 4.31 (2H, q, $J = 7.2$ Hz, OCH$_2$CH$_3$), 6.64 (2H, d, $J = 8.7$ Hz, H-3', H-5') and 7.86 (2H, d, $J = 8.7$ Hz, H-2', H-6'); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 14.4 (CH$_3$), 60.3 (CH$_2$), 113.8 (CH), 120.2 (C), 131.5 (CH), 150.7 (C) and 166.7 (C); MS (ESI, 70 eV) $m/z$ 166 (M$^+$, 100%). Found (M$^+$, 166.0860), C$_9$H$_{12}$NO$_2$ requires 166.0863.

7.2.4.2. 3-Substituted-1,2,4-oxadiazoles 144-149

(Z)-Acetamidoxime (150)

Compound 150 was prepared by a procedure similar to that of Chiou and co-workers. A solution of acetonitrile (2.00 g, 48.72 mmol), hydroxylamine hydrochloride (3.55 g, 51.16 mmol) and sodium hydroxide (2.14 g, 53.59 mmol) in ethanol (20 mL) and water (5 mL) was heated under reflux for 36 h. The solvent was removed in vacuo and the
residue was diluted with water (100 mL), extracted with ethyl acetate (3 × 100 mL), dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo to afford 150 as a white solid (1.60 g), which was used without further purification. mp 132-136 °C [ lit.403 mp 133-135 °C]; $^1$H NMR (400 MHz; CDCl$_3$) $\delta_{H}$ 1.85 (3H, s, CH$_3$) and 4.54 (2H, s, NH$_2$); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta_C$ = 17.0 (CH$_3$) and 151.2 (C).

(Z)-Propamidoxime (151)

A similar procedure$^{254}$ to that previously described for the preparation of 150 was followed using propionitrile (2.00 g, 36.31 mmol), hydroxylamine hydrochloride (2.65 g, 38.13 mmol) and sodium hydroxide (1.60 g, 39.94 mmol) in ethanol (16 mL) and water (4 mL) to afford 151 as a pale brown oil (0.63 g), which was used without further purification. $^1$H NMR (400 MHz; CDCl$_3$) $\delta_{H}$ 1.15 (3H, t, $J$ = 7.8 Hz, CH$_2$CH$_3$), 2.19 (2H, q, $J$ = 7.8 Hz, CH$_2$CH$_3$) and 4.56 (2H, s, NH$_2$). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_C$ 10.9 (CH$_3$), 24.6 (CH$_2$) and 154.9 (C); IR ($\nu_{\text{max/cm}}$) 866, 1072 (C–N), 1649 (N-H), 2853, 2922, 3184 (N-H) and 3382 (O-H); MS (ESI, 70 eV) $m/z$ 111 (MNa$^+$, 100%); Found (MNa$^+$, 111.0532), C$_3$H$_8$N$_2$NaO requires 111.0529.

(Z)-Butyramidoxime (152)

A similar procedure$^{254}$ to that previously described for the preparation of 150 was followed using butyronitrile (2.00 g, 28.94 mmol), hydroxylamine hydrochloride (2.11 g, 30.39 mmol) and sodium hydroxide (1.27 g, 31.83 mmol) in ethanol (16 mL) and water (4 mL) to afford 152 as a pale brown oil (0.81 g), which was used without further purification. $^1$H NMR (400 MHz; CDCl$_3$) $\delta_{H}$ 0.95 (3H, t, $J$ = 7.4 Hz, CH$_2$CH$_2$CH$_3$), 1.57 (2H, m, CH$_2$CH$_2$CH$_3$), 2.11 (2H, t, $J$ = 7.4 Hz, CH$_2$CH$_2$CH$_3$) and 4.67 (2H, s, NH$_2$); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_C$ 13.5 (CH$_3$), 19.9 (CH$_2$), 33.0 (CH$_2$) and 153.9 (C); IR
(ν_{max}/cm^{-1}) 867, 1166 (C-N), 1651 (N-H), 2874, 2963, 3180 (N-H) and 3323 (O-H); MS (ESI, 70 eV) m/z 125 (MNa^+, 100%); Found (MNa^+, 125.0687), C_{4}H_{10}N_{2}NaO requires 125.0685.

(Z)-Isobutyramidoxime (153)^{404}

A similar procedure^{254} to that previously described for the preparation of 150 was followed using isobutyronitrile (2.0 g, 28.94 mmol), hydroxylamine hydrochloride (2.11 g, 30.39 mmol) and sodium hydroxide (1.27 g, 31.83 mmol) in ethanol (16 mL) and water (4 mL) to afford 153 as a pale brown oil (0.74 g), which was used without further purification. \(^1\)H NMR (400 MHz; d_6-DMSO) δ_H 1.08 (6H, d, J = 7.0 Hz, CH(CH_{3})_{2}), 2.30 (1H, m, CH(CH_{3})_{2}), 4.67 (2H, s, NH_{2}), and 8.74 (1H, s, OH); \(^13\)C NMR (100 MHz, d_6-DMSO) δ_C 21.1 (CH_{3}), 31.3 (CH) and 158.0 (C).

(Z)-Benzamidoxime (154)^{405}

A similar procedure^{254} to that previously described for the preparation of 150 was followed using benzonitrile (5.00 g, 48.44 mmol), hydroxylamine hydrochloride (3.54 g, 50.09 mmol) and sodium hydroxide (2.13 g, 53.28 mmol) in ethanol (40 mL) and water (10 mL) to afford 154 as a pale brown oil (3.06 g), which was used without further purification. R_f = 0.50 (hexane:ethyl acetate, 1:1); \(^1\)H NMR (400 MHz; d_6-DMSO) δ_H 4.91 (2H, s, NH_{2}), 7.38-7.43 (3H, m, ArH) and 7.62-7.66 (2H, m, ArH); \(^13\)C NMR (100 MHz, d_6-DMSO) δ_C 125.8 (CH), 127.3 (C), 128.6 (CH), 132.5 (CH) and 152.6 (C).
(Z)-Phenylacetamidoxime (155)

A similar procedure to that previously described for the preparation of 150 was followed using benzyl cyanide (2.00 g, 17.07 mmol), hydroxylamine hydrochloride (1.25 g, 17.92 mmol) and sodium hydroxide (0.75 g, 18.78 mmol) in ethanol (16 mL) and water (4 mL) to afford 155 as a pale brown oil (2.01 g), which was used without further purification. \( R_f = 0.60 \) (hexane:ethyl acetate, 1:1); \( ^1H\text{ NMR} \) (400 MHz; CDCl\(_3\)) \( \delta H \) 3.30 (2H, s, CH\(_2\)), 5.41 (2H, s, NH\(_2\)), 7.30-7.33 (5H, m, ArH) and 8.93 (1H, s, OH); \( ^{13}C\text{ NMR} \) (100 MHz, CDCl\(_3\)) \( \delta C \) 43.2 (CH\(_2\)), 127.1 (CH), 129.0 (CH), 129.6 (CH), 138.9 (C) and 152.9 (C).

3-Methyl-5-(4'-nitrophenyl)-1,2,4-oxadiazole (157)

Compound 157 was prepared by a procedure similar to that of Chiou and co-workers. To a stirring suspension of crude (Z)-acetamidoxime (150) (1.60 g) in pyridine (25 mL) at room temperature was added slowly 4-nitrobenzoyl chloride (156) (8.03 g, 43.20 mmol), and the mixture heated under reflux for 30 min. The solution was allowed to cool and diluted with water (50 mL). The resulting precipitate was collected by filtration, washed with water (10 mL) and dried \textit{in vacuo}. Purification by flash chromatography (hexane:ethyl acetate 9:1) afforded 157 as a pale brown solid (0.77 g, 3.31 mmol, 7% over two steps). \( R_f = 0.80 \) (hexane:ethyl acetate, 1:1); mp 145-149 °C [lit.\textsuperscript{407} mp 146 °C]; \( ^1H\text{ NMR} \) (400 MHz; CDCl\(_3\)) \( \delta H \) 2.52 (3H, s, CH\(_3\)), 8.31 (2H, d, \( J = 8.0 \) Hz, H-2', H-6') and 8.39 (2H, d, \( J = 8.0 \) Hz, H-3', H-5'); \( ^{13}C\text{ NMR} \) (100 MHz, CDCl\(_3\)) \( \delta C \) 11.6 (CH\(_3\)), 124.3 (CH), 129.0 (CH), 129.4 (C), 150.1 (C), 168.3 (C) and 173.3 (C).
3-Ethyl-5-(4'-nitrophenyl)-1,2,4-oxadiazole (158)

A similar procedure to that previously described for the preparation of 157 was followed using crude (Z)-propamidoxime (151) (0.63 g) and 4-nitrobenzoyl chloride (156) (2.66 g, 14.30 mmol) in pyridine (15 mL). Purification by flash chromatography (hexane:ethyl acetate 9:1) afforded 158 as a pale brown solid (0.58 g, 2.64 mmol, 7\% over two steps). \( R_f = 0.75 \) (hexane:ethyl acetate, 2:1); mp 108-113 °C; \(^1\)H NMR (400 MHz; CDCl\(_3\)) \( \delta_H \) 1.41 (3H, t, \( J = 7.6\) Hz, CH\(_2\)C\(_3\)H\(_3\)), 2.88 (2H, q, \( J = 7.6\) Hz, CH\(_2\)CH\(_3\)), 8.33 (2H, d, \( J = 9.0\) Hz, H-2', H-6') and 8.39 (2H, d, \( J = 9.0\) Hz, H-3', H-5'); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta_C \) 11.3 (CH\(_3\)), 19.8 (CH\(_2\)), 124.3 (CH), 129.0 (CH), 129.6 (C), 150.0 (C), 172.7 (C) and 173.3 (C); IR (\( \nu_{\text{max}}/\text{cm}^{-1} \)) 718, 866, 1103 (C-N), 1287, 1413, 1515 (N-O), 1562, 2932, 2976 and 3121; MS (ESI, 70 eV) \( m/z \) 220 (M\(^+\), 100\%); Found (M\(^+\), 220.0719), C\(_{10}\)H\(_{10}\)N\(_3\)O\(_3\) requires 220.0717.

3-Propyl-5-(4'-nitrophenyl)-1,2,4-oxadiazole (159)

A similar procedure to that previously described for the preparation of 157 was followed using crude (Z)-butyramidoxime (152) (0.80 g) and 4-nitrobenzoyl chloride (156) (2.91 g, 15.67 mmol) in pyridine (15 mL). Purification by flash chromatography (hexane:ethyl acetate 9:1) afforded 159 as a pale brown solid (0.77 g, 3.31 mmol, 11\% over two steps). \( R_f = 0.75 \) (hexane:ethyl acetate, 2:1); mp 84-89 °C; \(^1\)H NMR (400 MHz; CDCl\(_3\)) \( \delta_H \) 1.05 (3H, t, \( J = 7.6\) Hz, CH\(_2\)CH\(_2\)C\(_3\)H\(_3\)), 1.86 (2H, m, CH\(_2\)C\(_2\)CH\(_3\)), 2.82 (2H, t, \( J = 7.6\) Hz, CH\(_2\)CH\(_2\)CH\(_3\)), 8.32 (2H, d, \( J = 9.0\) Hz, H-2', H-6') and 8.40 (2H, d, \( J = 9.0\) Hz, H-3', H-5'); IR (\( \nu_{\text{max}}/\text{cm}^{-1} \)) 711, 866, 1100 (C-N), 1284, 1411, 1513 (N-O), 1567, 2930, 2976 and 3121; MS (ESI, 70 eV) \( m/z \) 220 (M\(^+\), 100\%); Found (M\(^+\), 220.0719), C\(_{10}\)H\(_{10}\)N\(_3\)O\(_3\) requires 220.0717.
7. Experimental

7.2. Synthesis

Hz, H-3', H-5'); $^{13}\text{C} \text{NMR}$ (100 MHz, CDCl$_3$) $\delta$C 13.6 (CH$_3$), 20.4 (CH$_2$), 27.9 (CH$_2$), 124.3 (CH), 129.0 (CH), 129.6 (C), 150.0 (C), 171.6 (C) and 173.2 (C); $\text{IR}$ ($\nu_{\max}$/cm$^{-1}$) 722, 864, 1091 (C-N), 1309, 1521 (N-O), 1570, 2868, 2971 and 3115; $\text{MS}$ (ESI, 70 eV) $m/z$ 256 (MNa$^+$, 100%). Found (MNa$^+$, 256.0699), C$_{11}$H$_{11}$N$_3$NaO$_3$ requires 256.0693.

3-Isopropyl-5-(4'-nitrophenyl)-1,2,4-oxadiazole (160)

A similar procedure$^{254}$ to that previously described for the preparation of 157 was followed using crude (Z)-isobutyramidoxime (153) (0.74 g) and 4-nitrobenzoyl chloride (156) (2.70 g, 14.49 mmol) in pyridine (15 mL). Purification by flash chromatography (hexane:ethyl acetate 9:1) afforded 160 as a pale brown solid (0.24 g, 1.03 mmol, 4% over two steps). $R_f = 0.80$ (hexane:ethyl acetate, 2:1); mp 110-115 °C; $^1\text{H} \text{NMR}$ (400 MHz; CDCl$_3$) $\delta$H 1.42 (6H, d, $J = 7.2$ Hz, CH(CH$_3$)$_2$), 3.21 (1H, m, CH(CH$_3$)$_2$), 8.33 (2H, d, $J = 9.0$ Hz, H-2', H-6') and 8.39 (2H, d, $J = 9.0$ Hz, H-3', H-5'); $^{13}\text{C} \text{NMR}$ (100 MHz, CDCl$_3$) $\delta$C 20.5 (CH$_3$), 26.8 (CH), 124.2 (CH), 129.1 (CH), 129.7 (C), 150.0 (C), 173.2 (C) and 176.2 (C); $\text{IR}$ ($\nu_{\max}$/cm$^{-1}$) 705, 866, 1061 (C-N), 1346, 1524 (N-O), 1569, 2876, 2979 and 3121; $\text{MS}$ (ESI, 70 eV) $m/z$ 234 (M$^+$, 100%); Found (M$^+$, 234.0878), C$_{11}$H$_{12}$N$_3$O$_3$ requires 234.0873.
3-Phenyl-5-(4'-nitrophenyl)-1,2,4-oxadiazole (161)\textsuperscript{408}

A similar procedure\textsuperscript{254} to that previously described for the preparation of 157 was followed using crude (Z)-benzamidoxime (154) (2.00 g) and 4-nitrobenzoyl chloride (156) (5.46 g, 29.40 mmol) in pyridine (35 mL). Purification by flash chromatography (hexane:ethyl acetate 9:1) afforded 161 as a pale brown solid (1.40 g, 5.23 mmol, 18% over two steps). $R_f = 0.90$ (hexane:ethyl acetate, 2:1); $^1\text{H NMR}$ (400 MHz; CDCl$_3$) $\delta$H 7.51-7.56 (3H, m, ArH), 8.17 (2H, m, ArH) and 8.40-8.42 (4H, m, H-2', H-3', H-5', H-6'); $^{13}\text{C NMR}$ (100 MHz, CDCl$_3$) $\delta$C 124.4 (CH), 126.3 (C), 127.5 (CH), 129.0 (CH), 129.2 (CH), 129.5 (C), 131.6 (CH), 150.2 (C), 169.4 (C) and 173.6 (C).

3-Benzyl-5-(4'-nitrophenyl)-1,2,4-oxadiazole (162)\textsuperscript{408}

A similar procedure\textsuperscript{254} to that previously described for the preparation of 157 was followed using crude (Z)-phenylacetamidoxime (155) (1.00 g) and 4-nitrobenzoyl chloride (156) (2.51 g, 13.5 mmol) in pyridine (15 mL). Purification by flash chromatography (hexane:ethyl acetate 9:1) afforded 162 as a pale brown solid (60 mg, 0.21 mmol, 2% over two steps). $R_f = 0.90$ (hexane:ethyl acetate, 2:1); $^1\text{H NMR}$ (400 MHz; CDCl$_3$) $\delta$H 4.17 (2H, s, CH$_2$), 7.26-7.40 (5H, m, ArH), 8.29 (2H, d, $J = 8.8$
Hz, H-2', H-6') and 8.35 (2H, d, J = 8.8 Hz, H-3', H-5'); $^{13}$C NMR (100 MHz, CDCl₃) $\delta_C$

32.4 (CH₂), 124.3 (CH), 127.3 (CH), 128.8 (CH), 129.0 (CH), 129.1 (CH), 129.5 (C), 135.1 (C), 150.1 (C), 170.6 (C) and 173.5 (C).

3-Methyl-5-(4'-aminophenyl)-1,2,4-oxadiazole (144)²⁵⁵

Compound 144 was prepared by a procedure similar to that of Lin and co-workers.²⁵⁵ To a stirring solution of 3-methyl-5-(4'-nitrophenyl)-1,2,4-oxadiazole (162) (50 mg, 0.24 mmol) in 1,4-dioxane (1 mL) at 80 °C was added a hot solution (75 °C) of sodium sulfide nonahydrate (140 mg, 0.58 mmol) in water (1 mL), and the reaction mixture was stirred at 80 °C for 45 min. The solution was allowed to cool and the solvent removed in vacuo. The residue was diluted with ethyl acetate (50 mL), washed with water (50 mL), the separated aqueous layer further extracted with ethyl acetate (3 × 50 mL) and the combined organic phases dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate 9:1) afforded 144 as a pale brown solid (37 mg, 0.21 mmol, 87%). $R_f = 0.45$

(hexane:ethyl acetate, 1:1); mp 185-190 °C [lit.²⁵⁵ mp 186-188 °C]; $^1$H NMR (300 MHz; CDCl₃) $\delta_H$ 2.42 (3H, s, CH₃), 4.13 (2H, s, NH₂), 6.72 (2H, d, J = 8.7 Hz, H-3', H-5') and 7.90 (2H, d, J = 8.7 Hz, H-2', H-6'); $^{13}$C NMR (75 MHz, CDCl₃) $\delta_c$ 11.7 (CH₃), 113.8 (C), 114.5 (CH), 129.8 (CH), 150.5 (C), 167.4 (C) and 175.7 (C); MS (ESI, 70 eV) m/z 176 (M⁺, 100%). Found (M⁺, 176.0822), C₉H₁₀N₃O requires 176.0818.
3-Ethyl-5-(4'-aminophenyl)-1,2,4-oxadiazole (145)

A similar procedure to that previously described for the preparation of 144 was followed using 3-ethyl-5-(4'-nitrophenyl)-1,2,4-oxadiazole (158) (0.58 g, 2.64 mmol) in 1,4-dioxane (10 mL) and sodium sulfide nonahydrate (1.52 g, 6.34 mmol) in water (10 mL). Purification by flash chromatography (hexane:ethyl acetate 4:1) afforded 145 as a pale brown solid (0.40 g, 2.13 mmol, 81%). $R_f = 0.30$ (hexane:ethyl acetate, 2:1); mp 100-105 °C; $^1$H NMR (400 MHz; CDCl₃) $\delta$ 1.37 (3H, t, $J = 8.0$ Hz, CH₂CH₃), 2.79 (2H, q, $J = 8.0$ Hz, CH₂CH₃), 4.22 (2H, s, NH₂), 6.71 (2H, d, $J = 8.8$ Hz, H-3', H-5') and 7.90 (2H, d, $J = 8.8$ Hz, H-2', H-6'); $^{13}$C NMR (100 MHz, CDCl₃) $\delta$ C 11.4 (CH₃), 19.8 (CH₂), 113.7 (C), 114.3 (CH), 129.8 (CH), 150.6 (C), 171.8 (C) and 175.6 (C); IR (νmax/cm⁻¹) 683, 751, 825, 1254 (C-N), 1322, 1484, 1581 (N-H), 1645, 2926, 2980, 3228, 3334 (N-H) and 3446; MS (ESI, 70 eV) m/z 190 (M⁺, 100%). Found (M⁺, 190.0976), C₁₀H₁₂N₃O requires 190.0975.

3-Propyl-5-(4'-aminophenyl)-1,2,4-oxadiazole (146)

A similar procedure to that previously described for the preparation of 144 was followed using 3-propyl-5-(4'-nitrophenyl)-1,2,4-oxadiazole (159) (770 mg, 3.30 mmol) in 1,4-dioxane (10 mL) and sodium sulfide nonahydrate (1.90 g, 7.92 mmol) in water (10 mL). Purification by flash chromatography (hexane:ethyl acetate 4:1) afforded 146 as a
pale brown solid (0.46 g, 2.26 mmol, 69%). $R_f = 0.32$ (hexane:ethyl acetate, 2:1); mp 96-100 °C; $^1$H NMR (400 MHz; CDCl$_3$) $\delta_H$ 1.02 (3H, t, $J = 7.3$ Hz, CH$_2$CH$_2$CH$_3$), 1.82 (2H, m, CH$_2$CH$_2$CH$_3$), 2.74 (2H, t, $J = 7.3$ Hz, CH$_2$CH$_2$CH$_3$), 4.13 (2H, s, NH$_2$), 6.71 (2H, d, $J = 8.4$ Hz, H-3', H-5') and 7.91 (2H, d, $J = 8.4$ Hz, H-2', H-6'); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_C$ 13.7 (CH$_3$), 20.5 (CH$_2$), 28.0 (CH$_2$), 113.8 (C), 114.4 (CH), 129.8 (CH), 150.5 (C), 170.7 (C) and 175.5 (C); IR ($\nu_{\text{max}}$/cm$^{-1}$) 709, 759, 839, 1179, 1230 (C-N), 1326, 1491, 1578 (N-H), 1628, 2872, 2961, 3205, 3324 (N-H) and 3473; MS (ESI, 70 eV) m/z 204 (M$,^+$, 100%); Found (M$,^+$, 204.1134), C$_{11}$H$_{14}$N$_3$O requires 204.1131.

