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The Development of Metallohydroxamates as Novel Anti-Bacterial and Anti-Leishmanial Agents

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The Development of Metallohydroxamates as Novel Anti-Bacterial and Anti-Leishmanial Agents

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Under the supervision of Dr. Darren Griffith

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DECLARATION

I declare that this thesis, which I submit to RCSI for examination in consideration of the award of a higher degree Doctor of Philosophy, is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research programme this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work. Furthermore, I took reasonable care to ensure that the work is original, and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

This thesis represents a multidisciplinary project that comprises organic and inorganic synthesis and microbiology, all of which has been undertaken by the author except where otherwise stated.

Signed__________________________________________________

RCSI Student Number________________________________________

Date_______________________________________________________
PUBLICATIONS


ACKNOWLEDGEMENTS

Ultimately, this is the page that most read part of any thesis.

Partaking in a PhD has always been an ambition of mine and I was lucky enough to get an opportunity at the Royal College of Surgeons. It has opened many doors for me in my professional career, and I have many people to thank for making this journey an enjoyable and memorable one.

The first person I would like to thank is my supervisor, Dr. Darren Griffith. His guidance, support, and expertise in the area throughout my time studying was invaluable, in the greatest sense of the word. He was always on hand to discuss my work and brainstorm any issues and I am so grateful for the help he offered in the completion of this PhD. Darren, I will always count myself lucky to have had you as a supervisor. Thank you. I would like to thank all the collaborators in this project, namely Dr. Deirdre Fitzgerald-Hughes and her research group in Beaumont Hospital. All their help with the microbiology work afforded me the opportunity to learn an abundance of new skills and techniques. In addition, the help given to me by Dr. Brid Quilty, Dr. Éva A. Enyedy Prof. Dos Santos, Dr. Brendan Twamley, Dr. Helge Muller-Bunz and Ann Conolly will not be forgotten. Finally, none of this would have been possible without the funding awarded to us by the Irish Research Council.

Huge thanks also to my examiners, I appreciate the effort of taking time out of their hectic work schedules to read this thesis and travelling to Ireland to examine me on it.

Embarking on this new journey, I was very lucky to have joined a hardworking, supportive, and friendly group of researches in RCSI. In particular, The Griffith Group, consisting of Aoife, Eolann and Aisling, were fantastic company inside and outside the lab. Thank you all for the chats at the fume hoods and the sinks, along with the numerous cups of tea and the very rare alcoholic beverage. I wish each of you the best of luck in your future careers and thank you for your friendship, advice and knowledge over the past three years. A special mention also goes out to the Chemistry department in RCSI and the members of the Microbiology department in Beaumont. You have all had a helping hand in the completion of this thesis and I
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Rarely did a week go by in the lab where something did not break or a problem needed resolving. However, the lab is very lucky to have a fantastic Lab Manager (Emmet) and Lab Technician (Graeme) on hand to keep the lab running smoothly. I hope that you find someone to give you a hand with the liquid nitrogen and the gas cylinders now.

One vital group of people who I would like to thank for helping me keep my sanity is the tea group; Emmet, Suzzie, Graeme, Eanna, Alan, Harrison, Aoife, Eolann, Aisling, Hugo, Scott, Ruairí, Shona and Siobhan. Countless cups of tea (some often quite dodge in appearance, Hugo) helped to break up the workload during the day and keep me sane. If it were not for you all, I probably would have finished a lot sooner. Thank you all for the laughs, the slaggings and the numerous conversations about the films I have not seen.

You can often get very frustrated during the course of a PhD. The best way to get over this is to remove yourself from the situation for a while, unwind and reset the mind. I want to thank Rob for being a welcome distraction, for bringing me for pints in the Lough, coffee in Checkovs and snooker/pool matches in the Emerald. To the CSGB lads, the many pointless conversations and banter provided a welcomed distraction from the day-to-day lab work. I’m glad I never listened to your advice Mitch, “If at first you don’t succeed, quit. Then pints.”

All throughout my PhD and my life, football has played a significant role. My teammates and management in Meath and Rathkenny, past and present, provided a welcome relief from the lab environment. Playing football allowed me to switch off from my PhD and view it from a different perspective. Chatting with the lads during and after training and matches was refreshing and Thank you all for being great friends. Thankfully, the management were accommodating and understanding during my studies, which eased some of the pressure. I am also grateful to the Gaelic Players Association who were a very valuable service and resource for me in the final few months of the PhD.
Luckily, I have a great network of family and friends. I would not have gotten through this PhD without the help of Mum, Dad, Sinead, Niamh, Denis and Bruce. Those long dinners, spending hours chatting around the kitchen table were better therapy that anyone could pay for. Thank you for not really understanding what it is I was doing for the past three years but still asking me how work was going. Even though I treat you all like mushrooms, I did appreciate your support throughout the journey. Thank you and FTB.

So, this brings me to my final word of appreciation. To Ms. Aoife Ryan, Thank you for putting up with me, my mood swings, my meltdowns and everything in between for the past three years. I am indebted to you for all the support and encouragement you gave me. When all I wanted to do was quit and pack it in, you persuaded me to keep going. You were always a beam of reassurance and you never doubted me, even when I doubted myself. Apologies for the numerous stress induced migraines, which I have been undoubtedly responsible for. You will never truly know how much you helped me through this PhD but I am forever appreciative of your support.

Thank you all,

Donal.
ABSTRACT

Since the initial development and clinical use of antibiotics, there has been a steady and alarming increase in the levels of antibiotic resistant bacterial infections arising worldwide. The reduced efficacy of antibiotics coupled to the lack of new classes of antibiotics being developed, may result in antibiotic resistance infections rising to epidemic levels.

The decrease in new antibiotic discovery has resulted in increased interest in repurposing and repositioning of previously developed drugs as effective antibacterial treatments. The use of metals in medicine is not a new phenomenon as they have been used in medicine for thousands of years. Nonetheless the scientific community has not realised the full medicinal potential of metal complexes. Novel complexes will offer new treatment to current diseases and provide alternatives to purely organic treatments.

Bismuth had been used in the 18th Century for the treatment of many diseases and its anti-bacterial properties were well documented. It has also been shown to have antifungal, anti-leishmanial and anti-bacterial properties and is currently now used as part of the new treatment for H. pylori infections of the stomach. Gallium compounds are currently being used clinically as diagnostic and therapeutic agents. Gallium’s similarities to iron allows for its incorporation into certain iron binding proteins, disrupting the natural function of the proteins and leading to adverse downstream effects. Antimony containing drugs have become the first line drugs used in the clinic for the treatment of leishmaniasis.

Hydroxamic acids are a family of important bio-ligands of general formula RCONR’OH. They are known for their ability to chelate to metals and metalloenzymes, often inhibiting many important biological processes involving urease, lipoxygenases and peptide deformylase enzymes. They have also been exploited as siderophores, due to their ability to efficiently bind iron. For these reason they have shown activity against a range of conditions including cancer, malaria, tuberculosis, cardiovascular disease, HIV, Alzheimer disease and fungal infections.
Chapter 1 provides an introduction into the role some of the previously mentioned metals play in medicine currently and in the past. The ever increasing problem of bacterial resistance will be discussed, with some of the important mechanisms of resistance and acquiring of resistance explained. An introduction to some important bacteria and the diseases they cause will also be described and hydroxamic acids as an important class of bioligands will be discussed.

*H. pylori* is a microaerophilic bacteria which causes dyspepsia, peptic ulcer and is one of the main causative agents for the development of gastric cancer. Due to bacterial resistance to many antibiotics, current treatment is failing and consequently, the use of bismuth containing quadruple therapies are increasing as a first-line treatment recommendation in many countries. Chapter 3 focuses on development of novel bismuth hydroxamate complexes as structurally well-defined potential treatments for *H. pylori*.

Chapter 4 looks to further develop a novel class of bismuth hydroxamate complexes which have activity against a broader range of bacteria, beyond *H. pylori*.

Chapter 5 describes the synthesis of novel bismuth and gallium retro hydroxamate complexes which may be used as anti-bacterial agents with activity against both Gram-positive and Gram-negative bacteria. These compounds are also developed to investigate if they have activity against current antibiotic resistant bacteria.

Finally chapter 6 details the development of novel antimony hydroxamate compounds and an investigation of their potential to act as anti-leishmanial agents.
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<tr>
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<td>Degree Celsius</td>
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<tr>
<td>α</td>
<td>Alpha</td>
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<td>Å</td>
<td>Angström</td>
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<tr>
<td>a.m.u.</td>
<td>Atomic mass unit</td>
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<td>Abs</td>
<td>Absorbance</td>
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<td>Aha</td>
<td>Acetohydroxamic acid</td>
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<td>AMP</td>
<td>Antimicrobial Peptides</td>
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<td>APT</td>
<td>Antimony potassium tartrate</td>
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<td>AR</td>
<td>Antibiotic resistance</td>
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<td>ARB</td>
<td>Antibiotic resistant bacteria</td>
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<td>β</td>
<td>Beta</td>
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<tr>
<td>b</td>
<td>Broad</td>
</tr>
<tr>
<td>Bha</td>
<td>Benzohydroxamic acid</td>
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<td>BP</td>
<td>Base pairs</td>
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<td>BSS</td>
<td>Bismuth Subsalicylate</td>
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<td>CagA</td>
<td>Cytotoxin-associated gene A</td>
</tr>
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<td>CBS</td>
<td>Colloidal Bismuth Citrate</td>
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<td>CL</td>
<td>Cutaneous leishmaniasis</td>
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<td>CLO</td>
<td><em>Campylobacter</em>-like Organism</td>
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<td>CLSI</td>
<td>Clinical &amp; Laboratory Standards Institute</td>
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<td>d</td>
<td>Doublet</td>
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<tr>
<td>DCM</td>
<td>Dichloromethane</td>
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<td>DMF</td>
<td>Dimethylformamide</td>
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<td>Dimethyl Sulfoxide</td>
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<td>Deoxyribonucleic acid</td>
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<td>EA</td>
<td>Elemental analysis</td>
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<td>Extended spectrum β-lactamases</td>
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<td>Electron Spray Ionisation</td>
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<td>Fetal Bovine Serum</td>
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<td>FT-IR</td>
<td>Fourier-transform infrared spectroscopy</td>
</tr>
<tr>
<td>GC</td>
<td>Gastric Cancer</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
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<td>------------------------------------------------</td>
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<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>GR</td>
<td>Glutathione Reductase</td>
</tr>
<tr>
<td>h</td>
<td>Hour (s)</td>
</tr>
<tr>
<td>HA</td>
<td>Hospital-Acquired</td>
</tr>
<tr>
<td>HDAC</td>
<td>Histone deacetylase</td>
</tr>
<tr>
<td>HGT</td>
<td>Horizontal Gene Transfer</td>
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<tr>
<td>Hpn</td>
<td>Nickel Binding protein</td>
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<tr>
<td>Hsp</td>
<td>Heat Shock Protein</td>
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<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>IR</td>
<td>Infra-red</td>
</tr>
<tr>
<td>J</td>
<td>Coupling constant</td>
</tr>
<tr>
<td>K</td>
<td>kelvin</td>
</tr>
<tr>
<td>m</td>
<td>Multiplet</td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>Matrix-assisted laser desorption/ionization - time of flight</td>
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<tr>
<td>MBC</td>
<td>Minimum Bactericidal Activity</td>
</tr>
<tr>
<td>MDR</td>
<td>Multi-Drug Resistant</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
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<tr>
<td>MGE</td>
<td>Mobile Genetic Element</td>
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<tr>
<td>MIC</td>
<td>Minimum Inhibitory Concentration</td>
</tr>
<tr>
<td>min</td>
<td>minutes</td>
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<td>mL</td>
<td>Millilitre</td>
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<tr>
<td>ML</td>
<td>Mucocutaneous leishmaniasis</td>
</tr>
<tr>
<td>MOA</td>
<td>Mechanism Of Action</td>
</tr>
<tr>
<td>MRSA</td>
<td>Methicillin resistant S. aureus</td>
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<tr>
<td>MS</td>
<td>Mass spectrometer</td>
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<td>MTT</td>
<td>3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide</td>
</tr>
<tr>
<td>NCI</td>
<td>National Cancer Institute</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
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<tr>
<td>NTD</td>
<td>Neglected Tropical Disease</td>
</tr>
<tr>
<td>PBP</td>
<td>Penicillin binding protein</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered solution</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>pKa</td>
<td>Proton dissociation constant</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<td>---------</td>
<td>------------------------------------</td>
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<tr>
<td>PPI</td>
<td>Proton Pump Inhibitor</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>rt</td>
<td>Room temperature</td>
</tr>
<tr>
<td>s</td>
<td>Singlet</td>
</tr>
<tr>
<td>Sha</td>
<td>Salicylhydroxamic acid</td>
</tr>
<tr>
<td>t</td>
<td>Triplet</td>
</tr>
<tr>
<td>TCM</td>
<td>Traditional Chinese Medicine</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
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<td>TLC</td>
<td>Thin Layer Chromatography</td>
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<tr>
<td>TNF</td>
<td>Tumour Necrosis Factor</td>
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<tr>
<td>TR</td>
<td>Trypanothione Reductase</td>
</tr>
<tr>
<td>tRNA</td>
<td>Transfer-RNA</td>
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<tr>
<td>μg</td>
<td>Microgram</td>
</tr>
<tr>
<td>μL</td>
<td>Microliter</td>
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<tr>
<td>UV</td>
<td>Ultraviolet</td>
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<tr>
<td>VacA</td>
<td>Vacuolating cytotoxin A</td>
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<td>VL</td>
<td>Visceral leishmaniasis</td>
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<td>WHO</td>
<td>World Health Organisation</td>
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<td>γ</td>
<td>Gamma</td>
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Chapter 1

Introduction
1. Drug Resistance

1.1 History of antibiotics

Pre antibiotic era
Antimicrobial agents are one of the most successful chemotherapy stories in the history of modern medicine. It is impossible to put a figure on the number of lives saved as a result of the introduction of antibiotics. In addition to this, they have significantly contributed to a better quality of life, almost the world around. They have assisted greatly in the controlling of infectious diseases, a leading cause of morbidity and mortality for most of human existence. Research has revealed that the use of antibiotics dates back further than what is now perceived as the modern “antibiotic era”. Traces of tetracycline have been detected in the human skeletal remains of an ancient Sudanese Nubia population, originating in 350–550 AD and likewise from the femoral mid-shaft of remains in Egypt which originated from the Roman period.1,2 Tetracycline compounds are strong metal chelators, and as a result, incorporated well into the mineral portion of bones and tooth enamel, providing permanent markers of tetracycline exposure.

Exposure to tetracycline-containing materials most likely occurred through the diet of this civilisation. This could be responsible for the unusually low infectious disease rates documented in the Sudanese Nubian and Egyptian population.3 Exposure to other antibiotics in ancient populations are much more difficult to detect, relying heavily on anecdotal evidence. For instance, the perceived anti-bacterial properties of red soil in Jordan, subsequently led to the discovery of Actinomycin producing bacteria in these soils.4 Exposure to antimicrobial compounds could also have occurred through traditional/alternative medicine, such as traditional Chinese medicine (TCM), e.g. use of the Artemisia plant to treat many illnesses, which was later discovered to possess the potent anti–malarial drug Artemisinin, Figure 1.1.5

Interestingly, however, the use of antimicrobials in TCM and small-scale exposure may have been a contributing factor for the accumulation of antibiotic resistance genes in human populations, due to selective pressures imposed on these antimicrobials over time. Studies of β-lactamases established that these enzymes originated over two billion years ago, with some β-lactamases evident on plasmids
for millions of years. These findings imply that the evolutionary period of resistance genes has not been a relatively recently phenomenon, but has in fact been in existence for over 100 million years.

Figure 1.1. The structure of Artemisinin, a component of the Artemisia plant.

Antibiotic era

The modern “antibiotic era” is usually associated with the names of Paul Ehrlich and Alexander Fleming. Ehrlich first proposed the idea of the “magic bullet”, an agent that selectively targeted disease-causing microbes only and caused no harm to the host. He based this theory on the fact that some dyes stained only bacterial cells and no other cell types. In turn, Ehrlich hypothesised that certain chemical compounds would “exert their full action exclusively on the parasite living within an organism.”

With this theory in mind, he began a large-scale screening program in 1904, in the hope of discovering a drug to treat syphilis, a disease that was endemic and almost incurable at that time. Treponema palladium, the causative agent of this sexually transmitted disease, was inefficiently treated with inorganic mercury salts, resulting in severe side effects. In his screening, he synthesized hundreds of derivatives of a highly toxic arsenic drug Atoxyl and tested them against syphilis-infected rabbits. Over 5 years later, in 1909, he discovered Salvarsan, a compound which cured syphilis-infected rabbits and showed promise for the treatment in humans patients. Neosalvarsan, became available in 1912 and superseded the more toxic and less water-soluble Salvarsan as an effective treatment of syphilis. Both these drugs were hugely successful and were the most frequently prescribed drugs until their replacement by penicillin in 1940. Amazingly, their modes of action are still unknown, even 100 years after their discovery and the chemical structure was solved only recently, Figure 1.2. Paul Ehrlich’s systematic screening approach became fundamental to drug discovery strategies in pharmaceutical industries and many drugs were identified using this method.
Figure 1.2. Salvarsan, thought only to be present in its dimeric form (A), but recent elucidation of its structure revealed it exists also as a trimer (B) and pentamer (C). Neosalvarsan (D) is a safer and more water-soluble replacement.

Penicillin, one of the most famous medicines and treatments for bacterial infections was serendipitously discovered on September 3, 1928, by Alexander Fleming, Figure 1.3. The anti-bacterial properties of mould had been known from ancient times. However, it was Fleming’s formidable persistence, which made the difference. For 12 years after the initial observation, Fleming persevered to try overcome purification and stability obstacles but abandoned his efforts in 1940. In the same year, Howard Florey and Ernest Chain in Oxford University, published a paper describing the purification of penicillin in quantities sufficient for clinical testing, leading to penicillin mass production and distribution. Fleming’s method of anti-bacterial testing, which monitored growth inhibition zones of pathogenic bacteria on agar plates, became widely used in screening potential antibiotic-producing microorganisms.
The discovery of Salvarsan and penicillin set the precedent for future drug discovery. The golden era of antibiotic discovery was between the 1950s and 1970s and, significantly, no new classes of antibiotics have been discovered since. As a result, attention has now switched to the modification and repurposing of existing antibiotics as the best route to developing new drugs.\textsuperscript{13}

\section*{1.2 Antibiotic Resistance}

Antibiotic resistance (AR) is a serious worldwide problem that occurs as a result of the ineffective elimination of bacteria in a population leading to the domination of the non-sensitive bacteria. AR had been observed by Abraham \textit{et al.} in 1940, even before the widespread use of penicillin. It was noted that there was evidence of enzymatic degradation of this antimicrobial agent.\textsuperscript{14} The outlook was, however, more or less optimistic. An early study into resistance emergence concluded that, as syphilis had been treated for about 40 years without any indications of arsenic-resistance, it was highly probable that this would also be the case with penicillin.\textsuperscript{15} Fleming also warned of the possibilities of penicillin resistance during his Nobel Prize acceptance speech, in 1945. World health leaders now describe the treatment of antibiotic resistant bacteria (ARB) as a nightmare scenario, posing a catastrophic threat to people in every country in the world.\textsuperscript{16}

The mortality rates due to multidrug-resistant bacterial infections from hospital-acquired bacterial are estimated to be about 25,000 deaths in the EU and more than 63,000 deaths in the United States per year.\textsuperscript{8} The estimated economic burden of healthcare costs and productivity loss is in the region of billions of dollars each year.\textsuperscript{17} The treatment of hospital-acquired (HA) infections from just six species of ARB was calculated to cost €1.6 billion in the EU, which was more than the annual spending on influenza.\textsuperscript{18} Antibiotic resistance is rising to dangerously high levels in all parts of the world. A World Economic Forum Global Risks report listed AR as

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{penicillin_core.png}
\caption{Penicillin core with a variable R group.}
\end{figure}
one of the greatest threats to human health. A review on AR reported that if the current trend of AR continues in the future, by 2050, ARB would be responsible for 10 million deaths every year and a reduction of 2-4% in Gross Domestic Product (GDP), costing the world up to 100 trillion USD.

**How Bacterial Resistance Occurs**

A mutation confers a spontaneous change in the DNA sequence of a gene, resulting in a change in its activity or function. These mutations arise in many forms. In prokaryotic genomes, the mutation rate is 0.0033 per DNA replication, and may be introduced in the form of exogenous agents, DNA polymerase errors, deletions, insertions, and duplications. These mutations cause a change in the amino acid sequence. This small change may result in a large alteration in the enzyme or cell structure, consequently effecting the affinity of the target for its antimicrobial agent.

More recently bacterial pathogens with multiple-drug resistance traits are being reported worldwide. This phenomenon is resulting in human and animal pathogens such as *Mycobacterium* and *Salmonella* becoming more difficult/impossible to treat, resulting in large fatalities across the world.

In order to understand AR, we must first understand that bacteria have been genetically evolving since their existence, as a survival mechanism for overcoming naturally occurring antibiotics. Thus, these organisms are often considered to be “intrinsically” resistant to one or more antimicrobials. However, when discussing AR, often we are not talking about this intrinsic resistance and we are more focused on the expression of acquired resistance mechanisms of bacteria. This deals with how resistance develops in once susceptible bacteria. Resistance generally occurs by a mutation at the level of the target of the drug, or a mutation which affects the drug itself.

**Genetic Basis of Bacterial Resistance**

Bacteria, having been in existence for thousands of years, have demonstrated amazing genetic plasticity and are capable of responding rapidly and efficiently to a variety of environmental stress. Two strategies commonly utilised by bacteria in evading and surviving in the presence of antibiotics are gene mutations and acquisition of resistant DNA by horizontal gene transfer (HGT).
Gene Mutation

In many cases, a susceptible bacterial population may develop AR through a genetic mutation which results in increased cell survival in the presence of the antimicrobial agent. The mutation provides enhanced selectivity and survival in these bacteria, while the antibiotic eliminates the rest of the susceptible population. Once this advantageous mutation is established, these resistant bacteria can replicate and become the dominant population, Figure 1.4. Resistance due to mutational changes are diverse and vary in complexity. Table 1.1, outlines and explains some of the possible mutations which may arise in bacterial population.

Figure 1.4. (A) A bacterial population with a cell harbouring an advantageous resistant mutation. (B) An antibiotic kills the susceptible bacteria but is ineffective in killing the resistant strain. (C) These resistant bacteria replicate and become the primary strain of bacteria. (D) The resistant genes can be passed from one bacterial strain to another and resistance can spread.

Table 1.1. Mutational changes which may occur in bacteria.22

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Point mutations</td>
<td>Change of a single base pair resulting in ‘single nucleotide polymorphism’ in populations.</td>
</tr>
<tr>
<td>Insertions/deletions</td>
<td>Single or a number of base pairs introduced or removed</td>
</tr>
<tr>
<td>Transpositions</td>
<td>The movement of a sequence of DNA</td>
</tr>
<tr>
<td>Inversions</td>
<td>Changing of the orientation of a stretch of DNA</td>
</tr>
<tr>
<td>Chromosome mutation</td>
<td>Results in irregular pieces of visible DNA</td>
</tr>
<tr>
<td>Changes of chromosomes number</td>
<td>Whole copies of DNA are gained or lost</td>
</tr>
</tbody>
</table>
Horizontal Gene Transfer (HGT)

HGT is an important and frequent driver of bacterial evolution and resistance. Classically, acquisition of external genetic material can occur through three main strategies; transformation, transduction and conjugation, Figure 1.5. Transformation is the simplest form of HGT, in which naked DNA is incorporated into the chromosome. However, very few bacterial species are capable of transformation as they lack the cellular machinery to do so.

Conjugation involves transfer of genetic information in areas where the numbers of bacteria are high and direct contact is attainable. This is the most common method used in the gastrointestinal tract of humans and in hospitals. As a general rule, conjugation uses mobile genetic elements (MGEs) as transport vehicles for this genetic information, such as plasmids and transposons, both of which play a crucial role in the development and dissemination of antimicrobial resistance.

Finally, one of the most efficient mechanisms for accumulating antimicrobial resistance genes is represented by integrons, through transduction. Integrons are site-specific recombination pieces of genetic information capable of recruiting open reading frames in the form of mobile gene cassettes. They are an efficient and rather
simple mechanism for the incorporation of new genes and possess the necessary machinery to ensure their expression into bacterial chromosomes. This method is one of the main drivers of bacterial evolution.26

Be it from gene mutation or HGT, the result is the development of mutations that can lead to antibiotic resistance, through modification of the target, decreased drug uptake, efflux mechanisms or development of a new pathway.

**Bacterial Resistance Strategies**

*Target site modifications*

1. **Target Protection**

A target is anywhere an antibiotic exerts its activity. As a general rule, proteins and molecules involved in bacterial structure and assembly, which are absent or differ sufficiently in the host, provide the best targets for anti-bacterial agents.27 The main targets include inhibition of proteins and enzymes involved in cell wall synthesis, transcription, chromosome segregation and integrity and folic acid metabolism, **Figure 1.6**. Mutational changes may reduce the effectiveness of the anti-bacterial agent by altering the structure of the target, **Figure 1.7**.

---

Figure 1.6. Targets for anti-bacterial agents including cell wall synthesis, protein synthesis, folic acid metabolism and DNA replication.
Targets may also be altered enzymatically. For example, resistance has arisen in Gram-positive and Gram-negative bacteria, due to methylation or dimethylation of key adenine bases at the 50S subunit target site by peptidyl transferase.\textsuperscript{28}

Figure 1.7. A susceptible target (A) is inhibited by an antibiotic. Mutation of the target site (B) results in a functional target, with reduced affinity for the antibiotic resulting in negligible inhibitory effect. Enzymatic modification/alteration of the target (C), ineffective antibiotic activity ensues.\textsuperscript{29}

2. Target Replacement or Bypass
Bacteria can acquire new proteins that can carry out the same biochemical functions of an original antibiotic target but is not inhibited by this antibiotic. For example, methicillin resistant \textit{S. aureus} (MRSA) have acquired an exogenous penicillin binding protein (PBP), namely PBP2a. β-lactam drugs disrupt cell wall synthesis through inhibition of PBPs, preventing transpeptidation and transglycosylation of peptidoglycan. PBP2a is encoded for by a gene called \textit{mecA} which can be incorporated into a bacterial genome. PBP2a has a low affinity for all β-lactam antibiotics. The use of PBP2a results in normal cell wall synthesis in the presence of these antibiotics, allowing for the survival of the bacteria and giving rise to MRSA. Limitations arise with PBP2a, however, and it requires additional enzymes to complete the synthesis of fully cross linked peptidoglycan. To date, 11 different \textit{mec} allotypes have been discovered with varying degrees of genetic homology.\textsuperscript{30} Importantly, these differing homologies give rise to different MRSA strains, from
community-associated MRSA to hospital-associated (HA) MRSA, and each respond differently to treatment regimens.31

Another route to avoid inhibition by antibiotics is to “bypass” or overproduce the biological target. Resistance to trimethoprim-sulfamethoxazole (TMP-SMX) arises as a result of this method.32 TMP-SMX impairs bacterial synthesis of purines by altering the production of folate. Most bacteria are unable to utilise folate from external sources and rely solely on their own biochemical machinery for folate synthesis. The synthetic biochemical process of folate synthesis involves two major enzymes; i) dihydropteroic acid synthase (DHPS), inhibited by SMX, and ii) dihydrofolate reductase (DHFR), inhibited by TMP. TMP-SMX resistance develops by overproduction of DHFR or DHPS, through mutations in the promoter region of the DNA encoding these enzymes. The antibiotic cannot accumulate at high enough concentrations to inhibit the enzymes and folate is synthesised, Figure 1.8.32-34

Figure 1.8. (A) A susceptible host inhibited by an antibiotic. (B) The antibiotic inhibits the intended target, but a new target, not inhibited by the antibiotic, carries out the same functions. (C) Overproduction of the target renders the antibiotic useless.

3. Target Site Exclusion
Preventing the anti-bacterial agent from reaching its target, mainly through decreased antibiotic cell uptake or penetration, is an effective resistance technique.
Almost all antimicrobial agents require entry into the bacterial cell to exert their activity, as the majority of targets are located within the cytoplasm. The usual route of entry for these agents is through porin channels. Bacterial protection can occur, especially in Gram-negative bacteria, via inhibition of entry into the cell by modifying the cell membrane porin channel frequency, size, and selectivity. This strategy has been observed in *Pseudomonas aeruginosa* against Imipenem, a β-lactam antibiotic.\(^{35}\)

**Antibiotic modification**

1. Loss of activity by chemical alteration of drug

Bacteria can produce enzymes capable of chemically altering the antibiotic molecule, resulting in AR in Gram-negative and Gram-positive bacteria.\(^ {36}\) The modifications in question include acetylation (catalysed by acetyltransferase), phosphorylation (catalysed by phosphotransferase) and adenylation (catalysed by adenyltransferase) and result in decreased drug-target binding due to steric hindrance at the active site. The group of aminoglycoside modifying enzymes are responsible for the development of aminoglycoside resistance worldwide and have been detected in many different strains of bacteria, Figure 1.9.\(^ {37, 38}\)

![Figure 1.9](image-url)

**Figure 1.9.** (A) Susceptible host efficiently inhibited by an antibiotic. (B) Modified antibiotic by acquired modifying enzymes resulting in loss of activity.\(^ {29}\)
2. Elimination of Drug

β-lactam resistance has occurred mainly due to the destruction of the compounds by β-lactamases, which destroy the amide bond of the β-lactam ring. Elimination of the drug is a common form of resistance. Figure 1.10. This alteration renders the antimicrobial agent ineffective. As previously mentioned, warnings were issued by individuals who worked with penicillin and observed some evidence of resistance in the early 1940s, one year before penicillin was introduced to the market. It is now known, that β-lactamases have been in existence for millions of years. Penicillin-resistant *S. aureus* infections became clinically relevant soon after penicillin introduction. β-lactamases are encoded by a readily transmitted plasmids, resulting in rapid dissemination of resistance.

![Figure 1.10](image)

**Figure 1.10** (A) Susceptible target inhibited by an antibiotic. (B) Acquisition of an enzyme capable of destroying the antibiotic, resulting in loss of activity.

To overcome this problem, new generations of β-lactam compounds were developed which encompassed a wider spectrum of activity but it was not long before a new plasmid-encoded β-lactamase was capable of hydrolysing this new generation. This trend of producing antibiotics, followed by the rapid appearance of resistance became the normal sequence of events, Table 1.2. Extended Spectrum β-lactamase (ESBL) enzymes hydrolyse a large number of drugs, while carbapenemases knock out the activity of carbapenems, the most potent β-lactams available. To date, over
1,000 different β-lactamases have been described with more likely to be reported in the future, as part of the normal process of bacterial evolution.\(^8\)

<table>
<thead>
<tr>
<th>Year</th>
<th>Introduction</th>
<th>Significant Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001-present</td>
<td>Fluoroquinolones, tigecycline</td>
<td>Vancomycin resistance, ESBL spread among Gram-negative.</td>
</tr>
<tr>
<td>1981-1990</td>
<td>Amoxicillin</td>
<td>Methicillin resistant <em>S. aureus</em> spreads and emergence of AIDS.</td>
</tr>
<tr>
<td>1979-1980</td>
<td>Carbenicillin, Cefoxitin, quinolones</td>
<td>Ampicillin resistant infections become frequent.</td>
</tr>
<tr>
<td>1961-1970</td>
<td>Gentamicin, Ampicillin, Cefalothin</td>
<td>Emergence of gentamicin resistance <em>Pseudomonas</em> as well as methicillin resistant <em>S. aureus</em> infections.</td>
</tr>
<tr>
<td>1951-1960</td>
<td>Erythromycin, Vancomycin, Meticillin</td>
<td>Penicillin resistant infections become clinically significant.</td>
</tr>
<tr>
<td>1941-1950</td>
<td>Streptomycin, Chloramphenicol</td>
<td>Penicillin made available to the public, and widespread use.</td>
</tr>
<tr>
<td>1930-1940</td>
<td>Sulfonamides</td>
<td>Penicillin efficacy shown.</td>
</tr>
<tr>
<td>Pre 1930</td>
<td>Discovery of Penicillin</td>
<td></td>
</tr>
</tbody>
</table>

3. **Removal of drug from the cell.**

Antimicrobial agents are required at the target in sufficient concentration to exert an activity. However, some bacteria possess membrane bound proteins which quickly and efficiently export antibiotics out of the cell, **Figure 1.11.** This results in low intracellular concentrations at the target incapable of inhibiting activity. Efflux pumps are generally specific for an antibiotic, but a class of efflux pumps, called multiple drug resistance pumps, which expel a variety of structurally diverse antibacterial agents have been identified in *E. coli* and other Enterobacteriaceae. As a result, Enterobacteriaceae have developed resistance against a wide variety of antibiotics.\(^41\)
Figure 1.11. An overview of resistance caused by poor intracellular concentration. Antibiotic A enters the cell via a membrane-spanning porin protein, reaches and inhibits its target. Antibiotic B also enters the cell via a porin, but is efficiently removed by efflux pump. Antibiotic C cannot cross the outer membrane and so is unable to access the target.

1.3 Prevention and control of antibiotic resistance

As previously mentioned, bacteria have incredible genetic plasticity properties. Resistance to treatments often develops quickly, as was observed in the case for Penicillin. So how can we protect and prolong the power of antibiotics?

Many different factors contribute to the emergence and dissemination of antibiotic resistance. The approach, therefore, is not straight forward but rather complex. New resistance mechanisms are constantly emerging and spreading globally, threatening our ability to treat common infectious diseases. A growing list of common infections are becoming harder, and sometimes impossible to treat.

Individuals

As the saying goes, ‘Prevention is better than the cure’ and this is very true in relation to bacterial infections. By preventing infection, no consumption of antibiotics are required, reducing the possibility of AR developing. Immunization, safe food preparation and good sanitization and living conditions all contribute to the prevention of bacterial infection.
Antibiotics are among the most commonly prescribed drugs in human medicine, with some antibiotics being obtained without a valid prescription. Antibiotics should only be used when prescribed by a certified health professional. Patients are demanding antibiotics in the case of viral infections and other non-bacterial related ailments. The limitations of antibiotics needs to be better explained to patients and it is this lack of knowledge which ultimately correlates with a higher prevalence of resistance. Improper diagnosis, unsuitable antibiotic choice, incorrect usage, non-compliance, poor treatment monitoring and self-medication further compounds the problem and can result in rapid dissemination of resistance.

Drug regimen compliance is an important factor in the development of bacterial resistance. This is hugely problematic in the case of infections requiring long-term therapy with multiple antibiotics, as in the case with TB. Even if the symptoms are noticed to have eased, this is not confirmation that the bacteria are completely eradicated and patients are required to complete the full course of antibiotics.

**Health professionals**

To prevent and control the spread of antibiotic resistance, health professionals should ensure hands, instruments, and environment are clean. As stated previously they should also only prescribe and dispense antibiotics when they are absolutely required, in accordance with current guidelines. The practice of empirically prescribing antibiotics, which accounts for the majority of prescriptions, has a major role to play in AR development. It is the duty of health professionals to report any case of AR to the surveillance teams. It is also important that the healthcare professionals educate the patient about the importance of adhering to antibiotic regimes and the dangers of misuse. The commitment to always use antibiotics appropriately and safely, only when required and to choose the right antibiotics and to administer, is known as antibiotic stewardship.

**Research**

There is a constant necessity for research and development of new antibiotics, vaccines, diagnostics and other tools to battle the problem of AR. Recently there were 1243 anti-bacterial resistance research projects being under-taken, with total investment of €1.3 billion across 19 countries and at EU level. New antibiotics will always be required, to keep up with resistant bacteria. Improved diagnostic tests to
track the development of resistance would be an effective monitoring technique also. However, no matter how successful research is in this area, the reality is that bacteria often rapidly develop resistance.

**Agricultural sector**

Antimicrobials are now ubiquitous in the environment. They enter the environment by a number of routes; by veterinarians treating diseases in animals, in cleaning products and other consumer products, in aquaculture, and in animal agriculture.\(^{47}\) The largest use of antimicrobial agents outside human medicine is in livestock, with an estimation that millions of pounds of antimicrobials are used each year in animal agriculture.\(^{48}\) The use of antibiotics in agricultural and horticulture as growth promoters and for prophylactic purposes in livestock, has been a major source of resistance. The use of antimicrobials in animal production can result in the spread of resistant organisms to humans.\(^{49}\) Even with this knowledge, little is being done in an attempt to change these practices. Vaccination could be used as an alternative to antibiotics but is not always a viable option.

In 2006, proper measures were implemented in Scandinavian countries, and the use of antibiotics in agriculture was closely monitored.\(^{50}\) A ban on the use of antibiotics for growth promotion purposes was established in these countries and has led to other EU countries implementing similar measures to limit the occurrence and spread of antibiotic resistance from agricultural sources.

**Tracking**

The Centre for Disease Control and Prevention (CDC) has gathered data to try develop specific strategies to prevent infections and in particular resistant bacteria spreading. They investigate such areas as which bacteria cause the most antibiotic-resistant infections, the exact causes of infections and whether there are particular risk factors that caused some people to develop resistant infections.\(^{51}\)

A rapid test which could identify the most efficient therapy to be administered while overcoming possible hurdles with antibiotic resistance would be a very helpful tool, but one which as of yet has not been developed.
1.4 New targets for antimicrobials

Efflux Pump Inhibitors

One approach to overcome the problem of AR is to eliminate the source of the resistance, for example the MDR efflux pumps. These pumps are primarily responsible for Gram-negative associated resistance.52,53 An effective inhibitor of an MDR was discovered, which, when administered to a strain of fluoroquinolones resistant Pseudomonas aeruginosa, could restore susceptibility to the antibiotic both in vitro and in vivo infection. However the essential poly-cationic property of the drug meant it was nephrotoxic and therefore unable to be used on large scale. Further research is required in this area.

The in situ Screening Platform: Back to Domagk

It is possible that current screening techniques are actually ruling out some very good drug candidates, including pro-drugs. For example, one of the very first antimicrobial drugs, the sulphanilamide drug Prontosil, discovered by Gerhard Domagk in the 1930s, was identified having screened only a few hundred (at most) compounds.54 Contrast that with screening of current libraries, consisting of over $10^7$ compounds which hasn’t produced a single hit. It is possible that every time “improvements” were made to the screening process valuable compounds were discarded? Modern in vitro screening would have missed Prontosil, as it acts as a pro-drug and is only activated in the gut. In theory using in vivo testing would be a very accurate way to screen drugs but this approach is not feasible. It would, of course, require a prohibitive number of test subjects and large amounts of test compounds compared to in vitro testing. A hybrid testing technique between in situ and in vitro testing has been developed and it involves the use of Caenorhabditis elegans, a small worm that can be readily infected with human diseases. Some hits were identified, which exhibited activity in the worm but didn’t show activity in in vitro testing. This could be utilised in the future with additional parameters to obtain more accurate results.55

Untapped Sources of Natural Compounds

Penicillin was the first reported naturally synthetic commercially available antibiotic. Naturally occurring antibiotics have been discovered in soils and flora in the past. These methods of discovering natural products did not continue due to the rise in the
golden antibiotic era where there were more lucrative means of antibiotic discovery. However, since this has stalled, there has been a concerted effort to re-examine different areas of nature for metabolites which could be used as anti-bacterial agents. Marine invertebrates, such as sponges and corals, have revealed a plethora of secondary metabolites. Corals themselves are made by bacteria colonising the organisms. Some have displayed antimicrobial properties however reducing toxicity of these agents is the primary challenge.\(^5^6\)

**Silent Operons**

Another untapped source of antimicrobials is the silent operons of microorganisms. The genome sequencing of *Streptomyces coelicolor* showed that it encodes 20 secondary metabolites but only produces three antimicrobial compounds.\(^5^7\) The challenge in this field is to manipulate the bacteria to produce these secondary metabolites and then investigate if they possess any useful activity.\(^5^8\) Secondary metabolite production is not a very well understood or investigated area, but bioengineering and manipulation of operons is an ever increasing discipline in science. It is estimated that 99% of all microbial species on the planet are ‘uncultured’; i.e. they do not grow under laboratory conditions.\(^5^9\), \(^6^0\) Several groups in academia, biotechnology and industry have realised the potential here and are carrying out different experiments to advance this area.\(^6^1\), \(^6^2\)

**Antimicrobial Peptides**

Antimicrobial peptides (AMPs) are oligopeptides that kill cells by a number of ways. They can disrupt membrane integrity, inhibit proteins, DNA and RNA synthesis, or interact with certain intracellular targets. AMPs have activity against parasites, viruses, bacteria and fungi. The discovery of AMPs dates back to 1939, and since then, more than 5,000 AMPs have been discovered or synthesized to date.\(^6^3\) AMPs can be found naturally. Frog skin, for example, is a source of over 300 different AMPs.\(^6^4\) Most AMPs are produced by specific cells at all times, while the production of some AMPs is inducible.

AMPs can be characterized into four types based on their secondary structures: \(\beta\)-sheet, \(\alpha\)-helix, extended, and loop. They have a rapid killing effect, with some AMPs causing death within seconds of contact with cell membrane.\(^6^5\) AMPs can be used synergistically with antibiotics.
Challenges such as host cytotoxicity, sensitivity to conditions (extreme pH) and lack of selectivity against specific strains are associated with AMPs. Also their cost of production, due to their complexity, can be very high.66

Certain factors must be considered when designing new AMPs;

(i) The length of an AMP is important. 7–8 amino acids are required to form amphipathic structures and 22 amino acids are required for an α-helix to transverse the lipid bilayer of bacteria.

(ii) The net charge of AMPs can vary from negative to positive. The charge is responsible for the main interactions of the AMP with the cell membrane. Altering the charge can provide an element of selectivity.

(iii) Hydrophobicity influences the activity and selectivity of AMP molecules. Increases in hydrophobicity affords increased antimicrobial activity.

(iv) Helicity represents the ability of an AMP to form helical structure. It has less of an effect on activity than other factors. D-amino acids reduce helicity.

AMPs are a diverse group of exciting and relatively new anti-bacterial agents, which allow for high levels of functionalization and modifications resulting in diverse activities. More precision in targeting pathogens and limiting the indiscriminate use of antibiotics is essential for their longevity. Other mal-practices accelerate the emergence of resistance among bacteria. This arms race is fragile and must be handled and approached with careful considerations.

**Perturbing metal homeostasis**

Metal ions are an essential component for life and are involved in many central biological processes including respiration, photosynthesis and nitrogen fixation. Bacteria metal homeostasis is also vital for survival. Disruption of this sensitive process by means of inhibiting metalloenzymes or replacing metals in functionally important moiety’s can have a devastating effect on the bacteria.67 Research has been conducted in identification of new targets to perturb metal homeostasis.
Recently the WHO have developed a list of twelve very common drug resistant bacteria which it is prioritising the development of new treatments for. These bacteria pose a catastrophic threat to mankind and urgent, effective treatments are required to be developed. I am interested in the development of novel treatments for many of the bacteria in this list including *H. pylori, E. coli* and *S. aureus*. I am also interested in tackling the neglected tropical parasitic disease Leishmaniasis which affects a huge number of people in the world. Outlined below is a brief introduction into each of these diseases.

## 2. *Helicobacter pylori*

First identified by Barry Marshall and Robin Warren in 1984, *Helicobacter pylori* are microaerophilic and neutrophilic bacteria. They are spiral bacillus shaped Gram-negative species and have adapted the ability to not only survive in the human stomach but also to colonise it. In 2005, the Nobel Prize in Physiology and Medicine was awarded to Marshall and Warren, for their discovery of this pathogen.\(^6^8\) Upon breach and colonisation of the lining of the stomach, *H. pylori* can cause the development of diseases such as idiopathic gastritis, inflammation of the lining of the stomach,\(^6^9\) peptic ulcers and dyspepsia, **Figure 1.12**. Significantly it is thought to be one of the strongest known risk factors for development of gastric cancer, and in 1994 the World Health Organisation (WHO) classified *H. pylori* as a Class 1 carcinogen.\(^7^0\)

**Figure 1.12** Some diseases associated with *H. pylori* infection.\(^7^1\)
Chapter 1

Infection rates vary across the world, with rates of 4.9% in Czech Republic, 13% in the USA and 78.1% in areas of Nepal. Gender is not thought to be a risk factor, however, when socioeconomic status and geographical factors are taken into account there is a clear disparity between infection rates in developing versus industrialised countries.\textsuperscript{72, 73} It is perceived that overcrowding and unsanitary living conditions contribute significantly to high infection rates of \textit{H. pylori}. Though 50\% of the world’s population are hosts to \textit{H. pylori}, 80\% of those are asymptomatic, and remain unaffected by the bacteria. The bacteria can be spread via oral-oral or oral-faecal interaction, hence, sanitary conditions have an important part to play in the control and spread of the disease.\textsuperscript{74}

2.1 Survival and Infection in the Stomach

The natural acidic environment of the human stomach makes it a very inhospitable environment for bacteria. It acts as a natural immunity barrier against most pathogens, preventing their access to the gut. Most of the protective nature of the stomach comes from the parietal cells, which secret large amounts of hydrochloric acid, maintaining the stomach at a low pH and killing most of the bacteria that enter.\textsuperscript{75} However, \textit{H. pylori} is a bacteria which has developed means to both survive and colonise the stomach.

Flagella

\textit{H. pylori} is a spiral bacterium and it is this shape along with its flagella which gives rise to high mobility within the stomach.\textsuperscript{76-78} Flagella are complex organs, composed of multiple protein subunits.\textsuperscript{79} Each flagellum consists of a basal body, hook, and filament.\textsuperscript{80, 81} Further observation also identified a sheath and a terminal bulb, however the exact role and composition of the sheath is unknown.\textsuperscript{82, 83} \textit{H. pylori} have 4–8 unipolar flagella which, depending on their environment, can produce different movement patterns; swimming motility (in liquid media), spreading motility, (in soft agar) and swarming motility (on solid media). Specific assembly and arrangement of the associated proteins is required for these flagella to function correctly. There are four receptors, Tlp A, B, C and D, which respond to chemotactic cues. Mutation studies show that Tlp D is an essential element for infection within the inflamed atrium.\textsuperscript{84} \textit{H. pylori} flagella have a role in colonization, inflammation, and immune evasion. Studies show that flagella deficient bacteria possess a weak spread-ability
and reduced colonisation of the stomach compared to that of the wild type bacteria.\(^8^5\).\(^8^6\)

\textit{H. pylori} adhere to the epithelial and gastric mucosal cells of the stomach, preventing them being swept away and allowing direct access to nutrients through the host cells, Figure 1.13. Although this is the most economical and efficient way of availing of nutrients, there have been suggestions that this may also have a detrimental effect on the bacteria allowing for an intimate interaction with the hosts defence.\(^8^6\)

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.13}
\caption{Process of adhesion of \textit{H. pylori} to epithelial cells. Acid in the stomach forms a gastric mucin gel on the epithelial cell face. Upon an increase of the pH via urease activity, this gel becomes solvated and the bacteria can swim freely through and attach to the epithelial cells, assisted by the flagella.}
\end{figure}

\textbf{Acid acclimation}

Once acquired, \textit{H. pylori} has the ability to survive for years, decades, or even for life within the stomach. Long term infection has be associated with development of adenocarcinoma of the stomach and gastric lymphomas.\(^8^7\) This survival is due to a process called acid acclimation, in which the enzyme urease plays an important role. \textit{H. pylori} produces an extremely high amount of neutral pH optimum urease, more than any other know bacteria.\(^8^8\) This increased production is vitally important for \textit{H. pylori} colonization of the human gastric mucosal cells. The bacteria also produce alpha carbonic anhydrase, another essential component in the acid acclimation process.\(^8^9\) Upon entering the stomach the pH drops from the conventional range of 6.8-7.2 to a lower pH range of 1-4.
**The mechanism of action of Acid Acclimation**

When *H. pylori* enter the acidic medium of the stomach, protons can permeate the cell wall, acidifying the intracellular environment. This acidification results in activation of the gated urea transporter, UreI. Urea is transported into the cytoplasmic domain of the cell where it is converted by urease to NH$_3$ and CO$_2$.$^{90}$

These gaseous products can readily pass through the membranes into the periplasm. Here they exert their activity by neutralising incoming protons through the formation of NH$_4^+$ from NH$_3$, or using a stronger buffer HCO$_3^-$, generated from CO$_2$ by alpha carbonic anhydrase activity, Figure 1.14.$^{89, 91}$ The ammonia forms a thin protective acid-neutralizing layer around the bacterium protecting the bacterium from destruction and allowing for survival.$^{92}$ Kinetic studies of the urease enzyme revealed a low Michaelis–Menten constant ($K_m$) (0.17-0.8 mM) meaning urea has a high affinity for *H. pylori* urease. It was also found that urease has a high specific activity ($V_{max}$) of 1100 to 1700 tmol of urea hydrolysed per minute per milligram of enzyme.$^{93, 94}$

![Diagram](image)

**Figure 1.14.** An overview of the Acid Acclimation process. *H. pylori* utilise this process in order to survive and colonise the stomach. Acidification of the environment results in an influx of urea through the UreI channels. This urea is converted to NH$_3$ and CO$_2$ by urease, which are effective buffering agents, neutralising the microenvironment and allowing for bacterial survival.$^{90}$
**Urease Structure**

*H. pylori* urease has been purified, with the enzyme comprising of two subunits; an α subunit and a β subunit. These exist as dimers, in a 1:1 molar ratio, and as four trimers of the dimers, \((αβ)_3\), **Figure 1.15**. The active site is located in the α subunit, giving a total of 12 active sites.\(^{95}\) 10-15\% of the total mass of protein in *H. pylori* is accounted for by urease.

![A crystal structure of the urease enzyme.\(^{96}\)](image)

Like other urease producing bacteria, it is encoded for by a gene cluster. The structural subunit of the enzyme are coded for by *ureA* and *ureB*.\(^{97, 98}\) Expression of all the genes in the genome is not necessarily required for urease functional activity.\(^{91, 99}\) Urease is a di-nickel(II) nuclear enzyme, i.e. it possesses two Ni atoms in its active site, **Figure 1.16**. The active site is a highly conserved structural moiety, showing considerable homology among different strains of bacteria.\(^{91}\) The two nickel atoms are separated by 3.5-3.7 Å. Each nickel is bound to the oxygen atom of the same lysine residue and to nitrogen atoms of two separate histidine residues. One nickel atom is bound to an additional oxygen of an aspartate amino acid. The coordination sphere of each nickel atom is completed by the binding of two water molecules, of which one is shared between the two atoms, **Figure 1.16**. Therefore one nickel is penta-coordinate while the other one is hexa-coordinate. A lid at the entrance to the active sites switches between the opened and closed conformations, regulating substrate entry.
Bacillus pasteurii urease is used as a model to study the mechanism of action of urease. The mechanism proposed by Zambelli et al. is in agreement with the available data available, in particular pH-dependence and non-competitive inhibition studies.

An overview of the process is provided in Scheme 1.1. Urea gains entry to the native hydrated enzyme when the flap is open only (A), giving the substrate bound intermediate (B). Urea binds to Ni(1) via the carbonyl oxygen, resulting in the displacement of three water molecules from the active site.\(^\text{100}\) Computational findings suggest that urea coordinates to the second Ni(2) via its NH\(_2\), facilitated by flap closure (C). Crystal structures of the interaction of urease with B(OH)\(_3\), an inert analogue of urea, has supported the hypothesis of bi-dentate binding, with the expulsion of water molecules.\(^\text{101}\)

The next step involves nucleophilic attack on urea by the Ni\(^{2+}\) bound hydroxide, made possible only by the weakening of the bonds from binding of the substrate. This results in the formation of a tetrahedral intermediate (D). The nickel-bridged OH group is now very acidic and the hydrogen can transfer to the distal urea NH\(_2\) group via a nearby oxygen atom of the Ni(2)-bound aspartate. The neutral imidazole side chain of the histidine residue stabilizes the reactive C-NH\(_3^+\) group (E). This C-N bond is broken and ammonia is released. The release of carbamate, the resulting side product, is facilitated by the flap opening. Carbamate can then itself decomposes into NH\(_3\) and CO\(_2\). Rehydration of the active site then occurs, returning the enzyme to its native state (A).

Figure 1.16  The coordination environment of the di-nickel nuclear active site of the urease enzyme.
Scheme 1.1 The proposed mechanism of action of hydrolysis of urea at the active site of the urease enzyme.

Localisation

*H. pylori* urease localisation is unique. It is found both in the intercellular cytoplasm and on the surface of the bacteria, as identified by some simple experiments such as recovery of urease from washing the intact cells, or extraction of the enzyme with detergents such as n-octylglucoside.\(^{102, 103}\) In all other bacteria, urease is localised in the cytoplasm only. The mechanism by which *H. pylori* obtain surface acquired urease was investigated. In addition, further analysis of surface acquired proteins highlighted other cytoplasmic components were also present.\(^{104, 105}\) It is not possible that exporter proteins are responsible for this localisation, as those genes are absent in the genome.\(^{106}\)

This has been experimentally investigated and it was observed that in early log (one day) and mid log phase (two days) of growth, urease is located strictly within the cytoplasm. Only during the late phase of growth (three days), or subsequent growth from subculture, is urease located on the surface and in the cytoplasm of *H. pylori*.\(^{104}\) These results strongly support the hypothesis that the acquisition of surface associated urease is as a result of a mechanism termed ‘bacterial autolysis’ or ‘altruistic autolysis’. Bacterial autolysis followed by adsorption is widely accepted as
the mechanism for surface association of urease. Urease, along with other cytoplasmic proteins, are released as a result of this programmed autolysis of a portion of the bacteria. Released urease then adsorb onto the surface of intact neighbouring bacteria. Figure 1.17.

![Figure 1.17. Overview of the process of altruistic autolysis. Urease is found only in the cytoplasm of early log phase bacteria, (A). Following altruistic autolysis, (B), cytoplasmic urease is identified on the surface of the bacteria (C).](image)

2.2 Development of Gastric Cancer

As mentioned previously, *H. pylori* is one of the major risk factors associated with development of gastric cancer (GC). Many genetic factors have an important impact on the clinical outcome of *H. pylori* infection, and for the development of GC. In this section some of the different virulence factors associated with *H. pylori* infection and progression to GC will be discussed.

Cytotoxin-Associated Gene (cag)

There is a region in *H. pylori* chromosomal DNA which encodes a 31 gene secretory system. CagA, an onco-protein, is delivered to the cytosol of host gastric epithelial cells through this secretion system. Upon delivery into host cells, CagA activates...
dephosphorylation and cellular morphological changes of host cell proteins.\textsuperscript{110} CagA has been reported to induce the production of interleukin-8 (IL-8) a pro-inflammatory cytokine, resulting in increased inflammation.\textsuperscript{111, 112} Individuals infected with cagA-positive strains of \textit{H. pylori} are at a much higher risk of development of GC than those infected with cagA-negative strains.\textsuperscript{113} Generally the process of GC development proceeds via non-atrophic chronic gastritis followed by multifocal atrophic gastritis, intestinal metaplasia, low-grade non-invasive dysplasia, high-grade non-invasive dysplasia, and invasive adenocarcinoma.\textsuperscript{114} The progression from non-atrophic chronic gastritis is an important step in progression to GC, and virulence factors can influence this step.

**Vacuolating Cytotoxin Gene (VacA)**

VacA, a large 140-kDa polypeptide cytotoxin, is secreted from bacteria and delivered in its active state to host cells.\textsuperscript{115} The VacA gene in \textit{H. pylori} strains displays allelic diversity within three main regions, the \textit{s} (signal), the \textit{i} (intermediate), and the \textit{m} (middle) regions. Different combinations of alleles lead to much variety among vac genes.\textsuperscript{116} VacA plays an important role in multiple cellular activities, the best studied of which is a process which leads to epithelial cell vacuolation. Many different strains with varying allele combinations have been highly associated with ulcers and GC.\textsuperscript{117} In Western countries, individuals infected with \textit{s}1 or \textit{m}1 strain of \textit{H. pylori}, compared with \textit{s}2 or \textit{m}2 strains have experienced an increased risk of peptic ulcer or GC.\textsuperscript{118}

VacA and CagA, although located in different genomic regions, play an important relationship in GC development.\textsuperscript{119} \textit{H. pylori} expressing the \textit{vacA s1m1} alleles along with cagA are the most virulent strains, associated with severe gastric diseases and cancer.\textsuperscript{120}

### 2.3 Identification and Detection of \textit{H. pylori}

**Urease Biopsy Test**

\textit{H. pylori} express high levels of urease which makes for simple detection in gastric biopsies. Endoscopy samples are tested for the presence of \textit{H. pylori} by a colorimetric assay, monitoring the conversion of urea to ammonia, detectable by a
change in colour relating to the pH. Phenol Red is an indicator used to detect the pH changes. The CLO (Campylobacter-like organism) test first utilised this process for detection of *H. pylori*.

Recently, new detection method has been developed which consists of a urease sensor embedded to the end of an endoscopy scope. It carries a urea solution which allows for quick on-site detection of *H. pylori* with satisfactory sensitivity and specificity.

### Urease-Positive Colonies after Culture

Freshly cultured endoscopic biopsies on serum based medium generally always provide a strong urease positive test after 3 to 5 days of culture. Oxidase and catalase positive tests further confirm the presence of *H. pylori*.

### Urea Breath Test

Even though the two previous tests are highly accurate, the process involve invasive procedures. The urease breath test has been developed as an alternative, non-invasive test. This test is a sensitive and specific indicator of the presence of *H. pylori*. Labelled urea, either $^{13}$C urea or $^{14}$C urea is administered and the detection of $^{13}$CO$_2$ or $^{14}$CO$_2$ in the breath of the patient, as a by-product of the acid acclimation process, is a positive result for the presence of *H. pylori*. Of course a number of gut anaerobic flora may also liberate $^{13}$CO$_2$ or $^{14}$CO$_2$ but so far the results indicate a rare occurrence of false-positive reactions.

### MALDI-TOF MS

The use of matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF), coupled with MS, is a rapid method to identify and distinguish different strains of bacteria by the use of peptide fingerprints. Each different strain of *H. pylori*, for example, has its own fingerprint when subjected to MALDI-TOF. By analysing a sample of bacteria and comparing it to a standard fingerprint, easy identification can be carried out. Positively charged ions are resolved after laser irradiation of intact microorganisms in the presence of a saturated solution of UV-absorbing matrix. This is a quick and accurate method of detection from live populations of bacteria. This method is often used in clinical setting where the rapid identification of specific strains of bacteria are required in order to choose the appropriate treatment regimen.
2.4 Current treatment

**Triple Therapy**
Currently the first line of treatment for *H. pylori* associated gastritis is the administration of a triple therapy treatment of two antibiotics and a proton pump inhibitor (PPI). The most common antibiotics used are clarithromycin, amoxicillin, trimethoprim or tetracycline. This triple therapy combination treatment works by a dual mode of action. On one hand, the antibiotics exert their activity against the *H. pylori* bacteria, while on the other hand, the PPI works to prevent the production of acid by the parietal cells in the stomach to reduce inflammation.\(^{124}\)

**Quadruple Therapy**
In an attempt to overcome the problem of AR in *H. pylori* treatment, new drug combinations are being developed from existing formulas. A quadruple therapy is now available in areas where triple therapy has failed in the eradication of *H. pylori*. Quadruple therapy involves the administration of triple therapy, but with an added component, a bismuth based drug. These drugs can be administered either sequentially or concomitantly. More recently, this therapy has become the treatment of choice due to the high levels of AR to clarithromycin. Quadruple therapy involves administration of a bismuth salt, tetracycline HCl, tinidazole/metronidazole, and a PPI, three or four times daily for 7-14 days.\(^ {125}\)

2.5 *H. pylori* Resistance
AR has affected the efficacy of *H. pylori* triple therapy treatment. For instance in Europe, the resistance levels of essential components of triple therapy such as clarithromycin are 17.5% and metronidazole are 34.9%, while in Asia the levels are even higher with resistance rates of 21.5% and 95.4% reported respectively.\(^{126}\) As a result of this AR, bismuth containing drugs now play a pivotal role in the newly implemented quadruple therapy for the treatment of *H. pylori*.\(^ {127}\)

Insufficient concentration of antibiotics at the site of infection is one of the main problems for ineffective eradication of *H. pylori*. The concentrations are not sufficient to overcome the minimal bactericidal concentration (MBC) and minimum
inhibitory concentrations (MIC) of the bacterium. Other reasons for ineffective treatment and bacterial resistance are outlined below.

Lack of patient compliance
Most people at some point in time will take some sort of drug, generally administered once daily, as is the preferred treatment. However, for *H. pylori*, the usual treatment involves taking three/four different drugs, numerous times daily. These drugs work synergistically together and the effect on symptoms may not be immediately obvious. With this treatment, side effects are common, with 1 in 3 people experiencing diarrhoea and a bad taste in the mouth among others. The failure to take a few doses of drug, even on a minimal length 7 day recommended treatment, may lead to the incomplete eradication of the bacteria. Low compliance levels resulted in a poor prognosis, often resulting in reinfection within six months.

Selection of resistant mutants
*H. pylori*, like any other bacteria, can develop resistance to antibiotics by acquisition of a spontaneous mutation which occur primarily during replication. Transformations are the most regularly documented. Subtle changes in the antibacterial target, resulting in reduced efficacy of binding of the antibiotic can result in resistance. Clarithromycin resistance comes about as a result of a point mutation on the 23S rRNA protein, resulting in an increased MIC/MBC. These concentrations are unattainable in the gastric mucosa.

Low gastric pH
A unique characteristic of *H. pylori*, is its ability to survive in acidic environments of the stomach. The MBCs and MICs of most anti-bacterial agents are determined at physiological pH. It is seen that at lower pH, the MIC tend to increase. As part of the treatment for *H. pylori*, proton pump inhibitors are used to try raise the pH of the stomach to a level which allows the drugs function better. However, the variability between individuals regarding the gastric pH levels is quite vast. Some people are hyper-secretors and have very low pH levels in the stomach. Other environmental factors which may affect the pH of the individual need to be considered also.
High bacterial load

High bacterial load can result in poor efficacy of the administered drug.\textsuperscript{132} Unfortunately, there are no tests which can quantify bacterial load in the stomach. The breath test and histology tests are only semi-quantitative. With higher loads of bacteria, more anti-bacterial agents will be required to kill all the bacteria. Surprisingly there has been some evidence to support the benefits of alcohol on \textit{H. pylori} treatment, which may be due to alcohol decreasing the bacterial load.\textsuperscript{133}

Sanctuaries not accessible to antibiotics

This phenomenon of intracellular \textit{H. pylori} was first documented in 1990 but is considered a rare phenomenon. Intact and degenerated bacteria were observed in the cytoplasm of epithelial, parietal and chief cells of patients.\textsuperscript{134} Some strains are capable of invading epithelial cells by receptor-mediated endocytosis.\textsuperscript{135} These intravacuolar deposits of bacteria could explain the difficulties encountered in complete eradication, given that many of the antibiotics, for example amoxicillin, do not penetrate the cells.

2.6 Additional Treatment Options

New and alternative treatment regimen are constantly being developed along with modification of older treatments for the effective eradication of \textit{H. pylori}.