3-Isopropyl-5-(4'-aminophenyl)-1,2,4-oxadiazole (147)

A similar procedure$^{255}$ to that previously described for the preparation of 144 was followed using 3-isopropyl-5-(4'-nitrophenyl)-1,2,4-oxadiazole (160) (0.24 g, 1.01 mmol) in 1,4-dioxane (5 mL) and sodium sulfide nonahydrate (0.58 g, 2.42 mmol) in water (5 mL). Purification by flash chromatography (hexane:ethyl acetate 2:1) afforded 147 as a pale brown solid (0.13 g, 0.64 mmol, 63%). $R_f = 0.30$ (hexane:ethyl acetate, 2:1); mp 90-95 °C; $^1$H NMR (400 MHz; CDCl$_3$) $\delta_H$ 1.38 (6H, d, $J = 7.2$ Hz, CH(CH$_3$)$_2$), 3.12 (1H, m, CH(CH$_3$)$_2$), 4.15 (2H, s, NH$_2$), 6.70 (2H, d, $J = 8.6$ Hz, H-3', H-5') and 7.91 (2H, d, $J = 8.6$ Hz, H-2', H-6'); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_C$ 20.5 (CH$_3$), 26.8 (CH$_2$), 113.8 (C), 114.3 (CH), 129.8 (CH), 150.5 (C), 175.3 (C) and 175.5 (C); IR ($\nu_{\text{max}}$/cm$^{-1}$) 661, 766, 837, 837, 1162, 1252 (C-N), 1387, 1490, 1583 (N-H), 1626, 2873, 2927, 3192, 3301 (N-H) and 3470; MS (ESI, 70 eV) m/z 204 (M$,^+$, 100%). Found (M$,^+$, 204.1127), C$_{11}$H$_{14}$N$_3$O requires 204.1131.
3-Phenyl-5-(4'-aminophenyl)-1,2,4-oxadiazole (148)

A similar procedure to that previously described for the preparation of 144 was followed using 3-phenyl-5-(4'-nitrophenyl)-1,2,4-oxadiazole (161) (0.35 g, 1.32 mmol) in 1,4-dioxane (3 mL) and sodium sulfide nonahydrate (0.76 g, 3.17 mmol) in water (3 mL). Purification by flash chromatography (hexane:ethyl acetate 2:1) afforded 148 as a pale brown solid (0.26 g, 1.10 mmol, 83%). Rf = 0.40 (hexane:ethyl acetate, 1:1); mp 165-170 °C; 1H NMR (400 MHz; CDCl3) δH 4.15 (2H, s, NH2), 6.76 (2H, d, J = 8.8 Hz, H-3’, H-5’), 7.49-7.51 (3H, m, ArH), 8.02 (2H, d, J = 8.8 Hz, H-2’, H-6’) and 8.15 (2H, m, ArH); 13C NMR (100 MHz, CDCl3): δC = 114.0 (C), 114.5 (CH), 127.3 (C), 127.5 (CH), 128.8 (CH), 130.0 (CH), 130.9 (CH), 150.6 (C), 168.6 (C) and 176.0 (C); IR (νmax/cm-1) 689, 748, 1217 (C-N), 1366, 1500, 1604 (N-H), 1739, 2970, 3221, 3324 (N-H) and 3392; MS (ESI, 70 eV) m/z 238 (M+, 100%). Found (M+, 238.0970), C14H12N3O requires 238.0975.

3-Benzyl-5-(4'-aminophenyl)-1,2,4-oxadiazole (149)

A similar procedure to that previously described for the preparation of 144 was followed using 3-benzyl-5-(4'-nitrophenyl)-1,2,4-oxadiazole (162) (60 mg, 0.21 mmol) in 1,4-dioxane (5 mL) and sodium sulfide nonahydrate (0.12 g, 0.51 mmol) in water (5 mL).
Purification by flash chromatography (hexane:ethyl acetate 4:1) afforded 149 as a pale brown solid (25 mg, 0.10 mmol, 47%). \( R_f = 0.38 \) (hexane:ethyl acetate, 2:1); \(^1\)H NMR (400 MHz; CDCl\(_3\)) \( \delta_H \) 4.10 (4H, s, \( CH_2, NH_2 \)), 6.67 (2H, d, \( J = 8.8 \) Hz, H-3', H-5'), 7.25-7.38 (5H, m, ArH) and 7.88 (2H, d, \( J = 8.8 \) Hz, H-2', H-6'); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta_C \) 32.4 (CH\(_2\)), 113.8 (C), 114.4 (CH), 126.9 (CH), 128.6 (CH), 129.0 (CH), 129.9 (CH), 135.8 (C), 150.6 (C), 169.7 (C) and 176.0 (C); IR (\( \nu_{\text{max}}/\text{cm}^{-1} \)) 706, 754, 1181, 1289 (C-N), 1364, 1488, 1607 (N-H), 2924, 3029, 3217, 3345 (N-H) and 3427; MS (ESI, 70 eV) \( m/z \) 273 (MNa\(^+\), 100%). Found (MNa\(^+\), 274.0950), C\(_{15}\)H\(_{13}\)N\(_3\)NaO requires 274.0951.

7.2.4.3. 5-Substituted-1,3,4-oxadiazoles 163-169

4'-Nitrobenzohydrazide (171)

\[
\begin{align*}
\text{O} & \text{O} \\
\text{N} & \text{N} \\
\text{H} & \text{H} \\
\text{NH}_2 & \text{NH}_2
\end{align*}
\]

Compound 171 was prepared by a procedure similar to that of Tasaganva and co-workers.\(^{256}\) To a stirring solution of 4-nitrobenzoyl chloride (156) (7.50 g, 40.35 mmol) in methanol (20 mL) at room temperature was added dropwise hydrazine hydrate (6.22 g, 80.71 mmol, 65% v/v) and mixture heated under reflux for 30 min. The reaction mixture was allowed to cool and the resulting precipitate was collected by filtration, washed with water (50 mL) and dried \textit{in vacuo}. Purification by recrystallization (ethanol) afforded 171 as a white solid (4.00 g, 22.08 mmol, 55%). \( R_f = 0.05 \) (hexane:ethyl acetate, 1:1); \(^1\)H NMR (400 MHz; d\(_6\)-DMSO) \( \delta_H \) 4.73 (2H, s, NH\(_2\)), 8.08 (2H, d, \( J = 8.8 \) Hz, H-2', H-6'), 8.33 (2H, d, \( J = 8.8 \) Hz, H-3', H-5') and 10.18 (1H, s, NH); \(^{13}\)C NMR (100 MHz, d\(_6\)-DMSO) \( \delta_C \) 124.5 (CH), 129.4 (CH), 140.0 (C), 149.9 (C) and 164.8 (C).
\(N'^{-}\text{Acetyl-4'\text{-nitrobenzohydrazide (172)}^{256}\)

[Image]

Compound 172 was prepared by a procedure similar to that of Tasaganva and co-workers.\textsuperscript{256} To a stirring solution of 4-nitrobenzohydrazide (171) (1.02 g, 5.63 mmol) in dimethylacetamide (5 mL) at 0 °C was added dropwise acetyl chloride (0.48 mL, 6.19 mmol), and the mixture stirred at 0 °C for 6 h. The reaction mixture was then quenched through the addition of pyridine (0.68 mL, 8.48 mmol) and the resulting mixture poured onto ice-cold water (50 mL). The resulting precipitate was collected by filtration, washed with water (25 mL) and dried \textit{in vacuo}. Purification by recrystallization (ethanol) afforded 172 as a white solid (0.40 g, 1.79 mmol, 31%). \(R_f = 0.25\) (ethyl acetate); \(^1\text{H NMR}\) (400 MHz; \(d_6\)-DMSO) \(\delta_H\) 1.98 (3H, s, \(CH_3\)), 8.12 (2H, d, \(J = 8.8\) Hz, H-2', H-6'), 8.38 (2H, d, \(J = 8.8\) Hz, H-3', H-5'), 10.04 (1H, s, NH) and 10.68 (1H, s, NH); \(^{13}\text{C NMR}\) (100 MHz, \(d_6\)-DMSO) \(\delta_C\) 21.5 (CH\(_3\)), 124.6 (CH), 129.9 (CH), 139.1 (C), 150.3 (C), 164.8 (C) and 169.3 (C).

\(N'^{-}\text{Propionyl-4'\text{-nitrobenzohydrazide (173)}\)

[Image]

A similar procedure\textsuperscript{256} to that previously described for the preparation of 172 was followed using 4-nitrobenzohydrazide (171) (0.50 g, 2.76 mmol) and propionic anhydride (0.39 mL, 3.04 mmol) in dimethylacetamide (3 mL), quenching with pyridine (0.33 mL, 4.14 mmol). Purification by recrystallization (ethanol) afforded 173 as a white solid (0.19 g, 0.80 mmol, 29%). \(R_f = 0.33\) (ethyl acetate); mp 203-205 °C; \(^1\text{H NMR}\) (400 MHz; \(d_6\)-DMSO) \(\delta_H\) 1.11 (3H, t, \(J = 7.2\) Hz, CH\(_2\)CH\(_3\) ), 2.25 (2H, q, \(J = 7.2\) Hz, CH\(_2\)CH\(_3\)), 8.13 (2H, d, \(J = 9.0\) Hz, H-2’, H-6’), 8.38 (2H, d, \(J = 9.0\) Hz, H-3’, H-5’), 10.00 (1H, s, NH) and
10.68 (1H, s, NH); $^{13}$C NMR (100 MHz, d$_6$-DMSO) $\delta$C 10.6 (CH$_3$), 27.4 (CH$_2$), 124.6 (CH), 129.9 (CH), 139.1 (C), 150.3 (C), 164.8 (C) and 172.3 (C); IR ($\nu_{\text{max}}$/cm$^{-1}$) 856, 869, 1317 (C-N), 1497, 1519 (N-O), 1573 (N-H), 1648, 2961 and 3195 (N-H); MS (ESI, 70 eV) $m/z$ 260 (MNa$^+$, 100%); Found (MNa$^+$, 260.0645), C$_{10}$H$_{11}$N$_3$NaO$_4$ requires 260.0641.

$N'$-Butyryl-4'-nitrobenzohydrazide (174)

A similar procedure $^{256}$ to that previously described for the preparation of 172 was followed using 4-nitrobenzohydrazide (171) (0.50 g, 2.76 mmol) and butyric anhydride (0.50 mL, 3.04 mmol) in dimethylacetamide (3 mL), quenching with pyridine (0.33 mL, 4.14 mmol). Purification by recrystallization (ethanol) afforded 174 as a white solid (70 mg, 0.28 mmol, 10%). $R_f = 0.40$ (ethyl acetate); mp 188-192 °C; $^1$H NMR (300 MHz; d$_6$-DMSO) $\delta$H 0.97 (3H, t, $J = 7.4$ Hz, CH$_2$CH$_2$CH$_3$), 1.62 (2H, m, CH$_2$CH$_2$CH$_3$), 2.22 (2H, t, $J = 7.4$ Hz, CH$_2$CH$_2$CH$_3$), 8.13 (2H, d, $J = 9.0$ Hz, H-2', H-6'), 8.38 (2H, d, $J = 9.0$ Hz, H-3', H-5'), 10.00 (1H, s, NH) and 10.68 (1H, s, NH); $^{13}$C NMR (100 MHz, d$_6$-DMSO) $\delta$C 14.4 (CH$_3$), 19.4 (CH$_2$), 36.1 (CH$_2$), 124.6 (CH), 129.9 (CH), 139.1 (C), 150.3 (C), 164.8 (C) and 172.3 (C); IR ($\nu_{\text{max}}$/cm$^{-1}$) 834, 869, 1224, 1345 (C-N), 1496, 1517 (N-O), 1574 (N-H), 1670, 2984 and 3182 (N-H); MS (ESI, 70 eV) $m/z$ 274 (MNa$^+$, 100%); Found (MNa$^+$, 274.0805), C$_{11}$H$_{13}$N$_3$NaO$_4$ requires 274.0798.
**N'-Isobutyryl-4'-nitrobenzohydrazide (175)**

![Chemical structure of N'-Isobutyryl-4'-nitrobenzohydrazide (175)](image)

A similar procedure to that previously described for the preparation of 172 was followed using 4-nitrobenzohydrazide (171) (0.50 g, 2.76 mmol) and isobutyryl chloride (0.32 mL, 3.04 mmol) in dimethylacetamide (3 mL), quenching with pyridine (0.33 mL, 4.14 mmol). Purification by recrystallization (ethanol) afforded 175 as a white solid (0.25 g, 1.00 mmol, 36%).

\[ R_f = 0.42 \text{ (ethyl acetate); mp 186-190 °C; } \]

\[ ^1H \text{ NMR (400 MHz; } d_6\text{-DMSO) } \delta_H 1.13 \text{ (6H, d, } J = 6.8 \text{ Hz, } \text{CH}(CH_3)_2), \]

\[ 2.55 \text{ (1H, m, } \text{CH}(CH_3)_2), \]

\[ 8.14 \text{ (2H, d, } J = 8.6 \text{ Hz, H-2', H-6'), 8.38 \text{ (2H, d, } J = 8.6 \text{ Hz, H-3', H-5'), 10.00 \text{ (1H, s, NH) and } 10.68 \text{ (1H, s, NH); } ^13C \text{ NMR (100 MHz, } d_6\text{-DMSO) } \delta_C 20.3 \text{ (CH}_3), 33.1 \text{ (CH), } 124.6 \text{ (CH), } 129.8 \text{ (CH), } 139.1 \text{ (C), } 150.3 \text{ (C), } 164.8 \text{ (C) and } 176.4 \text{ (C); } \]

\[ ^1\text{H NMR (300 MHz; } d_6\text{-DMSO) } \delta_H 0.94 \text{ (3H, t, } J = 7.5 \text{ Hz, } \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3), 1.38 \text{ (2H, m, } \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3), 1.59 \text{ (2H, m, } \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3), 2.24 \text{ (2H, t, } J = 7.5 \text{ Hz, }} \]

\[ \text{IR (v max/cm}^{-1}) \text{ 713, 868, 1195, 1320 (C=N), 1496, 1514 (N-O), (N-H), 1627 and 3200 (N-H); MS (ESI, 70 eV) m/z 274 (MN}^+\text{, } 100\%); \]

\[ \text{Found (MN}^+,274.0795), \text{ C}_{11}\text{H}_{13}\text{N}_3\text{NaO}_4 \text{requires 274.0798.} \]

**N'-Valeroyl-4'-nitrobenzohydrazide (176)**

A similar procedure to that previously described for the preparation of 172 was followed using 4-nitrobenzohydrazide (171) (1.00 g, 5.52 mmol) and valeroyl chloride (0.73 mL, 6.07 mmol) in dimethylacetamide (10 mL), quenching with pyridine (0.67 mL, 8.28 mmol). Purification by recrystallization (ethanol) afforded 176 as a white solid (1.21 g, 4.56 mmol, 83%).

\[ R_f = 0.45 \text{ (hexane:ethyl acetate, 1:3); mp 188-190 °C; } \]

\[ ^1H \text{ NMR (300 MHz; } d_6\text{-DMSO) } \delta_H 0.94 \text{ (3H, t, } J = 7.5 \text{ Hz, } \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3), 1.38 \text{ (2H, m, } \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3), 1.59 \text{ (2H, m, } \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3), 2.24 \text{ (2H, t, } J = 7.5 \text{ Hz, }} \]

\[ \text{IR (v max/cm}^{-1}) \text{ 713, 868, 1195, 1320 (C-N), 1496, 1514 (N-O), (N-H), 1627 and 3200 (N-H); MS (ESI, 70 eV) m/z 274 (MN}^+\text{, } 100\%); \]

\[ \text{Found (MN}^+,274.0795), \text{ C}_{11}\text{H}_{13}\text{N}_3\text{NaO}_4 \text{requires 274.0798.} \]
**7. Experimental**  

**7.2. Synthesis**

CH₂CH₂CH₂CH₃, 8.13 (2H, d, J = 8.7 Hz, H-2', H-6'), 8.38 (2H, d, J = 8.7 Hz, H-3', H-5'), 10.00 (1H, s, NH) and 10.68 (1H, s, NH); **¹³C NMR** (75 MHz, d₆-DMSO) δC 14.6 (CH₃), 22.6 (CH₂), 28.1 (CH₂), 33.9 (CH₂), 124.6 (CH), 129.9 (CH), 139.1 (C), 150.3 (C), 164.8 (C) and 172.4 (C); **IR** (νmax/cm⁻¹) 715, 846, 867, 1218, 1348 (C-N), 1508 (N-O), 1593 (N-H), 1610, 2962, 3027 and 3194 (N-H); **MS** (ESI, 70 eV) m/z 288 (MNa⁺, 100%); Found (MNa⁺, 288.0953), C₁₂H₁₅N₃NaO₄ requires 288.0955.

**N'-Benzoyl-4'-nitrobenzohydrazide (177)**

![N'-Benzoyl-4'-nitrobenzohydrazide (177)](image)

A similar procedure to that previously described for the preparation of 172 was followed using 4-nitrobenzohydrazide (171) (0.50 g, 2.76 mmol) and benzoyl chloride (0.35 mL, 3.04 mmol) in dimethylacetamide (4 mL), quenching with pyridine (0.33 mL, 4.14 mmol). Purification by recrystallization (ethanol) afforded 177 as a white solid (0.30 g, 2.10 mmol, 76%). Rf = 0.30 (hexane:ethyl acetate, 1:3); **¹H NMR** (400 MHz; d₆-DMSO) δH 7.56-7.67 (3H, m, ArH), 7.87 (2H, d, J = 7.2 Hz, ArH), 8.20 (2H, d, J = 8.6 Hz, H-2', H-6'), 8.42 (2H, d, J = 8.6 Hz, H-3', H-5'), 10.68 (1H, s, NH) and 10.91 (1H, s, NH); **¹³C NMR** (100 MHz, d₆-DMSO) δC 124.6 (CH), 129.9 (CH), 128.4 (C), 132.9 (C), 133.3 (C), 139.1 (C), 150.4 (C), 165.2 (C) and 166.7 (C).

**N'-Phenylacetyl-4'-nitrobenzohydrazide (178)**

![N'-Phenylacetyl-4'-nitrobenzohydrazide (178)](image)

A similar procedure to that previously described for the preparation of 172 was followed using 4-nitrobenzohydrazide (171) (1.00 g, 5.52 mmol) and phenylacetyl chloride (0.80 mL, 6.07 mmol) in dimethylacetamide (10 mL), quenching with pyridine.
(0.67 mL, 8.28 mmol). Purification by recrystallization (ethanol) afforded 178 as a white solid (1.44 g, 4.81 mmol, 87%). \( R_f = 0.50 \) (hexane:ethyl acetate, 1:3); \(^1\text{H} \) NMR (300 MHz; \( \text{d}_6\)-DMSO) \( \delta_H 3.60 \) (2H, s, CH\(_2\)), 7.27-7.41 (5H, m, ArH), 8.13 (2H, d, \( J = 9.0 \) Hz, H-2', H-6'), 8.37 (2H, d, \( J = 9.0 \) Hz, H-3', H-5'), 10.36 (1H, s, NH) and 10.78 (1H, s, NH); \(^{13}\text{C} \) NMR (100 MHz, \( \text{d}_6\)-DMSO) \( \delta_C 39.6 \) (CH\(_2\)), 124.6 (CH), 127.5 (CH), 129.2 (CH), 129.9 (CH), 130.0 (CH), 136.5 (C), 139.0 (C), 150.3 (C), 164.9 (C) and 170.3 (C); IR (\( \nu_{\text{max}}/\text{cm}^{-1} \)) 730, 862, 1217, 1350 (C-N), 1469 (N-O), 1588 (N-H), 1606, 1738, 3128 and 3197 (N-H); MS (ESI, 70 eV) \( m/\text{z} \) 322 (MNa\(^+\), 100%). Found (MNa\(^+\), 322.0804), C\(_{15}\)H\(_{13}\)N\(_{3}\)O\(_{4}\) requires 322.0798.

### 5-Methyl-2-(4'-nitrophenyl)-1,3,4-oxadiazole (179)

![5-Methyl-2-(4'-nitrophenyl)-1,3,4-oxadiazole](image)

Compound 179 was prepared by a procedure similar to that of Tasaganva and co-workers.\(^{256}\) A solution of \( N'\)-acetyl-4-nitrobenzohydrazide (172) (0.15 g, 0.67 mmol) in phosphorus oxychloride (3 mL) was heated at 80 °C for 20 h. The excess phosphorus oxychloride was removed \textit{in vacuo} and the residue carefully poured onto ice-cold water (50 mL), extracted with dichloromethane (3 × 25 mL), dried over anhydrous sodium sulfate, filtered and the solvent removed \textit{in vacuo}. Purification by flash chromatography (hexane:ethyl acetate 4:1) afforded 179 as a white solid (60 mg, 0.29 mmol, 44%). \( R_f \) = 0.23 (hexane:ethyl acetate 4:1); \(^1\text{H} \) NMR (400 MHz; CDCl\(_3\)) \( \delta_H 2.67 \) (3H, s, CH\(_3\)), 8.23 (2H, d, \( J = 8.0 \) Hz, H-2', H-6') and 8.38 (2H, d, \( J = 8.0 \) Hz, H-3', H-5'); \(^{13}\text{C} \) NMR (100 MHz, CDCl\(_3\)) \( \delta_C 11.1 \) (CH\(_3\)), 124.3 (CH), 127.6 (CH), 129.4 (C), 149.4 (C), 163.2 (C) and 164.7 (C).
5-Ethyl-2-(4'-nitrophenyl)-1,3,4-oxadiazole (180)

A similar procedure\(^\text{256}\) to that previously described for the preparation of 179 was followed using \(N^\prime\)-propyl-4-nitrobenzohydrazide (173) (0.19 g, 0.78 mmol) in phosphorus oxychloride (5 mL). Purification by recrystallization (ethanol:water, 1:1 v/v) afforded 180 as a white solid (35 mg, 0.16 mmol, 20%). \(R_t = 0.80\) (hexane:ethyl acetate, 1:1); mp 131-135 °C; \(^1\)H NMR (400 MHz; CDCl\(_3\)) \(\delta_H 1.48\) (3H, t, \(J = 7.6\) Hz, \(CH_2CH_3\)), 3.01 (2H, q, \(J = 7.6\) Hz, \(CH_2CH_3\)), 8.24 (2H, d, \(J = 8.8\) Hz, H-2', H-6') and 8.37 (2H, d, \(J = 8.8\) Hz, H-3', H-5'); \(^13\)C NMR (100 MHz, CDCl\(_3\)) \(\delta_C 10.7\) (CH\(_3\)), 19.2 (CH\(_2\)), 124.3 (CH), 127.6 (CH), 129.5 (C), 149.4 (C), 163.0 (C) and 168.9 (C); IR \((\nu_{\text{max}}/\text{cm}^{-1})\) 708, 1205, 1365, 1515 (N-O), 1739, 2971 and 3461; MS (ESI, 70 eV) \(m/z 242\) (MNa\(^+\), 100%). Found (MNa\(^+\), 242.0543), C\(_{10}\)H\(_9\)N\(_3\)NaO\(_3\) requires 242.0536.