Probiotics

The use of probiotics as a large-scale and low-cost alternative in the prevention and decrease of \textit{H. pylori} colonization has been investigated.\textsuperscript{136} Probiotics can have health benefits that cannot be acquired from basic good nutrition.\textsuperscript{137} It has been suggested that probiotics can modulate \textit{H. pylori} activity by immunological or non-immunological means. Dajani \textit{et al.} showed that using probiotics in conjunction with a drug regimen lead to an increase in \textit{H. pylori} eradication while also reducing some of the adverse side effects.\textsuperscript{138} However, some studies claim that no difference in the side effect were apparent with the use of probiotics.

Herbal remedy

Recently many plant extracts have been reported to have anti-\textit{H. pylori} activity, with one study in particular demonstrating that extracts from \textit{Mirtus communis} and
Teucrium polium were especially effective in preventing *H. pylori* resistance development. This treatment in combination with a traditional treatment could be a possible way to prevent the rise of resistance. Question marks remain over the mode of action, cytotoxicity and true benefits of these treatments.

**Photodynamic Therapy**

Photodynamic therapy combines the use of a sensitizer or photo sensitizer with harmless visible light of a certain wavelength. A cascade pathways can be initiated and this can result in the production of reactive oxygen species (ROS) which are harmful to bacteria. Recently, some success has been reported for *in vitro* and *in vivo* studies for anti-*H. pylori* photodynamic therapy. An *in vitro* study, combined a photosensitizer derived from chlorophyll with optimal irradiation, resulting in an inactivation of *H. pylori*. It was noticed that ten *H. pylori* infected patients responded well to this therapy, exhibiting a decline in bacterial numbers.

**Vaccine**

There is a real need for the development of a vaccine to eradicate *H. pylori* in areas where antibiotic resistance is high and complete eradication of the bacteria is seldom. The high treatment costs of this infection makes vaccine development a sensible alternative. Vaccines that could cure/prevent the spread of this infection are desirable.

Efforts to obtain a *H. pylori* vaccine have focused on finding the best delivery route to introduce protective immunity. Mucosal immunisation has been the main method developed as *H. pylori* is an extracellular bacteria. Several routes of mucosal vaccination and different antigens and adjuvants, have been tested. In animal models, either cholera toxin (CT) or the *E. coli* heat-labile toxin (LT) are strong adjuvants that induce significant protection resulting in a decrease in bacterial load. However, these adjuvants are not transferrable to a clinical setting as they have been shown to induce adverse effects in humans. An alternative is aluminium hydroxide, which is already approved for use in human studies by the United States Food and Drug Administration (FDA). The introduction of attenuated *Salmonella* expressing *H. pylori* antigens is an alternative adjuvant free strategy but studies in humans did not produce satisfactory results. Different antigens modelled on antibodies such as CagA and VacA are the best candidates for vaccine formulations.
Although the initial results of single antigen vaccination were disappointing, the combination of antigens conferred better protection. Sometimes one single antigen is not enough to illicit a response but combinations of antigens or incorporation of adjuvants may be the key to development of an effective vaccine. This search for a novel antigen lead to the development of a genetically fused HspA, UreB and HpaA multivalent vaccine showing significant protection against *H. pylori* in infected mice.

Although some promising results have been obtained, mainly in animal models, more clinical trials are needed to develop a secure and effective vaccine for human use. Investigation are ongoing in an attempt to provide a further understanding regarding formulations, antigens, adjuvants, and delivery systems.

**New Approach to Drug Discovery**

Given the dramatic decrease in the discovery of novel anti-bacterial agents, many research groups have turned their attention to the area of repurposing previous drug candidates. There have been a number of reports where by the linking of an inorganic metal with a drug candidate or failed drug was fruitful in the search of a new treatment for bacteria, viruses and cancers. Many of these treatments include the inhibition of enzymes. They possess the possibility of forming strong interactions with the target by combining the coordination ability of the metal with the unique sterio-electronic properties of the organic ligand.

The repurposing of some drugs by the incorporation of bismuth into a regimen is routinely used in the treatment of gastrointestinal disorders. Bismuth is a component of the quadruple therapy used for the treatment of *H. pylori* infections. Bismuth itself has useful anti-microbial, anti-leishmanial and anti-cancer properties, making it a good candidate for the use in repurposing of current but failing chemotherapeutics. Much research has been undertaken recently in relation to the synthesis, characterisation and evaluation of novel Bi-based compounds as potential anti-microbial, anti-leishmanial and anti-cancer agents, with some promising results.

One such area we hope to exploit is the use of bismuth hydroxamic acid complexes to target the urease enzyme in the treatment of *H. pylori*. Given the importance of urease in *H. pylori* survival, it is a good target. Bismuth likely binds cysteine rich
domains of proteins. Metallomic/metalloproteomic approaches can help elucidate bismuths mechanism of action, resistance, biological uptake, storage and removal.

Bismuth is only one example of where metals can by utilised for the repurposing of organic ligands as drug candidates. Given the notable resurgence of interest in the bioinorganic chemistry of many metals, it is becoming increasingly apparent that the use of metals in medicinal chemistry has the potential to overcome a lot of the problems associated with resistance to current treatments.

2.7 Strains

Independent *H. pylori* isolates can exhibit extensive genetic diversity, resulting in differences in pathogenesis and symptomology.\(^{148}\) In this study we use three different strains of *H. pylori*, Table 1.3. All strains were purchased from ATCC.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Application</th>
<th>Source</th>
<th>Genotype</th>
<th>Genome Sequenced</th>
</tr>
</thead>
<tbody>
<tr>
<td>J99</td>
<td>Enteric Research</td>
<td>Patient with a duodenal ulcer, United States, 1994</td>
<td>cagA+ vacA+</td>
<td>Yes</td>
</tr>
<tr>
<td>60190</td>
<td>Enteric Research</td>
<td>N/A</td>
<td>Cytotoxin activity and serologic response</td>
<td>N/A</td>
</tr>
<tr>
<td>26695</td>
<td>Enteric Research</td>
<td>Stomach of a human patient with gastritis, United Kingdom</td>
<td>N/A</td>
<td>Yes</td>
</tr>
</tbody>
</table>
3. *Escherichia coli*

In his publication in 1885, Theodor Escherich reported the isolation and characterization of slender short rods from an infant stool, which he named *Bacterium coli commune*. In 1954 however, the name *Escherichia coli* was now the common name for this bacteria. We now know that *E. coli* is a harmless commensal of the gastrointestinal tract in warm-blooded animals. However, through gene mutation, *E. coli* have become highly diverse and adapted pathogens causing a broad range of human diseases. These include diseases of the gastrointestinal tract to extraintestinal sites such as the urinary tract, bloodstream, and central nervous system.

*E. coli* has been highlighted for its significant pathogenic role in diarrheal disease, a major cause of death in children worldwide, particularly in infants under the age of 5 and in sub-Saharan Africa and South Asia.

A lot of research has been carried out in relation to understanding the microbiology, pathogenesis, ecology, and interaction of *E. coli* with its host. As a result, novel treatments and vaccines for *E. coli*-induced diarrheal illness have been developed. Various strains of *E. coli* have been identified, making its treatment problematic.

*E. coli* is a Gram-negative, rod-shaped bacterium from the Enterobacteriaceae family. It may grow aerobically and anaerobically, preferably at 37°C. It can be non-motile or motile, with peritrichous flagella and is isolated from faecal samples. Although this method of isolation is effective, further morphological, phenotypic, and genotypic characteristics need to be carried out for verification of pathotypes, generally, by rapid molecular techniques such as PCR or enzyme immunoassays. Different serotypes can be identified due to the major surface expressed antigen, lipopolysaccharide (O type antigen), flagellin (H type antigen) and capsule (K type antigen) Figure 1.18.

There are 174 *E. coli* O and 53 *E. coli* H antigens recognized. Pulsed-field gel electrophoresis (PFGE) is considered the gold standard for typing and even though it is very accurate, it is time consuming and laborious.
Figure 1.18. The antigens expressed by *E. coli*.

### 3.1 Pathogenesis

*E. coli* must adhere to cells in order to infect the host. Different strains have unique methods of infection outlined in Table 1.4.

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Method of Pathogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enteropathogenic <em>E. coli</em> (EPEC)</td>
<td>Form lesions and micro-colonies on the surfaces of intestinal epithelial cells due to bundle-forming pili.</td>
</tr>
<tr>
<td>Enterotoxigenic <em>E. coli</em> (ETEC)</td>
<td>Utilise colonization factors (CFs) for attachment to host intestinal cells.</td>
</tr>
<tr>
<td>Enteroinvasive <em>E. coli</em> (EIEC)</td>
<td>Penetrate intestinal epithelium through M-cells and escape submucosal macrophages by inducting macrophage cell death. Spread by basolateral invasion.</td>
</tr>
<tr>
<td>Enteroinvasive <em>E. coli</em> (EIEC)</td>
<td>Penetrate intestinal epithelium through M-cells and escape submucosal macrophages by inducting macrophage cell death. Spread by basolateral invasion.</td>
</tr>
<tr>
<td>Diffusely adherent <em>E. coli</em> (DAEC)</td>
<td>Disperse over the surfaces of intestinal cells, resulting in a diffuse adherence (DA) pattern.</td>
</tr>
<tr>
<td>Adherent invasive <em>E. coli</em> (AIEC)</td>
<td>Colonizes the intestinal mucosae of Crohn’s disease patients.</td>
</tr>
<tr>
<td>Adherent invasive <em>E. coli</em> (AIEC)</td>
<td>Can invade epithelial cells and replicate within macrophages.</td>
</tr>
<tr>
<td>Enteroinvasive <em>E. coli</em> (EIEC)</td>
<td>Penetrate intestinal epithelium through M-cells and escape submucosal macrophages by inducting macrophage cell death. Spread by basolateral invasion.</td>
</tr>
</tbody>
</table>
3.2 Treatment

Antimicrobials

In most cases, EPEC-induced diarrhoea is self-limiting, effectively treated with oral rehydration therapy. However, antimicrobials may be required for persistent infections, but the efficacy of these treatments are based on the profile of EPEC. Several clinical isolates exhibit increasing resistance to standard antibiotic treatments.\textsuperscript{154} The cost and supply of antimicrobials is also a limiting factor in successful treatment of \textit{E. coli} in developing countries.\textsuperscript{155} \textit{E. coli} displays resistance to penicillins, cephalosporins, and aminoglycosides.\textsuperscript{154} In a recent study, markers for a multidrug resistance plasmid were detected in 30\% of atypical isolates.\textsuperscript{156} The mechanism by which \textit{E. coli} has become resistant to antibiotics is through the spread of ESBL’s, resulting in the destruction of the antibiotics originally used for their treatment.

Vaccines

The spread of infections due to EPEC has been well documented however, no vaccines are currently available to control its spread. Antibodies against EPEC O antigens and outer membrane proteins have been found in breast milk.\textsuperscript{157} Various attempts to devise a vaccine from the antibodies in maternal colostrum and serum samples have been explored, but with limited success in humans. There is a suggestion that more than one antigen may be required for successful vaccine development.

Given then the rise in resistance to the current treatments for \textit{E. coli} and the lack of progression on the vaccine front, there is a need for a new treatment which can effectively treat \textit{E. coli} infections.
**4. Staphylococcus aureus**

*Staphylococcus aureus* is a Gram-positive, round-shaped bacterium that is a member of the phylum *Firmicutes*. It is a facultative anaerobe which can grow without the need for oxygen but is a member of the normal bacteria of the body with 20-30% of the human population long term carriers. It is frequently found in the nose, respiratory tract, and on the skin. Staphylococci are the most studied of skin flora due to their ubiquitous colonization of human skin and the wide spectrum of diseases they cause. *S. aureus* is not always pathogenic and can commonly be found existing as a commensal. However, upon infection which generally occurs through a break in the skin, *S. aureus* can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteraemia, and sepsis.

Pathogenic strains produce virulence factors that can bind and inactivate antibodies. The emergence of methicillin-resistant *S. aureus* (MRSA) and other antibiotic-resistant strains of *S. aureus* is a worldwide problem. No vaccines have yet been developed which are successful in prevention of infection. It is one of the five most common causes of hospital-acquired infections. Each year, over 500,000 patients contract a staphylococcal infection in hospitals of the United States, resulting in up to 50,000 deaths.

*S. aureus* exemplifies better than any other pathogen the genetic plasticity which we have come to associate with bacteria. It has demonstrated a unique and rapid ability to respond to stresses. The original antibiotics such as penicillin and methicillin were rendered inactive due to the production of an extracellular designated penicillinase enzyme that hydrolysed the important amide bond of the drug. It was acquired by a mobile plasmid which is expressed at high levels following introduction of penicillin or penicillin like complexes. Alteration of the target with decreased affinity for the antibiotic for example in PBP2a is another effective means of resistance development. Trapping of the antibiotic and efflux pumps are also alternative means which *S. aureus* circumvents the action of the antibiotics. Complex genetic elements have been acquired by *S. aureus* through HGT, which provide for the resistance,
while AR can also be acquired through spontaneous mutations and positive selection.

Details pertaining to other pathogenic or environmental bacterial strains that were investigated throughout this research are listed below in Table 1.5.

**Table 1.5. Additional bacteria utilised in this work.**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>DSMZ code</th>
<th>Species Type</th>
<th>Isolated from:</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>DSM10</td>
<td>Environmental</td>
<td>N/A</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>DSM30053</td>
<td>Pathogenic or biohazard class II</td>
<td>Sputum</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>DSM1103</td>
<td>Pathogenic or biohazard class II</td>
<td>Clinical isolate</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>DSM498</td>
<td>Environmental</td>
<td>N/A</td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em></td>
<td>DSM50090</td>
<td>Environmental</td>
<td>Pre-filter tanks</td>
</tr>
<tr>
<td><em>Pseudomonas putida</em></td>
<td>DSM6125</td>
<td>Environmental</td>
<td>N/A</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>DSM799</td>
<td>Pathogenic or biohazard class II</td>
<td>Human lesion</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>T37-1</td>
<td>Environmental</td>
<td>In-house</td>
</tr>
<tr>
<td><em>Pseudomonas putida</em></td>
<td>CP1</td>
<td>Environmental</td>
<td>In-house</td>
</tr>
</tbody>
</table>
5. Leishmaniasis

Leishmaniasis is a class of neglected tropical disease (NTD) that occurs in tropical and subtropical areas. A recent report, generated by the WHO, indicates around 955 million people, in 98 countries, are at risk of contracting the disease and it is the second largest parasitic killer in the world after malaria. It is estimated that two million new cases occur every year.

The causative agent for leishmaniasis are protozoan parasites of the genus *Leishmania*, which is part of the *Trypanosomatidae* family. There are currently 21 different species of this genus. There are two forms of the parasite; the promastigote and the amastigote, Figure 1.19. The promastigote form is transmitted by the bite of a female phlebotomine sandfly. The epidemiology of leishmaniasis depends on the characteristics of the parasite species, the local ecological characteristics of the transmission sites, human behaviour and current and past exposure of the human population to the parasite. Some 70 animal species, including humans and especially wild dogs, have been found as natural reservoir hosts of *Leishmania*. This promastigote can develop into the disease-causing amastigote, which can live and multiply within the host and the life cycle of both are shown in Figure 1.20, and Table 1.6.

![Figure 1.19. Promastigote and amastigote forms of Leishmania.](image)

<table>
<thead>
<tr>
<th>Table 1.6. Features of the promastigote and the amastigote.</th>
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<tbody>
<tr>
<td><strong>Promastigotes</strong></td>
</tr>
<tr>
<td>Flagellar stage</td>
</tr>
<tr>
<td>Occurs in the Sand Fly</td>
</tr>
<tr>
<td>Divide by binary fission at 27 °C</td>
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<tr>
<td>Spindle shaped</td>
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<tr>
<td>Smaller Nucleus</td>
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<tr>
<td><strong>Amastigotes</strong></td>
</tr>
<tr>
<td>Aflagellar Stage</td>
</tr>
<tr>
<td>Occurs in Vertebrate host</td>
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<tr>
<td>Divide by binary fission at 37 °C</td>
</tr>
<tr>
<td>Round or oval shaped</td>
</tr>
<tr>
<td>Large Nucleus</td>
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Figure 1.20. The life cycle and infection routes of the promastigote in the sand fly and the amastigote in the vertebrate host.  

5.1 Forms of the disease

Visceral leishmaniasis (VL)

This is the most deadly form of the disease and if not treated promptly and completely can result in rapid death in over 95% of cases. It is caused by *L. donovani* and *L. infantum*. It is reported that 300,000 cases result in about 20,000 deaths every year. Its symptoms include irregular bouts of fever, weight loss, enlargement of the spleen and liver, and anaemia. Endemic of Indian subcontinent and East Africa, it is estimated that 50,000 to 90,000 new cases of VL occur worldwide per annum. Seven countries (Brazil, Ethiopia, India, Kenya, Somalia, South Sudan and Sudan) account for more than 90% of new cases reported to WHO in 2015.

Cutaneous leishmaniasis (CL)

This is the most common form of leishmaniasis, causing skin lesions and ulcers on exposed parts of the body. Untreated, these lesions can result in life-long scars and serious disability. About 95% of CL cases occur in the Americas, the Mediterranean basin, the Middle East and Central Asia. It is estimated there are 0.6 million to 1 million new cases annually worldwide of which six countries (Afghanistan, Algeria, Brazil, Colombia, Iran and Syria) are responsible for two thirds.
Mucocutaneous Leishmaniasis (ML)
This form of the disease leads to partial or total destruction of mucous membranes of the bodily cavities (nose, mouth and throat). Over 90% of ML cases occur in Bolivia, Brazil, Ethiopia and Peru.

5.2 Risk Factors
There has been a huge increase in the number of infections over the past decade and some of the common risk factors are outlined below.

Socioeconomic conditions
In many infected countries, housing standards and domestic sanitary conditions are poor and inadequate. Waste management often consists of open sewerage systems in these areas. This offers a breeding ground for the sandfly and facilitates more frequent sandfly-humans interactions. Human behaviour, such as sleeping outside or on the ground, increases the risk of being bitten by a sandfly, due to inadequate protection by bed nets or insecticide.

Malnutrition
Poor diets, such as those lacking in protein, iron, vitamin A and zinc, increase the risk of rapid disease progressing to the VL stage.163

Population mobility
Epidemics of both CL and VL are often associated with migration of non-immune populations into areas where infection is rampant. Widespread deforestation remains an important factors as people are now living in areas where sand flies reside leading to a sharp increase in the numbers of cases.

Climate change
Leishmaniasis is climate-sensitive, and strongly affected by changes in rainfall, temperature and humidity. Rainfall can have a strong effect by disturbing and altering their survival and numbers. Small fluctuations in temperatures can greatly affect the developmental cycle of *Leishmania* promastigotes, resulting in transmission in areas that would not be effected usually. Famine and drought can
lead to the mass movement of people into previously unoccupied areas which might host the sandfly.\textsuperscript{167, 168}

5.3 Detection and identification

Diagnosis of leishmaniasis is difficult due to the varied species and symptoms involved.\textsuperscript{169} There are many reliable laboratory diagnostic methods such as direct smear examination, culture, immunologic and molecular techniques. \textsuperscript{170-172} The sensitivities of these different techniques and their success can vary, often determined by the type of infection. Direct microscopy and culture are not highly sensitive. Given this varied diagnosis, there is an urgent need to develop a technique which accurately identifies the vast leishmanial infections.\textsuperscript{173}

5.4 Current treatment of Leishmaniasis

Although leishmaniasis has a number of treatment options, the complexity of the disease and its various forms means that development of an effective antileishmanial therapy has been problematic.\textsuperscript{174} The available therapies are far from ideal, primarily due to extensive toxicity, parenteral route of administration affecting regime adherence, high cost, emerging drug resistance, lack of access in regional areas and large variety in efficacies depending upon the species and geographical regions of the parasite.\textsuperscript{174} The severity and risk to benefit ratio often determines treatment regimens. None the less, current treatments are considered limited and unsatisfactory, irrespective of which form of leishmaniasis is being discussed

Mono Therapy

Antimonials

Pentavalent antimonial drugs such as sodium stibogluconate and meglumine antimoniate, along with their cheaper generic forms, have been the first-line treatment for VL in many areas over the past 70 years, Figure 1.2.\textsuperscript{175, 176} A downfall in this type of drug is antimonial toxicity. Side effects include cardiac arrhythmia and acute pancreatitis, affecting in particular patients under the age of 2 and over the age of 45 who suffer from malnutrition.\textsuperscript{177} In India, antimonials
treatments are reaching high failure levels (> 60%), but elsewhere antimonials are still used as the first line treatment.

Figure 1.21. Pentavalent antimonial drugs sodium stibogluconate (left) and meglumine antimoniate (right), for treatment of leishmaniasis.

Even though these antimonials are associated with high toxicity, it must also be stated that they are hugely effective and efficient in the eradication of leishmaniasis and so still to this day represent a viable class of drugs that can be improved.\(^{178}\)

**Amphotericin B**

In the case of antimonial resistant areas, the first line treatment now involve administration of conventional amphotericin B, originally designed for its antifungal properties, Figure 1.22.\(^{179}\) Treatment with expensive amphotericin B is not straight forward and involves a complicated regimen of 15 slow infusions on alternating days. Infusion-related fever, chills, rigor, hypokalaemia, nephrotoxicity and first-dose anaphylaxis are not uncommon as potentially life-threatening side effects. Research is continuously ongoing into developing other therapies and improving the current drugs.

Some alternative treatment modes of amphotericin B have been developed such as administration in the form of liposomal amphotericin B in Europe and the United States. This treatment is limited by its price, costing US$ 2,800 per treatment.\(^{180}\)

Figure 1.22. The structure of amphotericin B, original an antifungal agent.
**Miltefosine**

Originally an anticancer drug, Miltefosine, Figure 1.23, became the first orally effective drug for leishmaniasis, with very interesting phase IV clinical trial results showing only 3 deaths in 1132 patients, and a cure rate of 95%, but again its use is limited as it is a teratogenic drug and can therefore not be used in pregnant women.\(^{181}\) Non-adherence to the recommended regimen could lead to widespread parasite resistance. Due to the ease at which resistance to this drug may occur, a strictly supervised public distribution of this drug is performed using direct observed therapy (DOT).

**Sitamaquine**

Sitamaquine, an 8-aminoquinoline, is another oral drug which has been studied for its activity as an anti-leishmaniasis drug, Figure 1.23. The progress of this drug is slow however due to problems with toxicity. Resistance is a risk also and this has to be taken into account also.\(^{182}\)

**Paromomycin**

Paromomycin, an aminoglycoside antibiotic, is another drug with good activity against leishmaniasis, Figure 1.23.\(^{183}\) Phase III trials showed excellent efficacy and safety with no nephrotoxicity observed and as a result is now a viable treatment of leishmaniasis.\(^{184}\)

![Figure 1.23. Miltefosine (A), Sitamaquine (B) and Paromomycin (C).](image)
Combination therapy

Increase in resistance to anti-leishmanial drugs has resulted in the use of monotherapies being reviewed. Combination therapy aims to increase treatment efficacy while preventing parasite resistance. Lower dose requirements and a reduction in the duration of therapy may be possible due to synergistic effects, thereby resulting in less toxic side effects. \footnote{185} Ideally, the approach taken for combination treatment of leishmaniasis is the use of a potent drug with a short half-life combined with a second drug having an extended half-life to clear the remaining parasites. \footnote{186} Some examples of combination treatments include combination of the monotherapies discussed above, for example, the use of sodium stibogluconate with paromomycin. This treatment is used as the standard treatment in East Africa by WHO and a cure rate of 93\% has been achieved. \footnote{187} In another case, a phase III study tested the administration of L-AmB, miltefosine and paromomycin and it resulted in 97\% efficacy. \footnote{188} Now however, pentavalent substitutes such as sodium stibogluconate and meglumine antimoniate are used as first-choice anti-leishmaniasis drugs in many countries due to their lower toxicity and more efficient therapeutic indices.

5.5 Leishmaniasis resistance

In particular, Leishmania has evolved mechanisms to subvert both innate and adaptive immune responses ultimately allowing parasite proliferation and persistence within the mammalian host. \footnote{189}

Given that the mechanism of action of antimonials is still not completely understood, the exact mechanism by which resistance occurs is a mystery. Pentavalent administered antimonial drugs require reduction \textit{in situ} to their active trivalent form. The site and the mechanism of this reduction is unclear. \footnote{190, 191}

Diminished biological reduction of Sb(V) to Sb(III) has been observed in some species of Leishmania which may account for some of the resistance to antimonial drugs. \footnote{191} Many other studies have been carried out in an effort to understand the mechanism of action of the different anti–leishmanial drugs and in turn, how the resistance develops. Very limited success has been achieved. \footnote{192-195}
5.6 Current Leishmaniasis Drug Discovery

Drug discovery and development has evolved into an interdisciplinary scientific field, incorporating areas such as biology, chemistry, molecular modelling and pharmacokinetics. As with many other diseases, the discovery phase often begins with the identification of a ‘hit’ molecule using High Throughput Screening (HTS) in vitro assays. Lead compounds are optimised to improve various physicochemical parameters, such as solubility, metabolic stability, biological activity and selectivity. Further in vitro evaluation of molecules is assessed during optimisation to evaluate the pharmacokinetic and pharmacodynamic properties of the compound and in vivo efficacy is also undertaken.

Repurposing of existing drugs have been the main strategies to replace antimonials as first-line treatment of leishmaniasis. In addition, the parasites response to the drugs can vary substantially. For example, the cure rate of paromomycin treatment for VL ranging from 14.3% to 93.1% in Sudanese and Ethiopian patients, respectively. Relapse can occur, even months after therapy concludes.

Current efforts towards new anti-leishmanial drug discovery focus on the identification of target molecules that present significant structural and/or functional differences to their mammalian hosts and are involved in essential parasite pathways. Some key enzymes and pathways could be interesting drug targets in the fight against leishmaniases, identified outlined below.

**Enzymes of Polyamine Biosynthesis and Transport**

Polyamine plays a vital role in the life cycle of the promastigote and amastigote. Many of the enzymes involved in polyamine metabolism are over expressed, and provide a possibility to act as drug target. Inhibitors of the polyamine biosynthetic pathway, along with polyamine transporters have given rise to useful anti-leishmanial activity. Further investigations are required.

**Targeting Peptidases**

Peptidases as drug targets have been successfully exploited in the past to treat HIV, hypertension, pancreatitis and multiple myeloma. Many metallopeptidases are identified in the *Leishmania major* genome, which are essential for the survival of the parasite and may serve as valuable targets for treatment of leishmaniasis.
Targeting Enzymes of Glycosomal Machinery

*Leishmania* depend completely on their host to fulfil their energy requirement. The parasite receives blood, glucose and other essential components from the mammalian blood stream. Glycosomes are the main components for metabolic activities like glycolysis, oxidation of fatty acid, lipid biosynthesis and purine salvage pathways. Superoxide radicals are generated in large amount as side products of these processes. Fe-superoxide dismutase (FeSOD) evolved to prevent the radicals from damaging the glycosomes in the parasite.\(^{203}\) More importantly, FeSOD is absent in mammalian counterpart and therefore provides a possible effective drug target. Despite the success of biochemical, pharmacological, and genetic approaches to validate a potential drug targets, any new drug that directly targets the parasite will likely have only a short therapeutic use, given the capacity of *Leishmania* to rapidly develop resistant phenotypes through remarkable genome plasticity.\(^{204}\)

**Vaccine approach**

The simple lifecycle of this parasite, along with the fact that, after eradication of an original infection, reinfection is impossible, means that development of a vaccine to protect against this disease should be possible. The success of this will come down to a number of factors;

i) results from animal studies in the area,\(^{205}\)

ii) further understanding of *Leishmania*–host interactions, pathogenesis and protective immunity,\(^{206}\)

iii) the complete *Leishmania major* genome sequencing.\(^{207}\)

So far, development of a successful vaccine has not materialised. Previously, a vaccination study in Uzbekistan, Iran and Israel was discontinued, as it was deemed unachievable for large scale use or in areas where HIV is endemic.\(^{208}\) Work is currently on going in order to develop an effective vaccine against leishmaniasis.\(^{209}\)

### 5.7 Strains Used

The testing of compounds against two different strains of *Leishmania* was carried out in Brazil by Leandro Sangenito in the research group of Professor André Luis Souza dos Santos. The strains used were *Leishmania amazonensis* and *Leishmania chagasi*. These strains give different infection outcomes. The genome of the *L.*
*amazonensis* parasite has been sequenced completely, together with functional annotations and extended analyses focused on host-parasite interactions. 210 This parasite is a main causative agents of CL.

*Leishmania chagasi*, also known as *Leishmania infantum* in the Mediterranean region of the world, is the main cause of infantile VL. It has also be linked to some CL diseases. Wild and domestic dogs are the natural reservoir of this organism. *L. chagasi* is a closely related strain to *L. donovani*, often describes as subspecies of each other, but phylogenetic analyses can easily distinguish between the two groups.211
6. Metals in Medicine

The use of metals in medicine is not a new phenomenon. It has a rich and varied tradition. The records for the use of mercury date back 3500 years, where Hg₂Cl₂ was utilised as a diuretic agent in China and the Middle East.²¹², ²¹³ Silver has been utilised in Greek, Roman and Egyptian civilisations as food a preservative and for healing wounds and ulcers.²¹⁴ At the beginning of the 20th century, Paul Ehrlich discovered that arsenic may be used as a treatment for syphilis, in the form of Salvarsan.²¹⁵ Currently, some of the best and well-known metal-based chemotherapy agents are platinum based metal drugs.

The application of metal based drugs in clinical environments is diverse. They act as therapeutic or diagnostic agents, Figure 1.24.

![Figure 1.24. Some metal based therapeutics used in medicine, Cisplatin (A), Carboplatin (B), Oxaliplatin (C) and bismuth subsalicylate (BSS) (D).](image)

We are only scratching the surface in relation to understanding the roles and potential that metals provide in the therapeutic field.²¹⁶ They may offer a new treatment to current diseases and provide alternatives to purely organic treatments.²¹⁷

The rational design of metal-based drugs is desirable. Careful selection of metals and manipulation of geometries, coordination numbers and redox states by selection of appropriate ligands, can lead to the regulation of electronic, chemical and photophysical properties. Individual ligands also contribute greatly to structural diversity and modulate stability, ligand exchange kinetics and second coordination sphere interactions. In turn the prediction and control of the pharmacodynamics and pharmacokinetics of compounds, provide the opportunity to develop metal-based drugs with bespoke mechanisms of action.²¹⁵
6.1 Bismuth

Bismuth is a pnictogen, group 15 element, which has medical references dating back to the 18th century. The name bismuth is thought to have originated from the German word “wismuth” meaning white mass. It is the least abundant of the pnictogen group elements and is commonly found as bismite and bismuthinite or as a by-product of mining of lead, tin and copper. Bismuth is mainly monoisotopic (\(^{209}\text{Bi}\)) with a half-life of about \(1.9 \times 10^9\) years in comparison to its radioactive constituent’s \(^{212}\text{Bi}\) and \(^{213}\text{Bi}\) with half-lives of 60 and 45 minutes respectively, making them useful agents in radiopharmaceuticals.

As the metallic character increases down the pnictogen group, bismuth is considered semi-metallic. Arsenic and Antimony are considered to be metalloids. Bismuth has a ground state electronic configuration of [Xe]\(^{4f^{14}5d^{10}6s^26p^3}\). Trivalent, \(\text{Bi(III)}\), and pentavalent bismuth, \(\text{Bi(V)}\), are the predominant oxidation states. Given \(\text{Bi(V)}\) is a very strong oxidant in aqueous solutions, \(\text{Bi(V)}\) is generally not stable in biological solutions.

\(\text{Bi(III)}\) is classified as a Lewis acid according to the Hard Soft Acids Bases (HSAB) theory and can form weak complexes with hard Lewis bases for example. In general though \(\text{Bi(III)}\) is known to have a high affinity for oxygen, nitrogen and sulphur atoms, which is important when considering it’s biological activity. \(\text{Bi(III)}\) also has a relatively large ionic radius (1.16 Å), which facilitates formation of interesting complexes with high levels of coordination and interesting geometries.