5-Propyl-2-(4'-nitrophenyl)-1,3,4-oxadiazole (181)

A similar procedure\(^\text{256}\) to that previously described for the preparation of 179 was followed using \(N^\prime\)-butyryl-4-nitrobenzohydrazide (174) (55 mg, 0.22 mmol) in phosphorus oxychloride (3 mL). Purification by recrystallization (ethanol:water, 1:1 v/v) afforded 181 as a white solid (29 mg, 0.12 mmol, 57%). \(R_t = 0.65\) (hexane:ethyl acetate, 1:1); mp 110-112 °C; \(^1\)H NMR (400 MHz; CDCl\(_3\)) \(\delta_H 1.09\) (3H, t, \(J = 7.5\) Hz, \(CH_2CH_2CH_3\)), 1.92 (2H, m, \(CH_2CH_2CH_3\)), 2.96 (2H, t, \(J = 7.5\) Hz, \(CH_2CH_2CH_3\)), 8.23 (2H, d, \(J = 9.0\) Hz, H-2', H-6') and 8.37 (2H, d, \(J = 9.0\) Hz, H-3', H-5'); \(^13\)C NMR (100 MHz, CDCl\(_3\)) \(\delta_C 13.6\) (CH\(_3\)), 20.0 (CH\(_2\)), 27.3 (CH\(_2\)), 124.3 (CH), 127.6 (CH), 129.5 (C), 149.4 (C), 163.0 (C) and 168.9 (C); IR \((\nu_{\text{max}}/\text{cm}^{-1})\) 708, 1205, 1365, 1515 (N-O), 1739, 2971 and 3461; MS (ESI, 70 eV) \(m/z 242\) (MNa\(^+\), 100%). Found (MNa\(^+\), 242.0543), C\(_{10}\)H\(_9\)N\(_3\)NaO\(_3\) requires 242.0536.
129.6 (C), 149.4 (C), 163.0 (C) and 168.0 (C); IR ($\nu_{\text{max}}$/cm$^{-1}$) 711, 862, 1216, 1366, 1514 (N-O), 1738, 2970, 3104 and 3457; MS (ESI, 70 eV) $m/z$ 256 (MNa$^+$, 100%). Found (MNa$^+$, 256.0697), C$_{11}$H$_{11}$N$_3$NaO$_3$ requires 265.0693.

5-Isopropyl-2-(4'-nitrophenyl)-1,3,4-oxadiazole (182)

A similar procedure$^{256}$ to that previously described for the preparation of 179 was followed using N'-isobutyryl-4-nitrobenzohydrazide (175) (0.25 g, 1.00 mmol) in phosphorus oxychloride (5 mL). Purification by recrystallization (ethanol:water, 1:1 v/v) afforded 182 as a white solid (27 mg, 0.12 mmol, 12%). $R_f$ = 0.65 (hexane:ethyl acetate, 1:1); mp 138-141 °C; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.48 (6H, d, $J$ = 6.9 Hz, CH(CH$_3$)$_2$), 3.31 (1H, m, CH(CH$_3$)$_2$), 8.24 (2H, d, $J$ = 9.0 Hz, H-2', H-6') and 8.37 (2H, d, $J$ = 9.0 Hz, H-3', H-5'); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ C 20.0 (CH$_3$), 26.5 (CH), 124.3 (CH), 127.7 (CH), 129.7 (C), 149.4 (C), 162.9 (C) and 172.0 (C); IR ($\nu_{\text{max}}$/cm$^{-1}$) 706, 855, 1217, 1365, 1516 (N-O), 1739, 2970 and 3115; MS (ESI, 70 eV) $m/z$ 256 (MNa$^+$, 100%). Found (MNa$^+$, 256.0696), C$_{11}$H$_{11}$N$_3$NaO$_3$ requires 265.0693.

5-Butyl-2-(4'-nitrophenyl)-1,3,4-oxadiazole (183)

A similar procedure$^{256}$ to that previously described for the preparation of 179 was followed using N'-valeroyl-4-nitrobenzohydrazide (176) (1.00 g, 3.77 mmol) in phosphorus oxychloride (25 mL). Purification by recrystallization (ethanol:water, 1:1 v/v)
afforded 183 as a white solid (0.23 g, 0.93 mmol, 25%). $R_t = 0.65$ (hexane:ethyl acetate, 1:1); mp 178-182 °C; $^1$H NMR (400 MHz; CDCl$_3$) $\delta$H 1.00 (3H, t, $J = 7.2$ Hz, CH$_2$CH$_2$CH$_2$CH$_3$), 1.50 (2H, m, CH$_2$CH$_2$CH$_2$CH$_3$), 1.88 (2H, m, CH$_2$CH$_2$CH$_2$CH$_3$), 3.00 (2H, t, $J = 7.6$ Hz, CH$_2$CH$_2$CH$_2$CH$_3$), 8.25 (2H, d, $J = 9.2$ Hz, H-2', H-6') and 8.37 (2H, d, $J = 9.2$ Hz, H-3', H-5'); $^{13}$C NMR (100 MHz; CDCl$_3$) $\delta$C 13.4 (CH$_3$), 22.0 (CH$_2$), 24.9 (CH$_2$), 28.3 (CH$_2$), 124.1 (CH), 127.5 (CH), 129.4 (C), 149.2 (C), 162.8 (C) and 168.0 (C); IR ($\nu_{\text{max}}$/cm$^{-1}$) 653, 711, 867, 1106 (C-O), 1221 (C-N), 1340, 1495, 1520 (N-O), 1567, 1609, 1707, 2873, 2935, 2961 and 3193; MS (ESI, 70 eV) $m/z$ 270 (MNa$^+$, 100%); Found (MNa$^+$, 270.0842), C$_{12}$H$_{13}$N$_3$NaO$_3$ requires 270.0846.

**5-Phenyl-2-(4'-nitrophenyl)-1,3,4-oxadiazole (184)**

A similar procedure to that previously described for the preparation of 179 was followed using N'-benzoyl-4-nitrobenzohydrazide (178) (0.16 g, 0.56 mmol) in phosphorus oxychloride (5 mL). Purification by recrystallization (ethanol:water, 1:1 v/v) afforded 184 as a white solid (52 mg, 0.19 mmol, 35%). $R_t = 0.50$ (hexane:ethyl acetate, 2:1); $^1$H NMR (400 MHz; CDCl$_3$) $\delta$H 7.59-7.63 (3H, m, ArH), 8.18 (2H, m, ArH), 8.34 (2H, d, $J = 8.8$ Hz, H-2', H-6') and 8.41 (2H, d, $J = 8.8$ Hz, H-3', H-5'); $^{13}$C NMR (100 MHz; CDCl$_3$) $\delta$C 123.3 (C), 124.4 (CH), 127.2 (CH), 127.8 (CH), 129.3 (CH), 129.4 (C), 132.3 (CH), 149.6 (C), 162.9 (C) and 165.6 (C).
5-Benzyl-2-(4'-nitrophenyl)-1,3,4-oxadiazole (185)\textsuperscript{411}

A similar procedure\textsuperscript{256} to that previously described for the preparation of 179 was followed using \textit{N}'-phenylacetyl-4-nitrobenzohydrazide (178) (1.00 g, 3.34 mmol) in phosphorus oxychloride (25 mL). Purification by recrystallization (ethanol:water, 1:1 v/v) afforded 185 as a white solid (0.50 g, 1.99 mmol, 60%). \( R_f = 0.60 \) (hexane:ethyl acetate, 1:1); mp 143-146 °C [lit.\textsuperscript{411} mp 141.0-141.5 °C]; \textit{\textsuperscript{1}H NMR} (400 MHz; d\textsubscript{6}-DMSO) \( \delta_H \) 4.45 (2H, s, CH\textsubscript{2}), 7.35-7.43 (5H, m, ArH), 8.25 (2H, d, \( J = 9.0 \text{ Hz, H-2', H-6'} \)) and 8.43 (2H, d, \( J = 9.0 \text{ Hz, H-3', H-5'} \)); \textit{\textsuperscript{13}C NMR} (100 MHz, d\textsubscript{6}-DMSO) \( \delta_C \) 31.7 (CH\textsubscript{2}), 125.6 (CH), 128.2 (CH), 128.7 (CH), 129.7 (CH), 129.8 (C), 129.9 (CH), 135.1 (C), 150.1 (C), 163.9 (C) and 167.5 (C).

5-Methyl-2-(4'-aminophenyl)-1,3,4-oxadiazole (163)

A similar procedure\textsuperscript{255} to that previously described for the preparation of 144 was followed using 2-methyl-5-(4'-nitrophenyl)-1,3,4-oxadiazole (179) (47 mg, 0.23 mmol) in 1,4-dioxane (1 mL) and sodium sulfide nonahydrate (0.13 g, 0.55 mmol) in water (1 mL). Purification by flash chromatography (hexane:ethyl acetate 2:1) afforded 163 as a pale brown solid (22 mg, 0.13 mmol, 55%). \( R_f = 0.15 \) (hexane:ethyl acetate, 2:1); mp 190-194 °C; \textit{\textsuperscript{1}H NMR} (400 MHz; CDCl\textsubscript{3}) \( \delta_H \) 2.57 (3H, s, CH\textsubscript{3}), 4.05 (2H, s, NH\textsubscript{2}), 6.72 (2H, d, \( J = 8.6 \text{ Hz, H-3', H-5'} \)) and 7.81 (2H, d, \( J = 8.6 \text{ Hz, H-2', H-6'} \)); \textit{\textsuperscript{13}C NMR} (75 MHz, CDCl\textsubscript{3}) \( \delta_C \) 11.0 (CH\textsubscript{3}), 113.7 (C), 114.6 (CH), 129.4 (CH), 149.5 (C), 162.6 (C) and
165.2 (C); IR (ν<sub>max</sub>/cm<sup>-1</sup>) 701, 827, 1170 (C-O), 1349 (C-N), 1494, 1557 (N-H), 1600, 2927, 3214, 3335 (N-H) and 3399; MS (ESI, 70 eV) m/z 176 (M<sup>+</sup>, 100%); Found (M<sup>+</sup>, 176.0822), C<sub>9</sub>H<sub>10</sub>N<sub>3</sub>O requires 176.0818.

5-Ethyl-2-(4'-aminophenyl)-1,3,4-oxadiazole (164)

![Chemical structure of 5-Ethyl-2-(4'-aminophenyl)-1,3,4-oxadiazole (164)]

A similar procedure<sup>255</sup> to that previously described for the preparation of 144 was followed using 3-ethyl-5-(4'-nitrophenyl)-1,3,4-oxadiazole (180) (50 mg, 0.23 mmol) in 1,4-dioxane (1 mL) and sodium sulfide nonahydrate (0.13 g, 0.55 mmol) in water (1 mL). Purification by flash chromatography (hexane:ethyl acetate 1:1) afforded 164 as a pale brown solid (30 mg, 0.16 mmol, 70%). R<sub>f</sub> = 0.15 (hexane:ethyl acetate, 2:1); mp 138-142 °C; <sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>) δ<sub>H</sub> 1.41 (3H, t, J = 7.6 Hz, CH<sub>2</sub>C<sub>H</sub><sub>3</sub>), 2.91 (2H, q, J = 7.6 Hz, C<sub>H</sub><sub>2</sub>CH<sub>3</sub>), 4.05 (2H, s, NH<sub>2</sub>), 6.72 (2H, d, J = 8.8 Hz, H-3', H-5') and 7.82 (2H, d, J = 8.8 Hz, H-2', H-6'); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ<sub>C</sub> 10.9 (CH<sub>3</sub>), 19.1 (CH<sub>2</sub>), 113.8 (C), 114.6 (CH), 128.8 (CH), 149.5 (C), 165.0 (C) and 166.8 (C); IR (ν<sub>max</sub>/cm<sup>-1</sup>) 834, 1175 (C-O), 1366 (C-N), 1497, 1574 (N-H), 1608, 1738, 2924, 3216, 3332 (N-H) and 3446; MS (ESI, 70 eV) m/z 190 (M<sup>+</sup>, 100%); Found (M<sup>+</sup>, 190.0975), C<sub>10</sub>H<sub>12</sub>N<sub>3</sub>O requires 190.0975.

5-Propyl-2-(4'-aminophenyl)-1,3,4-oxadiazole (165)

![Chemical structure of 5-Propyl-2-(4'-aminophenyl)-1,3,4-oxadiazole (165)]

A similar procedure<sup>255</sup> to that previously described for the preparation of 144 was followed using 3-propyl-5-(4'-nitrophenyl)-1,3,4-oxadiazole (181) (27 mg, 0.12 mmol) in
1,4-dioxane (1 mL) and sodium sulfide nonahydrate (67 mg, 0.28 mmol) in water (1 mL). Purification by flash chromatography (hexane:ethyl acetate 1:1) afforded 165 as a pale brown solid (23 mg, 0.11 mmol, 98%). $R_f = 0.15$ (hexane:ethyl acetate, 1:1); mp 127-132 °C; $^1H$ NMR (400 MHz; CDCl$_3$) $\delta$ 1.05 (3H, t, $J = 7.5$ Hz, CH$_2$CH$_2$CH$_3$), 1.86 (2H, m, CH$_2$CH$_2$CH$_3$), 2.86 (2H, t, $J = 7.5$ Hz, CH$_2$CH$_2$CH$_3$), 4.09 (2H, s, NH$_2$), 6.72 (2H, d, $J = 8.4$ Hz, H-3', H-5') and 7.82 (2H, d, $J = 8.4$ Hz, H-2', H-6'); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 13.6 (CH$_3$), 20.1 (CH$_2$), 27.2 (CH$_2$), 113.7 (C), 114.6 (CH), 128.4 (CH), 149.5 (C), 165.0 (C) and 165.8 (C); IR ($\nu_{\text{max}}$/cm$^{-1}$) 826, 1172 (C-O), 1315 (C-N), 1498, 1569 (N-H), 1607, 1739, 2970, 3207, 3324 (N-H) and 3425; MS (ESI, 70 eV) $m$/z 204 (M$^+$, 100%); Found (M$^+$, 204.1131), C$_{11}$H$_{14}$N$_3$O requires 204.1131.

5-Isopropyl-2-(4'-aminophenyl)-1,3,4-oxadiazole (166)

A similar procedure$^{255}$ to that previously described for the preparation of 144 was followed using 3-isopropyl-5-(4'-nitrophenyl)-1,3,4-oxadiazole (182) (25 mg, 0.11 mmol) in 1,4-dioxane (1 mL) and sodium sulfide nonahydrate (62 mg, 0.26 mmol) in water (1 mL). Purification by flash chromatography (hexane:ethyl acetate 2:1) afforded 166 as a pale brown solid (18 mg, 0.09 mmol, 83%). $R_f = 0.15$ (hexane:ethyl acetate, 1:1); mp 110-114 °C; $^1H$ NMR (400 MHz; CDCl$_3$) $\delta$ 1.42 (6H, d, $J = 7.2$ Hz, CH(CH$_3$)$_2$), 3.23 (1H, m, CH(CH$_3$)$_2$), 4.15 (2H, s, NH$_2$), 6.73 (2H, d, $J = 8.8$ Hz, H-3', H-5') and 7.82 (2H, d, $J = 8.8$ Hz, H-2', H-6'); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 20.1 (CH$_3$), 26.3 (CH), 113.8 (C), 114.6 (CH), 128.4 (CH), 149.5 (C), 164.9 (C) and 169.8 (C); IR ($\nu_{\text{max}}$/cm$^{-1}$) 834, 1176 (C-O), 1365 (C-N), 1498, 1566 (N-H), 1608, 1738, 2971, 3220, 3328 (N-H) and 3407; MS (ESI, 70 eV) $m$/z 204 (M$^+$, 100%); Found (M$^+$, 204.1133), C$_{11}$H$_{14}$N$_3$O requires 204.1131.
5-Butyl-2-(4’-aminophenyl)-1,3,4-oxadiazole (167)

A similar procedure to that previously described for the preparation of 144 was followed using 3-butyl-5-(4’-nitrophenyl)-1,3,4-oxadiazole (183) (0.23 g, 0.93 mmol) in 1,4-dioxane (5 mL) and sodium sulfide nonahydrate (0.54 g, 2.23 mmol) in water (5 mL). Purification by flash chromatography (hexane:ethyl acetate 1:1) afforded 167 as a pale brown solid (69 mg, 0.30 mmol, 31%). $R_f = 0.40$ (hexane:ethyl acetate, 1:1); mp 119-122 °C; $^1H$ NMR (400 MHz; CDCl$_3$) $\delta$ 0.97 (3H, t, $J = 7.5$ Hz, CH$_2$CH$_2$CH$_2$C$_2$H$_3$), 1.45 (2H, m, CH$_2$CH$_2$CH$_2$C$_2$H$_3$), 1.81 (2H, m, CH$_2$CH$_2$CH$_2$C$_2$H$_3$), 2.88 (2H, t, $J = 7.5$ Hz, CH$_2$CH$_2$CH$_2$C$_2$H$_3$), 4.05 (2H, s, NH$_2$), 6.72 (2H, d, $J = 8.8$ Hz, H-3’, H-5’) and 7.81 (2H, d, $J = 8.8$ Hz, H-2’, H-6’); $^{13}C$ NMR (100 MHz, CDCl$_3$) $\delta_C$ 13.6 (CH$_3$), 22.1 (CH$_2$), 25.1 (CH$_2$), 28.7 (CH$_2$), 113.9 (C), 114.6 (CH), 128.4 (CH), 149.5 (C), 165.0 (C) and 166.0 (C); IR ($\nu_{\text{max}}$/cm$^{-1}$) 828, 1173 (C-O), 1314 (C-N), 1498, 1572 (N-H), 1606, 1738, 2863, 2962, 3212, 3323 (N-H) and 3428; MS (ESI, 70 eV) $m/z$ 218 (M$^+$, 100%). Found (M$^+$, 218.1279), C$_{12}$H$_{16}$N$_3$O requires 218.1288.

5-Phenyl-2-(4’-aminophenyl)-1,3,4-oxadiazole (168)

A similar procedure to that previously described for the preparation of 144 was followed using 3-phenyl-5-(4’-nitrophenyl)-1,3,4-oxadiazole (184) (52 mg, 0.19 mmol) in 1,4-dioxane (3 mL) and sodium sulfide nonahydrate (0.11 g, 0.47 mmol) in water (3 mL). Purification by flash chromatography (hexane:ethyl acetate 2:1) afforded 168 as a pale
brown solid (30 mg, 0.13 mmol, 65%). $R_f = 0.25$ (hexane:ethyl acetate, 2:1); $^1$H NMR (400 MHz; CDCl$_3$) $\delta_H$ 4.11 (2H, s, NH$_2$), 6.76 (2H, d, $J = 8.8$ Hz, H-3', H-5'), 7.51-7.53 (3H, m, ArH), 7.93 (2H, d, $J = 8.8$ Hz, H-2', H-6') and 8.12 (2H, m, ArH); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_C$ 113.7 (C), 114.7 (CH), 124.3 (C), 126.7 (CH), 128.7 (CH), 129.0 (CH), 131.3 (CH), 149.7 (C), 163.7 (C) and 165.0 (C); MS (ESI, 70 eV) $m/z$ 238 (M$^+$, 100%); Found (M$^+$, 238.0973), C$_{14}$H$_{12}$N$_3$O requires 238.0975.

5-Benzyl-2-(4'-aminophenyl)-1,3,4-oxadiazole (169)

A similar procedure$^{255}$ to that previously described for the preparation of 144 was followed using 3-benzyl-5-(4'-nitrophenyl)-1,3,4-oxadiazole (185) (0.25 g, 0.89 mmol) in 1,4-dioxane (5 mL) and sodium sulfide nonahydrate (0.51 g, 2.13 mmol) in water (5 mL). Purification by flash chromatography (hexane:ethyl acetate 1:1) afforded 169 as a pale brown solid (20 mg, 0.08 mmol, 9%). $R_f = 0.30$ (hexane:ethyl acetate, 1:1); mp 131-135 °C; $^1$H NMR (400 MHz; CDCl$_3$) $\delta_H$ 4.03 (2H, s, NH$_2$), 4.24 (2H, s, CH$_2$), 6.69 (2H, d, $J = 8.4$ Hz, H-3', H-5'), 7.28-7.35 (5H, m, ArH) and 7.78 (2H, d, $J = 8.4$ Hz, H-2', H-6'); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_C$ 31.9 (CH$_2$), 113.6 (C), 114.6 (CH), 127.4 (CH), 128.5 (CH), 128.8 (CH), 134.2 (C), 149.6 (C), 164.2 (C) and 165.5 (C); IR ($\nu_{\max}$/cm$^{-1}$) 723, 841, 1179 (C-O), 1371 (C-N), 1498, 1571 (N-H), 1608, 1702, 2924, 3216, 3354 (N-H) and 3455; MS (ESI, 70 eV) $m/z$ 274 (M$^{+Na^+}$, 100%); Found (M$^{+Na^+}$, 274.0942), C$_{15}$H$_{13}$N$_3$NaO requires 274.0951.
7. Experimental

7.2. Synthesis

7.2.4.4. 5-Methyl-1,2,4-oxadiazole 186

4-Nitrobenzonitrile (191)

![Chemical Structure](image)

Compound 191 was prepared by a procedure similar to that of Chill and co-workers.257 To a stirring solution of hydroxylamine hydrochloride (8.50 g, 122.41 mmol) in dimethyl sulfoxide (40 mL) at room temperature was added 4-nitrobenzaldehyde (69) (10.00 g, 66.17 mmol), and the mixture heated at 100 °C for 3 h. The solution was allowed to cool, diluted with water (50 mL), extracted with ethyl acetate (3 × 50 mL), the combined organic phases washed with water (3 × 50 mL), dried over anhydrous sodium sulfate, filtered and the solvent removed *in vacuo*. Purification by flash chromatography (hexane:ethyl acetate 2:1) afforded 191 as a yellow-brown solid (9.17 g, 61.90 mmol, 94%).

\[ R_f = 0.60 \text{ (hexane:ethyl acetate, 2:1)} \]

\[ \textsuperscript{1}H \text{ NMR (400 MHz; CDCl}_3\text{)} \delta_H 7.89 (2H, d, J = 8.8 Hz, H-2, H-6) \text{ and } 8.36 (2H, d, J = 8.8 Hz, H-3, H-5); \textsuperscript{13}C \text{ NMR (100 MHz, CDCl}_3\text{)} \delta_C 116.8 (C), 118.4 (C), 124.3 (CH), 133.5 (CH) \text{ and } 150.1 (C). \]

(Z)-4-Nitrobenzamidoxime (190)

![Chemical Structure](image)

A similar procedure\textsuperscript{254} to that previously described for the preparation of 150 was followed using 4-nitrobenzonitrile (191) (9.17 g, 61.90 mmol), hydroxylamine hydrochloride (4.52 g, 68.09 mmol) and sodium hydroxide (2.72 g, 65.00 mmol) in ethanol (80 mL) and water (20 mL). Purification by flash chromatography (hexane:ethyl acetate 2:1) afforded 190 as a red-brown solid (1.20 g, 6.62 mmol, 11%). \[ R_f = 0.35 \]
(hexane:ethyl acetate, 1:1); \(^1\)H NMR (400 MHz; d\textsubscript{6}-DMSO) \(\delta_H\) 6.09 (2H, s, NH\textsubscript{2}), 7.89 (2H, d, \(J = 9.6\) Hz, H-2, H-6), 8.26 (2H, d, \(J = 9.6\) Hz, H-3, H-5) and 10.17 (1H, s, OH); \(^{13}\)C NMR (100 MHz, d\textsubscript{6}-DMSO) \(\delta_C\) 124.3 (CH), 127.3 (CH), 140.5 (C), 148.4 (C) and 150.3 (C).

5-Methyl-3-(4'-nitrophenyl)-1,2,4-oxadiazole (192)

A similar procedure\(^{254}\) to that previously described for the preparation of 157 was followed using (Z)-4-nitrobenzamidoxime (190) (0.15 g, 0.83 mmol) and acetyl chloride (0.12 mL, 1.66 mmol) in pyridine (3 mL). Purification by flash chromatography (hexane:ethyl acetate 9:1) afforded 192 as a pale brown solid (36 mg, 0.18 mmol, 21%). \(R_f = 0.70\) (hexane:ethyl acetate, 2:1); \(^1\)H NMR (300 MHz; CDCl\textsubscript{3}) \(\delta_H\) 2.70 (3H, s, CH\textsubscript{3}), 8.26 (2H, d, \(J = 9.0\) Hz, H-2', H-6') and 8.34 (2H, d, \(J = 9.0\) Hz, H-3', H-5'); \(^{13}\)C NMR (75 MHz, CDCl\textsubscript{3}) \(\delta_C\) 12.4 (CH\textsubscript{3}), 124.1 (CH), 128.3 (CH), 132.7 (C), 149.4 (C), 166.9 (C) and 177.4 (C).

5-Methyl-3-(4'-aminophenyl)-1,2,4-oxadiazole (186)

A similar procedure\(^{255}\) to that previously described for the preparation of 144 was followed using 5-methyl-3-(4'-nitrophenyl)-1,2,4-oxadiazole (192) (36 mg, 0.18 mmol) in 1,4-dioxane (1 mL) and sodium sulfide nonahydrate (0.10 g, 0.42 mmol) in water (1 mL). Purification by flash chromatography (hexane:ethyl acetate 2:1) afforded 186 as a yellow-brown solid (13 mg, 0.07 mmol, 42%). \(R_f = 0.20\) (hexane:ethyl acetate, 2:1); \(^1\)H
NMR (400 MHz; CDCl₃) δH 2.62 (3H, s, CH₃), 3.96 (2H, s, NH₂), 6.72 (2H, d, J = 8.4 Hz, H-3', H-5') and 7.85 (2H, d, J = 8.4 Hz, H-2', H-6'); ¹³C NMR (100 MHz, CDCl₃): δC 12.4 (CH₃), 114.7 (CH), 116.6 (C), 128.8 (CH), 149.1 (C), 168.3 (C) and 175.9 (C); MS (ESI, 70 eV) m/z 176 (M⁺, 100%); Found (M⁺, 176.0823), C₉H₁₀N₃O requires 176.0818.

7.2.4.5. 5-Alkyl-1,3,4-thiadiazoles 196 and 197, and 3-ethyl-1,2,4-thiadiazole 198

5-Propyl-2-(4'-nitrophenyl)-1,3,4-thiadiazole (199)

Compound 199 was prepared by a procedure similar to that of Gierczyk and co-workers.²⁶¹ A solution of N'-butyryl-4'-nitrobenzohydrazide (174) (0.20 g, 0.40 mmol) and Lawesson's reagent (0.24 g, 0.60 mmol) in toluene (8 mL) was heated under reflux for 5 h. The solvent was removed in vacuo and the resulting residue was taken up in dichloromethane (25 mL), washed with water (25 mL), the separated aqueous layer further extracted with ethyl acetate (2 × 10 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate 4:1) afforded 199 as a yellow solid (56 mg, 0.23 mmol, 56%). Rf = 0.70 (hexane:ethyl acetate, 1:1); mp 126-130 °C; ¹H NMR (400 MHz; CDCl₃) δH 1.09 (3H, t, J = 7.6 Hz, CH₂CH₂CH₃), 1.91 (2H, m, CH₂CH₂CH₃), 3.17 (2H, t, J = 7.6 Hz, CH₂CH₂CH₃), 8.13 (2H, d, J = 8.8 Hz, H-2', H-6') and 8.33 (2H, d, J = 8.8 Hz, H-3', H-5'); ¹³C NMR (100 MHz, CDCl₃) δC 13.5 (CH₃), 23.4 (CH₂), 32.1 (CH₂), 124.4 (CH), 128.6 (CH), 136.0 (C), 149.0 (C), 165.9 (C) and 171.8 (C); IR (νmax/cm⁻¹) 687, 751, 859, 984, 1345 (C-N), 1516 (N-O), 1599, 1736, 2869, 2962, 3086 and 3287; MS (ESI, 70 eV) m/z 272 (MNa⁺, 100%). Found (MNa⁺, 272.0475), C₁₁H₁₁N₃NaO₃S requires 272.0464.
5-Butyl-2-(4'-nitrophenyl)-1,3,4-thiadiazole (200)

A similar procedure\textsuperscript{261} to that previously described for the preparation of \textit{199} was followed using \textit{N}'-valeroyl-4'-nitro-benzohydrazide (176) (0.50 g, 1.89 mmol) and Lawesson's reagent (1.14 g, 2.83 mmol) in toluene (20 mL). Purification by flash chromatography (hexane:ethyl acetate 9:1) afforded 200 as a yellow solid (0.35 g, 1.31 mmol, 70\%). \(R_f = 0.75\) (hexane:ethyl acetate, 1:1); mp 175-180 °C; \textsuperscript{1}H NMR (400 MHz; CDCl\textsubscript{3}) \(\delta_H\) 0.99 (3H, t, \(J = 7.6\) Hz, CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 1.49 (2H, m, CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 1.86 (2H, m, CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 3.19 (2H, t, \(J = 7.6\) Hz, CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 8.13 (2H, d, \(J = 9.0\) Hz, H-2', H-6') and 8.33 (2H, d, \(J = 9.0\) Hz, H-3', H-3'); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \(\delta_C\) 13.6 (CH\textsubscript{3}), 22.1 (CH\textsubscript{2}), 30.0 (CH\textsubscript{2}), 32.1 (CH\textsubscript{2}), 124.3 (CH), 128.6 (CH), 136.0 (C), 149.0 (C), 165.8 (C) and 172.0 (C); IR (\(\nu_{\text{max}}/\text{cm}^{-1}\)) 713, 852, 867, 1008, 1106, 1339 (C-N), 1517 (N-O), 1563, 1738, 2872, 2935, 3110 and 3184; MS (ESI, 70 eV) \(m/z\) 360 (100%).

5-Propyl-2-(4'-aminophenyl)-1,3,4-thiadiazole (196)

A similar procedure\textsuperscript{255} to that previously described for the preparation of \textit{144} was followed using 2-propyl-5-(4'-nitrophenyl)-1,3,4-thiadiazole (199) (50 mg, 0.20 mmol) in 1,4-dioxane (2.5 mL) and sodium sulfide nonahydrate (0.12 g, 0.48 mmol) in water (2.5 mL). Purification by flash chromatography (hexane:ethyl acetate 2:1) afforded 196 as a light brown solid (20 mg, 0.09 mmol, 46%). \(R_f = 0.15\) (hexane:ethyl acetate, 2:1); mp
102-107 °C; $^1$H NMR (400 MHz; CDCl$_3$) $\delta_H$ 1.04 (3H, t, $J = 7.5$ Hz, CH$_2$CH$_2$CH$_3$), 1.84 (2H, m, CH$_2$CH$_2$CH$_3$), 3.06 (2H, t, $J = 7.5$ Hz, CH$_2$CH$_2$CH$_3$), 4.04 (2H, s, NH$_2$), 6.70 (2H, d, $J = 8.8$ Hz, H-3', H-5') and 7.72 (2H, d, $J = 8.8$ Hz, H-2', H-6'); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_C$ 13.5 (CH$_3$), 23.4 (CH$_2$), 32.0 (CH$_2$), 114.8 (CH), 120.4 (C), 129.3 (CH), 149.1 (C), 168.5 (C) and 168.7 (C); IR (v$_{max}$/cm$^{-1}$) 842, 1174 (C-O), 1306 (C-N), 1416, 1450, 1602 (N-H), 1623, 2852, 2961, 3211, 3322 (N-H) and 3414; MS (ESI, 70 eV) m/z 220 (M$^+$, 100%); Found (M$^+$, 220.0906), C$_{11}$H$_{14}$N$_3$S requires 220.0903.

5-Butyl-2-(4'-aminophenyl)-1,3,4-thiadiazole (197)

A similar procedure$^{255}$ to that previously described for the preparation of 144 was followed using 2-butyl-5-(4-nitrophenyl)-1,3,4-thiadiazole (200) (0.25 g, 1.06 mmol) in 1,4-dioxane (5 mL) and sodium sulfide nonahydrate (0.61 g, 2.54 mmol) in water (5 mL). Purification by flash chromatography (hexane:ethyl acetate 2:1) afforded 197 as a light brown solid (0.40 g, 0.60 mmol, 56%). $R_f = 0.20$ (hexane:ethyl acetate, 2:1); mp 115-119 °C; $^1$H NMR (400 MHz; CDCl$_3$) $\delta_H$ 0.97 (3H, t, $J = 7.5$ Hz, CH$_2$CH$_2$CH$_2$CH$_3$), 1.46 (2H, m, CH$_2$CH$_2$CH$_2$CH$_3$), 1.80 (2H, m, CH$_2$CH$_2$CH$_2$CH$_3$), 3.09 (2H, t, $J = 7.5$ Hz, CH$_2$CH$_2$CH$_2$CH$_3$), 3.98 (2H, s, NH$_2$), 6.70 (2H, d, $J = 8.6$ Hz, H-3', H-5') and 7.73 (2H, d, $J = 8.6$ Hz, H-2', H-6'); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_C$ 13.7 (CH$_3$), 22.1 (CH$_2$), 29.9 (CH$_2$), 32.1 (CH$_2$), 114.8 (CH), 120.6 (C), 129.3 (CH), 149.0 (C), 168.7 (C) and 168.8 (C); IR (v$_{max}$/cm$^{-1}$) 842, 1174 (C-O), 1307 (C-N), 1421, 1456, 1604 (N-H), 1635, 2867, 2946, 3211, 3321 (N-H) and 3472; MS (ESI, 70 eV) m/z 234 (M$^+$, 100%); Found (M$^+$, 234.1059), C$_{12}$H$_{16}$N$_3$S requires 234.1059.
$N,N$-Dimethylpropionamide (206)$^{415}$

Compound 206 was prepared by a procedure similar to that of Claydon and co-workers.$^{262}$ To a stirring solution of dimethylamine hydrochloride (205) (26.11 g, 320.2 mmol) in benzene (200 mL) at 0 °C were added triethylamine (56.00 mL, 404.0 mmol) and propionic anhydride (10.50 mL, 81.5 mmol), and the mixture stirred at room temperature for 10 h. The resulting solution was then cooled to 0 °C and quenched through the careful addition of hydrochloric acid (5 mL, 2 M), extracted with ethyl acetate (3 × 50 mL), washed with a sat. sodium hydrogen carbonate (50 mL), then brine (50 mL), dried over anhydrous magnesium sulfate, filtered and the solvent removed \textit{in vacuo} to afford 206 as a pale yellow oil (3.72 g), which was used without further purification. $R_f = 0.20$ (hexane:ethyl acetate, 4:1); $^1$H NMR (300 MHz; CDCl$_3$) $\delta_H$ 1.14 (3H, t, $J = 7.5$ Hz, CH$_2$C$_3$), 2.33 (2H, q, $J = 7.5$ Hz, C$_2$H$_2$CH$_3$) 2.95 (3H, s, NCH$_3$) and 3.00 (3H, s, NCH$_3$); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta_C$ 9.3 (CH$_3$), 26.5 (CH$_2$), 35.4 (CH$_3$), 37.1 (CH$_3$) and 173.8 (C).

$N,N$-Dimethylpropionamide dimethyl sulfate complex (207)$^{416}$

Compound 207 was prepared by a procedure similar to that of Salomon and co-workers.$^{263}$ To a stirring solution of dimethyl sulfate (7.53 mL, 79.50 mmol) at room temperature was added dropwise crude $N,N$-dimethylpropionamide (206) (7.35 g), and the mixture heated at 80 °C for 5 h. The reaction was then cooled to 0 °C, washed with dry benzene (20 mL), then ether (2 × 20 mL), and the solvent removed \textit{in vacuo} to afford
as colorless viscous oil (12.57 g), which was used without further purification; \(^1\)H NMR (300 MHz; CDCl\(_3\)) \(\delta_H\) 1.31 (3H, t, \(J = 7.1\) Hz, \(\text{CH}_2\text{C}_3\)), 3.02 (2H, q, \(J = 7.1\) Hz, \(\text{CH}_2\text{C}_3\)), 3.32 (3H, s, NCH\(_3\)), 3.51 (3H, s, NCH\(_3\)), 3.70 (3H, s, OCH\(_3\)) and 4.33 (2H, s, SO\(_3\)OCH\(_3\)); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta_C\) 8.7 (CH\(_3\)), 21.2 (CH\(_2\)), 39.4 (CH\(_3\)), 41.1 (CH\(_3\)), 54.5 (CH\(_3\)), 60.3 (CH\(_3\)) and 179.7 (C); IR (\(\nu_{\text{max}}/\text{cm}^{-1}\)) 732, 908, 1201 (C-O), 1399, 1631, 1732, 2243, 2930 and 2986.

\(N,N\)-Dimethylpropionamide dimethyl acetal (201)

\[\begin{align*}
\text{O} & \quad \text{O} \\
\text{N} & \\
\end{align*}\]

Compound 201 was prepared by a procedure similar to that of Salomon and co-workers.\(^{263}\) To a vigorously stirring solution of anhydrous methanol (20 mL) was added batchwise sodium metal (1.91 g, 79.45 mmol). To the resulting freshly prepared methanolic solution of sodium methoxide at -5 °C was added dropwise crude \(N,N\)-dimethylpropionamide dimethyl sulfate complex (207) (12.57 g), and the resulting mixture stirred at room temperature for 20 h. Purification by distillation at reduced pressure (bp 120-130 °C/200 Torr) afforded 201 as a colourless oil (2.50 g, 16.98 mmol, 10% over three steps). \(^1\)H NMR (400 MHz; CDCl\(_3\)) \(\delta_H\) 0.88 (3H, t, \(J = 7.4\) Hz, \(\text{CH}_2\text{C}_3\)), 1.78 (2H, q, \(J = 7.4\) Hz, \(\text{CH}_2\text{C}_3\)) 2.43 (6H, s, N(CH\(_3\))\(_2\)) and 3.18 (6H, s, (OCH\(_3\))\(_2\)); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta_C\) 8.1 (CH\(_3\)), 23.8 (CH\(_2\)) 37.7 (CH\(_3\)), 48.5 (CH\(_3\)) and 109.5 (C); IR (\(\nu_{\text{max}}/\text{cm}^{-1}\)) 687, 751, 853, 1039, 1345 (C-N), 1516 (N-O), 1605, 1739, 2869 and 2964; MS (ESI, 70 eV) \(m/z\) 124 (100%).

4-Nitrobenzamide (208)\(^{417}\)

\[\begin{align*}
\text{O}_2\text{N} & \quad \text{NH}_2 \\
\end{align*}\]
Compound 208 was prepared by a procedure similar to that of Kakuta and co-workers.\textsuperscript{264} A solution of 4-nitrobenzoyl chloride (156) (2.50 g, 13.45 mmol) in aqueous ammonia (25 mL, 15 M) was stirred at room temperature for 30 min. The mixture was then extracted with ethyl acetate (3 × 100 mL), washed with water (100 mL), then brine (100 mL), dried over anhydrous magnesium sulfate, filtered and the solvent removed \textit{in vacuo}. Purification by flash chromatography (hexane:ethyl acetate 1:1) afforded 208 as a white solid (2.20 g, 13.22 mmol, 98%). $R_f = 0.30$ (hexane:ethyl acetate, 1:1); $^1H$ NMR (400 MHz; d$_6$-DMSO) $\delta_H$ 7.75 (1H, s, NH), 8.13 (2H, d, $J = 8.8$ Hz, H-2', H-6') and 8.32-8.35 (3H, m, H-3', H-5', NH); $^{13}C$ NMR (100 MHz, d$_6$-DMSO) $\delta_C$ 124.4 (CH), 129.8 (CH), 140.9 (C), 150.0 (C) and 167.1 (C).

\textbf{4-Nitrothiobenzamide (202)}\textsuperscript{266}

\begin{center}
\includegraphics[width=1in]{4-nitrothiobenzamide}
\end{center}

Compound 202 was prepared by a procedure similar to that of Colabufo and co-workers.\textsuperscript{265} A solution 4-nitrobenzamide (208) (1.50 g, 9.01 mmol) and Lawesson’s reagent (4.01 g, 9.92 mmol) in anhydrous tetrahydrofuran (50 mL) was heated under reflux for 5 h. The solvent was removed \textit{in vacuo} and the resulting residue taken up in dichloromethane (50 mL), washed with water (50 mL), the separated aqueous layer further extracted with ethyl acetate (2 × 50 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed \textit{in vacuo}. Purification by flash chromatography (hexane:ethyl acetate 1:1) afforded 202 as a yellow solid (0.96 g, 5.22 mmol, 58%). $R_f = 0.20$ (hexane:ethyl acetate, 1:1); $^1H$ NMR (400 MHz; d$_6$-DMSO) $\delta_H$ 8.06 (2H, d, $J = 8.8$ Hz, H-2', H-6'), 8.28 (2H, d, $J = 8.8$ Hz, H-3', H-5'), 9.84 (1H, s, NH) and 10.25 (1H, s, NH); $^{13}C$ NMR (100 MHz, d$_6$-DMSO) $\delta_C$ 124.1 (CH), 129.3 (CH), 146.1 (C), 149.4 (C) and 199.3 (C).
(E)-N-[1-(Dimethylamino)propylidene]-4-nitrobenzenecarbothioamide (203)

Compound 203 was prepared by a procedure similar to that of Lin and co-workers.266 A solution of 4-nitrothiobenzamide (202) (0.15 g, 0.82 mmol) in N,N-dimethylpropionamide dimethyl acetal (201) (2.00 mL, 13.6 mmol) was stirred at room temperature for 1 h, and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate 1:1) afforded 203 as red-orange solid (0.20 g, 0.75 mmol, 92%). $R_f = 0.05$ (hexane:ethyl acetate, 2:1); mp 112-117 °C; $^1$H NMR (400 MHz; d$_6$-DMSO) $\delta$H 1.31 (3H, t, $J = 7.5$ Hz, CH$_2$CH$_3$), 2.97 (2H, q, $J = 7.5$ Hz, CH$_2$CH$_3$), 3.25 (3H, s, NCH$_3$), 3.36 (3H, s, NCH$_3$), 8.17 (2H, d, $J = 7.8$ Hz, H-2', H-6') and 8.38 (2H, d, $J = 7.8$ Hz, H-3', H-5'); $^{13}$C NMR (100 MHz, d$_6$-DMSO) $\delta$C 11.0 (CH$_3$), 24.6 (CH$_2$), 39.6 (CH$_3$), 40.0 (CH$_3$), 122.7 (CH), 129.1 (CH), 146.9 (C), 148.8 (C), 176.7 (C) and 193.1 (C); IR ($\nu_{\text{max}}$/cm$^{-1}$) 693, 850, 957, 1192 (C-N), 1341 (C-N), 1515 (N-O), 1598 and 2982; MS (ESI, 70 eV) m/z 288 (MNa$^+$, 100%); Found (MNa$^+$, 288.0783), C$_{12}$H$_{15}$N$_3$NaO$_2$S requires 288.0777.

3-Ethyl-5-(4'-nitrophenyl)-1,2,4-thiadiazole (204)

Compound 204 was prepared by a procedure similar to that of Lin and co-workers.266 To a stirring suspension of (E)-N-[1-(dimethylamino)propylidene]-4-
nitrobenzenecarbothioamide (203) (60 mg, 0.23 mmol) in pyridine (0.36 mL, 0.45 mmol) and ethanol (1.5 mL) at room temperature was added rapidly a solution of hydroxylamine-O-sulfonic acid (30 mg, 0.25 mmol) in methanol (2.25 mL), and the mixture stirred for 1 h. The solvent was removed in vacuo and the resulting residue taken up in dichloromethane (25 mL), washed with an aqueous solution of sodium hydroxide (25 mL, 1 M), the separated aqueous layer further extracted with ethyl acetate (2 × 10 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate 4:1) afforded 204 as a pale brown solid (50 mg, 0.21 mmol, 70%). \( R_f = 0.65 \) (hexane:ethyl acetate, 2:1); mp 106-110 °C; \( \text{^1H NMR} \) (400 MHz; CDCl\(_3\)) \( \delta_H 1.46 \) (3H, t, \( J = 7.6 \) Hz, CH\(_2\)CH\(_3\)), 3.10 (2H, q, \( J = 7.6 \) Hz, CH\(_2\)CH\(_3\)), 8.14 (2H, d, \( J = 8.8 \) Hz, H-2’, H-6’) and 8.35 (2H, d, \( J = 8.8 \) Hz, H-3’, H-5’); \( \text{^13C NMR} \) (100 MHz, CDCl\(_3\)) \( \delta_C 12.3 \) (CH\(_3\)), 26.6 (CH\(_2\)), 124.5 (CH), 128.3 (CH), 135.9 (C), 149.4 (C), 179.8 (C) and 185.0 (C); \( \text{IR} \) (\( \nu_{\text{max}}/\text{cm}^{-1} \)) 689, 851, 988, 1105, 1127, 1227, 1346 (C-N), 1481, 1536 (N-O), 1605, 2850, 2972, 3063 and 3097; \( \text{MS} \) (ESI, 70 eV) \( m/z 360 \) (100%).