Bismuth can interact with: (i) nucleosides/nucleotides; (ii) amino acids/peptides and in turn (iii) proteins/enzymes. For example, crystal structures have elegantly shown the binding of bismuth to two adenosine residues of calf thymus DNA, and bismuth interactions with human serum transferrin protein.

**Bismuth in Medicine**

Bismuth was originally used in medicine as a treatment against syphilis and dyspepsia in the early 18th Century. However the discovery of antibiotics, which were more effective in the treatments of these diseases, resulted in a decreased interest in bismuth based therapies. In 1980, however, there was a re-emergence of its use in medicine when it was discovered that it could be utilised in the treatment of
*H. pylori*, as well as other invasive gastrointestinal (GI) disorders. It exhibits GI protective qualities, as well as anti-fungal, anti-leishmanial and anti-bacterial properties.\(^7\)

**Gastrointestinal Diseases**

Bismuth is a component of quadruple therapy treatment regimen for *H. pylori*. It is widely accepted that the efficacy of bismuth in the treatment of GI diseases is due to its bactericidal activity against *H. pylori* and its interesting protective GI properties.\(^2\) Some examples of bismuth drugs used in this treatment include Bismuth Subsalicylate (Pepto Bismol, BSS), Colloidal Bismuth Subcitrate (De-Nol, CBS) and Ranitidine Bismuth Citrate (Pylorid, RBC). Figure 1.25. BSS has been in use since the early 20\(^{th}\) Century.\(^2\) The exact structure and precise mechanism of action (MOA) of these drugs are not completely known, despite bismuth based treatments being in use for almost 100 years.

![Predicted structures of RBC (A), CBS (B) and BSS (C).](image)

Figure 1.25.  Predicted structures of RBC (A), CBS (B) and BSS (C).

Structures of BSS recrystallized from acetone and CBS recrystallized at pH 3 have been proposed but these structures are unlikely the correct form of the active drug.\(^2\) Andrews *et al.* identified two clusters of high bismuth nuclearity, ranging from Bi\(_9\)O\(_7\) to Bi\(_{34}\)O\(_{44}\) and surrounded by ligands, Figure 1.26. The smaller crystal cluster predominated when the growth was for a short period but, upon leaving the crystals to grow for longer periods, the larger, less soluble cluster dominated. Water in the mixture causes hydrolysis, as tested on samples over different time scales confirmed. The indication of mixtures of clusters, further emphasises how difficult it is to produce one single crystal form in 100% purity.\(^2\)
Figure 1.26. Proposed crystal structures for BSS showing both the Bi$_{34}$O$_{44}$ (left) and the Bi$_{9}$O$_{7}$ (right) clusters.$^{227}$

Similarly CBS also presents challenges in regards to its structural characterization. Jin et al. solved the structure of CBS in dilute HCl (pH = 3), but it was proposed that CBS may rearrange from colloidal particles at neutral pH to 3-D polymers and sheets at low pH. Therefore, given the uncertainty regarding the structure of bismuth drugs on the market at the minute, there is a need for structurally well-defined and well characterised bismuth compounds.

Bismuth carboxylate compounds, such as BSS, CBS, or RBC, hydrolyse in the stomach forming insoluble bismuth salts or polymers, which are free to exert their bactericidal effects. BSS for example hydrolyses to release salicylic acid with the concomitant formation of bismuth oxychloride in gastric juice at pH 3.

**Anti-bacterial**

Question marks remain regarding bismuth’s interaction with bacteria in the stomach, its interaction with urease and localisation in the gastric regions.$^{227}$ As a result, in recent years there has been a lot of work conducted in the area of metallomics and metalloproteomics, which involves cellular tracking of metals in the body. The initial and most significant results of these early experiments has been identifying bismuths affinity for thiols and in particular, histidine and cysteine rich proteins, due to the strong interactions of bismuth and sulphur. Bismuth can interact with heat shock protein (Hsp) and nickel storage protein (Hpn) which play an important role in nickel homeostasis and energy supply in *H. pylori*.$^{228}$ Bismuth disrupts these processes upon binding.
Beyond *H. pylori*, the anti-bacterial properties of bismuth containing compounds have been demonstrated against a range of bacterial associated infections. Examples include, potassium bismuth tartrate, bismuth quinine iodide (Figure 1.27) and Iodobismitol in the early treatment of syphilis, bismuth subnitrate and bismuth citrate in the treatment of colitis, bismuth nitrate and BSS for effective and well documented treatment against enterotoxigenic *E. coli*, which is the principal bacterial cause of diarrhoea and bismuth oxide in wound infections medication. BSS has been proposed to significantly reduce the toxin secretory activity of *E. coli* resulting in the effective eradication and prevention of ‘traveller's diarrhoea’. BSS has also been seen to be effective in the treatment of another enteropathogen, *C. difficile*, having an *in vitro* minimum inhibitory concentration of 90% of growth of 128 μg mL⁻¹, while BSS exhibited noteworthy activity in an *in vivo* hamster model of *C. difficile* colitis.

**Figure 1.27.** Structures of bismuth containing antimicrobial agents: Potassium bismuth tartrate (A) and Bismuth quinine iodide (B).

Many studies have been carried out in an attempt to understand further the interactions and modes of actions of bismuth drugs the various bacteria. These interactions are varied and the outcomes based on environmental factors such as pH, pre-treatment, and the presence of other co administered agents.

The number of reports in relation to bismuth as an anti-bacterial agent in the literature however is low in comparison to other metal-based compounds of gold and silver, for example. Perhaps bismuth has become the forgotten metal, maybe due in part to the lack of understanding of many of its functions.

Regardless, recently there has been a resurgence of interest in the development of novel bismuth based compounds as anti-bacterial agents due to the fact that no new
antibiotics have come to market in recent years and the fact that bismuth has natural anti-bacterial activity.

**Anti-cancer Complexes**

Cancer is a major disease and cause of death worldwide. According to WHO, cancer related deaths are predicted to rise to over 21 million by 2030.²³² Platinum compounds have predominated as therapeutic and chemotherapy agents over the past 30 years. In spite of the success of platinum compounds, drawbacks such as toxicity, limited activity and resistance have also developed.²¹⁶ As a result, a vast amount of research has been undertaken in relation to development of non-platinum-based compounds as anti-cancer agents. Apart from radiometals, arsenic is the only other metal whose compound, Arsenic trioxide, is approved for treatment of cancer.²¹² It may also be surprising, given the success of this anti-cancer treatment, that there is a lack of reports into the development of novel Bi-based anti-cancer chemotherapeutics, given their general chemical similarities.

Bismuth thiosemicarbazone or thiocarbonohydrazone complexes (Figure 1.28) have been investigated as anti-cancer agents.²²¹, ²³³, ²³⁴ These agents are also reported to have anti-parasitic and anti-bacterial properties. The X-ray crystal structure of these bismuth complexes were solved, but they were found to be significantly less active (IC₅₀ = 46.2 μM) than cisplatin (1.2 μM) or the free ligand (12.3 μM) against leukaemia cells.²³⁵ Similar bismuth complex exhibited good cytotoxicity (IC₅₀ <10 μM) against colorectal, hepatocellular and cervical cancer cell lines.²³⁶

![Figure 1.28. Bismuth thiosemicarbazone (A) and bismuth thiocarbonohydrazone (B) complexes, developed as anti-cancer agents.](image)
Anti-leishmaniasis

Leishmaniasis is generally treated using antimonials as the first line of treatment. This treatment has given rise to high levels of resistance and problems with dosage associated toxicity. These problems have driven the discovery of new compounds for the treatment of leishmaniasis.\textsuperscript{237} The reasoning behind the use of bismuth as a potential metal based anti-leishmaniasis compound was due to its close proximity in the periodic table and similar biological chemistry to antimony. It was thought that bismuth would be an ideal candidate to target the same biomolecular targets as antimony. However, there are few bismuth based compounds, which have been developed and evaluated as potential anti-leishmanial drugs.\textsuperscript{238-240}

Andrews \textit{et al.} have explored and investigated the activity of a number of bismuth compounds for their activity against leishmaniasis.\textsuperscript{217} They hypothesised that bismuth complexes with a more thermodynamically stable bonds would be less labile and would result in better activity in biological systems.\textsuperscript{237} The results of these studies shows that bismuth could potentially be utilised as a metal based anti-leishmanial compound but properties are modulated by ligand choice.

6.2 Antimony

Antimony (Sb) is found in the pnictogen group (group 15) in the periodic table of elements. It is a lustrous grey metalloid, found mainly in nature as the sulphide mineral stibnite (Sb\textsubscript{2}S\textsubscript{3}). China has been the largest producer of antimony in the world, with most production from the Xikuangshan Mine in Hunan. Antimony is incorporated with lead and tin into alloys, and used in batteries, bullets, and plain bearings. Many common fire retardants in commercial and domestic environments comprise of antimony compounds.

Antimony is more electronegative than bismuth but less so than arsenic. The ground state electron configuration of neutral antimony is [Kr]\textsuperscript{4}d\textsuperscript{10}s\textsuperscript{2}p\textsuperscript{3}. Antimony has two stable isotopes, \textsuperscript{121}Sb and \textsuperscript{123}Sb, with natural abundance of 57.36\% and 42.64\% respectively. There are 35 radioisotopes of antimony, with \textsuperscript{125}Sb the longest-living having a half-life of 2.75 years. Like bismuth, antimony has two common oxidation states, Sb(III) and the more stable Sb(V).\textsuperscript{241}
Antimony in medicine

The first use of antimony in medicinal practices can be tracked back to the 16th century where it was used as an emetic drug. More recently, antimony containing drugs have become the first line drug used in the clinic for the treatment of leishmaniasis.

Anti-leishmaniasis

Originally Sb(III) compounds such as Tartar emetic (antimony potassium tartrate, APT) Figure 1.29, developed in 1910, were used as anti-leishmanial compounds but now pentavalent antimony-containing prodrugs, such as pentosam (sodium stibogluconate) and glucantine (meglumine antimoniate) are used as first-choice anti-leishmanial drugs in many countries due to their lower toxicity and more efficient therapeutic indices. It is likely that the Sb(V) serves only as the prodrug and is reduced to Sb(III) at, or near, the site of action. Detection of Sb(III) at the site of action when Sb(V) was administered, further confirms this theory. Little is understood about these drugs and how they exert their activity. It is quite remarkable that even after 50 years of clinical use, the MOA of antimony based drugs is still unknown. Like many treatments, resistance to this class of drug has arisen worldwide, and even to epidemic proportions in parts of India. The mechanism of resistance is also largely unknown due to the uncertainty of the MOA. Tackling Leishmania and resistance to antimonials is a priority for the WHO.

Other uses of antimonial drugs include APT for treatment of rough cough due to inhibition of septum excretion, antimony sodium suballate in the treatment of schitosomasis and antimony tartrate which is as active as Cisplatin and doxorubicin against various lung cancers in vitro. A series of Sb(V) compounds were observed to have anti-HIV properties, the activity of which is due to the disruptive interactions between viral glycoproteins and cell surface receptors.

Figure 1.29. Structure of Antimony Potassium Tartrate, APT.
The Mode of Action of Antimonials

Pentavalent antimonials have been used for more than six decades. It is uncertain if the active form is Sb(V) or Sb(III), but much evidence suggests that the formation of Sb(III) is required for toxicity.

Prodrug Model.

Pentavalent antimony Sb(V) behaves as a prodrug, undergoing biological reduction to its more toxic trivalent form of antimony, Sb(III). The site and mechanism of reduction remain disputed. Amastigotes, but not promastigotes, can reduce Sb(V) to Sb(III), hence, amastigotes are more susceptible to Sb(V).\(^{249}\) Other studies suggest the macrophage as the site of reduction.\(^{178}\) Generally, acidic pH and elevated temperature favour reduction of Sb(V) to Sb(III).\(^{250}\)

Trypanothione reductase (TR) and zinc-finger proteins are likely molecular targets of Sb(III) due to the thiophilic property of the metal. The TR system has a disulphide bond, T(SH)\(_2\) which it keeps in its reduced state, protecting the parasites from oxidative damage.\(^{251}\) Antimonials are selective for TR over the structural and mechanistically similar glutathione reductase (GR), due to differences in the disulphide binding sites. Trivalent antimonials interfere with T(SH)\(_2\) metabolism and prevents it from protecting the parasite from oxidative stress.\(^{251}\)

It has been shown that Sb(III) can replace Zn(II) in zinc-finger peptides.\(^{252}\) Given zinc finger peptides are involved in DNA replication and repair, this can have major implications on the viability of the *Leishmania* parasite.

Furthermore, it has been suggested that Sb(V) has anti-leishmanial activity by inhibiting macromolecular biosynthesis and disrupting energy metabolism.\(^{253}\) However, the specific targets in these pathways have not been identified.\(^{254}\)

Resistance to Antimonials

Resistance to pentavalent antimonial has arisen in some parts of the world. Resistance has been linked to the miss-use of the drug. Like most mechanisms, reduction of drug concentration within the parasite by decreased uptake or increased efflux of the drug, are the main reasons for antimonial resistance. Other potential resistance mechanisms include inhibition of drug reduction, inactivation of active drug, and gene amplification.\(^{255-258}\)
6.3 Gallium

Gallium (Ga) is a metal with an atomic number of 31. Being in group 13 on the periodic table of elements, it has properties similar to aluminium, indium, and thallium. Gallium does not occur naturally in its metallic form, but as gallium(III) compounds in trace amounts, too rare to serve as a primary source with an abundance of 17 ppm.\textsuperscript{259} The majority of gallium is produced as a by-product during processing of the ores of other metals. The ground state electron configuration of neutral gallium is [Ar]3d\textsuperscript{10}4s\textsuperscript{2}4p\textsuperscript{1}. Natural gallium (\textsuperscript{69}Ga) consists of two stable isotopes, \textsuperscript{69}Ga and \textsuperscript{71}Ga and two medically and commercially important radioisotopes, \textsuperscript{67}Ga and \textsuperscript{68}Ga.

Elemental gallium, at standard temperature and pressure, is soft and silvery blue in appearance. At lower temperatures it is a brittle solid. At temperatures greater than 29.7 °C it is a liquid and for this reason gallium is used as a temperature reference point. It is one of few metals that are near liquid at room temperature and melt in the hand.

The existence of the element gallium was first predicted in 1871 by the chemist Dmitri Mendeleev, and later discovered in 1875 by French chemist Paul Emile LeCoq de Boisbaudran, using spectroscopy.

Gallium has been used as a semiconductor dopant. It is predominantly used as an alloy in electronic microwave circuits, high-speed switching circuits, and infrared circuits. In the USA, more than 95% of gallium is consumed for electronic devices and integrated circuits.\textsuperscript{260} Gallium nitride as well as indium gallium nitride emit blue and violet light and hence can be used as light emitting diodes (LEDs).

**Gallium in medicine**

Gallium has no known natural physiologic function in the human body. However, certain characteristics enable it to interact with cellular processes. This has led to gallium compounds being used as diagnostic and therapeutic agents in cancer, metabolic bone disease and infectious disease.
**Gallium as an Iron Mimic**

Gallium has many similar properties to iron, allowing for it to bind with high avidity for certain iron-binding proteins. Substitution of iron with gallium disrupts natural function and may lead to adverse downstream effects for cells. Gallium is transported into cells by transferrin. Given tumour cells have a higher level of transferrin receptors than normal cells, gallium accumulates in high concentration within tumour cells and incorporated into metalloproteins in place of iron. This disrupts and inhibits the normal metabolic activity of the cell and ultimately causes cell death.

**Radiogallium Compounds as Tumour Imaging Agents**

$^{67}$Ga is used in standard nuclear medical imaging procedures, usually referred to as “gallium scans” It is a gamma-emitting isotope with a half-life of 3.3 days. The shorter-lived $^{68}$Ga is a positron-emitting isotope and is used for a small minority of diagnostic PET scans with a half-life of 68 minutes.

Early studies carried out on radioactive gallium, indicated that it localised in malignant cells when injected into a tumour bearing animals. Over the past two decades, $^{67}$Ga scanning have become the most frequently used tool for detection of residual disease or relapse in patients with Hodgkin’s and non-Hodgkin’s lymphomas following treatment with chemotherapy or radiotherapy. The level of $^{67}$Ga incorporation into lymphoma cells also reflects the proliferative rate and hence the aggression of the tumour. More recently, $^{67}$Ga scan has been replaced by the Positron Emission Tomography (PET) scan. However, the $^{67}$Ga scan remains an important diagnostic tool due to some limitations of the PET scans, namely the poor uptake in slow progressing tumours or false size depiction in areas where the tumours are surrounded by highly metabolic cells.

**Gallium as an Anti-cancer Agent**

Gallium can bind to DNA phosphate at low gallium concentrations and bind to nucleic acids at higher gallium concentrations. This binding causes DNA helix destabilisation and this effect may have potential anti-cancer activity. Gallium induces chromatin condensation *in vitro*, which may suggest it has a part to play in initiating cell apoptosis.
High concentrations of gallium can induce, modify and inhibit protein synthesis, as well as interact with ATPase and DNA polymerase proteins which have important roles in cell survival. Interaction with these proteins in cancerous cells can result in cell death and cancer inhibition.\textsuperscript{266-268}

The fact that \textsuperscript{67}Ga was observed to localize in tumour cells prompted the National Cancer Institute (NCI) to investigate the antineoplastic activities of salts of aluminium, gallium, indium, and thallium in tumour-bearing mice and rats.\textsuperscript{269} Gallium nitrate proved to be both highly effective in tumour growth suppression and least toxic. As a result, it was advanced for assessment of antitumor activity in phase I and phase II clinical trials.\textsuperscript{269} An apparent and major advantage for the use of gallium nitrate is that it does not produce myelosuppression, a condition in which bone marrow activity is decreased. Gallium nitrate therefore can be used in patients with low white blood cell or platelet counts.

\textit{In vitro} tests shown gallium exhibits synergistic effects when combined with fludarabine, hydroxyurea and gemcitabine, among others, Figure 1.30. This combination may prove fruitful as an anti-cancer treatment in clinical non-responders to traditional anti-cancer treatments.\textsuperscript{270, 271} Importantly, gallium nitrate does not appear to share cross-resistance with conventional chemotherapeutic drugs.

\textbf{Gallium Compounds as Antimicrobial Agents}

The potential for gallium to act as an antimicrobial agent derives from its ability to interfere with iron utilization by certain microorganisms. Gallium nitrate and transferrin-gallium were shown to block the iron-dependent extracellular growth of \textit{Mycobacterium tuberculosis} and \textit{Mycobacterium avium}.\textsuperscript{272} Gallium nitrate also inhibited \textit{in vitro} bacterial growth and biofilm formation through interference with iron uptake and signalling processes.

\textit{P. aeruginosa} infected mice were treated with gallium maltolate, Figure 1.30, and showed 100\% survival compared with 100\% mortality of non-treated mice.\textsuperscript{273} Currently, gallium nitrate is in a Phase II clinical trial being assessed for its efficacy in the treatment of lung acquired \textit{Pseudomonas}, in cystic fibrosis (CF) patients.\textsuperscript{274} CF is often resistant to standard antibiotic treatment.
Figure 1.3. Hydroxyurea (A), Gemcitabine (B) and Fludarabine (C), which exhibit synergistic effects when administered with gallium nitrate and the antimicrobial agent gallium maltolate (D).

7. Hydroxamic acids

Since the discovery by Lossen of oxalohydroxamic acid, the first hydroxamic acid in 1869 from the reaction of ethyl oxalate with hydroxylamine, until the early 1980’s, very little was known about the chemistry and bioactivity of this class of compound. However since the 1980’s, much useful information about synthesis and the bioactivity profile of many structurally diverse members of these compounds have been reported. With a broad range of pharmacological, toxicological and pathological properties, they are a very important class of compounds. They can be produced both synthetically and naturally but it is their rich biochemistry which has been the driving force behind the development of efficient methods of synthesis.\textsuperscript{275}

They have also been exploited as bacterial siderophores, due to their ability to efficiently bind iron and in turn developed as iron chelators.\textsuperscript{276, 277} Hydroxamic acid can act as metalloenzyme inhibitors and inhibit important enzymes such as urease, lipoxygenases and peptide deformylase for example. Furthermore, have shown activity against a range of conditions including cancer, malaria, tuberculosis, cardiovascular disease, HIV, Alzheimer disease and fungal infections, Figure 1.31.\textsuperscript{278-282}
Figure 1.31. Some hydroxamic acids used in modern medicine; Deferasirox (A) used as an important iron chelating agent in the treatment of iron overdose and SAHA (B) which is used as an effective HDAC inhibitor.

7.1 Chemistry of Hydroxamic Acids

Hydroxamic acids are weak acids, with the general formula RCONHOH. They possess a pKa of about 9 but may range from 7 to 11, depending on substituents. They are weaker acids than their corresponding carboxylic acid, but coordinate stronger with metals due to the formation of stable five membered ring structures. They are formed generally by reaction of hydroxylamine, or protected hydroxylamine, with an activated acyl group, such as acid chloride, ester or amides, Figure 1.32.

Figure 1.32. Formation of hydroxamic acids from activated acyl.

Free hydroxylamine is typically generated in situ from its salt by reaction with base. This then allows the nucleophilic nitrogen to attack the activated carbonyl resulting in the loss of a leaving group and formation of the hydroxamic acid. Furthermore, ethyl chloroformate can be used for the direct conversion of carboxylic acids to hydroxamic acids without the isolation of the activated mixed anhydride intermediate. Solid phases synthesis has also been employed to synthesise hydroxamic acids.283
There are two possible tautomeric forms which are possible for hydroxamic acids, the keto and the enol forms. The keto form predominates in acidic conditions and the enol exists mainly in basic conditions. Each tautomer can exist also in its Z/E isomeric form as highlighted by NMR studies. The E-isomer predominates in the solid phase while the Z-isomer exists mainly in aqueous conditions. Figure 1.33.

![Figure 1.33. Keto/Enol and are E/Z-isomers forms of hydroxamic acids.](image)

### 7.2 Metal Interactions with Hydroxamic Acids

A very important characteristic of hydroxamic acids is their ability to bind strongly to metal ions. They have shown diverse coordination behaviours and as a result they have been the subject of a number of comprehensive reviews.

The most typical binding form is as the mono-deprotonated hydroxamate. This binding occurs via the deprotonated hydroxyl group of the hydroxamic acid and the carbonyl oxygen to give a stable five membered ring. Another mode of coordination which may be observed is via the doubly deprotonated hydroximate form, in which both the hydroxyl and nitrogen moiety’s of the hydroxamic acid are deprotonated.

Binding of the ligand to metal via a bidentate bridging (O, µ-O) mode of action can occur, whereby the hydroxyl oxygen is bound to the metal centre as well as an adjacent metal centre.

Secondary binding moieties in hydroxamic acids could potentially generate interesting hydroxamato complexes. These secondary binding groups may act
as anchors and encourage coordination giving rise to structurally diverse complexes. Example of binding are shown in **Figure 1.34**.

![Figure 1.34](image.png)

**Figure 1.34.** The possible modes of binding of metals to hydroxamic acids; traditional bidentate \((O, O')\) hydroxamate binding (A), bidentate \((O, O')\) hydroximate binding, hydroximate bridging \((O, \mu-O')\) (C), and secondary site hydroximate binding (D).

### 7.3 Application of Hydroxamic Acids in Medicine

Excellent work has been carried out on design, structure-activity relationships (SAR), utilization, and potential of the hydroxamic acids in medicine. Hydroxamic acids are capable of the inhibition of a variety of enzymes, including ureases, ACE, and matrix metalloproteinases.\(^{289, 290}\) They can also participate as siderophores for Fe(III).\(^{291}\) In the biomedical sciences, hydroxamic acids are used in the design of therapeutics targeting cancer, cardiovascular diseases, HIV, Alzheimer’s, malaria, allergic diseases, tuberculosis, metal poisoning, bacterial infections and iron overload.\(^{292-294}\) Therefore hydroxamic acids possess a broad and varied potential applications in medicine.

**Metalloenzymes Inhibitors**

**Matrix Metalloproteinases (MMPs)**

MMPs are a family of zinc-containing enzymes capable of degrading many proteinaceous components of the extracellular matrix and activation of MMPs have been implicated in numerous diseases including arthritis, multiple sclerosis and metastasis associated with various human cancers.\(^{295}\) With their involvement in such a wide variety of maladies, MMPs have become a favourable drug target for medical use. MMP inhibition interferes with both endothelial and tumour cell invasion. Selective and nonselective MMP hydroxamic acid inhibitors such as Marimastat,
Batimastat and Prinomastat have been developed and advanced to clinical trials but with varied success, Figure 1.35.\textsuperscript{296}

![Figure 1.35. Some hydroxamic acid based MMP inhibitors; Marimastat (A), Batimastat (B) and Prinomastat (C).](image)

The hydroxamic acid-based MMP inhibitors are the most extensively studied class of MMP inhibitors due to the ability of the hydroxamate group to efficiently complex with the catalytic zinc (II) ion.

**Angiotensin converting enzyme (ACE)**

This zinc metalloenzyme is responsible for the formation of the octapeptide angiotensin II. ACE is an integral membrane glycoprotein that has an important role in the control of blood pressure and electrolyte homoeostasis. Inhibition of ACE decreases levels of angiotensin II in the body leading to a decrease in blood pressure.\textsuperscript{297}

Since their introduction, ACE inhibitors have been increasingly prescribed for the treatment of hypertension and a variety of other cardiovascular disorders. The clinically useful inhibitors are effective zinc-binding ligands such as hydroxamic acids. Idrapril, batimastat and TAPI-2 were identified as ACE inhibitors, Figure 1.36.\textsuperscript{298}

![Figure 1.36. Idrapril (A) and TAPI-2 (B) hydroxamic acid inhibitors of ACE.](image)
Urease enzymes, as discussed previously, catalyse the hydrolysis of urea to ammonia, carbamate, and subsequently carbon dioxide and ammonia. This hydrolysis therefore results in an increase in pH, a requirement for survival of some bacteria such as *H. pylori*. The determination of the X-ray structure of urease from a bacterial source, *Klebsiella aerogenes*, provided the first three-dimensional structural model of the enzyme, identifying a di-nickel nuclear active site.

Hydroxamic acids were found to be potent and specific inhibitors of urease. The mode of the inhibition involves coordination of the inhibitor with the di-nickel centre in the enzyme. Extensive efforts have attempted to develop hydroxamic acids as therapeutic agents against urease-related diseases. Well-known hydroxamic acid urease inhibitor are shown in **Figure 1.37**.

![Figure 1.37. Hydroxamic acids inhibitors of urease enzyme. Acetohydroxamic acid (A), benzo hydroxamic acid (B) and salicyl hydroxamic acids (C).](image)

**Figure 1.37.** Hydroxamic acids inhibitors of urease enzyme. Acetohydroxamic acid (A), benzo hydroxamic acid (B) and salicyl hydroxamic acids (C).

**Peptide deformylase (PDF)**

Polypeptide deformylase (PDF) is a novel anti-bacterial biomolecular target. PDF activity was first described in 1968 and shows a high level of homology at the active site among bacterial genomes sequenced to date. The identification of bacterial, malarial and human PDF (HsPDF which is over expressed in cancer cells of breast, colon and lung, for example) provides a remarkable prospect to develop drugs for anti-bacterial, anti-malarial and anti-cancer use. PDF is one of the few post translational drug targets. PDF is an attractive anti-bacterial drug target, as it is present in all bacteria and is essential for bacterial growth. The methionine formylation and deformylation cycle is not involved in eukaryotic cytoplasmic protein synthesis, hence providing an element of selectivity.

Bacterial protein synthesis is initiated by formylation of methionyl tRNA (Met-tRNA) by methionyl tRNA formyltransferase (FMT) to produce formyl-methionyl
tRNA (F-Met-tRNA). The F-Met moiety is incorporated at the N-terminal of newly synthesized polypeptides. Polypeptide deformylase (PDF) then deformylates primary peptides to produce Met-polypeptides, which are further processed by methionine amino peptidase (MAP) to yield the mature peptide and free methionine, which is recycled.\textsuperscript{302} Therefore PDF and MAP are essential for bacterial growth. This series of reactions is referred to as the methionine cycle, Figure 1.38.

![Figure 1.38. Overview of the methionine cycle in which PDF plays an important role. PDF is required for MAP activity.](image)

Much is known about the active site of the enzyme, given its homology with other well studied metalloenzymes.\textsuperscript{303} PDF consists of a Fe\textsuperscript{2+} centred active site. The iron centre of the active site can be replaced by zinc (Zn-PDF) or nickel (Ni-PDF) but demonstrate dramatically less activity than the native iron deformylase (Fe-PDF), Figure 1.39.\textsuperscript{304} The ferrous ion in PDF is unstable, with easy oxidation to ferric ion resulting in inactivation of the enzyme.\textsuperscript{305}
Figure 1.39. General overview of the mechanism of deformylation of peptides. The process includes several steps including formation of an enzyme substrate complex, nucleophilic addition of the metal-coordinated water/hydroxide to the carbonyl carbon of the formyl group, proton transfer, C-N bond breaking, final release of the product and rehydration of the active site.\textsuperscript{304}

Studies have been carried out in an attempt to understand this metal dependence activity of PDF.\textsuperscript{306, 307} Overall, the tertiary structure of the different metal centred protein were identical and only minor differences were observed in the domain of the active site. Zn was found to bind more tightly than the other two metals. The difference in activity is likely a result of the tighter binding of the Zn$^{2+}$ ion as compared to Ni$^{2+}$ and Fe$^{2+}$, preventing its essential transition from a tetrahedral to a penta-coordinated metal centre.\textsuperscript{308}

Hydroxamic acids have been identified as effective inhibitors of PDF. Actinonin is a known hydroxamate containing inhibitor of PDF. The hydroxamate group of actinonin evidently acts as the chelating group to bind the metal ion of the enzyme and it is this moiety that is primarily responsible for the potent PDF inhibitory activity. It is not clear why hydroxamate is the preferred metal-binding group, but polar interactions between the hydroxamate and enzyme residues in the vicinity of the metal binding site could contribute to the high affinity.\textsuperscript{309, 310}
**Histone Deacetylases (HDACs)**

Protein acetylation balance is regulated by histone acetyltransferases (HAT) and deacetylases (HDACs), and plays an important part in post-translational modifications. HDACs play very important roles in the regulation of multiple processes, from gene expression to protein activity. The dysregulation of HDACs is implicated in many diseases, such as cancer, autoimmune and psychiatric diseases.\textsuperscript{311-313} To date, 18 HDACs have been identified in mammals, subdivided into four classes, I-IV. The pharmacophore model of HDACs inhibitors (HDACi) consists of three structural domains: a zinc binding group (ZBG) that interacts with the zinc ion at the catalytic pocket, a linker that occupies a hydrophobic channel and a cap group that interacts with the surface of the enzyme. So far, hydroxamic acid based broad-spectrum HDACi include, SAHA, belinostat and panobinostat (Figure 1.40) have been approved by FDA for the treatment of cutaneous T-cell lymphoma, T-cell lymphoma, and multiple myeloma.\textsuperscript{314}

![Figure 1.40. FDA approved hydroxamic acid based HDACi; SAHA (A), belinostat (B) and panobinostat (C).](image)

**Iron Chelators**

There has been much research into the natural uses of hydroxamic acids as siderophores. Fe(III) has poor solubility at physiological conditions, resulting in its decreased availability to bacteria. In the early log growth stages of bacteria, iron is required for many vital processes including electron and oxygen transport. To circumvent the problem of poor availability, bacteria and microbes generally obtain their iron in solubilised form through the use of siderophores.\textsuperscript{315} Siderophores have high affinity and selectivity for iron.\textsuperscript{316} When hydroxamic acids bind iron, cell surface receptors can recognise the functional groups of the hydroxamic acid,
allowing transport of the siderophore into the cell where it can release the iron and it can be utilised by the cell. Following on from this discovery that hydroxamic acids efficiently bind iron, their use as iron chelators was examined. Deferoxamine is a hydroxamic acid iron chelator, which has been used to combat iron overdose in diseases where blood transfusions are required, such as thalassaemia, a condition characterised by the inability of the body to produce enough haemoglobin. Deferoxamine chelates the excess iron which is seen to accumulate after blood transfusions. If left untreated this iron can have toxic implications. Deferoxamine, Figure 1.41, readily binds Fe(III) and is water soluble so is then excreted in the urine of patients.\textsuperscript{317}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{deferxamine.png}
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Chapter 1


Chapter 1


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Chapter 2

Materials and Methods
1. Instrumentation

Infrared Spectroscopy

Infrared spectroscopic (IR) measurements were obtained using a Nicolet iS10 Fourier-transform infrared spectroscopy (FT-IR) (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The infrared spectra were recorded from 400-4000 wavenumbers (cm\(^{-1}\)) and analysed using OMNIC software (version 9.6, Thermo Fisher Scientific). Signals are assigned using the following abbreviations; broad (br), strong (s), and weak (w).