3-Ethyl-5-(4’-aminophenyl)-1,2,4-thiadiazole (198)

A similar procedure\(^{255}\) to that previously described for the preparation of 144 was followed using 3-ethyl-5-(4’-nitrophenyl)-1,2,4-thiadiazole (204) (50 mg, 0.21 mmol) in 1,4-dioxane (2 mL) and sodium sulfide nonahydrate (0.12 g, 0.51 mmol) in water (2 mL). Purification by flash chromatography (hexane:ethyl acetate, 2:1) afforded 198 as a light brown solid (30 mg, 0.15 mmol, 69%). \( R_f = 0.25 \) (hexane:ethyl acetate, 2:1); mp 138-143 °C; \( \text{^1H NMR} \) (400 MHz; CDCl\(_3\)) \( \delta_H 1.42 \) (3H, t, \( J = 7.6 \) Hz, CH\(_2\)CH\(_3\)), 3.01 (2H, q, \( J = 7.6 \) Hz, CH\(_2\)CH\(_3\)), 4.06 (2H, s, NH\(_2\)), 6.70 (2H, d, \( J = 8.6 \) Hz, H-3’, H-5’) and 7.75 (2H, d, \( J = 8.6 \) Hz, H-2’, H-6’); \( \text{^13C NMR} \) (100 MHz, CDCl\(_3\)) \( \delta_C 12.3 \) (CH\(_3\)), 26.6 (CH\(_2\)), 114.3 (CH), 121.0 (C), 129.1 (CH), 149.8 (C), 178.6 (C) and 187.9 (C); \( \text{IR} \) (\( \nu_{\text{max}}/\text{cm}^{-1} \)) 819,
1174, 1302 (C-N), 1419, 1477, 1601 (N-H), 1650, 2972, 3328 (N-H) and 3386; **MS** (ESI, 70 eV) m/z 206 (M⁺, 100%); Found (M⁺, 206.0743), C₁₀H₁₂N₃S requires 206.0746.

### 7.2.4.6. 4-Ethyl-1,2,5-oxadiazole 240 and 3-ethoxy-1,2,5-oxadiazole 241

**NEW CMPND**

![Structure of compound 246](image)

Compound 246 was prepared by a procedure similar to that of Onda and co-workers.²⁹⁴ To a stirring solution of propyltriphenylphosphonium iodide (3.50 g, 8.09 mmol) in anhydrous tetrahydrofuran (20 mL) under nitrogen at -20 °C was added dropwise n-butyllithium (5.06 mL, 8.12 mmol, 1.6 M in n-hexane), and the mixture stirred at -20 °C for 45 min. To the resulting solution at -20 °C was added at dropwise 4-nitrobenzaldehyde (69) (2.64 g, 17.5 mmol) in anhydrous tetrahydrofuran (5 mL), and the mixture stirred at room temperature for 2 h. The solvent was then removed *in vacuo* and the residue taken up in ethyl acetate (100 mL), washed with water (100 mL), the separated aqueous layer further extracted with ethyl acetate (3 × 100 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo*. Purification by flash chromatography (hexane:ethyl acetate 49:1) afforded 246 as a mixture of (E):(Z) isomers (10:7) (yellow oil; 0.63 g, 3.53 mmol, 54%). \( R_f = 0.40 \) (hexane:ethyl acetate, 99:1); **(E) Isomer;** \(^1\)H NMR (400 MHz; CDCl₃) \( \delta_H \) 1.07 (3H, t, \( J = 7.5 \) Hz, CHCHCH₂CH₃), 2.33 (2H, m, CHCHCH₂CH₃), 5.83 (1H, m, CHCH₂CH₂CH₃), 6.40 (1H, d, \( J = 16.6 \) Hz, CHCH₂CH₂CH₃), 7.37 (2H, d, \( J = 8.8 \) Hz, H-2', H-6') and 8.14 (2H, d, \( J = 8.8 \) Hz, H-3', H-5'); **\(^{13}\)C NMR** (100 MHz, CDCl₃) \( \delta_C \) 13.9 (CH₃), 22.0 (CH₂), 123.2 (CH), 123.6 (CH), 128.3 (CH), 129.1 (CH), 138.4 (C) and 145.9 (C); **(Z) Isomer;** \(^1\)H NMR (400 MHz; CDCl₃) \( \delta_H \) 1.11 (3H, t, \( J = 7.2 \) Hz, CHCHCH₂CH₃), 2.26 (2H, m, CHCHCH₂CH₃), 5.83 (1H, m, CHCH₂CH₂CH₃), 6.43 (1H, d, \( J = 7.6 \) Hz, CHCH₂CH₂CH₃), 7.41 (2H, d, \( J = 8.8 \) Hz, H-2', H-6') and 8.10 (2H, d, \( J = 8.8 \) Hz, H-3', H-5'); **\(^{13}\)C NMR** (100 MHz, CDCl₃) \( \delta_C \) 12.9 (CH₃), 26.0 (CH₂), 123.2 (CH), 123.6 (CH), 128.3 (CH), 129.1 (CH), 137.7 (C) and 144.3 (C); **IR** (\( \nu_{\text{max}}/\text{cm}^{-1} \)) 862, 1108,
1201, 1340 (N-O), 1593, 1641 (C=C), 2874, 2933 (C-H) and 2966; MS (ESI, 70 eV) m/z 200 (MNa\(^+\), 100%); Found (MNa\(^+\), 200.0686), C\(_{10}\)H\(_{11}\)NNaO\(_2\) requires 200.0682.

### 3-Ethyl-4-(4'-nitrophenyl)-2-oxide-1,2,5-oxadiazole (247)

Compound 247 was prepared by a two-step procedure similar to that of Gasco and co-workers\(^{418}\) and Barnes and co-workers\(^{296}\). In the first step, to a stirring solution of 4'-nitro-β-ethylstyrene (246) (0.63 g, 3.52 mmol) in acetic acid (20 mL) at 5 °C was added dropwise over 30 min sodium nitrite (2.44 g, 35.27 mmol) in water (10 mL), and the mixture stirred at room temperature for 18 h. The resulting mixture was quenched through the addition of an aqueous solution of sodium hydroxide (100 mL, 2 M), extracted with ethyl acetate (3 × 100 mL), dried over anhydrous magnesium sulfate, filtered and the solvent removed \textit{in vacuo} to afford crude 1-ethyl-2-(4'-nitrophenyl)-1,2-pseudonitrosites 248 and 249 as a yellow solid (0.55 g), which was used without further purification. In the second step, to a stirring solution of crude 1-ethyl-2-(4'-nitrophenyl)-1,2-pseudonitrosites 248 and 249 (0.55 g) in dimethylformamide (12.5 mL) at 0 °C was added dropwise chlorosulfonic acid (4 mL), and the mixture heated at 100 °C for 30 min. The mixture was allowed to cool and poured onto ice-cold water, quenched through the addition of an aqueous solution of sodium hydroxide (100 mL, 2 M), extracted with dichloromethane (3 × 100 mL), washed with brine (3 × 100 mL), dried over anhydrous magnesium sulfate, filtered and the solvent removed \textit{in vacuo}. Purification by flash chromatography (hexane:ethyl acetate 9:1) afforded 247 as a bright yellow solid (0.35 g, 1.60 mmol, 45% over two steps); \(R_f = 0.25\) (hexane:ethyl acetate, 9:1); mp 75-80 °C; \(^1\)H NMR (400 MHz; CDCl\(_3\)) \(\delta_H\) 1.28 (3H, t, \(J = 7.6\) Hz, CH\(_2\)C\(_3\)H\(_2\)), 2.79 (2H, q, \(J = 7.6\) Hz, CH\(_2\)CH\(_3\)), 7.89 (2H, d, \(J = 8.6\) Hz, H-2', H-6') and 8.42 (2H, d, \(J = 8.6\) Hz, H-3', H-5'); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta_C\) 9.9 (CH\(_3\)), 16.8
(CH₂), 115.8 (C), 124.5 (CH), 128.5 (CH), 132.8 (C), 149.3 (C) and 154.8 (C); IR (ν max/cm⁻¹) 843, 861, 981, 1350 (N-O), 1446, 1523 (N-O), 1593, 1655, 2850, 2918, 3223 and 3094; MS (APCI, 70 eV) m/z 236 (M⁺, 100%). Found (M⁺, 236.0675), C₁₀H₁₀N₃O₄ requires 236.0666.

4-Ethyl-4-(4'-aminophenyl)-1,2,5-oxadiazole (240)

![Structure of 4-Ethyl-4-(4'-aminophenyl)-1,2,5-oxadiazole (240)]

Compound 240 was prepared by a procedure similar to that of Boiani and co-workers.²⁹⁷ To a stirring solution of 3-ethyl-4-(4'-nitrophenyl)-2-oxide-1,2,5-oxadiazole (247) (0.13 g, 0.59 mmol) and zinc dust (0.19 g, 2.97 mmol) in tetrahydrofuran (5 mL) at room temperature was added ammonium chloride (1.57 g, 59.36 mmol) in water (5 mL), and the mixture heated under reflux for 5 h. The solution was filtered through celite and the solvent removed in vacuo. The resulting residue was taken up in ethyl acetate (50 mL), washed with water (50 mL), the separated aqueous layer further extracted with ethyl acetate (2 × 50 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (dichloromethane:methanol 199:1) afforded 240 as a yellow solid (18 mg, 0.10 mmol, 16%). Rᵣ = 0.30 (dichloromethane:methanol 199:1); mp 51-54 °C; ¹H NMR (400 MHz; CDCl₃): δ H 1.38 (3H, t, J = 7.4 Hz, CH₂CH₃), 2.91 (2H, q, J = 7.4 Hz, CH₂CH₃), 3.94 (2H, s, NH₂), 6.76 (2H, d, J = 8.4 Hz, H-3', H-5') and 7.51 (2H, d, J = 8.4 Hz, H-2', H-6'); ¹³C NMR (100 MHz, CDCl₃) δ C 11.9 (CH₃), 18.2 (CH₂), 115.0 (CH), 115.8 (C), 129.3 (CH), 148.4 (C), 153.3 (C) and 154.4 (C); IR (ν max/cm⁻¹) 829, 1046, 1074, 1186, 1294, 1444, 1530 (N-O), 1608 (N-H), 1631, 2852, 2925, 3230, 3355 (N-H) and 3447; MS (ESI, 70 eV) m/z 190 (M⁺, 100%). Found (M⁺, 190.0975), C₁₀H₁₂N₃O requires 190.0975.
(1Z, 2Z)-α-Oximido-(4'-nitrophenyl)-acetamidoxime (252)

Compound 252 was prepared by a procedure similar to that of Sauerberg and co-workers. A mixture of (Z)-4-nitrobenzohydroximoyl cyanide (126) (5.90 g, 30.87 mmol), hydroxylamine hydrochloride (2.36 g, 33.96 mmol) and sodium acetate (5.57 g, 67.91 mmol) in ethanol (60 mL) was heated under reflux for 6 h. The solvent was removed in vacuo and the resulting mixture quenched with water (200 mL), extracted with ethyl acetate (3 × 200 mL), washed with brine (200 mL), dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate 1:1) afforded 252 as a light brown solid (3.40 g, 15.17 mmol, 49%). \( R_f = 0.25 \) (hexane:ethyl acetate, 1:2). mp 168-172 °C; \(^1\)H NMR (400 MHz; CDCl\(_3\)) \( \delta \) 5.93 (2H, s, NH\(_2\)), 7.85 (2H, d, \( J = 9.0 \) Hz, H-2', H-6'), 8.29 (2H, d, \( J = 9.0 \) Hz, H-3', H-5'), 9.64 (1H, s, OH) and 12.26 (1H, s, OH); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta \) 124.5 (CH), 128.5 (CH), 139.5 (C), 147.0 (C), 152.7 (C) and 165.6 (C); IR (\( \nu_{\text{max}}/\text{cm}^{-1} \)) 696, 823, 854, 949, 1041, 1107, 1189 (C-O), 1262 (C-N), 1333, 1411, 1500 (N-O), 1582, 1654, 3022, 3105, 3395 (N-H) and 3498 (O-H); MS (ESI, 70 eV) \( m/z \) 225 (M\(^+\), 100%). Found (M\(^+\), 225.0624), C\(_8\)H\(_9\)N\(_4\)O\(_4\) requires 225.0618.

3-Amino-4-(4'-nitrophenyl)-1,2,5-oxadiazole (253)

Compound 253 was prepared by a procedure similar to that of Lakhan and co-workers. A mixture of (1Z, 2Z)-α-oximido-(4'-nitrophenyl)-acetamidoxime (252) (0.25 g, 1.12
mmol) and sodium acetate (0.46 g, 5.58 mmol) in ethanol (20 mL) was heated under reflux for 120 h. The solvent was removed in vacuo and the resulting residue taken up in ethyl acetate (50 mL), washed with water (50 mL), the separated aqueous layer further extracted with ethyl acetate (2 × 50 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate 1:1) afforded 253 as a light brown solid (0.10 g, 0.49 mmol, 44%). $R_f = 0.50$ (hexane:ethyl acetate, 1:1); mp 70-75 °C [lit.419 mp 71-71 °C]; $^1$H NMR (400 MHz; CDCl$_3$) $\delta$H 4.26 (2H, s, NH$_2$), 7.98 (2H, d, $J = 8.8$ Hz, H-2', H-6') and 8.40 (2H, d, $J = 8.8$ Hz, H-3', H-5'); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 124.0 (CH), 128.4 (CH), 132.0 (C), 145.2 (C), 148.5 (C) and 154.5 (C).

3-Chloro-4-(4'-nitrophenyl)-1,2,5-oxadiazole (254)

![3-Chloro-4-(4'-nitrophenyl)-1,2,5-oxadiazole (254)](image)

Compound 254 was prepared by a three step procedure similar to that of Sauerberg and co-workers$^{155}$ and Marvel and co-workers.$^{260,299}$ To a stirring solution of 3-amino-4-(4'-nitrophenyl)-1,2,5-oxadiazole (253) (0.23 g, 1.11 mmol) in acetic acid (20 mL) and hydrochloric acid (5 mL, 10 M) at 0 °C was added dropwise sodium nitrite (80 mg, 1.10 mmol) in water (3 mL), and the mixture stirred at 0 °C for 30 min. To a solution of cuprous chloride (0.11 g, 1.10 mmol) in hydrochloric acid (5 mL, 10 M) at 0 °C was added dropwise a solution of the above preformed diazonium salt, and the mixture stirred at 0 °C for 5 min. The reaction was then allowed to warm to room temperature and stirred for a further 30 min. The resulting mixture was quenched through the addition of an aqueous solution of sodium hydroxide (100 mL, 2 M), extracted with ethyl acetate (3 × 100 mL), dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate 9:1) afforded 254 as a light brown solid (56 mg, 0.25 mmol, 22%). $R_f = 0.60$ (hexane:ethyl acetate, 9:1); mp 45-50 °C; $^1$H NMR (400 MHz; CDCl$_3$) $\delta$H 8.16 (2H, d, $J = 9.0$ Hz, H-2', H-6') and 8.42
Compound 241 was prepared by a two-step procedure similar to that of Sauerberg and co-workers\textsuperscript{155} and a similar procedure\textsuperscript{255} to that previously described for the preparation of 144. In the first step, to a stirring solution of sodium metal (10 mg, 0.26 mmol) in ethanol (2 mL) at room temperature was added 3-chloro-4-(4'-nitrophenyl)-1,2,5-oxadiazole (254) (20 mg, 0.09 mmol), and the reaction mixture stirred at room temperature for a further 2 h. The solvent was removed \textit{in vacuo} and the resulting residue was taken up in ethyl acetate (25 mL), washed with water (25 mL), the separated aqueous layer further extracted with ethyl acetate (2 × 25 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed \textit{in vacuo} to afford crude 3-ethoxy-4-(4'-aminophenyl)-1,2,5-oxadiazole (255) as a light brown solid (12 mg), which was used without further purification. In the second step, a similar procedure\textsuperscript{255} to that previously described for the preparation of 144 was followed using crude 3-ethoxy-4-(4'-aminophenyl)-1,2,5-oxadiazole (255) (12 mg) in 1,4-dioxane (1 mL) and sodium sulfide nonahydrate (25 mg, 0.10 mmol) in water (1 mL). Purification by flash chromatography (hexane:ethyl acetate 4:1) afforded 241 as a brown solid (10 mg, 0.05 mmol, 55% over two steps); $R_f = 0.35$ (hexane:ethyl acetate, 1:1); mp 80-85 °C; $^1$H NMR (400 MHz; CDCl$_3$): $\delta_H$ 1.53 (3H, t, $J = 7.2$ Hz, CH$_2$CH$_3$), 4.50 (2H, q, $J = 7.2$ Hz, CH$_2$CH$_3$), 6.73 (2H, d, $J = 8.6$ Hz, H-3', H-5') and 7.81 (2H, d, $J = 8.6$ Hz, H-2', H-6'); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_C$ 14.6 (CH$_3$), 68.5 (CH$_2$), 114.8 (CH), 115.1 (C), 128.9 (CH), 145.1 (C), 148.6 (C) and 163.4 (C); IR (v$_{\text{max/cm}}$) 573, 829, 988, 1031, 1189.
(C-O), 1285 (C-N), 1380, 1490 (N-O), 1564, 1606, 1723, 2924, 2997, 3214, 3320 (N-H) and 3435; MS (ESI, 70 eV) m/z 206 (M⁺, 100%). Found (M⁺, 206.0928), C₁₀H₁₂N₃O₂ requires 206.0924.

7.2.4.7. 2-Propyltetrazole 256 and 5-propyltetrazole 257

5-(4’-Nitrophenyl)-1H-tetrazole (258)

Compound 258 was prepared by a procedure similar to that of Katritzky and co-workers.³¹⁹ A mixture of 4-nitrobenzonitrile (191) (0.50 g, 3.78 mmol), sodium azide (0.74 g, 11.36 mmol), ammonium chloride (0.81 g, 15.14 mmol) and dimethylformamide (15 mL) was heated under reflux for 12 h. The reaction was allowed to cool, quenched with hydrochloric acid (50 mL, 2 M), extracted with ethyl acetate (3 × 50 mL), dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Purification by recrystallization (chloroform) afforded 258 as a yellow solid (0.67 g, 3.52 mmol, 93%). \( R_f = 0.05 \) (hexane:ethyl acetate, 1:1); \(^1\)H NMR (400 MHz; d₆-DMSO) \( \delta_H \) 7.99 (1H, s, NH), 8.34 (2H, d, \( J = 8.4 \) Hz, H-2, H-6) and 8.48 (2H, d, \( J = 8.4 \) Hz, H-3, H-5); \(^{13}\)C NMR (100 MHz, d₆-DMSO): \( \delta_C \) 125.8 (CH), 129.1 (CH), 131.6 (C), 149.7 (C) and 156.4 (C).
7. Experimental

7.2. Synthesis

2-Propyl-5-(4’-nitrophenyl)-2H-tetrazole (259)

Compound 259 was prepared by a procedure similar to that of Andrews and co-workers.\textsuperscript{320} To a stirring solution of sodium hydride (50 mg, 1.15 mmol, 60% w/w in mineral dispersion oil) and \textit{n}-propyl iodide (0.12 mL, 1.26 mmol) in dimethylformamide (1 mL) at 0 °C was added dropwise a solution of 5-(4’-nitrophenyl)-1H-tetrazole (258) (0.20 g, 1.05 mmol) in dimethylformamide (3 mL), and the mixture stirred at 5 °C for 1.5 h. The solution was diluted with ethyl acetate (50 mL), washed with water (50 mL), the separated aqueous layer further extracted with ethyl acetate (2 × 50 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed \textit{in vacuo}. Purification by flash chromatography (hexane:ethyl acetate 4:1) afforded 259 as a pale brown solid (0.11 g, 0.47 mmol, 45%). \textit{Rf} = 0.50 (hexane:ethyl acetate, 1:1); mp 124-127 °C; \textbf{\textit{1H NMR}} (400 MHz; CDCl\textsubscript{3}) \(\delta\) 1.03 (3H, t, \(J = 6.9\) Hz, CH\textsubscript{2}CH\textsubscript{2}C\textsubscript{4}H\textsubscript{3}), 2.14 (2H, m, CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 4.66 (2H, t, \(J = 6.9\) Hz, CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}) and 8.34-8.35 (4H, m, H-2’, H-3’, H-5’, H-6’); \textbf{\textit{13C NMR}} (100 MHz, CDCl\textsubscript{3}) \(\delta\) 11.0 (CH\textsubscript{3}), 22.9 (CH\textsubscript{2}), 55.1 (CH\textsubscript{2}), 124.2 (CH), 127.6 (CH), 133.4 (C), 148.9 (C) and 163.2 (C); \textbf{\textit{IR}} \(\nu_{\text{max}}/\text{cm}^{-1}\) 730, 853, 1042, 1104, 1279 (C -N), 1336, 1515 (N-O), 1605, 2852, 2943, 2975 and 3095; \textbf{\textit{MS}} (ESI, 70 eV) \textit{m/z} 256 (MNa\textsuperscript{+}, 100%); Found (MNa\textsuperscript{+}, 256.0807), C\textsubscript{10}H\textsubscript{11}N\textsubscript{5}NaO\textsubscript{2} requires 256.0805.
2-Propyl-5-(4'-aminophenyl)-2H-tetrazole (256)

A similar procedure\(^{255}\) to that previously described for the preparation of 144 was followed using 5-(4'-nitrophenyl)-2-propyl-2H-tetrazole (259) (0.11 g, 0.47 mmol) in 1,4-dioxane (2.5 mL) and sodium sulfide nonahydrate (0.26 g, 1.08 mmol) in water (2.5 mL). Purification by flash chromatography (hexane:ethyl acetate 2:1) afforded 256 as a pale brown solid (89 mg, 0.44 mmol, 93\%). \(R_f = 0.25\) (hexane:ethyl acetate, 2:1); mp 74-78 °C; \(^1\)H NMR (400 MHz; CDCl\(_3\)) \(\delta\) H 0.98 (3H, t, \(J = 7.4\) Hz, CH\(_2\)CH\(_2\)C\(_6\)H\(_5\)), 2.06 (2H, m, CH\(_2\)CH\(_2\)CH\(_3\)), 3.97 (2H, s, NH\(_2\)), 4.56 (2H, t, \(J = 7.4\) Hz, CH\(_2\)CH\(_2\)CH\(_3\)), 6.74 (2H, d, \(J = 8.4\) Hz, H-3', H-5') and 7.93 (2H, d, \(J = 8.4\) Hz, H-2', H-6'); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) C 10.9 (CH\(_3\)), 22.8 (CH\(_2\)), 54.4 (CH\(_2\)), 114.8 (CH), 117.4 (C), 128.0 (CH), 148.4 (C) and 165.2 (C); IR (\(\nu_{\text{max}}/\text{cm}^{-1}\)) 763, 838, 1173, 1303 (C-N), 1442, 1460, 1610 (N-H), 2878, 2967, 3225, 3331 (N-H), 3372, 3431 and 3475; MS (ESI, 70 eV) \(m/z\) 204 (M\(^+\), 100\%). Found (M\(^+\), 204.1239), C\(_{10}\)H\(_{14}\)N\(_5\) requires 204.1244.