Elemental Analysis

Elemental analysis (EA) was carried out on Carbon, Hydrogen, Nitrogen and Chlorine, as performed by Ms. Ann Connolly using an Exeter Analytical CE 440 elemental analyser (Exeter Analytical Ltd, University of Warwick Science Park, The Venture Centre, Coventry, UK) in the micro-analytical laboratory at the School of Chemistry and Chemical Biology, University College Dublin.

\(^1\)H and \(^{13}\)C Nuclear Magnetic Resonance Spectroscopy

\(^1\)H and \(^{13}\)C Nuclear magnetic resonance (NMR) experiments were performed using a Bruker DPX-400 spectrometer, (Bruker, Billerica, Massachusetts, USA) at room temperature (25 °C). The spectra recorded were analysed using MestRelvaNova software (version 6.0.2-5475, Mestrelab, Santiago de Compostela, Spain). Chemical shifts (\(\delta\)) are quoted in parts per million (ppm) using residual protons in the indicated solvents as internal standards and coupling constants (\(J\)) quoted in hertz (Hz). The residual undeuterated \(d_4\)-methanol (MEOD) signal at 3.31 ppm, \(d_6\)-dimethyl sulfoxide (DMSO-\(d_6\)) signal at 2.505 ppm, chloroform signal at 7.26 ppm and \(D_2O\) signal at 4.79 were used as internal references. Proton multiplicities are assigned using the following abbreviations; singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m).\(^1\)
Mass spectrometry

Electron spray ionisation (ESI) mass spectrometry (MS) experiments were performed using an Advion Expression CMS instrument (Advion, Ithaca, New York, USA) and analysed using Advion Data Express software (version 1.2.17.2, Advion, Ithaca, New York, USA) at the Pharmaceutical and Medicinal Chemistry Department, Royal College of Surgeons in Ireland. A sample (10 μL of concentrations of 0.1 mg mL\(^{-1}\)) was injected in a running buffer (MeOH:H\(_2\)O:CH\(_2\)O\(_2\), 90:9:1). The mass spectrometry data were acquired both in positive (+) and negative (-) ion modes.

Single crystal X-ray diffraction methods

X-ray crystallographic analysis was performed by Dr. Brendan Twamley at the School of Chemistry, Trinity College Dublin. The X-ray intensity data were measured at using an Oxford Cryosystems (Oxford Cryosystems Ltd, Oxford, UK) low temperature device using a MiTeGen micromount (MiTeGen, Itacha, New York, USA). Bruker APEX software was used to correct for Lorentz and polarization effects. Collection parameters and exposure time are highlighted in the appropriate sections.

X-ray crystallographic analysis was also performed by Dr. Helge Müller-Bunz at the School of Chemistry & Chemical Biology, University College Dublin. The results were collected using a Rigaku Oxford Diffraction (Rigaku Europe, Kent, England) Super-Nova A diffractometer fitted with an Atlas detector and times are highlighted in the appropriate sections.

General considerations and materials

All commercially available reagents and solvents were purchased from Sigma-Aldrich (Sigma-Aldrich Ireland Ltd., Arklow, Co Wicklow) with the exception of anhydrous THF which was supplied in house by a solvent purification system, Pure Solv™ Micro, also Sigma-Aldrich. All reagents were used as supplied without further purification unless otherwise stated. All reactions which are described as ‘anhydrous’ were conducted in over-dried glassware (> 120 °C), in anhydrous
solvents under an inert N\textsubscript{2} or Argon atmosphere. Deionised H\textsubscript{2}O is always used in the cases where H\textsubscript{2}O is mentioned.

Analytical thin layer chromatography (TLC) was carried out using pre-coated silica (\textit{Merck DC silica gel 60 F254}) plates and visualised under ultraviolet light (254 and 360 nm).
2. Organic Chemistry Synthesis

Synthesis of 2-NH$_2$-Pha$^2$ (1)

To a solution of methyl 2-aminobenzoate (5 g, 33 mmol) in MeOH (55 ml), was added a solution of hydroxylamine hydrochloride (4.65 g, 66.7 mmol) and NaOH (5.36 g, 133.5 mmol) in H$_2$O (33 mL). The resulting pale yellow solution was stirred at rt for 72 h. After this period the solution was carefully acidified using 6M HCl to pH 5.5. The solvent was removed in vacuo. MeOH (70 mL) was added and NaCl filtered off. The solution was brought to dryness in vacuo. The yellow solid was collected and purified by recrystallization from H$_2$O, filtered and dried under vacuum to give 1. Yield 3.6 g (70%).$^2$

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3404, 2964 (s, N-H), 1639 (s, C=O), 1559 (s, C-N). Elemental analysis; (C$_7$H$_8$N$_2$O$_2$) Calc (Found): C 55.25 (55.10), 5.30 (5.20), N 18.41 (18.39).

$^1$H NMR (400 MHz, DMSO-$d_6$, 25 °C): $\delta_H$ 10.91 (s, 1H, OH), 8.84 (s, 1H, NH), 7.31 (dd, $^3J = 7.8$, $^4J = 1.2$ Hz, 1H, ArH), 7.13 (m, 1H, ArH), 6.69 (dd, $^3J = 8.2$, $^4J = 1.0$ Hz, 1H, ArH), 6.56-6.35 (m, 1H, ArH), 6.23 (s, 2H, NH$_2$). $^{13}$C NMR (100 MHz, DMSO-$d_6$, 25 °C): $\delta_C$ 167.0, 149.2, 131.6, 127.5, 116.2, 114.7, 113.1. MS (ESI+) $m/z$: ([M+H]$^+$) 152.1.
Synthesis of 2-Pyha\(^{3}\) (5)

To a solution of ethyl-2-picolinate (5 g, 4.46 mL, 33 mmol) in MeOH (100 mL), was added a solution of hydroxylamine hydrochloride (4.6 g, 66 mmol) and NaOH (5.26 mg, 132 mmol) in H\(_2\)O (25 mL). The resulting solution was stirred at rt for a further 72 h after this period the solution was carefully acidified using 5% HCl to pH 5.5. The solvent was removed \textit{in vacuo}. MeOH (70 mL) was added and NaCl filtered off. The solution was brought to dryness \textit{in vacuo}. The white solid was collected and purified by recrystallization from H\(_2\)O water, filtered and dried under vacuum to give 5. Yield 3.6 g (80\%).\(^{3}\)

IR \(\nu_{\text{max}}\) (cm\(^{-1}\)): 1651 (C=O), 1567 (s, C-N). Elemental analysis; (C\(_6\)H\(_6\)N\(_2\)O\(_2\)) Calc. (Found): C 52.17 (52.02), H 4.38 (4.60), N 20.28 (20.01) \%.\(^{1}\)H NMR (400 MHz, DMSO-\(d_6\), 25 °C): \(\delta_H\) 11.44 (s, 1H, \(OH\)), 9.10 (s, 1H, \(NH\)), 8.60 (m, 1H, \(ArH\)), 7.99-7.95 (m, 2H, \(ArH\)), 7.60-7.53 (m, 1H, \(ArH\)). \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\), 25 °C): \(\delta_C\) 161.3, 150.1, 148.5, 137.6, 126.3, 121.8. MS (ESI+) \(m/z\): ([M+H]\(^{+}\)) 138.1.
Synthesis of \(N\)-hydroxy-\(N\)-phenylformamide (HFA)\(^4\) \((10)\)

\[
\begin{align*}
\text{H} & \quad \text{O} \\
\text{N} & \quad \text{OH}
\end{align*}
\]

To glacial acetic acid (20 mL) containing a nitrosobenzene (2.14 g, 20 mmol) was added glyoxylic acid monohydrate (4.6 g, 50 mmol) dissolved in glacial acetic acid (10 mL). The mixture was stirred at rt for 1 h. After treatment with \(\text{NH}_4\text{HCO}_3\) (50 mmol), the mixture was brought to dryness \textit{in vacuo}. The residue was dissolved in 1 M \(\text{NaOH}\) (50 mL) and the resulting solution was washed with \(\text{Et}_2\text{O}\) (3 x 20 mL). The pH of the aqueous phase was adjusted with 5 M \(\text{H}_3\text{PO}_4\) to pH 5.5 in an ice bath, followed by further extraction into \(\text{Et}_2\text{O}\) (3 X 40 mL). The combined ethereal fractions were dried over \(\text{Na}_2\text{SO}_4\) and again brought to dryness \textit{in vacuo} to give the crude product. Recrystallization from benzene:hexane (3:1, v/v) afforded \(10\) as colourless crystalline plates. Yield 2.4 g (90\%).\(^4\)

IR \(\nu_{\text{max}}\) (cm\(^{-1}\)): 1655 (s, C=O). Elemental analysis; \((\text{C}_7\text{H}_7\text{NO}_2)\) Calc. (Found): C 61.31 (61.09), H 5.15 (5.05), N 10.21 (10.02) %. \(^1\)H NMR (400 MHz, \(\text{D}_2\text{O}, 25^\circ\text{C}\)): \(\delta\) \(H\) 8.50 (s, 1H), 7.48-7.32 (m, Ar\(H\), 5H). \(^1\)H NMR (400 MHz, \(\text{DMSO-d}_6, 25^\circ\text{C}\)): \(\delta\) \(H\) 10.94 (s, 0.4H), 10.48 (s, 0.6H), 8.75 (s, 0.6H), 8.62 (s, 0.4H), 7.70 (s, 1H), 7.40 (s, 3H), 7.20 (s, 1H). \(^{13}\)C NMR (100 MHz, \(\text{D}_2\text{O}, 25^\circ\text{C}\)): \(\delta\) 159.7, 130.1, 128.0, 120.5.
Synthesis of Oxonane-2,9-dione \(^5\) \((16)\)

A solution of suberic acid (5 g, 28.7 mmol) in acetic anhydride (10 mL) was stirred at reflux for 1 h. After cooling to rt, the solvent was removed \textit{in vacuo}. The resulting pale yellow solid was recrystallized from anhydrous acetonitrile. The white solid was filtered, washed and dried under vacuum to give a moisture sensitive \(16\). Yield 4.3 g (95\%).\(^5\)

\(^1\)H NMR (400 MHz, CDCl\(_3\), 25 °C): \(\delta_H\) 2.44-2.33 (m, 4H, \(CH_2\)), 1.66-1.52 (m, 4H, \(CH_2\)), 1.40-1.26 (m, 4H, \(CH_2\)).

The compound was too unstable to perform any other analysis. Further reactions were carried out immediately.
Synthesis of 8-oxo-8-(phenylamino)octanoic acid

Aniline (600 mg, 6.4 mmol) was added to a stirred solution of 16 (1 g, 6.4 mmol) in anhydrous THF (10 mL). To the clear solution ca. 100 mL of H₂O was added and the solution stirred at rt for 30 min. The resulting white precipitate collected, washed with cold water and subsequently recrystallized from H₂O at rt to give 17, as a white solid. Yield 1 g (62%).

IR ν max (cm⁻¹): 3303 (s), 1689 (s, C=O), 1660 (s, C=O).

¹H NMR (400 MHz, DMSO-d₆, 25 °C): δ H 12.03 (s, 1H, OH), 9.89 (s, 1H, NH), 7.64 (d, ³J = 7.7 Hz, 2H, ArH), 7.33 (t, ³J = 7.9 Hz, 2H, ArH), 7.07 (t, ³J = 7.4 Hz, 1H, ArH), 2.40–2.22 (m, 4H, CH₂), 1.61 (m, 4H, CH₂), 1.45–1.30 (m, 4H, CH₂).

¹³C NMR (100 MHz, DMSO-d₆, 25 °C): δ C 174.5, 171.2, 139.3, 128.6, 122.9, 119.0, 36.3, 33.6, 28.4, 28.3, 25.0, 24.4. MS (ESI+) m/z: ([M+Na]⁺) 272.5.
To a 0 °C solution of 17 (470 mg, 1.89 mmol) in anhydrous THF (10 mL), was added ethyl chloroformate (0.25 mL, 2.52 mmol) and triethylamine (0.4 mL, 2.73 mmol). The mixture was stirred for 10 min. To this was added a solution of hydroxylamine hydrochloride (220 mg, 3.15 mmol) and KOH (180 mg, 3.15 mmol) in MeOH (10 mL). The resulting suspension was stirred at rt for 15 min. The solvent was removed in vacuo and the residue recrystallized from ACN. The white solid was filtered, washed and dried under vacuum to give 18. Yield 320 mg (64%).

IR ν max (cm⁻¹): 3190, 1654 (s, C=O), 1618 (s, C=O), 1597 (s, C-N), 1545 (s, C-N).

Elemental analysis; (C_{14}H_{20}N_{2}O_{3}) Calc. (Found): C 63.62 (62.79), H 7.63 (7.80), N 10.60 (10.34) %.

¹H NMR (400 MHz, DMSO-d₆, 25 °C): δH 10.30 (s, 1H, OH), 9.81 (s, 1H, NH), 8.62 (s, 1H, NH), 7.58 (d, ³J = 7.7 Hz, 2H, ArH), 7.28 (t, ³J = 7.9 Hz, 2H, ArH), 7.01 (t, ³J = 7.4 Hz, 1H, ArH), 2.29 (m, 2H, CH₂), 1.94 (m, 2H, CH₂), 1.62-1.45 (m, 4H, CH₂), 1.34–1.22 (m, 4H, CH₂).

¹³C NMR (100 MHz, DMSO-d₆, 25 °C): δC 171.2, 169.0, 139.3, 128.6, 122.9, 119.0, 36.3, 32.2, 28.4, 25.0. MS (ESI-) m/z: ([M-H]⁻) 263.4.
Methyl 8-((3-nitrophenyl)amino)-8-oxooctanoate (19)

![Chemical Structure]

To a boiling solution of 3-nitroaniline (290 mg, 2 mmol) in anhydrous DMF (15 mL) was added triethylamine (0.3 mL, 2.15 mmol). This solution was stirred for 20 min at reflux. To this solution was added methyl 8-chloro-8-oxooctanoate (0.3 mL, 2.11 mmol). The reaction was stirred at reflux for 3 h and cooled to rt. H₂O (50 mL) was added and the solution was stirred. A yellow solid was filtered from the solution, washed with water, and dried under vacuum to give 19. Yield 430 mg (70%).

IR ν_max (cm⁻¹): 1709 (s, C=O), 1691 (s, C=O). ¹H NMR (400 MHz, DMSO-d₆, 25 °C): δ_H 10.38 (s, 1H, NH), 8.65 (t, J= 2.1 Hz, 1H, ArH), 7.89 (dd, J= 8.1, J= 1.9 Hz, 2H, ArH), 7.59 (t, J= 8.2 Hz, 1H, ArH), 3.57 (s, 3H, OCH₃), 2.32 (m, 4H, CH₂), 1.66-1.45 (m, 4H, CH₂), 1.37-1.22 (m, 4H, CH₂). ¹³C NMR (100 MHz, DMSO-d₆, 25 °C): δ_C 173.3, 172.0, 147.9, 140.4, 130.1, 124.9, 117.5, 113.0, 51.1, 36.3, 33.2, 28.2, 24.7, 24.3. MS (ESI-) m/z: ([M-H]⁻) 307.5.
8-((3-nitrophenyl)amino)-8-oxooctanoic acid  (20)

To 19 (1 g, 3.25 mmol) in EtOH (50 mL) was added NaOH (520 mg, 13 mmol) in H₂O (10 mL). The reaction was monitored by TLC (10% MeOH in DCM). The reaction stirred at rt for 24 h. The solvent was removed in vacuo. The residue was dissolved in H₂O (50 mL) and washed with EtOAc (2 x 10 mL). The organic layers were discarded and the pH of the aqueous layer adjusted to pH 1.5 with 6M HCl. The solvent was reduced to ca. 50% in vacuo resulting in the precipitation of the product as an orange solid, which was filtered, washed and dried under vacuum to give 20. Yield 765 mg (80%).

IR ν max (cm⁻¹): 3343, 2980, 1683 (s, C=O).

¹H NMR (400 MHz, DMSO-d₆, 25 °C): δH 11.99 (s, 1H, OH), 10.38 (s, 1H, NH), 8.65 (t, ³J = 8.2 Hz, 1H, ArH), 7.89 (m, 2H, ArH), 7.59 (t, ³J = 8.2 Hz, 1H, ArH), 2.27 (m, 4H, CH₂), 1.69-1.42 (m, 4H, CH₂), 1.37-1.25 (m, 4H, CH₂).

¹³C NMR (100 MHz, DMSO-d₆, 25 °C): δC 174.4, 172.0, 147.9, 140.4, 130.1, 124.9, 117.5, 113.0, 36.3, 33.6, 28.3, 28.3, 24.7, 24.3. MS (ESI⁻) m/z: ([M-H⁻]) 293.4.
$N^{1}$-(benzyloxy)-$N^{8}$-(3-nitrophenyl)octanediamide \quad (21)

![Chemical Structure](image)

To **20** (230 mg, 0.92 mmol) in anhydrous THF (25 mL) was added ethyl chloroformate (0.115 mL, 1.12 mmol) and triethylamine (0.18 mL, 1.2 mmol) and stirred at rt for 1 h under Argon. To this was added a solution of $O$-benzylhydroxylamine hydrochloride (240 mg, 1.5 mmol) and 25% w/v Sodium methoxide (0.24 mL 1.5 mmol) in anhydrous MeOH (10 mL). This was stirred at rt for 24 h under Argon. The reaction was monitored by TLC (10% MeOH in DCM). The solvent was removed \textit{in vacuo}. The waxy solid was dissolved in saturated aqueous solution of Na$_2$CO$_3$ (15 mL) and extracted with EtOAc (2 x 30 mL). The organic layers were dried over Na$_2$SO$_4$ and the solvent removed \textit{in vacuo} to afford a solid. Recrystallization from EtOAc afforded **21**. Yield 220 mg (60%).

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3257, 2980, 1667 (s, C=O). $^1$H NMR (400 MHz, DMSO-$d_6$, 25 °C): $\delta$H 10.95 (s, 1H, NH), 10.38 (s, 1H, NH), 8.65 (t, $^3J$ =8.2 Hz, 1H, ArH), 7.94-7.86 (m, 2H, ArH), 7.59 (t, $^3J$ = 8.2 Hz, 1H, ArH), 7.44-7.31 (m, 5H, ArH), 4.76 (d, $^3J$ = 8.7 Hz, 2H, CH$_2$), 2.40-2.30 (m, 2H, CH$_2$), 1.96 (m, 2H, CH$_2$), 1.68-1.41 (m, 4H CH$_2$), 1.26 (m, 4H, CH$_2$). $^{13}$C NMR (100 MHz, DMSO-$d_6$, 25 °C): $\delta$C 172.0, 169.3, 147.9, 140.4, 136.1, 130.1, 128.7, 128.2, 124.9, 117.5, 113.0, 76.7, 36.3, 32.2, 28.3, 24.7. MS (ESI-) m/z: ([M-H]$^-$) 398.6.

Single crystals of $N^{1}$-(benzyloxy)-$N^{8}$-(3-nitrophenyl)octanediamide were isolated from slow evaporation of an EtOAc solution. These were analysed and characterised by X-ray crystallography.
Attempt to Synthesise $N^1$-(3-aminophenyl)-$N^8$-hydroxyoctanediamide (22)

![Chemical Structure](image)

To activated Pd/C 10% wt (100 mg, 1 mmol) in EtOH (10 mL) was added 21 (399 mg, 1 mmol) in EtOH (50 mL) under Argon. The reaction was stirred at reflux for 5 min. Hydrazine monohydrate (0.35 mL, 7 mmol) was added drop wise. The reaction stirred for 5 h and monitored by TLC (10% MeOH in DCM). The hot solution was filtered through celite and the solvent removed *in vacuo*. An attempted recrystallization was performed from EtOAc, however the product was not obtained in sufficient purity or yield.
Methyl 8-((2-nitrophenyl)amino)-8-oxooctanoate  (23)

\[
\begin{align*}
\text{Methyl 8-chloro-8-oxooctanoate (0.3 mL, 2.11 mmol) was added to a solution of 2-nitroaniline (276 mg, 2 mmol) in toluene (25 mL) at 0 °C. The solution was allowed to stir and warm slowly to rt and was then heated to 80 °C for 24 h. The reaction was monitored by TLC (10% EtOAc in DCM). The product was extracted with EtOAc and washed with water, brine and the combined organic layers were dried over MgSO}_4, \text{ and the solvent removed in vacuo. The white solid was washed and dried under vacuum to give 23. Yield 400 mg (65%).}
\end{align*}
\]

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3398, 2937, 1730 (s, C=O), 1705 (C=O).

$^1$H NMR (400 MHz, DMSO-$d_6$, 25 °C): $\delta_H$ 10.25 (s, 1H, NH), 7.92 (dd, $^3J = 8.2$, $^4J = 1.4$ Hz, 1H, ArH), 7.69 (m, 1H, ArH), 7.59 (m, 1H, ArH), 7.37-7.39 (m, 1H, ArH), 3.58 (s, 3H, OCH$_3$), 2.31 (m, 4H, CH$_2$), 1.54 (m, 4H, CH$_2$), 1.35-1.22 (m, 4H, CH$_2$).
Methyl 8-((2-aminophenyl)amino)-8-oxooctanoate (24)

Compound 23 (1.3 g, 4.2 mmol) in EtOH (75 mL) was degassed with Argon. To this was added activated Pd/C 10% wt (300 mg, 3 mmol) and the reaction brought to reflux. Hydrazine monohydrate (1.5 mL, 28.6 mmol) was added drop-wise. The reaction was stirred at reflux for 5 h under Argon and monitored by TLC (10% MeOH in DCM). Upon completion the hot solution was filtered through celite. The solvent was removed in vacuo and the residue recrystallized from EtOAc, the golden solid filtered, washed and dried under vacuum to give 24. Yield 1.025 g (85%).

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3281, 2980, 1721 (s, C=O), 1644 (s, C=O).

$^1$H NMR (400 MHz, DMSO-$d_6$, 25 °C): $\delta$H 9.13 (s, 1H, NH), 7.14 (d, $^3J = 7.6$ Hz, 1H, ArH), 6.91-6.86 (m, 1H, ArH), 6.71 (dd, $^3J = 8.0$, $^4J = 1.2$ Hz, 1H, ArH), 6.53 (m, 1H, ArH), 4.82 (s, 2H, $NH_2$), 3.58 (s, 2H, OCH$_3$), 2.31 (m, 3H, CH$_2$), 2.07-1.92 (m, 1H, CH$_2$), 1.53 (m, 4H, CH$_2$), 1.36-1.19 (m, 4H, CH$_2$).

$^{13}$C NMR (100 MHz, DMSO-$d_6$, 25 °C): $\delta$C 173.3, 171.1, 141.9, 125.7, 125.2, 123.5, 116.1, 115.8, 51.2, 35.6, 33.2, 28.3, 28.2, 25.1, 24.3. MS (ESI+) m/z: ([M+H]$^+$) 279.4, ([M+Na]$^+$) 301.4.

Crystals suitable for analysis by X-ray crystallography were isolated from slow evaporation of the EtOAc solution at rt.
3. Inorganic Chemistry Synthesis

Synthesis of \([\text{Bi}_2(\text{Bha}_{-1\text{H}})_2(\mu-\text{Bha}_{-1\text{H}})_2(\eta^2-\text{NO}_3)_2] \)  \(\text{(2)}\)

\[\text{Bi(NO}_3)_3\cdot5\text{H}_2\text{O} \text{ (485 mg, 1 mmol) and Bha (288 mg, 2.1 mmol) were dissolved in EtOH (50 mL) and the mixture was stirred for 30 min and subsequently heated to reflux for 5 h. The clear solution was allowed to cool and then filtered. The filtrate was condensed in vacuo to ca. 10 mL. On the addition of H}_2\text{O (5 mL) a white solid precipitated, which was filtered, and dried under vacuum to give 2. Yield 410 mg (77%).}\]

IR \(\nu_{\text{max}}\) (cm\(^{-1}\)): 1592 (s, C=O), 1559 (s, C–N), 1385 (s, N–O). Elemental analysis; (C\(_{28}\)H\(_{24}\)Bi\(_2\)N\(_6\)O\(_{14}\)) Calc. (Found): C 30.95 (30.84), H 2.23 (1.91), N 7.74 (7.45) %.

\(^1\text{H NMR}\) (400 MHz, CD\(_3\)OD, 25 °C): \(\delta_H\) 7.85 (m, 2H, Ar\(\text{H}\)), 7.56 (m, 1H, Ar\(\text{H}\)), 7.52-7.46 (m, 2H, Ar\(\text{H}\)). \(^{13}\text{C NMR}\) (100 MHz, CD\(_3\)OD, 25 °C): \(\delta_C\) 133.0, 131.5, 130.1, 128.0. MS (ESI+) \(m/\text{z}\): ([Bi(NO\(_3\))\(^2+\)] 271.8, ([Bi(Bha\(_{-1\text{H}}))\(^2+\)] 345.7, ([Bi(NO\(_3\))(Bha\(_{-1\text{H}}))]\(^+\)) 407.7, ([Bi(Bha\(_{-1\text{H}}))]\(^+\)) 480.7.

Single crystals of \([\text{Bi}_2(\text{Bha}_{-1\text{H}})_2(\mu-\text{Bha}_{-1\text{H}})_2(\eta^2-\text{NO}_3)_2]\) were isolated upon recrystallization of 2 from methanol.
Synthesis of [Bi(NO$_3$)$_3$(Sha$_{-1}$H)$_2$]$_n$ (3)

Bi(NO$_3$)$_3$·5H$_2$O (905 mg, 1.86 mmol) and Sha (597 mg, 3.9 mmol) were dissolved in EtOH (80 mL) and the mixture stirred for 30 min and subsequently heated to reflux for 5 h. The clear solution was allowed to cool and then filtered. The clear filtrate was condensed in vacuo to ca. 10 mL. On the addition of H$_2$O (5 mL) a white solid precipitated, which was filtered and dried under vacuum to give 3. Yield 750 mg (60%).

IR $\nu_{\text{max}}$ (cm$^{-1}$): 1604 (s, C=O), 1560 (s, C-N), 1383 (s, N-O). Elemental analysis; (C$_{14}$H$_{12}$BiN$_3$O$_9$0.5C$_2$H$_6$O) Calc. (Found); C 30.11 (30.25), H 2.53 (2.32), N 7.02 (6.72) %. $^1$H NMR (400 MHz, CD$_3$OD, 25 °C): $\delta$H 7.85 (d, $^3$J = 6.2 Hz, 1H, ArH), 7.31 (t, $^3$J = 6.9 Hz, 1H, ArH), 6.87 (m, 2H, ArH). $^{13}$C NMR (100 MHz, CD$_3$OD, 25 °C): $\delta$C 134.3, 130.4, 120.8, 117.2, 116.8. MS (ESI+) $m/z$: ([Bi(Sha$_{-1}$H)$_2$]$^+$) 513.4.

Crystals of [Bi$_6$(CH$_3$OH)$_2$(η$_1$-NO$_3$)$_2$(η$_2$-NO$_3$)$_2$(H$_2$O)$_2$(Sha$_{-1}$H)$_2$]·(CH$_3$OH)$_4$ (H$_2$O)$_4$(NO$_3$)$_2$ (3a) were isolated on recrystallization of 3 from methanol. Elemental analysis of these crystals was performed (C$_{86}$H$_{84}$Bi$_6$N$_{16}$O$_{52}$) Calc. (Found); C 30.14 (31.06), H 2.47 (2.08), N 6.54 (6.75) %.

120
Synthesis of [Bi(2-NH₂-Pha·1H)₂(NO₃)]ₙ (4)

Bi(NO₃)_3·5H₂O (457 mg, 0.95 mmol) and 2-NH₂-Pha (1) (424 mg, 2.78 mmol) were dissolved in EtOH (80 mL) and the mixture stirred for 30 min and subsequently heated to reflux for 5 h. The clear solution was allowed to cool and filtered. The clear filtrate was condensed *in vacuo* to ca. 10 mL. On the addition of H₂O (5 mL) a white solid precipitated which was filtered, washed and dried under vacuum to give 4. Yield 270 mg (55%).

IR ν<sub>max</sub> (cm<sup>−1</sup>): 1609 (s, C=O), 1558 (s, C-N), 1288 (s, N-O). Elemental analysis (C₁₄H₁₅BiN₆O₁₀) Calc. (Found): C 26.43 (26.85), H 2.38 (2.50), N 13.21 (12.07) %. ¹H NMR (400 MHz, MeOD, 25 °C): δ<sub>H</sub> 7.49 (d, ³J = 7.7 Hz, ArH, 1H), 7.38 (t, ³J = 7.7 Hz, ArH, 1H), 7.02 (t, ³J = 9.5 Hz, ArH, 1H), 6.98-6.89 (m, ArH, 1H). ¹³C NMR (100 MHz, CD₃OD, 25 °C): δ<sub>C</sub> 166.1, 133.9, 129.2, 122.0, 120.8. MS (ESI+) m/z: ([M+2Na]<sup>+</sup>) 619.0
Anhydrous THF (25 mL) was added to BiCl$_3$ (470 mg, 1.49 mmol) and Sha (260 mg, 1.7 mmol) under an atmosphere of Argon. The reaction was stirred at rt for 72 h. The reaction mixture was concentrated *in vacuo* to ca. 5 mL. H$_2$O (5 mL) was added resulting in precipitation of a light yellow solid, which was filtered and dried under vacuum to give 6. Yield 370 mg (88%).

IR $\nu_{\text{max}}$ (cm$^{-1}$): 1599 (s, C=O). 1564 (s, C-N). Elemental analysis; (C$_7$H$_6$BiCl$_2$NO$_3$) Calc. (Found); C 19.46 (19.40), H 1.4 (1.14), N 3.24 (2.84), Cl 16.41 (13.58) %. $^1$H NMR (400 MHz, CD$_3$OD, 25 °C): $\delta$H: 7.91 (d, $^3$J = 8.1 Hz, 1H, ArH), 7.40 (m, 1H, ArH), 6.93 (m, 2H, ArH). $^{13}$C NMR (100 MHz, CD$_3$OD, 25 °C): $\delta$C: 134.6, 129.2, 120.5, 117.8, 116.1. MS (ESI+) $m/z$: ([BiCl(Sha-1H)]$^+$) 396.1, ([Bi(Sha-1H)$_2$]$^+$) 512.9.

Single crystals of [BiCl$_2$(μ-Sha-1H)(THF)].THF$_6$ 6a, were formed upon standing of the filtrate. Elemental analysis of these crystals was performed (C$_{15}$H$_{22}$BiCl$_2$NO$_4$) Calc. (Found): C 31.27 (30.85), H 3.85 (3.63), N 2.43 (2.20), Cl 12.30 (10.26) %.
Anhydrous THF (35 mL) was added to BiCl$_3$ (490 mg, 1.55 mmol) and 2-NH$_2$-Pha (1) (269 mg, 1.76 mmol) under an atmosphere of argon. The reaction was stirred at rt for 72 h. A yellow precipitate was filtered and dried under vacuum to give 7. Yield 360 mg (49%).

IR $\nu_{\text{max}}$ (cm$^{-1}$): 1613 (s, C=O), 1541 (s, C=N). Elemental analysis; (C$_7$H$_8$BiCl$_3$N$_2$O$_2$·0.1C$_2$H$_4$O) Calc. (Found): C 18.72 (18.84), H 1.87 (1.72), N 5.90 (5.92), Cl 22.40 (22.46) %. $^1$H NMR (400 MHz, CD$_3$OD, 25 °C): $\delta$H 7.70 (d, $^3J = 8$ Hz, 1H, ArH), 7.60 (t, $^3J = 8$ Hz, 1H, ArH), 7.38 (m, 2H, ArH). $^{13}$C NMR (100 MHz, MeOD, 25 °C): $\delta$c 133.97, 129.42, 128.71, 125.06. MS (ESI+) $m/z$: ([BiCl$_3$(2-NH$_3$-Pha$_{1\text{H}}$)]$^+$) 430.9.
Chapter 2

Synthesis of \([\text{BiCl}_3(2-\text{Py}+1\text{ha}\cdot 1\text{H})_2.2\text{H}_2\text{O}]_n\) \hspace{1cm} (8)

Anhydrous THF (35 mL) was added to BiCl\(_3\) (315 mg, 1 mmol) and 2-Pyha (152 mg, 1.1 mmol) under Argon. The reaction was stirred at rt for 72 h. The clear solution was filtered and condensed in vacuo. H\(_2\)O (5ml) was added and a yellow precipitate was filtered and dried under vacuum to give 8. Yield 340 mg (50%).

IR \(\nu_{\text{max}}\) (cm\(^{-1}\)): 1602 (s, C=O), 1518 (s, C-N). Elemental analysis; \((\text{C}_{12}\text{H}_{16}\text{BiCl}_3\text{N}_4\text{O}_6)\) Calc. (Found): C 22.97 (23.05), H 2.57 (1.88), N 8.93 (8.58), Cl 16.95 (17.19) %; \(^1\text{H}\) NMR (400 MHz, MeOD, 25 °C): \(\delta_\text{H}\) 8.64 (m, 1H, ArH), 8.11-7.96 (m, 2H, ArH), 7.59 (m, 1H, ArH). MS (ESI+) \(m/z\): ([Bi(2-Py+1ha\cdot 1H)\(_2\)]) 483.2.
**Attempted synthesis of \([\text{BiCl}_2(\text{Bha} \cdot \text{H})]_n\)**

Reaction of Bha with BiCl$_3$, in anhydrous THF under an inert atmosphere for 72 hours at rt did not result in the isolation of desired bismuth benzohydroxamato chlorido product.

Clear crystals were isolated on standing of the filtrate at room temperature and analysed by X-ray crystallography. These crystals were identified as N-(benzoyloxy)benzamide, 9.

![Structure of 9](image)

**Synthesis of BiPh$_3$**

To a stirred suspension of triphenylbismuth dichloride (1.3 g, 2.5 mmol) in EtOH (30 mL) was added hydrazine hydrate (1.0 g, 1 mL). The reaction occurred immediately and the solution turned colourless. The solution stirred for 1 h at rt. The solution was poured into H$_2$O (200 mL) and the product extracted with diethyl ether (2 x 100 mL), dried over Na$_2$SO$_4$, and the solvent removed *in vacuo*, to yield a white solid, 11. Yield 800 mg (95%).

![Structure of 11](image)

Elemental analysis; (C$_{18}$H$_{15}$Bi) Calc. (Found): C 49.10 (48.98), H 3.43 (3.40) %. $^1$H NMR (400 MHz, DMSO-$d_6$, 25 °C): δ 7.73 (d, $^3$$J$ = 6.7 Hz, ArH, 6H), 7.39 (t, $^3$$J$ = 7.2 Hz, ArH, 6H), 7.31 (t, $^3$$J$ = 7.3 Hz, ArH, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$, 25 °C): δ 137.33, 130.31, 127.50. m.p. 75-76 °C.
Synthesis of [Bi(HFA-1H)3]  (12)

BiPh3 (11) (110 mg, 0.25 mmol) and HFA (10) (102 mg, 0.75 mmol) were ground together using a mortar and pestle. This mixture was heated to 90 °C in a test tube over an oil bath for 1 h. Elimination of benzene was indicated by condensation on top of the tube. The white product obtained was washed with toluene and ether to remove any unreacted BiPh3 and HFA and the white solid filtered and dried under vacuum to yield 12. Yield 118 mg (75%).

IR $\nu_{\text{max}}$ (cm$^{-1}$): 2939, 1607 (s, C=O), 1564. Elemental analysis; (C$_{21}$H$_{18}$BiN$_3$O$_6$) Calc. (Found): C 40.86 (40.48), H 2.94 (2.80), N 6.81 (6.60) %. $^1$H NMR (400 MHz, DMSO-$d_6$, 25 °C): $\delta^H$ 9.48 (s, 1H, OH), 7.50 (d, $^3J = 7.7$ Hz, 2H, ArH), 7.38 (t, $^3J = 7.2$ Hz, 1H, ArH). $^{13}$C NMR (100 MHz, DMSO-$d_6$, 25 °C): $\delta^C$ 152.1, 129.1, 128.4, 125.9, 119.2, 118.5, 112.9. MS (ESI+) m/z: ([Bi(HFA-1H)$_3$ + Na]) 640.6.
Synthesis of [Ga(HFA\textsubscript{1H})\textsubscript{3}].H\textsubscript{2}O \quad (13)

To a stirred solution of HFA (10) (140 mg, 1 mmol) in H\textsubscript{2}O (10 mL) was added Ga(NO\textsubscript{3})\textsubscript{3}.H\textsubscript{2}O (76 mg, 0.3 mmol) in H\textsubscript{2}O (10 mL). The initial solution was slightly cloudy. The pH of the stirred solution was adjusted to pH 6.2 and allowed to stir for 1 h at rt. The mixture was then filtered and the white solid collected, washed and dried under vacuum to give 13. Yield 80 mg (56%).

IR $\nu_{\text{max}}$ (cm$^{-1}$): 1604 (s, C=O), 1566 (s, C-N). Elemental analysis: (C\textsubscript{21}H\textsubscript{20}GaN\textsubscript{3}O\textsubscript{7})

Calc. (Found): C 50.84 (50.75), H 4.06 (3.14), N 8.47 (8.11) %.

$^1$H NMR (400 MHz, DMSO-$d_6$, 25 °C): $\delta$H 9.15 (s, 1H, OH), 7.65 (d, J = 7.7 Hz, 2H, ArH), 7.47 (t, J = 7.9 Hz, 2H, ArH), 7.34 (t, J = 7.4 Hz, 1H, ArH).

$^{13}$C NMR (100 MHz, DMSO-$d_6$, 25 °C): $\delta$C 153.7, 138.8, 129.3, 127.2, 118.1.
Attempted Synthesis of \([\text{Bi}(\text{GSK}_{\text{1H}})_3]\) \hspace{1cm} (14)

\[\text{[Bi}(\text{GSK}_{\text{1H}})_3]\]

(i) BiPh\(_3\) (11) (55 mg, 0.125 mmol) and GSK (320 mg, 0.375 mmol) were ground together using a mortar and pestle. This mixture was heated at various temperatures and for different durations. However no evidence of product was observed under any of the conditions.

(ii) To a stirred solution of GSK (320 mg, 0.375 mmol) in EtOH (10 mL) was added BiPh\(_3\) (11) (55 mg, 0.125 mmol) in EtOH (5 mL). The resulting clear solution was stirred at reflux for 8 h. No trace of product was evident.

Attempted Synthesis of \([\text{Ga}(\text{GSK}_{\text{1H}})_3]\) \hspace{1cm} (15)

\[\text{[Ga}(\text{GSK}_{\text{1H}})_3]\]

To a stirred solution of GSK (320 mg, 0.375 mmol) in 30\% w/w DMSO/H\(_2\)O (10 mL) was added Ga(NO\(_3\))\(_3\).H\(_2\)O (30 mg, 0.125 mmol) in 30\% w/w DMSO/H\(_2\)O (5 mL) and the reaction was stirred at rt. The pH of the stirred solution was adjusted to pH 6.2 and allowed to stir for a further 24 h at rt. A product could not be isolated for analysis.
Synthesis of [Sb(Bha\textsubscript{-1H})\textsubscript{2}Cl] (26)

Bha (806 mg, 5.87 mmol) and SbCl\textsubscript{3} (670 mg, 2.93 mmol) were stirred in EtOH (40 mL) for 3 days at rt. The reaction was concentrated \textit{in vacuo} and ca. 10 mL of H\textsubscript{2}O was added. The resulting solution was left stand in the fridge. A white crystalline solid was collected and dried under vacuum to give 26. Yield 510 mg (40.5%).

IR $\nu_{max}$ (cm\textsuperscript{-1}): 3218, 1592 (s, C=O), 1553 (vs, C-N). Elemental analysis; (C\textsubscript{14}H\textsubscript{12}ClN\textsubscript{2}O\textsubscript{4}Sb) Calc. (Found); C 39.15 (39.02), H 2.82 (2.54), N 6.52 (6.35), Cl 8.25 (8.13) %. $^1$H NMR (400 MHz, D\textsubscript{2}O, 25 °C): $\delta$\textsubscript{H} 7.68 (d, $^3$J = 8 Hz, 4H, ArH), 7.60 (t, $^3$J = 8 Hz, 2H, ArH) 7.50 (t, $^3$J = 8 Hz, 4H, ArH). $^{13}$C NMR (100 MHz, D\textsubscript{2}O, 25 °C): $\delta$C 132.4, 128.9, 126.9. MS (ESI+) $m/z$: ([Sb(Bha\textsubscript{-1H})\textsubscript{2}]\textsuperscript{+}) 393.5/395.5.

Single crystals of [Sb(Bha\textsubscript{-1H})\textsubscript{2}Cl] suitable for X-ray crystallography were isolated from the filtrate on standing of a concentrated solution at rt.
Synthesis of \([\text{Sb}_2(\mu\text{-Cl})_2(\text{Cl})_2(2\text{-Pyha}1\text{H})_2] \quad (27)\)

2-Phya (5) (690 mg, 5 mmol) and SbCl₃ (570 mg, 2.5 mmol) were stirred in EtOH (30 mL) at rt for 10 min. A yellow precipitate formed and was filtered, washed with EtOH and dried under vacuum to give 27. Yield 530 mg (32%).

IR νₘₐₓ (cm⁻¹) 1617 (s, C=O), 1603 (s, C=O), 1548 (s, C-N). Elemental analysis: (C₁₂H₁₀Cl₄N₄O₄Sb₂) Calc. (Found): C 21.85 (22.12), H 1.53 (1.46), N 8.49 (8.33), Cl 21.50 (20.84) %. ¹H NMR (400 MHz, D₂O, 25 °C): δH 8.74 (d, ³J = 8 Hz, 2H, ArH), 8.40 (t, ³J = 8 Hz, 2H, ArH), 8.19 (d, ³J = 8 Hz, 2H, ArH), 7.94 (t, ³J = 8 Hz, 2H, ArH). ¹³C NMR (100 MHz, D₂O, 25 °C): δC 129.0, 124.3. MS (ESI+) m/z: ([Sb(2-Phya1H)₂]⁺) 395.3/397.3.

Single crystals of \([\text{Sb}_2(\mu\text{-Cl})_2(\text{Cl})_2(2\text{-Pyha}1\text{H})_2].\text{H}_2\text{O}\) were isolated on recrystallization of white solid from methanol at rt.
Synthesis of \([\text{Sb}(2-\text{NH}_2-\text{Pha-H})(2-\text{NH}_3-\text{Pha-H})]\)Cl$_2$ \(28\)

2-NH$_2$-Pha (1) (520 mg, 3.39 mmol) and SbCl$_3$ (387 mg, 1.696 mmol) were stirred in EtOH (30 mL) for 30 min then brought to reflux for 1 h, giving a clear solution. The reaction was stirred overnight at rt. A white precipitate was filtered, washed with Et$_2$O and dried under vacuum to give \(28\). Yield 350 mg (38%).

IR $\nu_{\text{max}}$ (cm$^{-1}$): 1607 (s, C=O), 1582 (s, C=O) 1558 (s, C-N). Elemental analysis; (C$_{16}$H$_{21}$Cl$_2$N$_4$O$_5$Sb) Calc. (Found): C 35.46 (35.41), H 3.91 (3.81), N 10.34 (10.17), Cl 13.08 (12.72) %. $^1$H NMR (400 MHz, D$_2$O, 25 °C): $\delta$H 7.61 (2H, m, ArH), 7.40 (2H, m, ArH). $^{13}$C NMR (100 MHz, D$_2$O, 25 °C): $\delta$C 133.2, 128.4, 127.0, 123.3. MS (ESI+) $m/z$: ([Sb(2-NH$_2$-Pha-H)(2-NH$_3$-Pha-H)]$^{2+}$) 422.8/424.8.

Single crystals of [Sb(2-NH$_2$-Pha-H)(2-NH$_3$-Pha-H)]Cl$_2$ suitable for X-ray crystallographic analysis were collected from slow evaporation from an EtOH solution at rt.
Synthesis of [SbCl(Sha·1H)₂] \( (29) \)

Sha (536 mg, 3.5 mmol) and SbCl₃ (400 mg, 1.75 mmol) were stirred in EtOH (30 mL) overnight at rt. To the clear solution ca. 10 mL of H₂O was added and the resulting white precipitate collected, washed with cold water and dried under vacuum to give 29. Yield 230 mg (26.4%).

IR \( \nu_{\text{max}} \) (cm\(^{-1}\)): 1602 (vs, C=O), 1582, 1566 (s, C-N). Elemental analysis; (C\(_{14}\)H\(_{16}\)ClN\(_2\)O\(_8\)Sb) Calc (Found): C 33.80 (33.62), H 3.24 (2.73), N 5.63 (5.26), Cl 7.13 (7.58) %.

\(^1\)H NMR (400 MHz, D\(_2\)O, 25 °C): \( \delta_H 7.65 \) (d, \( ^3J = 8 \) Hz, 1H, ArH), 7.43 (t, \( ^3J = 8 \) Hz, 1H, ArH), 7.00 (m, 2H, ArH).

\(^{13}\)C NMR (100 MHz, D\(_2\)O, 25 °C): \( \delta_C 155.9, 134.0, 128.7, 120.3, 116.8 \).

MS (ESI+) \( m/z \): ([Sb(Sha·1H)₂]^+) 424.7/426.7.
Attempted synthesis of [SbCl(SAHA-1H)2] (30)

SAHA (18) (174 mg, 0.658 mmol) was added to SbCl₃ (75 mg, 0.328 mmol) dissolved in EtOH (30 mL). The suspension was stirred for 72h at rt. A white solid was filtered, washed with EtOH and dried under vacuum to give 30. Yield 55 mg (25%).

Microcrystals of [SbCl(SAHA-1H)2], which were not suitable for X-ray crystallography were isolated from the filtrate on standing and analysis carried out on this solid.

IR ν_max (cm⁻¹): 1658 (s, C=O), 1596 (s, C=O), 1530 (s, C-N); Elemental analysis: (C₂₈H₃₈ClN₄O₆Sb) Calc. (Found): C 49.18 (50.05), H 5.60 (5.70), N 8.19 (7.95), Cl 4.69 (3.88) %; ¹H NMR (400 MHz, CD₃OD, 25 °C): δ_H 7.52 (d, 3J = 8 Hz, ArH, 4H), 7.27 (t, 3J = 8 Hz, ArH, 4H), 7.06 (t, 3J = 8 Hz, ArH, 2H), 2.37 (m, 4H, CH₂), 1.69 (m, 4H, CH₂), 1.39 (m, 4H, CH₂). ¹³C NMR (100 MHz, CD₃OD, 25 °C): δ_C 128.3, 123.7, 119.8, 36.3, 28.35, 25.2, 24.8. MS (ESI-) m/z: ([Sb(SAHA-2H)2]⁻) 645.1/ 647.1.
Synthesis of \([\text{Sb(SAHA}-1\text{H})(\text{SAHA}-2\text{H})]\)  \(\text{(31)}\)

SAHA (18) (130 mg, 0.5 mmol) and Sb(OC\(_3\)Et\(_3\)) (28 \(\mu\)L, 0.165 mmol) were stirred in hexane (30 mL) for 30 h at rt. The solution was filtered of unreacted/undissolved SAHA and the clear filtrate concentrated \textit{in vacuo} resulting in precipitation of a white solid, which was filtered and dried under vacuum to give 31. Yield 69 mg (64.6%).

IR \(\nu_{\text{max}}\) (\(\text{cm}^{-1}\)): 1658 (s, C=O) 1628, (s, C=O), 1598 (s, C=O), 1545 (s, C-N). Elemental analysis; \((\text{C}_{28}\text{H}_{37}\text{N}_4\text{O}_6\text{Sb})\) Calc. (Found): C 51.95 (51.52), H 5.76 (6.06), N 8.65 (8.40) \%. \(^1\)H NMR (400 MHz, CD\(_3\)OD, 25 °C): \(\delta_H\) 7.53 (d, \(^3\)J = 8 Hz, 4H, ArH), 7.29 (t, \(^3\)J = 8 Hz, 4H, ArH), 7.08 (t, \(^3\)J = 8 Hz, 2H, ArH), 2.37 (m, 5H, \(\text{CH}_2\)), 2.09 (t, \(^3\)J = 8 Hz, 3H, \(\text{CH}_2\)), 1.66 (m, 8H, \(\text{CH}_2\)), 1.39 (m, 8H, \(\text{CH}_2\)). \(^{13}\)C NMR (100 MHz, CD\(_3\)OD, 25 °C): \(\delta_C\) 129.8, 125.1, 121.3, 37.9, 33.7, 29.9, 29.8, 26.7, 26.5. MS (ESI-) \(m/z\): [Sb(SAHA-2H)(SAHA-1H)] 645.3/647.3.
4. X-ray Crystallography

\[ \text{[Bi}_2\text{Bha-1H)}_2(\mu\text{-Bha-1H)}_2(\eta^2\text{-NO}_3)_2\cdot\text{CH}_3\text{OH} \quad (2) \]

A specimen of C\text{29}H\text{29}Bi\text{2}N\text{6}O\text{15}, approximate dimensions 0.030 mm × 0.050 mm × 0.210 mm, was used for the X-ray crystallographic analysis by Dr. Brendan Twamley in Trinity College Dublin. The X-ray intensity data were measured at 100 K using an Oxford Cryosystems Cobra low temperature device using a MiTeGen micromount. Bruker APEX software was used to correct for Lorentz and polarization effects.\(^7\)

A total of 1027 frames were collected. The total exposure time was 2.85 h. The frames were integrated with the Bruker SAINT Software package using a wide-frame algorithm. The integration of the data using a triclinic unit cell yielded a total of 32,284 reflections to a maximum \(\theta\) angle of 27.53° (0.77 Å resolution), of which 4213 were independent (average redundancy 7.663, completeness = 100.0%, \(R_{int} = 6.26\%\), \(R_{sig} = 4.44\%\)) and 3768 (89.44%) were greater than 2\(\sigma(F^2)\). The final cell constants of \(a = 4.8467(4)\) Å, \(b = 13.1798(12)\) Å, \(c = 15.0042(14)\) Å, \(\alpha = 74.217(3)\)°, \(\beta = 85.628(3)\)°, \(\gamma = 82.567(3)\)°, volume = 913.71(14) Å\(^3\), are based upon the refinement of the XYZ – centroids of 2486 reflections above 20 \(\sigma(I)\) with 4.823° < \(2\theta < 43.96\)°. Data were corrected for absorption effects using the multi-scan method (SADABS). The ratio of minimum to maximum apparent transmission was 0.620.
The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.4625 and 0.7456.

The structure was solved and refined using the Bruker SHELXTL Software Package\textsuperscript{8}, using the space group $P\bar{1}$, with $Z = 1$ for the formula unit, $C_{29}H_{28}Bi_2N_6O_{15}$. The final anisotropic full-matrix least-squares refinement on $F^2$ with 251 variables converged at $R_1 = 3.71\%$, for the observed data and $wR_2 = 9.35\%$ for all data. The goodness-of-fit was 1.084. The largest peak in the final difference electron density synthesis was $2.302 \text{ e}^- \text{ Å}^{-3}$ and the largest hole was $-1.477 \text{ e}^- \text{ Å}^{-3}$ with an RMS deviation of $0.227 \text{ e}^- \text{ Å}^{-3}$. On the basis of the final model, the calculated density was $2.033 \text{ g cm}^{-3}$ and $F(000), 530 \text{ e}^-$.

**Refinement note:** The phenyl ring systems were disordered 50 : 50\% and were modelled with restraints and constraints (ISOR and EADP). The disordered MeOH solvent molecule was modelled as 50\% occupied in three locations in the asymmetric unit. Restraints and constraints were applied to model the disorder (DFIX, EADP and ISOR). Each disordered MeOH moiety refined as 17 : 18 : 15\% occupied.

**Refinement note:** $N – H$ and $O – H$ hydrogen atoms were located and refined using a DFIX restraint: O5, O6, O16, O17, O18 and N9, N20, N31, N42, N53, N64.
A specimen of C₉₀H₁₀₈Bi₆N₁₈O₆₆, approximate dimensions 0.050 mm × 0.080 mm × 0.150 mm, was used for the X-ray crystallographic analysis by Dr. Brendan Twamley in Trinity College Dublin. The X-ray intensity data were measured at 100 K using an Oxford Cryosystems Cobra low temperature device using a MiTeGen micromount. Bruker APEX software was used to correct for Lorentz and polarization effects. A total of 1731 frames were collected. The total exposure time was 4.81 h. The integration of the data using a triclinic unit cell yielded a total of 111,802 reflections to a maximum θ angle of 30.14° (0.71 Å resolution), of which 17,529 were independent (average redundancy 6.378, completeness = 99.4%, R_int = 4.43%, R_sig = 3.33%) and 14,300 (81.58%) were greater than 2σ(F²). The final cell constants of \(a = 15.0432(6) \text{ Å}, \quad b = 15.2366(5) \text{ Å}, \quad c = 16.5434(6) \text{ Å}, \quad \alpha = 112.5660(10)°, \quad \beta = 116.5850(10)°, \quad \gamma = 94.4850(10)°, \) volume = 2984.48(19) Å³, are based upon the refinement of the XYZ centroids of reflections above 20 σ(I). Data were corrected for absorption effects using the numerical method (SADABS). The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.4747 and 0.7837.

\[
[\text{Bi}_6(\text{CH}_3\text{OH})_2(\eta^1-\text{NO}_3)_2(\eta^2-\text{NO}_3)_2(\text{H}_2\text{O})_2(\text{Sha}·\text{H})_2](\text{CH}_3\text{OH})_4(\text{H}_2\text{O})_4(\text{NO}_3)_2 \quad (3a)
\]
The structure was solved and refined using the Bruker SHELXTL Software Package\textsuperscript{8}, using the space group Pі, with $Z = 1$ for the formula unit, $C_{90}H_{108}Bi_6N_{18}O_{66}$. The final anisotropic full-matrix least-squares refinement on $F^2$ with 827 variables converged at $R_1 = 2.54\%$, for the observed data and $wR_2 = 5.33\%$ for all data. The goodness-of-fit was 1.004. The largest peak in the final difference electron density synthesis was 1.894 e\(^{-}\) Å\(^{-3}\) and the largest hole was $-1.451$ e\(^{-}\) Å\(^{-3}\) with an RMS deviation of 0.154 e\(^{-}\) Å\(^{-3}\). On the basis of the final model, the calculated density was 2.087 g cm\(^{-3}\) and $F(000), 1800$ e\(^{-}\).

\[ \text{[BiCl}_2(\mu-\text{Sha-1H})(\text{THF})].\text{THF}_\infty \quad (6a) \]

Crystal data for [BiCl\(_2(\mu\)-Sha-1H)(THF)].THF\(_\infty\) (6a) were collected with Mo-K\(_\alpha\) radiation (0.71073 Å) using an Rigaku Oxford Diffraction Super- Nova A diffractometer fitted with an Atlas detector. A five time's redundant dataset was collected, assuming that the Friedel pairs are not equivalent. An analytical absorption correction based on the shape of the crystal was performed.\textsuperscript{10} The structures were solved by Dr. Helge Müller Bunz in University College Dublin by direct methods using SHELXS-97 and refined by full matrix least-squares on F2 for all data using SHELXL-97.\textsuperscript{11} Hydrogen atoms were added at calculated positions and refined using a riding model. Their isotropic temperature factors were fixed to 1.2 times (1.5 times for methyl groups) the equivalent isotropic displacement parameters of the carbon atom the H-atom is attached to. Anisotropic thermal displacement parameters were used for all non-hydrogen atoms.
**N-(benzoyloxy)benzamide  (9)**

A specimen of C\textsubscript{14}H\textsubscript{11}NO\textsubscript{3}, approximate dimensions 0.37 x 0.11 x 0.09 mm, was used for the X-ray crystallographic analysis by Dr. Brendan Twamley in Trinity College Dublin. The X-ray intensity data were measured at 100 K using an Oxford Cryosystems Cobra low temperature device using a MiTeGen micromount. Bruker APEX software was used to correct for Lorentz and polarization effects.\(^7\)

A total of 360 frames were collected. The total exposure time was 2.05 hours. The integration of the data using an orthorhombic unit cell yielded a total of 9872 reflections to a maximum θ angle of 30.50° (0.70 Å resolution), of which 3504 were independent (average redundancy 2.817, completeness = 99.9%, R\textsubscript{int} = 3.72%, Rsig = 4.85%) and 2906 (82.93%) were greater than 2σ(F2). The final cell constants of a = 8.9401(5) Å, b = 9.1767(6) Å, c = 14.0341(8) Å, volume = 1151.37(12) Å\(^3\), are based upon the refinement of the XYZ-centroids of reflections above 20 σ(I). Data were corrected for absorption effects using the Multi-Scan method (SADABS). The ratio of minimum to maximum apparent transmission was 0.943. The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.7036 and 0.7461.

The structure was solved and refined using the Bruker SHELXTL Software Package, using the space group P2\textsubscript{1}2\textsubscript{1}2\textsubscript{1}, with Z = 4 for the formula unit, C\textsubscript{14}H\textsubscript{11}NO\textsubscript{3}. The final anisotropic full-matrix least-squares refinement on F\(^2\) with 168 variables converged at R1 = 4.30%, for the observed data and wR2 = 10.26% for all data. The goodness-of-fit was 1.041. The largest peak in the final difference electron density synthesis was 0.382 e/Å\(^3\) and the largest hole was -0.253 e/Å\(^3\) with an RMS deviation of 0.052 e/Å\(^3\). On the basis of the final model, the calculated density was 1.392 g/cm\(^3\) and F(000), 504 e\(^-\).
A clear colourless rod-like specimen of C_{21}H_{25}N_{3}O_{5}, approximate dimensions 0.070 mm x 0.080 mm x 0.310 mm, was used for the X-ray crystallographic analysis by Dr. Brendan Twamley in Trinity College Dublin. The X-ray intensity data were measured at 100 K with an Oxford Cryosystems low temperature device using a MiTeGen micromount. Bruker APEX software was used to correct for Lorentz and polarization effects.

A total of 723 frames were collected. The total exposure time was 6.28 h. The frames were integrated with the Bruker SAINT Software package using a narrow-frame algorithm. The integration of the data using a triclinic unit cell yielded a total of 10462 reflections to a maximum θ angle of 26.49° (0.80 Å resolution), of which 4016 were independent (average redundancy 2.605, completeness = 99.7%, R_{int} = 5.01%, R_{sig} = 6.13%) and 2684 (66.83%) were greater than 2σ(F^2). The final cell constants of a = 6.4325(4) Å, b = 9.3343(6) Å, c = 16.6717(10) Å, α = 81.219(2)°, β = 80.208(2)°, γ = 88.163(2)°, volume = 974.85(11) Å^3, are based upon the refinement of the XYZ-centroids of 3638 reflections above 2σ(I) with 6.168° < 2θ < 52.89°. Data were corrected for absorption effects using the Multi-Scan method (SADABS). The ratio of minimum to maximum apparent transmission was 0.859. The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.6405 and 0.7454.

The structure was solved using the Bruker APEX Software Package and refined with XL in Olex2, using the space group P\textoverline{1}, with Z = 2 for the formula unit, C_{21}H_{25}N_{3}O_{5}. The final anisotropic full-matrix least-squares refinement on F^2 with 270 variables
converged at R1 = 4.86%, for the observed data and wR2 = 11.40% for all data. The goodness-of-fit was 1.011. The largest peak in the final difference electron density synthesis was 0.219 e/Å³ and the largest hole was -0.265 e/Å³ with an RMS deviation of 0.055 e/Å³. On the basis of the final model, the calculated density was 1.361 g/cm³ and F(000), 424 e⁺.

**Methyl 8-((2-aminophenyl)amino)-8-oxooctanoate**  (24)

A clear colourless plate-like specimen of C₁₅H₂₂N₂O₃, approximate dimensions 0.120 mm x 0.250 mm x 0.390 mm, was used for the X-ray crystallographic analysis by Dr. Brendan Twamley in Trinity College Dublin. The X-ray intensity data were measured at 100 K with an Oxford Cryosystems low temperature device using a MiTeGen micromount. Bruker APEX software was used to correct for Lorentz and polarization effects.

A total of 1262 frames were collected. The total exposure time was 6.26 h. The frames were integrated with the Bruker SAINT Software package using a narrow-frame algorithm. The integration of the data using a monoclinic unit cell yielded a total of 22671 reflections to a maximum θ angle of 26.38° (0.80 Å resolution), of which 2970 were independent (average redundancy 7.633, completeness = 99.6%,
\[ R_{\text{int}} = 3.43\%, \ R_{\text{sig}} = 2.07\% \) and 2489 (83.80\%) were greater than 2σ(F²). The final cell constants of \( a = 11.7759(3) \ \text{Å}, \ b = 4.90770(10) \ \text{Å}, \ c = 25.3338(7) \ \text{Å}, \ \beta = 92.1329(14)°, \ \text{volume} = 1463.09(6) \ \text{Å}^3 \), are based upon the refinement of the XYZ – centroids of 9982 reflections above 20 σ(I) with \( 5.835° < 2\theta < 52.64° \). Data were corrected for absorption effects using the Multi-Scan method. The ratio of minimum to maximum apparent transmission was 0.910. The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.6782 and 0.7454.

The structure was solved with the XT structure solution program using Intrinsic Phasing and refined with the XL refinement package using Least Squares minimisation with Olex2, using the space group P2₁/n, with \( Z = 4 \) for the formula unit, \( \text{C}_{15}\text{H}_{22}\text{N}_{2}\text{O}_{3} \). The final anisotropic full-matrix least-squares refinement on F² with 193 variables converged at \( R1 = 4.14\% \), for the observed data and \( wR2 = 10.35\% \) for all data. The goodness-of-fit was 1.059. The largest peak in the final difference electron density synthesis was 0.215 e/Å³ and the largest hole was -0.218 e/Å³ with an RMS deviation of 0.048 e/Å³. On the basis of the final model, the calculated density was 1.264 g/cm³ and F(000), 600 e⁻.

\([\text{Sb(Bha-1H)}_{2}\text{Cl}] \) (26)

A clear colourless needle-like specimen of \( \text{C}_{14}\text{H}_{12}\text{ClN}_{2}\text{O}_{4}\text{Sb} \), approximate dimensions 0.080 mm x 0.100 mm x 0.500 mm, was used for the X-ray
crystallographic analysis by Dr. Brendan Twamley in Trinity College Dublin. The X-ray intensity data were measured at 100 K with an Oxford Cryosystems low temperature device using a MiTeGen micromount. Bruker APEX software was used to correct for Lorentz and polarization effects.