\(N\)-(4'-Nitrophenyl)butyramide (260)\(^{421}\)

Compound 260 was prepared by a procedure similar to that of Pokhodylo and co-workers.\(^{321}\) To a stirring solution of 4-nitroaniline (137) (0.50 g, 3.62 mmol) and triethylamine (0.38 mL, 0.36 mmol) in 1,4-dioxane (10 mL) at 0 °C was added dropwise butyric anhydride (0.59 mL, 3.62 mmol), and the mixture stirred at room temperature for 1 h. The resulting solution was diluted with ethyl acetate (50 mL), washed with water (50
mL), the separated aqueous layer further extracted with ethyl acetate (2 × 50 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Purification by recrystallization (ethanol) afforded 260 as a brown solid (0.60 g, 2.89 mmol, 80%). \( R_t = 0.70 \) (hexane:ethyl acetate, 1:1); \(^1\text{H} \text{ NMR} \) (400 MHz; CDCl\(_3\) \( \delta_H \) 1.03 (3H, t, \( J = 7.4 \) Hz, CH\(_2\)CH\(_2\)CH\(_3\)), 1.78 (2H, m, CH\(_2\)CH\(_2\)CH\(_3\)), 2.40 (2H, t, \( J = 7.4 \) Hz, CH\(_2\)CH\(_2\)CH\(_3\)), 7.71-7.75 (3H, m, NH, H-2', H-6') and 8.20 (2H, d, \( J = 9.0 \) Hz, H-3', H-5'); \(^{13}\text{C} \text{ NMR} \) (100 MHz, CDCl\(_3\) \( \delta_C \) 13.7 (CH\(_3\)), 18.8 (CH\(_2\)), 39.7 (CH\(_2\)), 114.3 (C), 118.9 (CH), 125.1 (CH), 143.9 (C) and 171.7 (C).

**5-Propyl-1-(4'-nitrophenyl)-1H-tetrazole (261)**

![Chemical structure of 5-Propyl-1-(4'-nitrophenyl)-1H-tetrazole (261)](image)

Compound 261 was prepared by a procedure similar to that of Pokhodylo and co-workers.\(^{321}\) A mixture of N-(4'-nitrophenyl)butyramide (260) (0.60 g, 2.88 mmol), sodium azide (0.38 g, 5.76 mmol) and phosphorus oxychloride (1.34 mL, 14.41 mmol) in acetonitrile (15 mL) was heated under reflux for 10 h. The solvent and excess phosphorus oxychloride were removed in vacuo. The residue was quenched with sat. sodium hydrogen carbonate (50 mL), extracted with ethyl acetate (3 × 50 mL), dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate 4:1) afforded 261 as a pale brown solid (0.11 g, 0.49 mmol, 17%). \( R_t = 0.75 \) (hexane:ethyl acetate, 1:1); \(^1\text{H} \text{ NMR} \) (400 MHz; CDCl\(_3\) \( \delta_H \) 1.02 (3H, t, \( J = 7.6 \) Hz, CH\(_2\)CH\(_2\)CH\(_3\)), 1.89 (2H, m, CH\(_2\)CH\(_2\)CH\(_3\)), 2.96 (2H, t, \( J = 7.6 \) Hz, CH\(_2\)CH\(_2\)CH\(_3\)), 7.78 (2H, d, \( J = 9.0 \) Hz, H-2', H-6') and 8.50 (2H, d, \( J = 9.0 \) Hz, H-3', H-5'); \(^{13}\text{C} \text{ NMR} \) (100 MHz, CDCl\(_3\) \( \delta_C \) 13.6 (CH\(_3\)), 20.5 (CH\(_2\)), 25.7 (CH\(_2\)), 125.4 (CH), 125.5 (CH), 138.6 (C), 148.3 (C) and 155.1 (C); \( \text{IR} \) (\( \nu_{\text{max}}/\text{cm}^{-1} \)) 688, 751, 854, 1342 (C-N), 1495, 1507 (N-O), 1526, 1598, 1614, 2875, 2962, 3093 and 3117; \( \text{MS} \) (ESI, 70 eV) \( m/z \) 256 (MNa\(^+\), 100%). Found (MNa\(^+\), 256.0809), C\(_{10}\)H\(_{11}\)N\(_5\)NaO\(_2\) requires 256.0805.
5-Propyl-1-(4'-aminophenyl)-1H-tetrazole (257)

A similar procedure to that previously described for the preparation of 144 was followed using 1-(4'-nitrophenyl)-5-propyl-1H-tetrazole (261) (0.11 g, 0.47 mmol) in 1,4-dioxane (3 mL) and sodium sulfide nonahydrate (0.27 g, 1.13 mmol) in water (3 mL). Purification by flash chromatography (hexane:ethyl acetate 2:1) afforded 257 as a pale brown solid (50 mg, 0.25 mmol, 52%). \( R_f = 0.15 \) (hexane:ethyl acetate, 2:1); mp 77-80 °C; \( ^1H \text{ NMR} \) (400 MHz; CDCl₃) \( \delta \) 0.96 (3H, t, \( J = 7.6 \) Hz, CH₂CH₂C₃H₇), 1.78 (2H, m, CH₂C₆H₄CH₃), 2.81 (2H, t, \( J = 7.6 \) Hz, CH₂CH₂CH₃), 4.18 (2H, s, NH₂), 6.80 (2H, d, \( J = 8.6 \) Hz, H-3', H-5') and 7.15 (2H, d, \( J = 8.6 \) Hz, H-2', H-6'); \( ^13C \text{ NMR} \) (100 MHz, CDCl₃) \( \delta \) C 13.6 (CH₃), 20.6 (CH₂), 25.3 (CH₂), 115.0 (CH), 123.7 (C), 126.2 (CH), 148.5 (C) and 155.2 (C); \( \text{IR} \) (\( \nu_{\text{max}}/\text{cm}^{-1} \)) 811, 830, 1172, 1304 (C-N), 1504, 1520, 1607 (N-H), 2873, 2960, 3235, 3348 (N-H) and 3443; \( \text{MS} \) (ESI, 70 eV) \( m/z \) 204 (M⁺, 100%). Found (M⁺, 204.1238), C₁₀H₁₄N₅ requires 204.1244.
7.2.4.8. 6-Membered heterocycles; 4-Pyridine 262, pyrimidines 263-265, pyrazine 266 and 3-pyridazine 267

4-(4'-Acetamidophenyl)pyridine (280)

Compound 280 was prepared by a procedure similar to that of Cai and co-workers. A mixture of 4-acetamidophenylboronic acid pinacol ester (276) (0.10 g, 0.38 mmol), 4-bromopyridine hydrochloride (277) (89 mg, 0.46 mmol) and sodium hydrogen carbonate (0.14 g, 1.61 mmol) in toluene (2 mL), ethanol (1 mL) and water (0.5 mL) was flushed with argon for 15 min. To this mixture was added bis(triphenylphosphine)palladium(II) chloride (14 mg, 0.02 mmol) and the resulting mixture heated under microwave irradiation (50 W) at 120 °C for 1 h. The reaction was allowed to cool, diluted with dichloromethane (50 mL), washed with water (50 mL), the separated aqueous layer further extracted with dichloromethane (2 × 50 mL), and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate, 4:1) afforded 280 as a pale brown solid (60 mg, 0.28 mmol, 74%). $R_f = 0.20$ (hexane:ethyl acetate, 1:4). mp 195-200 °C; $^1$H NMR (400 MHz; CDCl₃) $\delta_H$ 2.18 (3H, s, CH₃), 3.82 (1H, s, NH), 7.54 (2H, dd, $J = 6.4$ Hz, $J = 1.8$ Hz, H-3, H-5), 7.60-7.70 (4H, m, H-2', H-3', H-5', H-6') and 8.55 (2H, d, $J = 6.0$ Hz, H-2, H-6); $^{13}$C NMR (100 MHz, CDCl₃) $\delta_C$ 23.6 (CH₃), 120.1 (CH), 121.3 (CH), 127.2 (CH), 132.6 (C), 139.5 (C), 148.3 (C), 149.2 (CH) and 169.8 (C); IR ($\nu_{max}$/cm⁻¹) 651, 807, 1225, 1302 (C-N), 1324, 1489, 1530, 1556, 1596 (N-H), 1661 (C=O), 3034 and 3302 (N-H); MS (ESI, 70 eV) m/z 213 (M⁺, 100%); Found (M⁺, 213.1029), C₁₃H₁₃N₂O requires 213.1022.
2-(4'-Acetamidophenyl)pyrimidine (281)

A similar procedure\textsuperscript{330} to that previously described for the preparation of 280 was followed using 4-acetamidophenylboronic acid pinacol ester (276) (0.10 g, 0.38 mmol), 2-chloropyrimidine (274) (52 mg, 0.46 mmol), sodium hydrogen carbonate (97 mg, 1.15 mmol) and bis(triphenylphosphine)palladium(II) chloride (14 mg, 0.02 mmol) in toluene (2 mL), ethanol (1 mL) and water (0.5 mL). Purification by flash chromatography (hexane:ethyl acetate 1:1) afforded 281 as a pale brown solid (46 mg, 0.21 mmol, 56%). 

\[ R_f = 0.15 \] (hexane:ethyl acetate, 1:1); mp 116-121 °C; \textsuperscript{1}H NMR (400 MHz; CDCl\textsubscript{3}) \( \delta \)H 2.21 (3H, s, CH\textsubscript{3}), 7.15 (1H, t, \( J = 4.8 \) Hz, H-5), 7.41 (1H, s, NH), 7.65 (2H, d, \( J = 8.8 \) Hz, H-3', H-5'), 8.42 (2H, d, \( J = 8.8 \) Hz, H-2', H-6') and 8.78 (2H, d, \( J = 5.2 \) Hz, H-4, H-6); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \( \delta \)C 24.8 (CH\textsubscript{3}), 118.8 (CH), 119.3 (CH), 129.1 (CH), 132.0 (C), 140.3 (C), 157.2 (CH), 164.2 (C) and 168.3 (C); IR (\( \nu_{\text{max}}/\text{cm}^{-1} \)) 721, 795, 1116, 1171, 1262 (C-N), 1314, 1410, 1529, 1560, 1596 (N-H), 1695 (C=O), 3049, 3087, 3183 and 3241; MS (ESI, 70 eV) \( m/z \) 214 (M\textsuperscript{+}, 100%); Found (M\textsuperscript{+}, 214.0977), C\textsubscript{12}H\textsubscript{12}N\textsubscript{3}O requires 214.0975.

5-(4'-Acetamidophenyl)pyrimidine (282)

A similar procedure\textsuperscript{330} to that previously described for the preparation of 280 was followed using 4-acetamidophenylboronic acid pinacol ester (276) (0.10 g, 0.38 mmol),
5-bromopyrimidine (278) (73 mg, 0.46 mmol), sodium hydrogen carbonate (97 mg, 1.15 mmol) and bis(triphenylphosphine)palladium(II) chloride (14 mg, 0.02 mmol) in toluene (2 mL), ethanol (1 mL) and water (0.5 mL). Purification by flash chromatography (hexane:ethyl acetate 1:1) afforded 282 as a pale brown solid (65 mg, 0.30 mmol, 80%).

\[ R_f = 0.15 \text{ (hexane:ethyl acetate, 1:1); mp 185-190 °C; } \text{H NMR (400 MHz; CDCl}_3\text{)} \delta H 2.19 (3H, s, CH}_3, 3.71 (1H, s, NH), 7.55 (2H, d, } J = 8.6 \text{ Hz, H}-2', \text{ H}-6'), 7.74 (2H, d, } J = 8.6 \text{ Hz, H}-3', \text{ H}-5'), 8.94 (2H, s, H-4, H-6) \text{ and 9.13 (1H, s, H-2); C NMR (100 MHz, CDCl}_3\text{)} \delta C 23.7 \text{ (CH}_3, 120.4 \text{ (CH), 127.2 \text{ (CH), 128.8 \text{ (C), 134.0 \text{ (C), 139.5 \text{ (C), 154.3 \text{ (CH), 156.4 \text{ (CH} and 169.8 \text{ (C), IR (v}_\text{max/cm}^{-1}) 826, 841, 1194, 1325 \text{ (C-N), 1416, 1517, 1541, 1600 \text{ (N-H), 1667 \text{ (C}=\text{O), 2852, 2923, 3043, 3097 and 3238 \text{ (N-H); MS (ESI, 70 eV)} m/z 236 (MNa}_+^+, 100\%); Found (MNa}_+^+, 236.0794), C}_{12}\text{H}_{11}\text{N}_{3}\text{NaO requires 236.0794.} \]

4-(4'-Acetamidophenyl)pyrimidine (292)

Compound 292 was prepared by a two-step procedure similar to that of Cai and co-workers\textsuperscript{330} and Plate and co-workers.\textsuperscript{338} In the first step, a similar procedure\textsuperscript{330} to that previously described for 280 was followed, using 4-acetamidophenylboronic acid pinacol ester (276) (0.10 g, 0.38 mmol), 4,6-dichloropyrimidine (290) (0.18 g, 1.23 mmol), sodium hydrogen carbonate (97 mg, 1.15 mmol) and bis(triphenylphosphine)palladium(II) chloride (14 mg, 0.02 mmol) in toluene (2 mL), ethanol (1 mL) and water (0.5 mL). The reaction was allowed to cool, diluted with ethyl acetate (50 mL), washed with water (50 mL), the separated aqueous layer further extracted with ethyl acetate (2 × 50 mL), and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed \textit{in vacuo} to afford crude 6-chloro-4-(4'-acetamidophenyl)pyrimidine (291) as a light brown solid (31 mg), which was used without further purification. In the second step, to a stirring solution of crude 6-chloro-4-(4'-acetamidophenyl)pyrimidine (291) (31 mg) in tetrahydrofuran (5 mL) and aqueous ammonia (2.20 mL, 32.25 mmol, 15 M) was added zinc powder (0.22 g, 3.31
mmol), and the reaction mixture heated under reflux for 5 h. The solution was filtered through celite and the solvent removed in vacuo. The resulting residue was taken up in ethyl acetate (50 mL), washed with water (50 mL), the separated aqueous layer further extracted with ethyl acetate (2 × 50 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate 1:1) afforded 292 as a pale brown solid (12 mg, 0.06 mmol, 15% over two steps); $R_f = 0.15$ (hexane:ethyl acetate, 1:1); mp 206-210 °C [lit. $^{422}$ mp 213-214 °C]; $^1$H NMR (400 MHz; CDCl$_3$) $\delta_H$ 2.19 (3H, s, CH$_3$), 7.72-7.74 (3H, m, H-3', H-5', H-5), 8.05 (2H, d, $J = 8.4$ Hz, H-2', H-6'), 8.71 (1H, dd, $J = 5.2$ Hz, J = 1.2 Hz, H-6) and 9.18 (1H, s, H-2); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_C$ 23.9 (CH$_3$), 116.7 (CH), 119.6 (CH), 127.9 (CH), 131.1 (C), 141.4 (C), 156.9 (CH), 158.4 (CH), 163.6 (C) and 169.8 (C).

4'-Acetamidophenylpyrazine (283)

A similar procedure$^{330}$ to that previously described for the preparation of 280 was followed using 4-acetamidophenylboronic acid pinacol ester (276) (0.10 g, 0.38 mmol), chloropyrazine (279) (0.041 mL, 0.46 mmol), sodium hydrogen carbonate (97 mg, 1.15 mmol) and bis(triphenylphosphine)palladium(II) chloride (14 mg, 0.02 mmol) in toluene (2 mL), ethanol (1 mL) and water (0.5 mL). Purification by flash chromatography (hexane:ethyl acetate 1:1) afforded 283 as a pale brown solid (38 mg, 0.18 mmol, 47%). $R_f = 0.20$ (hexane:ethyl acetate, 1:1); mp 179-183 °C; $^1$H NMR (400 MHz; CDCl$_3$) $\delta_H$ 2.21 (3H, s, CH$_3$), 7.69 (2H, d, $J = 9.0$ Hz, H-3', H-5'), 7.81 (1H, s, NH), 7.99 (2H, d, $J = 9.0$ Hz, H-2', H-6'), 8.47 (1H, s, PyrH), 8.60 (1H, s, PyrH) and 9.00 (1H, s, PyrH); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_C$ 24.7 (CH$_3$), 119.9 (CH), 127.6 (CH), 132.1 (C), 139.7 (C), 141.8 (CH), 142.5 (CH), 144.1 (CH), 152.2 (C) and 168.6 (C); IR (ν$_{max}$/cm$^{-1}$) 828, 1015, 1116, 1369 (C-N), 1468, 1535, 1556, 1600 (N-H), 1668 (C=O), 3049 and 3307 (N-H);
MS (ESI, 70 eV) m/z 236 (MNa⁺, 100%); Found (MNa⁺, 236.0797), C_{12}H_{11}N_{3}NaO requires 236.0794.

3-Chloro-6-(4'-acetamidophenyl)pyridazine (294)

A similar procedure to that previously described for the preparation of 280 was followed using 4-acetamidophenylboronic acid pinacol ester (276) (0.10 g, 0.38 mmol), 3,6-dichloropyridazine (293) (0.18 g, 1.22 mmol), sodium hydrogen carbonate (97 mg, 1.15 mmol) and bis(triphenylphosphine)palladium(II) chloride (0.14 g, 1.61 mmol) in toluene (2 mL), ethanol (1 mL) and water (0.5 mL). Purification by flash chromatography (hexane:ethyl acetate 1:1) afforded 294 as a pale brown solid (24 mg, 0.10 mmol, 25%).

\[
R_f = 0.15 \text{ (hexane:ethyl acetate, 1:1); mp 235-240 °C; }^{1}H\text{ NMR (400 MHz; d}_6\text{-DMSO) } \delta_H 2.13 (3H, s, CH}_3, 7.80 (2H, d, J = 8.8 Hz, H-3', H-5'), 8.05 (1H, d, J = 9.2 Hz, H-5), 8.14 (2H, d, J = 8.8 Hz, H-2', H-6') 8.31 (1H, d, J = 9.2 Hz, H-6) \text{ and } 10.24 (1H, s, NH); ^{13}C\text{ NMR (100 MHz, d}_6\text{-DMSO) } \delta_C 25.1 \text{ (CH}_3, 120.0 \text{ (CH}, 127.8 \text{ (CH}, 128.5 \text{ (CH), 129.9} \text{ (C), 130.0} \text{ (CH), 142.4} \text{ (C), 155.6} \text{ (C), 158.7} \text{ (C) and 169.6} \text{ (C); IR (v}_{\text{max/cm}^{-1}}) 565 \text{ (C-Cl), 826, 1097, 1185, 1297 (C-N), 1416, 1528, 1599 (N-H), 1686 (C=O), 3056, 3185, 3258 and 3304; MS (ESI, 70 eV) m/z 248 (M^+, 100%); Found (M^+, 248.0592), C_{12}H_{11}ClN_{3}O requires 248.0592.}\]
4-(4’-Aminophenyl)pyridine (262)\textsuperscript{210}

A similar procedure\textsuperscript{210} to that previously described for the preparation of 104 was followed using 4-(4’-acetamidophenyl)pyridine (280) (55 mg, 0.26 mmol) in methanol (2.5 mL) and hydrochloric acid (2.5 mL, 2 M). Purification by flash chromatography (hexane:ethyl acetate 1:1) afforded 262 as a pale brown solid (39 mg, 0.23 mmol, 89%).

\[ R_f = 0.25 \text{ (hexane:ethyl acetate, 1:1); } ^1H \text{ NMR (400 MHz; CDCl}_3\text{) } \delta_H 6.78 \text{ (2H, d, } J = 8.4 \text{ Hz, H-3', H-5'), 7.48-7.51 \text{ (4H, m, H-3, H-5, H-2', H-6')} \text{ and 8.51 (2H, d, } J = 4.0 \text{ Hz, H-2, H-6); } ^{13}C \text{ NMR (100 MHz, CDCl}_3\text{) } \delta_C 115.3 \text{ (CH), 120.7 (CH), 127.2 (C), 127.9 (CH), 147.8 (C), 148.7 (C) and 149.2 (CH); MS (ESI, 70 eV) } m/\text{z} 171 \text{ (M+, 100%); Found (M^+ \text{, 171.0923), C}_{11}\text{H}_{11}\text{N}_2 \text{ requires 171.0917).} \]

2-(4’-Aminophenyl)pyrimidine (263)\textsuperscript{210}

A similar procedure\textsuperscript{210} to that previously described for the preparation of 104 was followed using 2-(4’-acetamidophenyl)pyrimidine (281) (40 mg, 0.19 mmol) in methanol (2.5 mL) and hydrochloric acid (2.5 mL, 2 M). Purification by flash chromatography (hexane:ethyl acetate 2:1) afforded 263 as a pale brown solid (31 mg, 0.18 mmol, 96%).

\[ R_f = 0.35 \text{ (hexane:ethyl acetate, 2:1); } ^1H \text{ NMR (400 MHz; CDCl}_3\text{) } \delta_H 3.95 \text{ (2H, s, NH}_2\text{), 6.75 (2H, d, } J = 8.8 \text{ Hz, H-3', H-5'), 7.05 (1H, t, } J = 4.6 \text{ Hz, H-5), 8.26 (2H, d, } J = 8.8 \text{ Hz, H-2', H-6')} \text{ and 8.70 (2H, d, } J = 4.8 \text{ Hz, H-4, H-6); } ^{13}C \text{ NMR (100 MHz, CDCl}_3\text{) } \delta_C 114.6 \text{ (CH), 117.8 (CH), 127.8 (C), 129.7 (CH), 149.1 (C), 157.0 (CH) and 164.8 (C);} \]
**7. Experimental**

**7.2. Synthesis**

**MS** (ESI, 70 eV) \(m/z\) 172 (M⁺, 100%); Found (M⁺, 172.0873), C\(_{10}\)H\(_{10}\)N\(_3\) requires 172.0869.

\[5-(4'-Aminophenyl)pyrimidine (265)^{424}\]

![Structure of 5-(4'-Aminophenyl)pyrimidine](image)

A similar procedure\(^{210}\) to that previously described for the preparation of 104 was followed using 5-(4'-acetamidophenyl)pyrimidine (282) (65 mg, 0.30 mmol) in methanol (2.5 mL) and hydrochloric acid (2 M, 2.5 mL). Purification by flash chromatography (hexane:ethyl acetate 1:1) afforded 265 as a pale brown solid (46 mg, 0.27 mmol, 88%). \(R_f = 0.30\) (hexane:ethyl acetate, 1:1); \(^1\)H NMR (400 MHz; CDCl\(_3\)) \(\delta_H\) 3.89 (2H, s, NH\(_2\)), 6.80 (2H, \(d, J = 8.8\) Hz, H-3', H-5'), 7.40 (2H, \(d, J = 8.8\) Hz, H-2', H-6'), 8.89 (2H, s, H-4, H-6) and 9.11 (1H, s, H-2); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta_C\) 115.6 (CH), 124.0 (C), 127.9 (CH), 134.2 (C), 147.4 (C), 154.1 (CH) and 156.4 (CH); MS (ESI, 70 eV) \(m/z\) 172 (M⁺, 100%); Found (M⁺, 172.0869), C\(_{10}\)H\(_{10}\)N\(_3\) requires 172.0869.

**4-(4'-Aminophenyl)pyrimidine (264)^{424}\]

![Structure of 4-(4'-Aminophenyl)pyrimidine](image)

A similar procedure\(^{210}\) to that previously described for the preparation of 104 was followed using 4-(4'-acetamidophenyl)pyrimidine (292) (11 mg, 0.06 mmol) in methanol (1.25 mL) and hydrochloric acid (1.25 mL, 2 M). Purification by flash chromatography (hexane:ethyl acetate 1:1) afforded 264 as a pale brown solid (9 mg, 0.06 mmol, 93%). \(R_f = 0.25\) (hexane:ethyl acetate, 1:1); mp 195-200 °C [ lit.\(^{424}\) mp 202-203 °C]; \(^1\)H NMR (300 MHz; CDCl\(_3\)) \(\delta_H\) 3.99 (2H, s, NH\(_2\)), 6.76 (2H, \(d, J = 8.7\) Hz, H-3', H-5'), 7.59 (1H, dd, \(J = 5.4\) Hz, \(J = 2.0\) Hz, H-5), 7.95 (2H, \(d, J = 8.7\) Hz, H-2', H-6'), 8.64 (1H, d, \(J = 5.4\) Hz, \(J = 2.0\) Hz, H-5).
7. Experimental                              7.2. Synthesis

13C NMR (75 MHz, CDCl₃) δC 114.9 (CH), 115.5 (CH), 126.3 (C), 128.7 (CH), 149.5 (C), 156.9 (CH), 158.9 (CH) and 163.6 (C); MS (ESI, 70 eV) m/z 172 (M⁺, 100%); Found (M⁺, 172.0871), C₁₀H₁₀N₃ requires 172.0869.

4'-Aminophenylpyrazine (266)

A similar procedure²¹⁰ to that previously described for the preparation of 104 was followed using 4'-acetamidophenylpyrazine (283) (35 mg, 0.19 mmol) in methanol (2.5 mL) and hydrochloric acid (2 M, 2.5 mL). Purification by flash chromatography (hexane:ethyl acetate 1:1) afforded 266 as a pale brown solid (22 mg, 0.13 mmol, 78%). Rᵣ = 0.30 (hexane:ethyl acetate, 1:1); mp 125-130 °C; ¹H NMR (400 MHz; CDCl₃) δH 3.94 (2H, s, NH₂), 6.78 (2H, d, J = 8.6 Hz, H-3', H-5'), 7.85 (2H, d, J = 8.6 Hz, H-2', H-6'), 8.47 (1H, d, J = 2.8 Hz, PyrH), 8.53 (1H, dd, J = 2.8 Hz, J = 1.2 Hz, PyrH) and 8.93 (1H, d, J = 1.6 Hz, PyrH); ¹³C NMR (100 MHz, CDCl₃) δC 115.1 (CH), 126.3 (C), 128.1 (CH), 141.2 (CH), 141.5 (CH), 143.8 (CH), 148.3 (C) and 152.8 (C); IR (νmax/cm⁻¹) 812, 1081, 1185, 1369 (C-N), 1473, 1512, 1603 (N-H), 1655, 2851, 2923, 3076, 3216, 3340 (N-H) and 3400; MS (ESI, 70 eV) m/z 172 (M⁺, 100%); Found (M⁺, 172.0870), C₁₀H₁₀N₃ requires 172.0869.

3-(4'-Aminophenyl)pyridazine (267)

Compound 267 was prepared by a two-step procedure similar to that of Guzzo and co-workers,³³⁹ and a similar procedure²¹⁰ to that previously described for the preparation.
of 104. In the first step, a mixture of 3-chloro-6-(4'-acetamidophenyl)pyridazine (294) (24 mg, 0.10 mmol), ammonium formate (32 mg, 0.50 mmol) and palladium-on-carbon (6 mg, 0.02 mmol, 10% w/w) in methanol (5 mL) was heated under reflux for 18 h. The reaction was allowed to cool, filtered through celite and the solvent removed in vacuo. The residue was diluted with ethyl acetate (50 mL), washed with an aqueous solution of sodium hydroxide (50 mL, 1 M), the separated aqueous layer further extracted with ethyl acetate (2 × 50 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo to afford crude 3-(4'-acetamidophenyl)pyridazine (295) as a light brown solid (12 mg), which was used without further purification. In the second step, a mixture of crude 3-(4'-acetamidophenyl)pyridazine (295) (12 mg) in methanol (2.5 mL) and hydrochloric acid (2 M, 2.5 mL) was heated under reflux for 1 h. The reaction was quenched with an aqueous solution of sodium hydroxide (20 mL, 1 M), extracted with ethyl acetate (2 × 20 mL), dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate 1:3) afforded 267 as an off white solid (10 mg, 0.06 mmol, 58% over two steps). \( R_f = 0.25 \) (hexane:ethyl acetate, 1:3); mp 168-173°C [ lit.\(^{425}\) mp 173-174 °C]; \(^1\)H NMR (400 MHz; CDCl\(_3\)) \( \delta \) 3.94 (2H, s, NH\(_2\)), 6.80 (2H, d, \( J = 8.8 \) Hz, H-3’, H-5’), 7.44 (1H, m, H-5), 7.75 (1H, dd, \( J = 8.8 \) Hz, \( J = 1.8 \) Hz, H-4), 7.94 (2H, d, \( J = 8.8 \) Hz, H-2’, H-6’) and 9.05 (1H, dd, \( J = 8.8 \) Hz, \( J = 1.4 \) Hz, H-6’); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta \) \(_C\) 115.1 (CH), 122.7 (CH), 126.3 (C), 126.5 (CH), 128.3 (CH), 148.5 (C), 149.1 (CH) and 159.2 (C); MS (ESI, 70 eV) \( m/z \) 172 (M\(^+\), 100%); Found (M\(^+\), 172.0871), C\(_{10}\)H\(_{10}\)N\(_3\) requires 172.0869.
7. Experimental  7.2. Synthesis

7.2.5. 4-Aminophenone ketoxime prodrugs 296-300

7.2.5.1. Ketoxime and O-methyl ketoxime PAVP prodrugs 296 and 297

(E/Z)-4’-Aminovalerophenone oxime (296)

A similar procedure to that previously described for the preparation of 117 was followed using 4’-aminovalerophenone (18) (2.00 g, 11.28 mmol) in ethanol (50 mL), and hydroxylamine hydrochloride (0.86 g, 12.42 mmol) in water (20 mL) and sodium carbonate (0.60 g, 5.64 mmol) in water (20 mL). Purification by flash chromatography (hexane:ethyl acetate 2:1) afforded 296 as a mixture of (E):(Z) isomers (6:1) (light brown solid; 1.93 g, 10.04 mmol, 89%). \( R_f = 0.15 \) (hexane:ethyl acetate, 2:1); mp 92-96 °C; (E) isomer; \(^1\)H NMR (400 MHz; CDCl\(_3\)) \( \delta_H 0.91 \) (3H, t, \( J = 7.6 \) Hz, CH\(_2\)CH\(_2\)CH\(_2\)C\(_6\)H\(_3\)), 1.39 (2H, m, CH\(_2\)CH\(_2\)CH\(_2\)C\(_6\)H\(_3\)), 1.54 (2H, m, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 2.76 (2H, t, \( J = 7.6 \) Hz, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 3.78 (2H, s, NH\(_2\)), 6.65 (2H, d, \( J = 8.6 \) Hz, H-3’, H-5’) and 7.42 (2H, d, \( J = 8.6 \) Hz, H-2’, H-6’); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta_C 13.8 \) (CH\(_3\)), 23.0 (CH\(_2\)), 25.7 (CH\(_2\)), 114.8 (CH), 125.9 (C), 127.5 (CH), 147.3 (C) and 159.5 (C); (Z) isomer; \(^1\)H NMR (400 MHz; CDCl\(_3\)) \( \delta_H 0.87 \) (3H, t, \( J = 7.4 \) Hz, CH\(_2\)CH\(_2\)CH\(_2\)C\(_6\)H\(_3\)), 1.36 (2H, m, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 1.54 (2H, m, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 2.53 (2H, t, \( J = 7.4 \) Hz, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 3.78 (2H, s, NH\(_2\)), 6.68 (2H, d, \( J = 8.8 \) Hz, H-3’, H-5’) and 7.37 (2H, d, \( J = 8.8 \) Hz, H-2’, H-6’); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta_C 13.7 \) (CH\(_3\)), 22.2 (CH\(_2\)), 29.0 (CH\(_2\)), 34.8 (CH\(_2\)), 114.3 (CH), 123.6 (C), 129.7 (CH), 147.1 (C) and 158.5 (C); IR (\( \nu_{\text{max}}/\text{cm}^{-1} \)) 840, 939, 1183, 1256 (C-N), 1453, 1514, 1603 (N-H), 2857, 2925, 2952, 3133 (O-H) and 3352 (N-H); MS (ESI, 70 eV) \( m/z \) 193 (M\(^+\), 100%). Found (M\(^+\), 193.1329), C\(_{11}\)H\(_{17}\)N\(_2\)O requires 193.1335.
(E/Z)-4'-Aminovalerophenone O-methyl ketoxime (297)

\[
\text{H}_2\text{N} \quad \text{O} 
\]

A similar procedure to that previously described for the preparation of 117 was followed using 4'-aminovalerophenone (18) (2.00 g, 11.28 mmol) in ethanol (50 mL) and O-methyl hydroxylamine hydrochloride (1.04 g, 12.41 mmol) in water (20 mL) and sodium carbonate (1.20 g, 11.28 mmol) in water (20 mL). Purification by flash chromatography (hexane:ethyl acetate 4:1) afforded 297 as a mixture of (E):(Z) isomers (9:1) (brown oil; 0.58 g, 2.77 mmol, 25%). \( R_f = 0.25 \) (hexane:ethyl acetate, 2:1); (E) isomer; \(^1\)H NMR (400 MHz; CDCl\(_3\)) \( \delta \) 0.89 (3H, t, \( J = 7.5 \) Hz, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 1.36 (2H, m, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 1.48 (2H, m, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 2.67 (2H, t, \( J = 7.5 \) Hz, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 3.77 (2H, s, NH\(_2\)), 3.92 (3H, s, OCH\(_3\)), 6.57 (2H, d, \( J = 8.8 \) Hz, H-3', H-5') and 7.43 (2H, d, \( J = 8.8 \) Hz, H-2', H-6'); \(^1^3\)C NMR (100 MHz, CDCl\(_3\)) \( \delta \) C 13.6 (CH\(_3\)), 22.7 (CH\(_2\)), 25.9 (CH\(_2\)), 28.7 (CH\(_2\)), 61.3 (CH\(_3\)), 114.5 (CH), 122.7 (C), 127.3 (CH), 147.3 (C) and 158.5 (C); (Z) isomer; \(^1\)H NMR (400 MHz; CDCl\(_3\)) \( \delta \) 0.87 (3H, t, \( J = 7.5 \) Hz, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 1.33 (2H, m, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 1.48 (2H, m, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 2.48 (2H, t, \( J = 7.5 \) Hz, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 3.78 (2H, s, NH\(_2\)), 3.81 (3H, s, OCH\(_3\)), 6.57 (2H, d, \( J = 8.8 \) Hz, H-3', H-5') and 7.28 (2H, d, \( J = 8.8 \) Hz, H-2', H-6'); \(^1^3\)C NMR (100 MHz, CDCl\(_3\)) \( \delta \) C 14.0 (CH\(_3\)), 20.8 (CH\(_2\)), 29.2 (CH\(_2\)), 34.6 (CH\(_2\)), 60.2 (CH\(_3\)), 113.9 (CH), 122.7 (C), 129.5 (CH), 146.9 (C) and 157.3 (C); IR (\( \nu_{\text{max}}/\text{cm}^{-1} \)) 757, 831, 884, 1050, 1181 (C-O), 1291 (C-N), 1336, 1464, 1517, 1622 (N-H), 2862, 2932, 2956, 3218, 3359 (N-H) and 3470; MS (ESI, 70 eV) \( m/z \) 207 (M\(^+\), 100%). Found (M\(^+\),207.1494), C\(_{12}\)H\(_{19}\)N\(_2\)O requires 207.1492.
7.2.5.2. Aminoalkyl ketoxime PAVP prodrugs 298 and 299

(E)-4'-Aminovalerophenone O-2-(dimethylamino)ethyl ketoxime (298)

Compound 298 was prepared by a procedure similar to that of Chen and co-workers. To a stirring solution of (E/Z)-4'-aminovalerophenone oxime (296) (0.75 g, 3.90 mmol) in tetrahydrofuran (40 mL) at room temperature was added potassium tert-butoxide (1.01 g, 8.58 mmol), and the mixture stirred at room temperature for 30 min. To the resulting solution was added (2-chloroethyl)dimethylamine hydrochloride (301) (0.67 g, 4.68 mmol), and the mixture heated under reflux for 2 h. The reaction was allowed to cool and the solvent removed in vacuo. The residue was taken up in ethyl acetate (100 mL), washed with water (100 mL), the separated aqueous layer further extracted with ethyl acetate (2 × 100 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (chloroform:methanol:ammonium hydroxide, 475:25:1) afforded 298 exclusively as the (E) isomer (brown oil; 0.40 g, 1.52 mmol, 23%). $R_f = 0.25$ (chloroform:methanol:ammonium hydroxide, 475:25:1); $^1$H NMR (400 MHz; CDCl₃) δH 0.90 (3H, t, $J = 7.2$ Hz, CH₂CH₂CH₂C₂H₃), 1.36 (2H, m, CH₂CH₂C₂H₂CH₃), 1.49 (2H, m, CH₂CH₂CH₂CH₃), 2.35 (6H, s, N(CH₃)₂), 2.65-2.70 (4H, m, OCH₂C₂H₃, CH₂CH₂CH₂CH₃), 3.84 (2H, s, NH₂), 4.26 (2H, t, $J = 5.8$ Hz, OCH₂C₂H₂N), 6.61 (2H, d, $J = 8.6$ Hz, H-3', H-5') and 7.43 (2H, d, $J = 8.6$ Hz, H-2', H-6'); $^{13}$C NMR (100 MHz, CDCl₃) δC 13.7 (CH₃), 22.8 (CH₂), 26.1 (CH₂), 28.7 (CH₂), 45.8 (CH₃), 58.5 (CH₂), 72.0 (CH₂), 114.5 (CH), 125.7 (C), 127.3 (CH), 147.3 (C) and 158.4 (C); IR (νmax/cm⁻¹) 832, 1032, 1181 (C-O), 1291 (C-N), 1337, 1465, 1518, 1610 (N-H), 2773, 2822, 2861, 2930, 2955, and 3343 (N-H); MS (ESI, 70 eV) m/z 175 (M⁺, 100%); Found (M⁺, 175.1234), C₁₁H₁₅N₂ requires 175.1230.
Benzophenone ketoxime (311)\textsuperscript{362}

![Benzophenone ketoxime structure](image)

Compound 311 was prepared by a procedure similar to that of Strawn and co-workers.\textsuperscript{362} To a stirring solution of benzophenone (309) (5.00 g, 27.44 mmol) and hydroxylamine hydrochloride (6.68 g, 80.00 mmol) in methanol (80 mL) at room temperature was added dropwise pyridine (7.63 g, 109.8 mmol), and the mixture stirred at room temperature for 18 h. The was solvent removed \textit{in vacuo} and the residue taken up in ethyl acetate (200 mL), washed with hydrochloric acid (1 M, 200 mL), water (200 mL), then brine (200 mL), the separated aqueous layer further extracted with ethyl acetate (2 x 200 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed \textit{in vacuo}. Purification by flash chromatography (hexane:ethyl acetate, 19:1) afforded 311 as a white solid (5.32 g, 27.00 mmol, 49%). $R_f = 0.20$ (hexane:ethyl acetate, 19:1); $^1\text{H NMR}$ (400 MHz; CDCl$_3$) $\delta$H 7.30-7.47 (10H, m, ArH) and 8.66 (1H, s, OH); $^{13}\text{C NMR}$ (100 MHz, CDCl$_3$) $\delta$C 127.8 (CH), 128.2 (CH), 128.3 (CH), 129.1 (CH), 129.2 (CH), 129.5 (CH), 132.7 (C), 136.2 (C) and 158.0 (C).

Benzophenone O-2-morpholinoethyl oxime (314)

![Benzophenone O-2-morpholinoethyl oxime structure](image)

Compound 314 was prepared by a two-step procedure similar to that of Latli and co-workers\textsuperscript{426} and Gaul and co-workers.\textsuperscript{365} In the first step, a mixture of 2-morpholinoethanol (313) (3.06 g, 23.30 mmol) and thionyl chloride (5 mL) in chloroform (25 mL) was heated under reflux for 4 h. The solution was allowed to cool and the resulting precipitate collected by filtration, washed with chloroform (5 mL) and dried \textit{in vacuo} to afford crude 4-(2-chloroethyl)morpholine hydrochloride (302) as a white solid (2.85 g), which was used without further purification. In the second step, to a
stirring suspension of potassium hydroxide (1.79 g, 31.83 mmol) and benzophenone oxime (311) (4.16 g, 21.22 mmol) in dimethylsulfoxide (50 mL) at room temperature was added crude 4-(2-chloroethyl)morpholine hydrochloride (302) (2.85 g), and the mixture stirred at room temperature for 18 h. The was solvent removed in vacuo and the residue taken up in ethyl acetate (100 mL), washed with water (100 mL), the separated aqueous layer further extracted with ethyl acetate (2 × 100 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo.

Purification by flash chromatography (hexane:ethyl acetate 1:1) afforded 314 as a yellow/brown oil (3.20 g, 10.35 mmol, 44% over two steps). $R_f = 0.35$ (hexane:ethyl acetate, 1:1); $^1$H NMR (400 MHz; CDCl$_3$) $\delta$H 2.45 (4H, t, $J = 3.4$ Hz, N(CH$_2$)$_2$(CH$_2$)$_2$O), 2.69 (2H, td, $J = 6.0$ Hz, OCH$_2$CH$_2$N), 3.66 (4H, t, $J = 3.8$ Hz, N(CH$_2$)$_2$(CH$_2$)$_2$O), 4.32 (2H, td, $J = 6.0$ Hz, $J = 1.2$ Hz, OCH$_2$CH$_2$N) and 7.29-7.48 (10H, m, ArH); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 53.8 (CH$_2$), 57.2 (CH$_2$), 66.8 (CH$_2$), 72.2 (CH$_2$), 127.6 (CH), 127.8 (CH), 128.0 (CH), 128.5 (CH), 129.0 (CH), 129.1 (CH), 133.2 (C), 136.2 (C) and 156.7 (C); IR ($\nu_{\text{max}}$/cm$^{-1}$) 697, 772, 982, 1117 (C-O), 1301 (C-N), 1444, 1494, 2808, 2853, 2932 and 3057; MS (ESI, 70 eV) $m/z$ 311 (M$^+$, 100%). Found (M$^+$, 311.1747), C$_{19}$H$_{23}$N$_2$O$_2$ requires 311.1754.

**Morpholinoethoxyamine dihydrochloride (306)**

![Morpholinoethoxyamine dihydrochloride](image)

Compound 306 was prepared by a procedure similar to that of Gaul and co-workers.$^{365}$ A solution of benzophenone O-2-morpholinoethyl oxime (314) (3.10 g, 10.03 mmol) in hydrochloric acid (40 mL, 6 M) was heated under reflux for 2 h. The reaction was allowed to cool, the aqueous phase washed with ethyl acetate (3 × 100 mL) and the separated aqueous phase then evaporated in vacuo to afford 306 as a white solid (1.79 g), which was used without further purification. mp 191-195 °C [lit.$^{427}$ mp 189-191 °C]; $^1$H NMR (400 MHz; d$_6$-DMSO) $\delta$H 2.58 (4H, s, N(CH$_2$)$_2$(CH$_2$)$_2$O), 3.53 (2H, t, $J = 6.0$ Hz, OCH$_2$CH$_2$N), 3.91 (4H, m, N(CH$_2$)$_2$(CH$_2$)$_2$O), 4.54 (2H, t, $J = 6.0$ Hz, OCH$_2$CH$_2$N) and
Compound 299 was prepared by a procedure similar to that of Chen and co-workers. A mixture of 4′-aminovalerophenone (18) (0.94 g, 5.30 mmol), morpholinoethoxyamine dihydrochloride (306) (2.40 g), and potassium carbonate (3.67 g, 26.52 mmol) in ethanol (50 mL) was heated under reflux for 4 h. The solution was allowed to cool and the solvent removed in vacuo. The residue was taken up in dichloromethane (100 mL), washed with water (100 mL), the separated aqueous layer further extracted with dichloromethane (2 × 100 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (hexane:ethyl acetate 1:1) afforded 299 exclusively as the (E) isomer (brown oil; 1.30 g, 3.87 mmol, 73%). \( R_f = 0.10 \) (hexane:ethyl acetate, 1:1); \(^1\text{H NMR}\) (400 MHz; CDCl\textsubscript{3}) \( \delta^H \) 0.90 (3H, t, \( J = 7.6 \) Hz, CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 1.35 (2H, m, CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 1.50 (2H, m, CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 2.55 (4H, t, \( J = 4.3 \) Hz, N(CH\textsubscript{2})\textsubscript{2}(CH\textsubscript{2})\textsubscript{2}O), 2.67 (2H, t, \( J = 7.6 \) Hz, CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 2.72 (2H, t, \( J = 6.0 \) Hz, OCH\textsubscript{2}CH\textsubscript{2}N), 3.71 (4H, t, \( J = 4.3 \) Hz, N(CH\textsubscript{2})\textsubscript{2}(CH\textsubscript{2})\textsubscript{2}O), 3.84 (2H, s, NH\textsubscript{2}), 4.28 (2H, t, \( J = 6.0 \) Hz, OCH\textsubscript{2}CH\textsubscript{2}N), 6.63 (2H, d, \( J = 8.4 \) Hz, H-3′, H-5′) and 7.43 (2H, d, \( J = 8.8 \) Hz, H-2′, H-6′); \(^{13}\text{C NMR}\) (100 MHz, CDCl\textsubscript{3}) \( \delta^C \) 13.7 (CH\textsubscript{3}), 22.8 (CH\textsubscript{2}), 26.2 (CH\textsubscript{2}), 28.8 (CH\textsubscript{2}), 53.4 (CH\textsubscript{2}), 57.5 (CH\textsubscript{2}), 66.9 (CH\textsubscript{2}), 71.6 (CH\textsubscript{2}), 114.5 (CH), 125.7 (C), 127.3 (CH), 147.5 (C) and 158.5 (C); IR (\( \nu_{\text{max}}/\text{cm}^{-1} \)) 755, 831, 1034, 1114 (C-O), 1181, 1294 (C-N), 1336, 1454, 1518, 1611 (N-H), 2812, 2860, 2929, 2956, 3221 and 3343 (N-H); MS (ESI, 70 eV) \( m/z \) 306 (M\textsuperscript{+}, 100%). Found (M\textsuperscript{+}, 306.2186), C\textsubscript{17}H\textsubscript{28}N\textsubscript{3}O\textsubscript{2} requires 306.2176.
7.2.5.3. Carboxymethyl PAVP prodrug 300

Acetone oxime (310)\textsuperscript{363}

\[
\text{\begin{array}{c}
\text{OH} \\
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\end{array}}
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Compound 310 was prepared by a procedure similar to that of Pohjakallio and co-workers.\textsuperscript{363} To a stirring solution of acetone (308) (20.00 g, 344 mmol) and hydroxylamine hydrochloride (35.90 g, 517 mmol) in water (500 mL) at room temperature was added portionwise sodium carbonate (65.63 g, 619 mmol), and the mixture stirred at room temperature for 16 h. The reaction mixture was diluted with ethyl acetate (250 mL), washed with water (500 mL), the separated aqueous layer further extracted with ethyl acetate (4 × 250 mL) and the combined organic phases dried over anhydrous magnesium sulfate, filtered and the solvent removed \textit{in vacuo}. Purification by recrystallization (hexane) afforded 310 as a white solid (20.77 g, 284 mmol, 83%). \textsuperscript{1}H NMR (400 MHz; CDCl\textsubscript{3}) \(\delta_H 1.89 (3\text{H, s, CH}_3), 1.90 (3\text{H, s, CH}_3)\) and 8.12 (1H, s, OH); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \(\delta_C 14.7 (\text{CH}_3), 21.8 (\text{CH}_3)\) and 155.7 (C).