A total of 3804 frames were collected. The total exposure time was 4.28 h. The frames were integrated with the Bruker SAINT Software package using a narrow-frame algorithm. The integration of the data using a monoclinic unit cell yielded a total of 50221 reflections to a maximum θ angle of 33.20° (0.65 Å resolution), of which 5612 were independent (average redundancy 8.949, completeness = 99.7%, Rint = 2.43%, Rsig = 1.29%) and 5355 (95.42%) were greater than 2σ(F²). The final cell constants of a= 10.9598(4) Å, b = 4.9157(2) Å, c = 27.3577(11) Å, β = 96.0053(16)°, volume = 1465.81(10) Å³, are based upon the refinement of the XYZ-centroids of 9420 reflections above 20 σ(I) with 5.989° < 2θ < 66.32°. Data were corrected for absorption effects using the Multi-Scan method. The ratio of minimum to maximum apparent transmission was 0.758. The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.5659 and 0.7465.

The structure was solved with the XT structure solution program using Intrinsic Phasing and refined with the XL refinement package using Least Squares minimisation with Olex2, using the space group P2₁/c, with Z = 4 for the formula unit, C₁₄H₁₂ClN₂O₄Sb. The final anisotropic full-matrix least-squares refinement on F² with 205 variables converged at R1 = 1.96%, for the observed data and wR2 = 4.50% for all data. The goodness-of-fit was 1.214. The largest peak in the final difference electron density synthesis was 0.656 e/Å³ and the largest hole was -0.866 e/Å³ with an RMS deviation of 0.095 e/Å³. On the basis of the final model, the calculated density was 1.946 g/cm³ and F(000), 840 e⁻.

**Refinement Note:** Donor H atoms located and refined with restraints (DFIX) Uiso = 1.2 x Ueq.
[\text{Sb}_2(\mu-\text{Cl})_2(\text{Cl})_2(2\text{-Pyha.1H})_2].\text{H}_2\text{O} \quad (27)

A clear pale yellow plate-like specimen of \text{C}_{12}\text{H}_{10}\text{Cl}_4\text{N}_4\text{O}_5\text{Sb}_2, approximate dimensions 0.040 mm x 0.300 mm x 0.340 mm, was used for the X-ray crystallographic analysis by Dr. Brendan Twamley in Trinity College Dublin. The X-ray intensity data were measured at 100 K with an Oxford Cryosystems low temperature device using a MiTeGen micromount. Bruker APEX software was used to correct for Lorentz and polarization effects.

A total of 1539 frames were collected. The total exposure time was 3.83 hours. The frames were integrated with the Bruker SAINT Software package using a wide-frame algorithm. The integration of the data using a triclinic unit cell yielded a total of 21969 reflections to a maximum θ angle of 36.54° (0.60 Å resolution), of which 4643 were independent (average redundancy 4.732, completeness = 99.7%, \(R_{\text{int}} = 3.08\%\), \(R_{\text{sig}} = 2.37\%\)) and 4339 (93.45%) were greater than 2σ(\(F^2\)). The final cell constants of \(a = 6.4349(2) \, \text{Å}, \ b = 7.7809(2) \, \text{Å}, \ c = 9.6588(3) \, \text{Å}, \ \alpha = 81.4760(10)°, \ \beta = 81.5970(10)°, \ \gamma = 86.7140(10)°, \) volume = 472.82(2) Å\(^3\), are based upon the refinement of the XYZ-centroids of 9908 reflections above 20 σ(I) with 6.337° < 2θ < 73.02°. Data were corrected for absorption effects using the Multi-Scan method.\(^9\) The ratio of minimum to maximum apparent transmission was 0.747. The calculated
minimum and maximum transmission coefficients (based on crystal size) are 0.5582 and 0.7471.

The structure was solved with the XT structure solution program using Intrinsic Phasing and refined with the XL refinement package using Least Squares minimisation with Olex2, using the space group P\(\bar{1}\), with \(Z = 1\) for the formula unit, \(\text{C}_{12}\text{H}_{10}\text{Cl}_{4}\text{Na}_{5}\text{O}_{5}\text{Sb}_{2}\). The final anisotropic full-matrix least-squares refinement on \(F^2\) with 129 variables converged at \(R1 = 2.06\%\), for the observed data and \(wR2 = 4.70\%\) for all data. The goodness-of-fit was 1.142. The largest peak in the final difference electron density synthesis was 0.736 e\(/\text{\AA}^3\) and the largest hole was -0.932 e\(/\text{\AA}^3\) with an RMS deviation of 0.126 e\(/\text{\AA}^3\). On the basis of the final model, the calculated density was 2.372 g/cm\(^3\) and \(F(000)\), 320 e\(^-\).

**Refinement Note:** Water molecule modelled as half occupied. Donor H atoms located and refined with restraints (DFIX ) with Uiso fixed to 1.5 times U carrier atom. Pyridine ring modelled in two positions with ca. 71:29% occupancy. Constraints used (EADP). Cl atoms disordered in two positions with ca. 58:42% occupancy.

\[\text{[Sb(2-NH}_2\text{-Pha}._1\text{H})(2-NH}_3\text{-Pha}._1\text{H})\text{Cl}_2\text{.C}_2\text{H}_5\text{OH} \ (28)\]
A specimen of $\text{C}_{15}\text{H}_{22}\text{Cl}_2\text{N}_4\text{O}_6.5\text{Sb}$, approximate dimensions 0.110 mm x 0.170 mm x 0.340 mm, was used for the X-ray crystallographic analysis by Dr. Brendan Twamley in Trinity College Dublin. The X-ray intensity data were measured at 100 K using an Oxford Cryosystems low temperature device using a MiTeGen micromount. Bruker APEX software was used to correct for Lorentz and polarization effects.

A total of 854 frames were collected. The total exposure time was 0.99 h. The integration of the data using a triclinic unit cell yielded a total of 34395 reflections to a maximum $\theta$ angle of 31.67° (0.68 Å resolution), of which 6864 were independent (average redundancy 5.011, completeness = 99.6%, $\text{R}_{\text{int}} = 2.75\%$, $\text{R}_{\text{sig}} = 2.42\%$) and 6272 (91.38%) were greater than 2$\sigma(F^2)$. The final cell constants of $a = 9.1379(3)$ Å, $b = 10.1593(4)$ Å, $c = 11.1638(4)$ Å, $\alpha = 82.915(2)^\circ$, $\beta = 84.2630(10)^\circ$, $\gamma = 86.2730(10)^\circ$, volume = 1021.87(6) Å$^3$, are based upon the refinement of the XYZ – centroids of reflections above 20 $\sigma(I)$. Data were corrected for absorption effects using the Multi-Scan method. The ratio of minimum to maximum apparent transmission was 0.878. The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.6549 and 0.7462.

The structure was solved and refined using the Bruker SHELXTL Software Package, using the space group $\text{P}\overline{1}$, with $Z = 2$ for the formula unit, $\text{C}_{15}\text{H}_{22}\text{Cl}_2\text{N}_4\text{O}_6.5\text{Sb}$. The final anisotropic full-matrix least-squares refinement on $F^2$ with 310 variables converged at $R_1 = 2.08\%$, for the observed data and $wR_2 = 4.34\%$ for all data. The goodness-of-fit was 1.083. The largest peak in the final difference electron density synthesis was 0.476 e/Å$^3$ and the largest hole was -0.590 e/Å$^3$ with an RMS deviation of 0.092 e/Å$^3$. On the basis of the final model, the calculated density was 1.804 g/cm$^3$ and F(000), 554 e-.

**Refinement Note:** EtOH molecule disordered over inversion and modelled as a complete molecule half occupied. Donor hydrogen’s located and refined.
5. Bacterial Evaluation

5.1 General considerations

Reagents

All chemicals, nutrients and agars were used as received from commercial suppliers. Isolated Jack bean (Canavalia ensiformis) urease, Columbia Blood and Tryptic Soy Broth, biological agar and Fetal Bovine Serum were purchased from Sigma-Aldrich (Sigma-Aldrich Ireland Ltd., Wicklow, Ireland). *H. pylori* strains were purchased from The American Type Culture Collection (ATCC, Rockville, MD, USA). Defibrinated Horse blood was purchased from Thermo Fischer Scientific (Fisher Scientific Ireland, Dublin, Ireland). GenBags, GenBoxes and microaerophilic environment producing sachets (Biomerieux) were purchased from Cruinn Ltd (Cruinn Diagnostics, Dublin, Ireland). All work with live *Helicobacter* cultures were performed in a biosafety level 2 laboratory in the RCSI microbiology laboratory, Royal College of Surgeons in Ireland, RCSI Education & Research Centre located at Beaumont Hospital.

Potassium phosphate buffer (pH 6.8) preparation

Potassium phosphate buffer solution (PBS) with a pH of 6.8 was required for a biological assay. A PBS tablet (Sigma-Aldrich) was dissolved with H$_2$O. A pH of 6.8 was required for the urease assay. This was achieved by slow addition of sterile aqueous 1M hydrochloric acid (HCl) to the PBS solution while closely monitoring the pH with a pH meter (Orion™ Versa Star Pro™ pH Benchtop Meter, ThermoFisher Scientific). The solution was then made up to the correct concentration and final volume using deionised water as per manufacturer’s guidelines. PBS contains sodium chloride, potassium chloride and phosphate buffer dissolved with a final concentration of 137 mM sodium chloride, 10 mM phosphate buffer and 2.7 mM potassium chloride per 200 mL of deionised water. PBS was stored at 10 °C until required.

Fetal Bovine Serum (FBS)

FBS is the supernatant fraction remaining after the natural coagulation of fetal calf blood. FBS is commonly used as a serum supplement for bacterial culture as it provides bacteria with nutrients for proliferation, as well as a number of hormones and growth factors. We used FBS to help with the growth of our bacteria in broth.
Chapter 2

medium. FBS was used without any further action as supplied by Sigma-Aldrich. Upon arrival it was dispensed in 50 mL aliquots and stored at -20 °C until required for addition to bacterial medium. It was then defrosted, heated to 37 °C in a water bath and added to bacterial growth medium in a 1:10 dilution.

**Phenol red indicator preparation**
Phenol red (2 mg) was dissolved in deionised water and made up to a volume of 100 mL, giving a 10x concentration, allowing for a 1 in 10 dilution in the test well.

**Urease inhibition Assay**
Assays were prepared in Corning 96 Well CellBIND Microplates (Sigma Aldrich). Jack Bean isolated Urease enzyme (5–15 I.U.) was pre-incubated at 25 °C for 30 min with inhibitors at 0.1, 1 and 10 mM in 100 mM potassium phosphate buffer, pH 6.8 in 50 µL volumes. Following pre-incubation, enzyme assay mixtures were added containing 100 µM potassium phosphate buffer, pH 6.8, 150 mM urea, 0.002% phenol red in a final volume of 200 mL. Linear changes in absorbance at 565 nm at 37 °C, associated with the change in absorption of phenol red due to production of ammonia, were measured at ten second intervals over a 15 min period. Rates of inhibition were determined and plotted as percentage inhibition relative to control.

### 5.2 *H. pylori* Techniques and Procedures
Four strains of *H. pylori* were used in this study, **Table 2.1.** *H. pylori* strains were purchased from The American Type Culture Collection (ATCC). Growth and manipulation of these strains, including the composition, make up and storage of agar plates required for the growth of these bacteria are highlighted below.

<table>
<thead>
<tr>
<th><em>H. pylori</em> Strain</th>
<th>Source</th>
<th>Official Code</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>J99</td>
<td>ATCC®</td>
<td>700824</td>
<td>Patient with a duodenal ulcer</td>
</tr>
<tr>
<td>60190</td>
<td>ATCC®</td>
<td>49503</td>
<td>Enteric Research <em>H. pylori</em> strain</td>
</tr>
<tr>
<td>26695</td>
<td>ATCC®</td>
<td>700392</td>
<td><em>H. pylori</em> genome complete</td>
</tr>
</tbody>
</table>
Identity of all the strains was confirmed by Matrix Assisted Laser Desorption Ionisation-Time of Flight (MALDI-TOF) mass spectrometry (Bruker, Germany) performed by in the Diagnostic Laboratory of Beaumont Hospital, Dublin, Ireland.

**Preparation of agar plates for growth of *H. pylori***

All work was carried out in the laminar flow cabinet to ensure aseptic conditions. Plates to be used for the growth of *H. pylori* were prepared in advance due to the specific nature of the plate composition required. All strains were cultured on tryptic soy agar (TSA) (Sigma-Aldrich) plates supplemented with 5% defibrinated sheep blood or on Columbia blood (CB, Oxoid) agar plates also supplemented with 10% defibrinated horse blood, as per ATCC guidelines. Good growth was observed on both plate compositions. These plates incorporate a higher level of nutrients to help sustain the growth and survival of bacteria over the unusual longer growth period.

In a dewar bottle, growth medium powder was dissolved in the required amount of water, using stirring where needed to aid the dissolution process. Once all the media was mixed thoroughly, the solution was autoclaved at 121 °C for 15 min in the Hi-Clave Autoclave (Hirayama, Hi Clave HVE 50, Japan). The sterilized medium was allowed to cool to ~ 47 °C. Aseptically an aliquot of room temperature defibrinated sheep blood was added as to make up the desired concentration of blood in the agar. This mixture was then gently mixed and dispensed (25-35 mL) into each empty sterile petri dish. The plates were then partially covered with the lid to avoid any contamination and preventing condensation building up on the plate. The plates can either be used directly or sealed and placed in the fridge. Fridge stored plates should be used within 1 month of pouring. *H. pylori* also grow in liquid media. Good growth was observed in Brucella Broth (BB, Oxoid) supplemented with 10% FBS.

**Preparation of agar plates for *H. pylori* anti-bacterial testing**

According to the CLSI, agar dilution testing is a standard test technique for the investigation of anti-bacterial properties of agents against *H. pylori*. In this method the anti-bacterial agent is incorporated into the agar on a plate and a solution of *H. pylori* is added to the surface of the plate. MIC is determination as the concentration of compound which totally inhibits the growth of bacteria on the plate.
The test compound to be incorporated into a plate was dissolved and mixed in sterile tryptic soy broth (TSB) (1 mL) giving 100x the required final concentration. Two sequential 1 in 10 dilutions were carried out into warm TSA + 5% SB to give the highest concentration of compound required. Serial dilutions affords the other concentrations required until the final concentration was 0.5 μg mL⁻¹, which can be incorporated into plates.

Control plates which incorporated BSS were also prepared in a similar method with the same concentrations for comparable analysis.

**General handling of *H. pylori***

*H. pylori* is a microaerophilic bacteria. They therefore require oxygen to survive but at a lower level than is present in our normal environment. If the bacteria are exposed to an oxygen environment for a long period of time this can cause bacterial death. For this reason all manipulations carried out as to minimise the time bacteria are exposed to oxygen. Typically the bacteria should not be exposed to non-microaerophilic conditions for more than 20 minutes. Manipulations or workings should also be done aseptic conditions.

All *H. pylori* cultures were maintained at 37 °C under microaerophilic conditions in the Genboxes and maintained with one MicroGen sachet per box (Biomerieux). Typically, bacteria took 5 days to revive from frozen and 3-4 days to replenish after re-seeding of plates. New MicroGen sachets were added every 72 h or after the container was opened in order to maintain the microaerophilic environment.

**H. pylori propagation**

All *H. pylori* strains arrived from ATCC in their freeze dried state. All *H. pylori* were propagated in the same manner. In order to propagate the growth of the bacteria, it is important to use freshly poured plates (TSA + 5% SB or CB + 10% SB) to give the best results.

The bacterial vial was opened and 0.5 mL of TSB was added. This was transferred onto two separate plates. These plates were incubated at 37 °C under microaerophilic conditions for 3 days, as described above. Propagation via broth or a biphasic slant is also an acceptable method. All tubes and slants should be incubated with caps
loosened if this is the case. Once good growth is obtained the culture was frozen or transferred for further growth as described below.

**Re-seeding *H. pylori* plates**

Note that re-seeding should be avoided wherever possible. It is better practice to grow stocks only when required. Prolonged re-seeding of the bacteria can result in slight deviations of their genetic properties.

Once good growth is observed, a sterile cotton wool swab was used to wipe some of the plate so that bacteria were taken up on the swab. This is then spread on a new plate, ensuring all the plate is covered by rotating the plate 120° three times and spreading in a lateral streaking motion.

**Freezing down stocks**

Freezing is an efficient and reliable way to store bacteria. A good supply of frozen stock solutions should be kept for use when required. A stock solution is made when a plate has good growth evident after 3-4 days. The freezing solution is a 10% glycerol solution in TSB. Glycerol reduces the harmful effect of ice crystals on bacteria which can damage cells by dehydration caused by a localized increase in salt concentration leading to denaturation of proteins. Additionally, ice crystals can also puncture cellular membranes. 0.5 mL of the freezing solution is added to a plate with a confluent law of growth. Using an inoculating loop, the bacteria are removed from the surface. The bacterial suspension is then removed from the plate using a Gilson pipette and placed in a sterile vial suitable for freezing. Additional washes of the plate can be performed to ensure complete removal of the bacteria, taking care not to dilute the stock too much.

The bacterial solution should then be placed at -20 °C for one hour and then transferred and stored until required at -80 °C.

**Revival of *H. pylori* from frozen stocks**

The frozen stocks, when required, were removed from storage at -80 °C. They were allowed to warm to room temperature slowly. Once thawed, the stock were agitated briefly, taken up, and spread on a pre-prepared plate. The plates were then placed in a GenBox with a MicroGen sachet and incubated at 37 °C for 4-5 days. It was
noticed that growth from frozen stocks took longer to reach a good confluent level compared to re-seeded growth.

5.3 In vitro testing

H. pylori MIC
Minimum inhibitory concentrations (MIC) of our complexes against H. pylori were determined using the agar dilution method, as per the Clinical and Laboratory Standards Institute (CLSI) guidelines. Doubling dilutions of compounds at concentration range of 0.5-64 μg mL\(^{-1}\) were incorporated into TSA + 5% SB plates.

A 4 McFarland standard suspension of H. pylori in 0.9% NaCl (approximately 12 x 10\(^8\) CFU mL\(^{-1}\)) measured using a Densicheck instrument, (Bioemerieux Ltd, Basingstoke, United Kingdom) was generated. Aliquots (5 μL) of these suspensions were dropped onto the TSA + 5% SB plates containing test compounds. Each compound was tested alongside BSS and their free ligand in comparable concentrations and a control plate. The MIC’s, defined as the lowest concentration at which no growth was observed, were determined by examination of the plates after incubation under microaerophilic conditions for 3–5 days at 37 °C.

Bacterial LC\(_{50}\) Determination
Anti-bacterial evaluation of our compounds was also carried out against a range of environmental and pathogenic strains of bacteria. LC\(_{50}\) work was carried out in the microbiology laboratory at the School of Biotechnology, Dublin City University. The bacteria used are outlined in the table below and were sourced from The Leibniz Institute DSMZ- German collection of microorganisms and cell cultures in-house clinical isolates, Table 2.2. The organisms were routinely grown at 37 °C overnight on nutrient agar (Sigma-Aldrich Ireland Ltd., Wicklow, Ireland) and maintained on the agar in sealed plates at 4 °C for up to 1 week.
Table 2.2. Strains of bacteria, beyond *H. pylori*, used in this research.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Bacteria</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSM10</td>
<td><em>Bacillus subtilis</em></td>
<td>Environmental</td>
</tr>
<tr>
<td>DSM30053</td>
<td><em>Enterobacter aerogenes</em></td>
<td>Biohazard II</td>
</tr>
<tr>
<td>DSM1103</td>
<td><em>Escherichia coli</em></td>
<td>Biohazard II</td>
</tr>
<tr>
<td>DSM498</td>
<td><em>Escherichia coli</em></td>
<td>Environmental</td>
</tr>
<tr>
<td>DSM50090</td>
<td><em>Pseudomonas fluorescens</em></td>
<td>Environmental</td>
</tr>
<tr>
<td>DSM6125</td>
<td><em>Pseudomonas putida</em></td>
<td>Environmental</td>
</tr>
<tr>
<td>DSM799</td>
<td><em>Staphylococcus aureus</em></td>
<td>Biohazard II</td>
</tr>
<tr>
<td>T37-1</td>
<td><em>Escherichia coli</em></td>
<td>Environmental</td>
</tr>
<tr>
<td>CP1</td>
<td><em>Pseudomonas putida</em></td>
<td>Environmental</td>
</tr>
</tbody>
</table>

Stock solutions of investigated compounds were freshly prepared and diluted with Mueller-Hinton broth to various working concentrations with a final DMSO concentration of ≤ 1%. LC$_{50}$ values for the compounds were determined using a modification of the broth micro dilution method described by Amsterdam.$^{16}$ Strains were grown in nutrient broth overnight, washed with phosphate buffered saline (PBS) and the cell number adjusted to give an optical density reading of 0.07 or 0.09, dependent on the bacterial strain, at 660 nm. Each well was inoculated with 5 μL of bacterial culture.

Qualitative agar incorporation tests indicated that the LC$_{50}$ of the test compound and control, BSS, would be in the 0-10 μg mL$^{-1}$ range against all bacteria and that Ampicillin had varying activities across the 0-500 μg mL$^{-1}$ range against all bacteria. In turn the LC$_{50}$ of test compound, BSS and Ampicillin were investigated using two fold dilutions in these concentration ranges. Wells containing only the stock solutions of investigated compounds in Mueller-Hinton broth were used as blanks and wells containing only 1% DMSO in Mueller-Hinton broth and culture were used as positive controls. The microplates were incubated overnight at 30 °C for all bacteria except *Enterobacter aerogenes* DSM30053, *Escherichia coli* DSM1103 and *Staphylococcus aureus* DSM799 which were incubated at 37 °C. The presence or absence of growth was determined by measuring the optical density of the wells at a wavelength of 660 nm using a plate reader. The LC$_{50}$ values were determined as the concentration or range of concentrations that caused a 50% reduction in cell growth.
Bacterial MIC Determination

MIC work was carried out in the microbiology laboratory in the RCSI Smurfit Research Institute at Beaumont Hospital. The bacteria used were sourced from American Type Culture Collection (ATCC) or in-house clinical isolates. The bacteria used in these tests are outlined below in Table 2.2.

Table 2.2. Pathogenic bacterial strains used in MIC determination.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Bacteria</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>25922</td>
<td><em>Escherichia coli</em></td>
<td>Biohazard I</td>
</tr>
<tr>
<td>25923</td>
<td><em>Staphylococcus aureus</em></td>
<td>Biohazard II</td>
</tr>
<tr>
<td>43300</td>
<td><em>MRSA S. aureus</em></td>
<td>Biohazard II</td>
</tr>
<tr>
<td>CL2</td>
<td><em>ESBL E. coli</em></td>
<td>Clinical Isolate</td>
</tr>
</tbody>
</table>

Stock solutions of investigated compounds were freshly prepared and diluted with Mueller-Hinton broth to various working concentrations with a final DMSO concentration of ≤ 2%. Minimum inhibitory concentrations (MIC) of test compound against *Escherichia coli* and *Staphylococcus aureus* were determined using broth micro dilution method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Briefly, doubling dilutions of test compounds were made in a sterile MH broth (Oxoid, UK) solution at concentration range of 1.56-200 µM. Strains were grown in nutrient broth overnight. A 1 McFarland (approximately 3 x 10⁸ CFU mL⁻¹) was generated using a Densichek meter (Biomerieux, Ireland) and were further diluted 1/50 in MH broth. Each well was inoculated with 50 µL of bacterial culture and 50 µL of test compound. Wells containing only the stock solutions of investigated compounds in Mueller-Hinton broth were used as negative controls and wells containing only 2% DMSO in Mueller-Hinton broth and culture were used as positive controls. The microplates were incubated overnight at 37 °C for all bacteria.

The presence or absence of growth was determined after centrifuging the 96 well plate, pelleting down the bacteria. The MIC values were determined as the concentration which caused complete inhibition of bacterial growth in the well.
6. Leishmanial Evaluation

Anti-proliferative Activity

Multiplication inhibition assay tests were carried out in the Department of Microbiology at the Federal University of Rio de Janeiro, Brazil. The tests were performed on *Leishmania amazonensis* (MHOM/BR/PH8) and *Leishmania chagasi* (NCL/UFRJ/IOC-L 3241 strain) promastigotes, obtained from Coleção de *Leishmania* from Fundação Oswaldo Cruz (*Leishmania* Type Culture Collection-LTTC-WDCM 731). These strains were maintained by weekly transfers in 25 cm³ culture flasks with Schneider's insect medium, pH 7.0, supplemented with 10% fetal bovine serum (FBS) at 28 ºC.

The activity of compounds were evaluated upon the growth rate of *L. amazonensis* and *L. chagasi* promastigote forms, by incubation in Schneider’s insect medium with 10% FBS at 28 ºC. Briefly, promastigotes were counted using a Neubauer chamber and re-suspended in fresh medium to a final concentration of 5×10⁵ viable promastigotes per mL. The viability was assessed by mobility and lack of staining after challenging with Trypan blue. Each compound was added to the culture at final concentrations in the 5-100 µM range and the antimonial was added to the culture at final concentrations in the 5-60 µM. After 24, 48, 72 and 96 h of incubation at 28 ºC, the number of viable, motile promastigotes was quantified. The IC₅₀, i.e. the minimum drug concentration that caused a 50% reduction in survival/viability was determined by linear regression analysis by plotting the number of viable promastigotes versus log drug concentration using Origin Pro 7.5 computer software.

Cell Viability Assays

Cell viability tests were also carried out in the Department of Microbiology at the Federal University of Rio de Janeiro, Brazil. RAW 264.7 (murine macrophage) cell lineage was used to test cytotoxic effect of our compounds. The cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) with supplemented with 10% FBS at 37 ºC in an atmosphere containing 5% CO₂.

The viability of RAW cells were evaluated by MTT assay.¹⁸ Firstly, the macrophages of density 10⁵ macrophages per mL were allowed to adhere in 96-well tissue culture plates for 12 h at 37 ºC, in a 5% CO₂ atmosphere. Non-adherent cells
were removed by washes with sterile DMEM and the wells refilled with DMEM medium supplemented with 10% FBS. After that, the cells were incubated or not (control) with increasing concentrations of the test compounds (2-500 µM) and then incubated for additional 24 hours at 37 °C, in a 5% CO₂ atmosphere. Subsequently, the culture medium was discarded and the formation of formazan was measured by adding MTT (5 mg mL⁻¹ in PBS) (Sigma-Aldrich Chemical Co., St Louis, USA) and incubating the wells for more 3 hours in the dark at 37 °C. The plates were subsequently centrifuged at 500 x g for 8 minutes, the supernatant was carefully removed, the pellet dissolved in 200 µL of DMSO and the absorbance measured in an ELISA reader at 570 nm (SpectraMax Gemini 190, Molecular Devices, CA, USA). The 50% cytotoxicity inhibitory concentration (CC₅₀) was determined by linear regression analysis. Pentavalent antimonial was also used as a positive control at the same conditions.
7. Potentiometry Study

Potentiometry Study was undertaken by Dr. Éva A. Enyedy at the Department of Inorganic and Analytical Chemistry, University of Szeged, Hungary.

Chemicals

GSK1322322 was obtained from GlaxoSmithKline (Pennsylvania, United States). Acetohydroxamic acid (Aha), KCl, HCl, KOH, 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS), DMSO, and GaCl₃ were purchased from Sigma-Aldrich and used without further purification. GaCl₃ stock solution was prepared in HCl and its concentration was determined by complexometry via the EDTA complex. Accurate strong acid content of the metal stock solution was determined by pH-potentiometric titrations. Doubly distilled Milli-Q water was used for sample preparation.

pH-Potentiometric measurements and calculations

The pH-potentiometric measurements for the determination of the proton dissociation constants of GSK1322322 and Aha, and the overall stability constants of the Ga(III) complexes were carried out at 25.0 ± 0.1 °C in DMSO : water 30 : 70 (w/w) as solvent and at an ionic strength of 0.10 M (KCl) used in order to keep the activity coefficients constant. The titrations were performed with carbonate-free KOH solution of known concentration (0.10 M). The concentrations of the base and the HCl were determined by pH-potentiometric titrations. An Orion 710A pH-meter equipped with a Metrohm combined electrode (type 6.0234.100) and a Metrohm 665 Dosimat burette were used for the titrations. The electrode system was calibrated to the pH = −log[H⁺] scale in the DMSO/water solvent mixture by means of blank titrations (strong acid vs. strong base: HCl vs. KOH), similar to the method suggested by Irving et al. in pure aqueous solutions. The average water ionization constant pKₐ was 14.52 ± 0.05, which corresponds well to the literature data. The reproducibility of the titration points included in the calculations was within 0.005 pKₐ. The pH-metric titrations were performed in the pH range 2.0-12.5. The initial volume of the samples was 5.0 mL. The ligand concentration was 1 or 0.5 mM and metal ion-to-ligand ratios of 1:1-1:4 were used. The accepted fitting of the titration curves was always less than 0.01 mL. Samples were deoxygenated by bubbling purified argon through them for approximately 10 min prior to the measurements. Argon was also passed over the solutions during the titrations.
concentration of the ligand stock solutions together with the proton dissociation constants were determined by pH-potentiometric titrations with the use of the computer program HYPERQUAD\textsuperscript{21} to establish the stoichiometry of the complexes and to calculate the stability constants (log(β(M\textsubscript{p}L\textsubscript{q}H\textsubscript{r})) employing literature data for Ga(III) hydroxido complexes.\textsuperscript{22} β(M\textsubscript{p}L\textsubscript{q}H\textsubscript{r}) is defined for the general equilibrium pM + qL + rH ⇌ M\textsubscript{p}L\textsubscript{q}H\textsubscript{r} as β(M\textsubscript{p}L\textsubscript{q}H\textsubscript{r}) = [M\textsubscript{p}L\textsubscript{q}H\textsubscript{r}]/[M]\textsuperscript{p}[L]\textsuperscript{q}[H]\textsuperscript{r}, where M denotes the metal ion and L the completely deprotonated ligand. In all calculations exclusively titration data were used from experiments in which no precipitate was visible in the reaction mixture.

**UV-vis spectrophotometric and 1H NMR measurements**

A Hewlett Packard 8452A diode array spectrophotometer was used to record the UV–vis spectra in the 200 to 950 nm window. The path length was 0.5 cm. Proton dissociation constants of GSK1322322 and the molar absorbance spectra of the individual species were calculated with the computer program PSEQUAD.\textsuperscript{23} The spectrophotometric titrations were performed on samples containing the ligand at 106 mM in the pH range from 2 to 12.5 at 25.0 ± 0.1 °C in DMSO:water 30:70 (w/w) at an ionic strength of 0.10 M (KCl).

\textsuperscript{1}H NMR spectroscopic titrations were carried out on a Bruker Ultrashield 500 Plus instrument. DSS was used as an internal NMR standard and WATERGATE method was used to suppress the solvent resonance. pH-dependency of the spectra of GSK1322322 was followed in a 30% (v/v) DMSO-d\textsubscript{6}/H\textsubscript{2}O mixture in a concentration of 0.96 mM at ionic strength of 0.10 M (KCl). The Ga(III)-to-ligand ratio was 1:3 in the Ga(III)-containing samples.
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Chapter 3

A Novel Class of Bi(III) Hydroxamato Nitrato Complexes; Synthesis and Investigation of Urease Inhibitory Activity and Anti-\textit{H. pylori} Activity.
1. Chapter Aims

- To design, synthesise and characterise a novel class of Bi(III) hydroxamato nitrato complexes.

- To elucidate the exact structure of novel Bi(III) complexes by X-ray crystallography.