Acetone carboxymethoxime (312)\textsuperscript{364}

\[
\text{\begin{array}{c}
\text{\_\_\_} \\
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\end{array}}
\]

Compound 312 was prepared by a procedure similar to that of Borek and co-workers.\textsuperscript{364} To a stirring suspension of bromoacetic acid (9.51 g, 68.41 mmol) and crushed ice (10 g) at 0 \textdegree C was added dropwise an aqueous solution of sodium hydroxide (40 mL, 2 M). To the resulting solution at 0 \textdegree C was then added acetone oxime (310) (5.00 g, 68.41 mmol) and an aqueous solution of sodium hydroxide (40 mL, 2 M), and the mixture stirred at room temperature for 3 h. The aqueous phase was washed with ethyl acetate (3 × 200
mL) and the separated aqueous layer cooled to 0 °C, adjusted to pH 1 through the addition of hydrochloric acid (20 mL, 10 M), extracted with ethyl acetate (3 × 200 mL), the combined organic phases dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo. Purification by recrystallization (acetone) afforded 312 as a white solid (2.40 g, 21.22 mmol, 31%). mp 75-78 °C [lit.364 mp 76-76.5 °C]; $^1$H NMR (400 MHz; CDCl$_3$) $\delta$H 1.89 (3H, s, CH$_3$), 1.93 (3H, s, CH$_3$), 4.61 (2H, s, CH$_2$) and 8.29 (1H, s, COOH); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 15.8 (CH$_3$), 21.6 (CH$_3$), 69.6 (CH$_2$), 155.7 (C) and 175.3 (C).

**Carboxymethoxylamine hemihydrochloride (307)**

\[ \text{HO}_2\text{C} \quad \text{HN} \quad \text{C} \quad \text{H}_2\text{Cl} \]

Compound 307 was prepared by a procedure similar to that of Borek and co-workers.364 A solution of acetone carboxymethoxime (312) (2.00 g, 17.69 mmol) in hydrochloric acid (25 mL, 6 M) was heated under reflux for 2 h. The reaction was allowed to cool and the water removed in vacuo. Purification by recrystallization (isopropanol) afforded 307 as a purple solid (1.60 g, 14.75 mmol, 83%). $^1$H NMR (400 MHz; CDCl$_3$) $\delta$H 4.65 (2H, s, CH$_2$); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 71.1 (CH$_2$) and 170.3 (C).

**(E)-4'-Aminovalerophenone carboxymethoxime (300)**

\[ \text{H}_2\text{N} \quad \text{O} \quad \text{C} \quad \text{OH} \]

Compound 300 was prepared by a procedure similar to that of Villani and co-workers.366 A mixture of 4'-aminovalerophenone (18) (0.87 g, 4.92 mmol) and carboxymethoxylamine hemihydrochloride (307) (1.60 g, 14.75 mmol) in pyridine (10 mL) and methanol (20 mL) was heated under reflux for 3 h. The solution was allowed to
cool to and the solvent removed in vacuo. The resulting residue was diluted with water (100 mL), the aqueous phase adjusted to pH 4 through the addition of acetic acid (5 mL), extracted with ethyl acetate (3 × 100 mL), the combined organic phases dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo. Purification by flash chromatography (dichloromethane:methanol:acetic acid, 475:25:1) afforded exclusively as the (E) isomer (pink solid; 0.70 g, 2.80 mmol, 57%). \( R_f = 0.15 \) (dichloromethane:methanol:acetic acid, 475:25:1); mp 128-132 °C; \( ^1H \) NMR (400 MHz; CDCl3) \( \delta \)H 0.91 (3H, t, \( J = 7.5 \) Hz, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 1.36 (2H, m, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 1.49 (2H, m, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 2.69 (2H, t, \( J = 7.5 \) Hz, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), 4.60 (2H, s, H-2"), 6.57 (2H, d, \( J = 8.6 \) Hz, H-3', H-5') and 7.34 (2H, d, \( J = 8.6 \) Hz, H-2', H-6'); \( ^{13}C \) NMR (100 MHz, CDCl3) \( \delta \)C 14.7 (CH\(_3\)), 23.7 (CH\(_2\)), 26.4 (CH\(_2\)), 29.4 (CH\(_2\)), 71.1 (CH\(_2\)), 114.3 (CH), 122.7 (C), 128.1 (CH), 151.0 (C) 159.8 (C) and 172.4 (C); IR (\( \nu_{\text{max}}/\text{cm}^{-1} \)) 705, 833, 878, 923, 1018, 1081, 1261 (C-N), 1399, 1514, 1536, 1574, 1598 (N-H), 2606 (O-H), 2859, 2932 and 2953; MS (ESI, 70 eV) \( m/z \) 251 (M\(^+\), 100%); Found (M\(^+\), 251.1393), C\(_{13}\)H\(_{19}\)N\(_2\)O\(_3\) requires 251.1390.
7.3. **General details – pharmacology**

7.3.1. **In vitro evaluation**

Microsomes pooled from male and female Sprague-Dawley rats (250-350 g) were prepared using the classical differential sedimentation method described by Gill *et al.* Microsomal protein concentration was determined by the method of Lowry *et al.* CYP1A, CYP2B and CYP2E1 microsomal activity was determined by Koop *et al.* and Pohl *et al.* Microsomes were diluted to 28 mg/mL, split into 300 μL aliquots and stored at -80 °C so that only one freeze-thaw cycle occurred prior to use.

Erythrocytes obtained from male Sprague-Dawley rats, were centrifuged at 4000 rpm for 5 min at 4 °C using an IEC Centra CL3R® refrigerated centrifuge, then washed with PBS (0.10 M, pH 7.4), centrifuged again and resuspended in PBS to approximately 50% hematocrit.

Standard MtHb assay conditions similar to that of Coleman *et al.* were followed: Washed erthyrocytes (100 μL) were incubated (37 °C, 1 h, n = 3) with compound (10 μM) in DMSO (1% v/v), microsomes (1 mg/ml) and NADPH (1 mM), with a final volume of 200 μL, diluted with PBS (0.1 M, pH 7.4), in an Eppendorf Thermomixer Compact. DMSO, PAPP and PAVP were used a standards to calibrate the assay. Subsequent to incubation, samples were immediately put on ice and a 35 μL aliquot of sample assayed for CO-oximetry parameters using an ABL700 series blood-gas analyzer, 100-240 V, 50-60 Hz, 90 W.

7.3.2. **Hydrolytic stability assay**

Compounds were subject to a 1 h hydrolytic stability appraisal (200 μL total volume, at a final concentration of 200 μM, 2.5% dimethyl sulfoxide overall, 37 °C, n = 3). Analysis was performed by RP-HPLC (HPLC section) at an injection volume of 5 μL.
7.3.3. Rat serum assay

Rat serum was obtained from Sigma-Aldrich and stored at -78 °C. Similar assay conditions to those reported by Di et al. were followed (200 µL total volume, 80% rat serum (diluting with phosphate buffer (0.1 M, pH 7.4)), at a final compound concentration of 200 µM, 2.5% dimethyl sulfoxide overall, 37 °C, 3 h, n = 3) using an Eppendorf Thermomixer Compact. Reactions were quenched through transferring 150 µL of the incubation mixture to 450 µL of ice-cold acetonitrile, affording a final compound concentration of 50 µM. Samples were centrifuged at 14,500 x g for 15 min using an Eppendorf Mini Spin Plus centrifuge, at ambient temperature. 400 µL of the supernatant was removed and transferred to clean tubes. Analysis was performed by RP-HPLC (HPLC section) at an injection volume of 20 µL.

7.3.4. Rat liver S9 fraction assay

Rat liver S9 fraction (20 mg/mL), pooled from male rat (Sprague-Dawley), was obtained from Sigma-Aldrich, diluted to 1 mg/mL with phosphate buffer (0.1 M, pH 7.4) and stored at -78 °C. Similar assay conditions to those reported by Di et al. were followed (200 µL total volume, 1 mg/mL rat liver S9 fraction (diluting with phosphate buffer (0.1 M, pH 7.4)), at a final prodrug concentration of 200 µM, 2.5% dimethyl sulfoxide overall, 37 °C, 6 h, n = 3). The assay protocol followed is as that described above for the rat serum assay, with an adjusted end-point of 6 h.

7.3.5. Simulated gastric fluid (SGF) assay

Compounds were subject to a 1 h hydrolytic stability appraisal in the presence of SGF, without pepsin (200 µL total volume, sodium chloride solution (0.03 M) acidified to pH 1.2 with hydrochloric acid (10 M, at a final compound concentration of 200 µM, 2.5% dimethyl sulfoxide overall, 37 °C, n = 3). The RP-HPLC analysis protocol followed is as that described above for the hydrolytic stability assay.
7.3.6. *In vivo evaluation*

Male and female Wistar rats (250 - 350 g) were used to determine the lethality of compounds selected for *in vivo* evaluation. Rats were individually housed in cages in temperature controlled rooms under natural day length lighting, and were fasted 12 hours prior to dosing. Briefly, prior to administration, the compounds were dissolved in hydrochloric acid (0.2 M, 5% dimethyl sulfoxide overall), to the desired concentration, and without delay dosed via oral gavage. Hydrochloric acid (0.2 M, 5% dimethyl sulfoxide overall) vehicle, PAPP and PAVP were used as standards. In some cases, when rats exhibited stress, they were lightly anaesthetized with CO₂/O₂ prior to dosing to reduce handling stress. The rats recovered from the effects of anaesthesia approximately 30 seconds after dosing. Rats were subsequently monitored every 15 min for rate of onset of symptoms, duration of symptoms, and time to death.
8. **APPENDIX**

8.1. *RP-HPLC purity of bioevaluated compounds*

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### 8.1. RP-HPLC purity of bioevaluated compounds

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<sup>a</sup>Peak observed was extremely broad, potentially due to acidic or basic character. These retention times were deemed unreliable.
8.2. Reference for numbering of compounds

**Substituted 4'-aminophenones 1, 16-19, 27-30**

4'-Aminochalcones 55, 58-62

4'-Aminodihydrochalcone (71) and heterocyclic derivatives 76-79

5-Aminoindanone (104)
8. Appendix

8.2. Reference for numbering of compounds

6-Aminoaurone (86)

Aminophenyl alkyl sulfoxide 129, sulfone 130 and sulfonamides 131 and 132

Ketoxime 117, chloro-oxime 118 and cyano-oximes 120 and 121
8. Appendix

8.2. Reference for numbering of compounds

**PAVP ketoxime prodrugs 296-300**
8.3. *NMR spectra of novel compounds*
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 32 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 32 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 33 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 33 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

1H NMR spectrum of 34 at 400 MHz, CDCl₃

13C NMR spectrum of 34 at 100 MHz, CDCl₃
8. Appendix

8.3. NMR spectra of novel compounds

\textbf{^1H NMR spectrum of 35 at 400 MHz, CDCl$_3$}

\textbf{^13C NMR spectrum of 35 at 100 MHz, CDCl$_3$}
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 36 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 36 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 37 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 37 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

\[ ^1H \text{ NMR spectrum of 46 at 400 MHz, CDCl}_3 \]

\[ ^13C \text{ NMR spectrum of 46 at 100 MHz, CDCl}_3 \]
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 47 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 47 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

\(^1\)H NMR spectrum of 48 at 400 MHz, CDCl\(_3\)

\(^{13}\)C NMR spectrum of 48 at 100 MHz, CDCl\(_3\)
8. Appendix

8.3. NMR spectra of novel compounds

\[ ^{1}H \text{ NMR spectrum of 49 at 400 MHz, CDCl}_3\]

\[ ^{13}C \text{ NMR spectrum of 49 at 100 MHz, CDCl}_3\]
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 71 at 300 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 71 at 100 MHz, CDCl$_3$
8.3. NMR spectra of novel compounds

**1H NMR spectrum of 73 at 300 MHz, CDCl₃**

![1H NMR spectrum of 73 at 300 MHz, CDCl₃](image)

**13C NMR spectrum of 73 at 100 MHz, CDCl₃**

![13C NMR spectrum of 73 at 100 MHz, CDCl₃](image)
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 76 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 76 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 77 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 77 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 78 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 78 at 100 MHz, CDCl$_3$
8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 79 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 79 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

\(^1\)H NMR spectrum of 86 at 400 MHz, CDCl\(_3\)

\(^1^3\)C NMR spectrum of 86 at 100 MHz, CDCl\(_3\)
8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 110 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 110 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

\(^1\text{H NMR spectrum of 112 at 400 MHz, CDCl}_3\)

\(^{13}\text{C NMR spectrum of 112 at 100 MHz, CDCl}_3\)
8. Appendix

8.3. NMR spectra of novel compounds

\[ ^1H \text{ NMR spectrum of 113 at 400 MHz, CDCl}_3 \]

\[ ^{13}C \text{ NMR spectrum of 113 at 100 MHz, CDCl}_3 \]
8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 117 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 117 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 118 at 400 MHz, CDCl$_3$

\[ \text{Chemical Structure} \]

$^{13}$C NMR spectrum of 118 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

\[^1H\] NMR spectrum of 121 at 400 MHz, CDCl\textsubscript{3}

\[^{13}C\] NMR spectrum of 121 at 100 MHz, CDCl\textsubscript{3}
8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 127 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 127 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 128 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 128 at 100 MHz, CDCl$_3$
8.3. NMR spectra of novel compounds

\[ ^{1}H \text{ NMR spectrum of 129 at 400 MHz, CDCl}_3 \]

\[ ^{13}C \text{ NMR spectrum of 129 at 100 MHz, CDCl}_3 \]
8. Appendix

8.3. NMR spectra of novel compounds

**1H NMR spectrum of 130 at 400 MHz, CDCl₃**

![1H NMR spectrum of 130 at 400 MHz, CDCl₃](image)

**13C NMR spectrum of 130 at 100 MHz, CDCl₃**

![13C NMR spectrum of 130 at 100 MHz, CDCl₃](image)
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 132 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 132 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 133 at 300 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 133 at 75 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 136 at 300 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 136 at 75 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 140 at 300 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 140 at 75 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 145 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 145 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 146 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 146 at 100 MHz, CDCl$_3$
8.3. NMR spectra of novel compounds

\[^{1}\text{H} \text{ NMR spectrum of 147 at 400 MHz, CDCl}_3\]

\[^{13}\text{C} \text{ NMR spectrum of 147 at 100 MHz, CDCl}_3\]
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 148 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 148 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 149 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 149 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

\(^1\)H NMR spectrum of 151 at 100 MHz, CDCl\(_3\)

\[^{13}\)C NMR spectrum of 151 at 100 MHz, CDCl\(_3\)
8. Appendix

8.3. NMR spectra of novel compounds

\( ^1H \) NMR spectrum of 153 at 400 MHz, CDCl\(_3\)

\( ^{13}C \) NMR spectrum of 153 at 100 MHz, CDCl\(_3\)
8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 158 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 158 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 159 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 159 at 100 MHz, CDCl$_3$
8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 160 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 160 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 163 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 163 at 75 MHz, CDCl$_3$
8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 164 at 300 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 164 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

**^1H NMR spectrum of 165 at 400 MHz, CDCl₃**

![^1H NMR spectrum of 165 at 400 MHz, CDCl₃](image)

**^13C NMR spectrum of 165 at 100 MHz, CDCl₃**

![^13C NMR spectrum of 165 at 100 MHz, CDCl₃](image)
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 166 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 166 at 100 MHz, CDCl$_3$
8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 167 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 167 at 100 MHz, CDCl$_3$
8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 169 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 169 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 173 at 400 MHz, $d_6$-DMSO

$^{13}$C NMR spectrum of 173 at 100 MHz, $d_6$-DMSO
8. Appendix  

8.3. NMR spectra of novel compounds

# H NMR spectrum of 174 at 300 MHz, d⁶-DMSO

![H NMR spectrum of 174 at 300 MHz, d⁶-DMSO]

# C NMR spectrum of 174 at 100 MHz, d⁶-DMSO

![C NMR spectrum of 174 at 100 MHz, d⁶-DMSO]
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 175 at 400 MHz, d$_6$-DMSO

$^{13}$C NMR spectrum of 175 at 100 MHz, d$_6$-DMSO
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 176 at 300 MHz, d$_6$-DMSO

$^{13}$C NMR spectrum of 176 at 75 MHz, d$_6$-DMSO
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 178 at 300 MHz, $d_6$-DMSO

$^{13}$C NMR spectrum of 178 at 100 MHz, $d_6$-DMSO
8. Appendix

8.3. NMR spectra of novel compounds

\[ ^1H \text{ NMR spectrum of 180 at 400 MHz, CDCl}_3 \]

\[ ^{13}C \text{ NMR spectrum of 180 at 100 MHz, CDCl}_3 \]
8. Appendix

8.3. NMR spectra of novel compounds

**1H NMR spectrum of 181 at 400 MHz, CDCl₃**

![1H NMR spectrum of 181 at 400 MHz, CDCl₃](image)

**13C NMR spectrum of 181 at 100 MHz, CDCl₃**

![13C NMR spectrum of 181 at 100 MHz, CDCl₃](image)
8.3. NMR spectra of novel compounds

1H NMR spectrum of 182 at 300 MHz, CDCl₃

13C NMR spectrum of 182 at 100 MHz, CDCl₃
8. Appendix

8.3. NMR spectra of novel compounds

\( ^1H \) NMR spectrum of 183 at 400 MHz, CDCl\(_3\)

\( ^13C \) NMR spectrum of 183 at 100 MHz, CDCl\(_3\)
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 196 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 196 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

**¹H NMR spectrum of 197 at 400 MHz, CDCl₃**

![¹H NMR spectrum of 197 at 400 MHz, CDCl₃](image1)

**¹³C NMR spectrum of 197 at 100 MHz, CDCl₃**

![¹³C NMR spectrum of 197 at 100 MHz, CDCl₃](image2)
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 198 at 400 MHz, CDCl$_3$

![1H NMR spectrum of 198 at 400 MHz, CDCl$_3$](image)

$^{13}$C NMR spectrum of 198 at 100 MHz, CDCl$_3$

![$^{13}$C NMR spectrum of 198 at 100 MHz, CDCl$_3$](image)
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 199 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 199 at 100 MHz, CDCl$_3$
\textbf{8.3. NMR spectra of novel compounds}

\textbf{\textsuperscript{1}H NMR spectrum of 200 at 400 MHz, CDCl\textsubscript{3}}

\textbf{\textsuperscript{13}C NMR spectrum of 200 at 100 MHz, CDCl\textsubscript{3}}
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 201 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 201 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 203 at 400 MHz, $d_6$-DMSO

$^{13}$C NMR spectrum of 203 at 100 MHz, $d_6$-DMSO
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 204 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 204 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 240 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 240 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 241 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 241 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

\[ ^1H \text{ NMR spectrum of 246 at 400 MHz, } \text{CDCl}_3 \]

\[ ^13C \text{ NMR spectrum of 246 at 100 MHz, } \text{CDCl}_3 \]
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 247 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 247 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$¹^H$ NMR spectrum of 252 at 400 MHz, CDCl$_3$

$¹³^C$ NMR spectrum of 252 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 254 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 254 at 100 MHz, CDCl$_3$
8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 256 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 256 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

1H NMR spectrum of 257 at 400 MHz, CDCl₃

13C NMR spectrum of 257 at 100 MHz, CDCl₃
8. Appendix

8.3. NMR spectra of novel compounds

**1H NMR spectrum of 259 at 400 MHz, CDCl₃**

![1H NMR spectrum of 259 at 400 MHz, CDCl₃]

**13C NMR spectrum of 259 at 100 MHz, CDCl₃**

![13C NMR spectrum of 259 at 100 MHz, CDCl₃]
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 261 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 261 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 262 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 262 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 266 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 266 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 267 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 267 at 100 MHz, CDCl$_3$
8.3. NMR spectra of novel compounds

1H NMR spectrum of 281 at 400 MHz, CDCl₃

13C NMR spectrum of 281 at 100 MHz, CDCl₃
8. Appendix

8.3. NMR spectra of novel compounds

1H NMR spectrum of 282 at 400 MHz, CDCl₃

13C NMR spectrum of 282 at 100 MHz, CDCl₃
8. Appendix

8.3. NMR spectra of novel compounds

**$^1$H NMR spectrum of 283 at 400 MHz, CDCl$_3$**

![1H NMR spectrum of 283 at 400 MHz, CDCl$_3$](image1)

**$^{13}$C NMR spectrum of 283 at 100 MHz, CDCl$_3$**

![13C NMR spectrum of 283 at 100 MHz, CDCl$_3$](image2)
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 294 at 400 MHz, $d_6$-DMSO

$^{13}$C NMR spectrum of 294 at 100 MHz, $d_6$-DMSO
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 296 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 296 at 100 MHz, CDCl$_3$
8.3. NMR spectra of novel compounds

**1H NMR spectrum of 298 at 400 MHz, CDCl₃**

**13C NMR spectrum of 298 at 100 MHz, CDCl₃**
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 299 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 299 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 300 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 300 at 100 MHz, CDCl$_3$
8. Appendix

8.3. NMR spectra of novel compounds

$^1$H NMR spectrum of 314 at 400 MHz, CDCl$_3$

$^{13}$C NMR spectrum of 314 at 100 MHz, CDCl$_3$
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