- To investigate the urease inhibitory activity of novel Bi(III) complexes.

- To determine the anti-\( H. \text{ pylori} \) activity of novel Bi(III) complexes against three strains of \( H. \text{ pylori} \).
2. Introduction

*Helicobacter pylori* is a microaerophilic and neutrophilic Gram-negative bacterial pathogen that colonises the human stomach. *H. pylori* infection can cause dyspepsia, peptic ulcer disease and GC and is the second highest cause of cancer related deaths in the world.¹,² The WHO classified *H. pylori* as a Class 1 Carcinogen in 1994 and significantly, eradication of *H. pylori* infection reduces the incidence of GC.³ More recently, *H. pylori* has been linked with extragastric diseases such as cardiovascular disease, diabetes mellitus, sideropenic anemia and gallbladder cancer, for example.⁴

*H. pylori* has developed a number of ways to survive and colonise the harsh acidic environment of the gastric mucosa and in turn induce chronic infection. One mechanism is “acid acclimation” whereby in the acidic environment of the stomach, *H. pylori* periplasmic pH is adjusted by regulation of urease enzymatic activity, UreI, and α-carbonic anhydrase.⁵ Urease, a dinuclear nickel(II) enzyme, catalyses the conversion of urea into ammonia and carbon dioxide.⁵ Upon a decrease in pH, UreI, a pH-gated urea channel in the cytoplasmic membrane, allows urea to be transported into the cytoplasm. A concurrent increase in bacterial urease activity, produces ammonia which can neutralise protons entering the cytoplasm and maintain the pH microenvironment at around pH 6.8.⁵ Urease, which accounts for 10–15% of total protein by weight,⁶ can be considered to be *H. pylori*’s Achilles heel, as its activity is vital to the survival of *H. pylori* and its ability to colonise the harsh acidic environment of the gastric mucosa, in turn inducing chronic infection.⁵

Standard triple therapy treatment comprising of the administration of two antibiotics in addition to a PPI, is the current first-line treatment for *H. pylori*.⁷ However, *H. pylori* infections are becoming increasingly difficult to eradicate due to AR. Antibacterial drug resistance is a serious global health problem and the prospect of untreatable bacterial infections is becoming a reality. AR rates in Europe and Asia for clarithromycin are 17.5% and 21.5% and for metronidazole are 34.9% and 95.4% respectively, resulting in poor *H. pylori* eradication.⁸,⁹

Consequently, the use of bismuth containing quadruple therapies are increasing as a first-line treatment recommendation in many countries, particularly in China where
antibiotic resistance is widespread and bismuth therapies achieve excellent eradication rates of > 95%\textsuperscript{,7, 10-12} Bismuth containing quadruple therapies therefore represent a successful strategy for overcoming \textit{H. pylori} resistance.\textsuperscript{13}

Clinically used bismuth subsalicylate (BSS), colloidal bismuth subcitrate (CBS) and the more recently developed ranitidine bismuth citrate (RBS), are administered orally. However their structure, behaviour in biological environment or indeed their MOA is not well understood.\textsuperscript{14-16} Recently innovative metallomic and metalloproteomic approaches have aided identification of bismuth targeting proteins in \textit{H. pylori} such as HspA, Ef-Tu, NapA and urease (UreA and UreB) and revealed that Bi(III) has a high selectivity for cysteine and histidine residues, particularly motif patterns of CX\textsubscript{n}C, CX\textsubscript{n}H and HX\textsubscript{n}H, where X is a cysteine or histidine amino acid. Evidence therefore suggests that bismuth interference with metal homeostasis and oxidation reduction processes may be important.\textsuperscript{17-21}

Significantly, it was recently demonstrated that glutathione and multidrug resistance protein transporters remove Bi from human cells, thereby protecting the human body from associated toxicity and also providing a level of selectivity between human and bacterial cells that lack glutathione such as \textit{H. pylori}.\textsuperscript{22} Orally delivered Bi(III) is known to be almost exclusively excreted and concentrated in urine and stool.\textsuperscript{23} Thus there is renewed interest in the development of novel Bi-based complexes as anti-\textit{H. pylori} agents and investigation of their mode of action.\textsuperscript{16, 24-29}

Hydroxamic acids are a family of important bio-ligands of general formula RCONHOH. They are known for their ability to chelate to metals and metalloenzymes.\textsuperscript{30-32} Significantly Aha, Bha and Sha are potent inhibitors of urease. Aha for example has been demonstrated to bridge the dinuclear Ni(II) active site of the urease enzyme as highlighted in \textbf{Figure 3.1}.\textsuperscript{6}

![Figure 3.1](image_url)  \textbf{Figure 3.1}  Aha (A), Bha (B) and Sha (C). The binding of Aha at the dinuclear Ni(II) active site of urease, displaying bidentate bridging (O, µ-O') chelation.\textsuperscript{33}
3. Project rational

The combination of the established anti-bacterial properties of bismuth with the urease inhibitory properties of hydroxamic acids could potentially develop structurally well-defined bismuth hydroxamic acid complexes as anti-\textit{H. pylori} agents, Figure 3.2.

It was hypothesised that such complexes could hydrolyse in the low pH of gastric juice releasing insoluble bismuth salts, such as BiOCl, and free hydroxamic acids. The bismuth salts and/or bismuth gastrointestinal biomolecular complexes will exhibit conventional anti-bacterial activity against \textit{H. pylori}. The free hydroxamic acids will inhibit the activity of \textit{H. pylori} urease drastically reducing its ability to buffer the pH of its microenvironment. Co-delivery of bismuth and hydroxamic acids and activation at the site of action could be effective strategy for \textit{H. pylori} eradication.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure3.2.png}
\caption{The proposed concept of this project. The Bi(III) hydroxamato complex dissociates in the acidic medium of the stomach. The hydroxamic acid is then free to inhibit urease enzymes and the Bi is free to disrupt metal homeostasis or exert its inherent gastroprotective qualities.}
\end{figure}

Bha, Sha and 2-aminophenylhydroxamic acid (2-NH$_2$-Pha) were selected as hydroxamic acid urease inhibitors and potential ligands for binding to bismuth. Hydroxamic acids typically coordinate metal centres in an (O, O’) bidentate chelating fashion, coordinating through the carbonyl oxygen atom and the
deprotonated hydroxyl group to form very stable five-membered hydroxamato chelates.

Sha and 2-NH$_2$-Pha could potentially generate interesting hydroxamato complexes. The hydroxyl and amino groups may act as secondary coordinating groups or anchors and encourage ($O_2$-$OH$, $O$) or ($N_{NH_2}$, $O$) hydroxamato coordination to Bi(III), Figure 3.3.

Figure 3.3. Possible binding modes for mononuclear coordination of bismuth to Sha: ($O$, $O'$), ($O_2$-$OH$, $O'$) and 2-NH$_2$-Pha: ($O$, $O'$), ($N_{NH_2}$, $O$).
4. Synthesis

Synthesis of Hydroxamic Acids

Bha and Sha were commercially available and used without further purification.

Synthesis of 2-NH$_2$-Pha (1)

As previously reported, synthesis of hydroxamic acids proceed via the reaction of hydroxylamine with an activated acyl carbonyl such as an acid chloride, amide or ester. 1 was synthesised from the corresponding methyl ester as shown in the reaction scheme below, Scheme 3.1.

![Scheme 3.1. Synthesis of 2-NH$_2$-Pha, 1, from methyl 2-aminobenzoate.](image_url)

The hydroxamic acid was obtained in good yield (70%) and excellent purity. This compound was characterised by EA, IR, MS, $^1$H and $^{13}$C NMR spectroscopy.

Elemental analysis was consistent with the formation of C$_7$H$_8$N$_2$O$_2$, with the full molecular ion present in ESI-MS in the positive mode at 152.1 a.m.u.

A significant and diagnostic feature of hydroxamic acids in general, is the frequency at which the $\nu$(C=O) appear in their IR spectra. Typically, as a result of intermolecular bonding, only one stretch is observed which encompasses the symmetric and asymmetric $\nu$(C=O) stretches. The $\nu$(C=O) generally occur between 1615 cm$^{-1}$ and 1660 cm$^{-1}$. The $\nu$(C=O) of 1 arises at 1639 cm$^{-1}$.

$^1$H NMR analysis for this compound was performed in DMSO-$d_6$. In this solvent observation and identification of the hydroxamic N-H and O-H signals is possible. The signal associated to N-H occurs at 8.84 ppm while the O-H signals appears at 10.91 ppm. Four signals in the aromatic region of this spectrum from 7.31-6.35 ppm, corresponding to and integrating well for the four protons of the ortho-substituted phenyl ring. A broad signal at 6.23 ppm integrates for two, representing the primary amine protons at the ortho-position. Other analysis further confirmed the production of 1.
Synthesis of Bismuth Hydroxamato Nitrato Complexes

Reaction of hydroxamic acids \(L\) with Bi(NO\(_3\))\(_3\), in ethanol, gave novel complexes of type [Bi(L\(-\text{H}\))\(_2\)NO\(_3\)], representing a novel class of Bi(III) hydroxamato nitrato complexes.

**Synthesis of \([\text{Bi}_2(\text{Bha-1H})_2(\mu-\text{Bha-1H})_2(\eta^2-\text{NO}_3)_2]\) (2)**

Reaction of Bha with Bi(NO\(_3\))\(_3\).5H\(_2\)O, in absolute ethanol, under reflux for 5 hours afforded the novel dinuclear Bi(III) complex \([\text{Bi}_2(\text{Bha-1H})_2(\mu-\text{Bha-1H})_2(\eta^2-\text{NO}_3)_2]\), 2. This product was obtained in 77% yield and high purity, **Scheme 3.2**.

![Scheme 3.2. Synthesis of \([\text{Bi}_2(\text{Bha-1H})_2(\mu-\text{Bha-1H})_2(\eta^2-\text{NO}_3)_2]\), 2 a novel Bi(III) benzohydroxamato nitrato complex.](image)

\([\text{Bi}_2(\text{Bha-1H})_2(\mu-\text{Bha-1H})_2(\eta^2-\text{NO}_3)_2]\) was characterised by EA, IR, \(^1\text{H}\) NMR, MS and X-ray crystallography.

Elemental analysis of 2 is consistent with the presence of two mono-deprotonated Bha ligands and one nitrate per bismuth centre.

The \(^1\text{H}\) NMR analysis of 2 was carried out in MeOD. No signals associated with the hydroxamic acid protons (N–H and O–H) are detected, as expected, due to the rapid exchange of protons with the solvent. In 2, three main aromatic signals are observed at 7.9-7.4 ppm, corresponding to and integrating well for the five aromatic protons of the ligand. The most significant shift is that of an aromatic doublet, corresponding to the protons closest to the hydroxamic acid group at the 2 and 6 positions on the phenyl ring. It shifts from 7.73 ppm in the free ligand to 7.84 ppm in complex 2, **Figure 3.4**.
Figure 3.4. The stacked $^1$H NMR spectra of Bha (top) and complex 2 (bottom). The expansion of the area of interest (insert), showing the shifts in the aromatic signals associated with coordination of Bi(III).

The IR spectra of 2 exhibits distinctive $\nu$(C=O) at 1595 cm$^{-1}$ displaying the characteristic shift associated with (O, O') hydroxamato coordination when compared to the corresponding $\nu$(C=O) of the uncoordinated ligand at 1647 cm$^{-1}$.

These findings are in accordance with earlier studies carried out by Deacon et al. which claim that ligands typically adopt a bi-dentate chelating mode if $\Delta \nu < 200$ cm$^{-1}$ and mono-dentate mode if $\Delta \nu > 200$ cm$^{-1}$.

The shift to lower wavenumber for the carbonyl suggests bonding to the bismuth atom via the formation of the thermodynamically preferred five membered bidentate chelate, via the hydroxamato carbonyl group and deprotonated hydroxyl group. A strong signal at 1383 cm$^{-1}$ is associated with the presence of a nitrate $\nu$(N-O) and the signal at 1560 cm$^{-1}$, is assigned to the $\nu$(C-N) of the hydroxamic acid.

ESI-MS in the positive mode assisted in the identification of $[\text{Bi}_2(\text{Bha} \cdot \text{H})_2(\mu-\text{Bha} \cdot \text{H})_2(\eta^2-\text{NO}_3)_2]$. The full molecular ion, ([M+H]$^+$), was not observed but molecular fragments associated with $[\text{BiNO}_3]^{2+}$ 271.8, $[\text{Bi}(\text{Bha} \cdot \text{H})]^{2+}$ 345.7, $[\text{Bi(NO}_3)(\text{Bha} \cdot \text{H})]^+$ 407.7 and $[\text{Bi}(\text{Bha} \cdot \text{H})_2]^+$ 480.7 a.m.u were evident.
Crystals suitable for X-ray diffraction were obtained of 1 from recrystallization in methanol, confirming the complex structure to be dinuclear and features both bidentate bridging (O, μ-O’) and bidentate non-bridging (O, O’) coordination modes.

Synthesis of [Bi(NO$_3$)$_3$(Sha$_{1H}$)$_2$]$_n$ (3)

Reaction of Sha with Bi(NO$_3$)$_3$·5H$_2$O, in absolute ethanol, under reflux for 5 hours afforded a Bi(III) complex of general formula [Bi(NO$_3$)(Sha$_{1H}$)$_2$]$_n$, 3, Scheme 3.3.

Scheme 3.3. Outline of the reaction scheme for complex 3, where n ≥ 1

3 was characterised fully by EA, IR, $^1$H NMR, MS and X-ray crystallography.

Elemental analysis of 3 is consistent with the presence of two hydroxamic acids and one nitrate per bismuth centre. We acknowledge that the carbon exceeds the 0.4% standard for EA. This however is an exception which we cannot explain. (It was accepted by peer review in Dalton Trans)

In the $^1$H NMR spectra of 3, carried out in MeOD, no hydroxamic acid protons signals are observed. Three main aromatic signals are observed in the region of 7.85-6.87 ppm. Those signals correspond and integrate well for the five aromatic protons of each ligand. The most significant shift is that of the protons closest to the hydroxamic acid group at the 6 positions on the aromatic ring. A shift is observed in the signal of the doublet at 7.65 ppm in the free ligand to 7.85 ppm in complex 3, Figure 3.5.
Figure 3.5. The stacked $^1$H NMR spectra of the Sha (top) and complex 3, (bottom), and the expansion of the area of interest (insert), showing the shifts in the aromatic signals associated with the binding of bismuth.

The IR spectra of 3 exhibits distinctive $\nu$(C=O) at 1604 cm$^{-1}$. This shift is characteristic of (O, O') hydroxamato coordination. The $\nu$(C=O) at 1604 cm$^{-1}$ is sufficiently different in comparison to the corresponding $\nu$(C=O) of the uncoordinated ligands at 1619 cm$^{-1}$. These findings are in accordance with previously mentioned studies carried out by Deacon et al. and predict a bi-dentate chelating mode of binding as was evident for 2. A strong signal at 1383 cm$^{-1}$ is representative of the present of a $\nu$(N-O) of a nitrate group and the stretch at 1560 cm$^{-1}$, also present in 2, represents the $\nu$(C-N) of the hydroxamic acid.

ESI-MS in the positive mode assisted in the characterisation of 3. The full molecular ion, ([M+H]$^+$), was not observed for this complex but significantly the molecular fragment associated with [Bi(Sha$_{111}H$)$_2$] was evident at 513.4 a.m.u.

Upon recrystallization of 3 from methanol, crystals of [Bi$_6$(CH$_3$OH)$_2$(η$_1$-NO$_3$)$_2$(η$_2$-NO$_3$)$_2$(H$_2$O)$_2$(Sha$_{111}$H)$_2$](CH$_3$OH)$_4$·(H$_2$O)$_4$, 3a were obtained. Elemental analysis carried out on these crystals were consistent with the crystal structure and formula of
Chapter 3

$\text{C}_{86}\text{H}_{84}\text{Bi}_{6}\text{N}_{16}\text{O}_{52}$. 3a is a hexanuclear bismuth complex that features only the bidentate bridging ($\text{O, } \mu\text{-O}')$ coordination modes for Sha.

**Synthesis of [Bi(2-NH$_2$-Pha$_{1\text{H}}$)$_2$(NO$_3$)$_2$]$_n$ (4)**

Reaction of 2-NH$_2$-Pha (1) with Bi(NO$_3$)$_3$.5H$_2$O in absolute ethanol at room temperature for 72 hours afforded a Bi(III) complex 4, [Bi(2-NH$_2$-Pha$_{1\text{H}}$)$_2$(NO$_3$)$_2$]$_n$ in a moderate yield of 49%, Scheme 3.4.

![Scheme 3.4. Outline of the reaction scheme for complex 4, where $n = \geq 1$.](image)

[Bi(2-NH$_2$-Pha$_{1\text{H}}$)$_2$(NO$_3$)$_2$]$_n$ was characterised fully by EA, IR, $^1$H NMR and MS.

Elemental analysis are reasonably consistent with the presence of two hydroxamic acid ligands and one nitrate per Bi(III) centre, although the $\Delta% C$ is 2.61 which far exceeds the 0.4% standard threshold for elemental analysis. Significantly the empirical formula associated with the elemental analysis, C$_{13}$H$_{14}$N$_5$, practically matches the required formula (C$_{14}$H$_{14}$N$_5$) for [Bi(2-NH$_2$-Pha$_{1\text{H}}$)$_2$(NO$_3$)$_2$]. Elemental analyses for 2, 3 and 4 are shown in Table 3.1 for purposes of comparison.

**Table 3.1. Summary of the EA results for complexes 2, 3 and 4.**

<table>
<thead>
<tr>
<th>Complex</th>
<th>% C</th>
<th>% H</th>
<th>% N</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2</strong></td>
<td><strong>Calculated</strong></td>
<td>30.95</td>
<td>2.23</td>
</tr>
<tr>
<td></td>
<td><strong>Found</strong></td>
<td>30.84</td>
<td>1.91</td>
</tr>
<tr>
<td><strong>3</strong></td>
<td><strong>Calculated</strong></td>
<td>30.14</td>
<td>2.47</td>
</tr>
<tr>
<td></td>
<td><strong>Found</strong></td>
<td>31.06</td>
<td>2.08</td>
</tr>
<tr>
<td><strong>4</strong></td>
<td><strong>Calculated</strong></td>
<td>29.33</td>
<td>2.46</td>
</tr>
<tr>
<td></td>
<td><strong>Found</strong></td>
<td>26.85</td>
<td>2.50</td>
</tr>
</tbody>
</table>

The following analysis is discussed to highlight that 2-NH$_2$-Pha does react with Bi(NO$_3$)$_3$.5H$_2$O though clearly acknowledging the product was not isolated in the required purity.
In the \(^1\)H NMR of complex 4, the four main resonance peaks in the aromatic region of 7.5-6.5 ppm are observed and correspond to the four aromatic protons of the ligand. When compared to the free ligand, a distinct downfield shift in the resonance signals on chelation of 2-NH\(_2\)-Pha to Bi(III) was noticed, Figure 3.6. Impurities are observed at 7.61 ppm, 7.27 ppm and 6.70 ppm, which are not associated with the free ligand.

**Figure 3.6.** The stacked \(^1\)H NMR spectra of the free ligand (top) and complex 4 (bottom), and the expansion of the area of interest (insert), which shows the shifts in the aromatic signals associated with bismuth binding.

The IR spectra of 4 exhibits a distinctive \(\nu(C=O)\) at 1609 cm\(^{-1}\) displaying the characteristic shift associated with (O, O') hydroxamato coordination when compared to the corresponding \(\nu(C=O)\) of the uncoordinated ligands at 1639 cm\(^{-1}\).\(^{35}\)

The shift to lower wavenumber for the carbonyl suggests chelation to the Bi(III) centre via the formation of the preferred five membered chelate. A strong signal at 1288 cm\(^{-1}\) is representative of the presence of the \(\nu(N-O)\) of the nitrate group. A summary of the IR frequency stretches of the ligand and the complexes are shown below in Table 3.2 for purposes of comparison.


Table 3.2. Overview of the stretches observed in IR spectroscopy.

<table>
<thead>
<tr>
<th>Complex</th>
<th>(\nu(C=O))</th>
<th>(\nu(C-N))</th>
<th>(\nu(N-O))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bha</td>
<td>1645 cm(^{-1})</td>
<td>1556 cm(^{-1})</td>
<td>---</td>
</tr>
<tr>
<td>2</td>
<td>1595 cm(^{-1})</td>
<td>1560 cm(^{-1})</td>
<td>1385 cm(^{-1})</td>
</tr>
<tr>
<td>Sha</td>
<td>1619 cm(^{-1})</td>
<td>1555 cm(^{-1})</td>
<td>---</td>
</tr>
<tr>
<td>3</td>
<td>1604 cm(^{-1})</td>
<td>1560 cm(^{-1})</td>
<td>1383 cm(^{-1})</td>
</tr>
<tr>
<td>2-NH(_2)-Pha (1)</td>
<td>1639 cm(^{-1})</td>
<td>1559 cm(^{-1})</td>
<td>---</td>
</tr>
<tr>
<td>4</td>
<td>1609 cm(^{-1})</td>
<td>1558 cm(^{-1})</td>
<td>1288 cm(^{-1})</td>
</tr>
</tbody>
</table>

ESI-MS in the positive mode assisted in characterising 4. The full molecular ion, ([M+H]\(^+\)) was not detected though a molecular fragment at 619.0 which is associated with [Bi(2-NH\(_2\)-Pha-H\(_2\))\(_2\)(NO\(_3\))Na\(_2\)] was observed.

Despite several attempts, single crystals of 4 suitable for X-ray structural characterisation could not be isolated. The predicted structure of complex 4 based on our analysis is shown in Figure 3.7.

![Proposed structure of 4](image)

Figure 3.7. Proposed structure of 4, based on the analysis of this complex.

Andrews and coworkers published work in relation to Bi(III) hydroxamato complexes at a similar time to this work being carried out. He described the synthesis of a number of Bi(III) hydroxamato and Bi(III) hydroxamato/hydroximato complexes from Sha, Bha, Aha, \(N\)-methylfurohydroxamic acid (Mfha) and \(N\)-benzoyl-\(N\)-phenylhydroxamic acid (Bpha) formed from two different bismuth precursors, BiPh\(_3\) and Bi(O\(_{i}\)Bu)\(_3\).\(^{28, 29, 37}\) A summary of the reactions and products are highlighted in Scheme 3.5 and Table 3.3.
Scheme 3.5. The different synthetic routes taken by Andrews et al. in the formation of Bi(III) complexes.

Table 3.3. Ligands, Bismuth precursor and reaction conditions for formation of Bi(III) hydroxamato and Bi(III) hydroxamato/hydroximato complexes published by Andrews et al.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Bismuth Precursor</th>
<th>Method Used</th>
<th>Product formed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BiPh₃</td>
<td>(i)</td>
<td>Bi(Sha₋₁H)₃</td>
</tr>
<tr>
<td></td>
<td>Bi(O'Bu)₃/KO'Bu</td>
<td>(ii)</td>
<td>[Bi(Sha₋₂H)]K</td>
</tr>
<tr>
<td></td>
<td>Bi(O'Bu)₃</td>
<td>(iii)</td>
<td>Bi(Sha₋₂H)(Sha₋₁H)</td>
</tr>
<tr>
<td></td>
<td>Bi(O'Bu)₃/KO'Bu</td>
<td>(ii)</td>
<td>[Bi(Bha₋₂H)]K</td>
</tr>
<tr>
<td></td>
<td>Bi(O'Bu)₃</td>
<td>(iii)</td>
<td>Bi(Bha₋₂H)(Bha₋₁H)</td>
</tr>
<tr>
<td></td>
<td>Bi(O'Bu)₃</td>
<td>(iii)</td>
<td>Bi(Aha₋₂H)(Aha₋₁H)</td>
</tr>
<tr>
<td></td>
<td>BiPh₃</td>
<td>(i)</td>
<td>Bi(Bpha₋₁H)₃</td>
</tr>
<tr>
<td></td>
<td>Bi(O'Bu)₃</td>
<td>(iii)</td>
<td>Bi(Bpha₋₁H)₃</td>
</tr>
<tr>
<td></td>
<td>BiPh₃</td>
<td>(i)</td>
<td>Bi(Mfha₋₁H)₃</td>
</tr>
<tr>
<td></td>
<td>Bi(O'Bu)₃</td>
<td>(iii)</td>
<td>Bi(Mfha₋₁H)₃</td>
</tr>
</tbody>
</table>

Methods (i) Solvent free: 120 °C or toluene at reflux, (ii)/(iii) THF -78 °C.

All these complexes were assessed for their anti-\textit{H. pylori} activities, and showed good inhibition in the low μg mL⁻¹ range.
Chapter 3

5. Crystal Structure Studies

The X-ray crystal structures described in this section were determined by Dr. Brendan Twamley, School of Chemistry, in Trinity College, Dublin.

\[
\text{[Bi}_2\text{(Bha}-\text{H})_2\text{(μ-Bha}-\text{H})_2\text{(η}_2\text{-NO}_3)_2]\quad (2)
\]

Crystals of \([\text{Bi}_2\text{(Bha}-\text{H})_2\text{(μ-Bha}-\text{H})_2\text{(η}_2\text{-NO}_3)_2]\) suitable for X-ray analysis were obtained by slow evaporation of a methanol solution, to yield 2. The X-ray intensity data were measured at 100 K using an Oxford Cryosystems Cobra low temperature device. A specimen, of approximate dimensions 0.030 mm x 0.050 mm x 0.210 mm, was used for refinement. A total of 1027 frames were collected and the total exposure time was 2.85 hours. The solid state structure of 2 is shown in Figure 3.8. The complex crystallizes in the \(\text{Pī}\) space group and is completed by inversion symmetry. Crystal data and details of data collection are summarised in Table 3.4.

![Figure 3.8. Solid state structure of 2, showing the coordination of the ligands around each bismuth atom, featuring two modes of the Bha ligand; (O, μ-O’) and (O, O’).](image-url)
Table 3.4. Table of collection parameters for 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Empirical formula</strong></td>
<td>$C_{29}H_{28}Bi_2N_6O_{15}$</td>
</tr>
<tr>
<td><strong>Formula weight</strong></td>
<td>1118.53</td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td>100 K</td>
</tr>
<tr>
<td><strong>Wavelength</strong></td>
<td>0.71073 Å</td>
</tr>
<tr>
<td><strong>Crystal system</strong></td>
<td>Triclinic</td>
</tr>
<tr>
<td><strong>Space group</strong></td>
<td>$P\bar{i}$</td>
</tr>
<tr>
<td><strong>Unit cell dimensions</strong></td>
<td>$a = 4.8467(4),\text{Å}$, $\alpha = 74.217(3)^\circ$</td>
</tr>
<tr>
<td></td>
<td>$b = 13.1798(12),\text{Å}$, $\beta = 85.628(3)^\circ$</td>
</tr>
<tr>
<td></td>
<td>$c = 15.0042(14),\text{Å}$, $\gamma = 82.567(3)^\circ$</td>
</tr>
<tr>
<td><strong>Volume</strong></td>
<td>$913.72(14) ,\text{Å}^3$</td>
</tr>
<tr>
<td><strong>Z</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>Density (calculated)</strong></td>
<td>2.033 Mg/m³</td>
</tr>
<tr>
<td><strong>Absorption coefficient</strong></td>
<td>9.692 mm$^{-1}$</td>
</tr>
<tr>
<td><strong>F(000)</strong></td>
<td>530</td>
</tr>
<tr>
<td><strong>Crystal size</strong></td>
<td>$0.210 \times 0.050 \times 0.030,\text{mm}^3$</td>
</tr>
<tr>
<td><strong>Theta range for data collection</strong></td>
<td>$1.412$ to $27.534^\circ$.</td>
</tr>
<tr>
<td><strong>Index ranges</strong></td>
<td>$-6 \leq h \leq 6$, $-17 \leq k \leq 17$, $-19 \leq l \leq 19$</td>
</tr>
<tr>
<td><strong>Reflections collected</strong></td>
<td>32284</td>
</tr>
<tr>
<td><strong>Independent reflections</strong></td>
<td>4213 [$R(\text{int}) = 0.0626$]</td>
</tr>
<tr>
<td><strong>Completeness to theta = 25.242^\circ</strong></td>
<td>100.0%</td>
</tr>
<tr>
<td><strong>Absorption correction</strong></td>
<td>Semi-empirical from equivalents</td>
</tr>
<tr>
<td><strong>Max. and min. transmission</strong></td>
<td>0.7456 and 0.4625</td>
</tr>
<tr>
<td><strong>Refinement method</strong></td>
<td>Full-matrix least-squares on $F^2$</td>
</tr>
<tr>
<td><strong>Data / restraints / parameters</strong></td>
<td>4213 / 94 / 251</td>
</tr>
<tr>
<td><strong>Goodness-of-fit on $F^2$</strong></td>
<td>1.084</td>
</tr>
<tr>
<td><strong>Final R indices [I&gt;2\sigma(I)]</strong></td>
<td>$R_1 = 0.0371$, $wR_2 = 0.0904$</td>
</tr>
<tr>
<td><strong>R indices (all data)</strong></td>
<td>$R_1 = 0.0437$, $wR_2 = 0.0935$</td>
</tr>
<tr>
<td><strong>Extinction coefficient</strong></td>
<td>0.0147(9)</td>
</tr>
<tr>
<td><strong>Largest diff. peak and hole</strong></td>
<td>2.302 and -1.477 e.Å$^{-3}$</td>
</tr>
</tbody>
</table>

The formula of 2 consists of a charge neutral complex. In the main core of the complex each Bi centre is chelated by two ligands, in a bi-dentate fashion, as predicted from IR data, via Bi-O bonds (Bi1-O1, Bi1-O4, Bi1-O11 and Bi1-O14). A bidentate nitrato group is also bound (Bi1-O19 and Bi1-O22). The bismuth atoms are linked by two $\mu$-oxo bridges via the benzohydroxamato groups forming a planar Bi2-$\mu$-O2 dimer oxygen. Significantly, 2 exhibits two distinct Bi hydroxamato binding modes: bidentate bridging (O, $\mu$-O’) and bidentate non-bridging (O, O’). Tables of significant bond lengths and bond angles are displayed, Tables 3.5 and Table 3.6.
Table 3.5. A list of the significant bond lengths of 2.

<table>
<thead>
<tr>
<th>Atoms</th>
<th>Dist (Å)</th>
<th>Atoms</th>
<th>Dist (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bi(1)-O(1)</td>
<td>2.201(5)</td>
<td>Bi(1)-O(19)</td>
<td>2.519(5)</td>
</tr>
<tr>
<td>Bi(1)-O(11)</td>
<td>2.234(4)</td>
<td>Bi(1)-O(11)#1</td>
<td>2.674(5)</td>
</tr>
<tr>
<td>Bi(1)-O(14)</td>
<td>2.276(5)</td>
<td>Bi(1)-O(22)</td>
<td>2.755(5)</td>
</tr>
<tr>
<td>Bi(1)-O(4)</td>
<td>2.409(5)</td>
<td>Bi(1)-O(1)*</td>
<td>3.034(5)</td>
</tr>
</tbody>
</table>

Table 3.6. A list of the significant bond angles of 2.

<table>
<thead>
<tr>
<th>Angle Atoms</th>
<th>Angle (°)</th>
<th>Angle Atoms</th>
<th>Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O(1)-Bi(1)-O(11)</td>
<td>76.45(17)</td>
<td>O(11)-Bi(1)-O(11)#1</td>
<td>66.77(19)</td>
</tr>
<tr>
<td>O(1)-Bi(1)-O(14)</td>
<td>85.54(19)</td>
<td>O(14)-Bi(1)-O(11)#1</td>
<td>103.39(17)</td>
</tr>
<tr>
<td>O(11)-Bi(1)-O(14)</td>
<td>71.06(16)</td>
<td>O(4)-Bi(1)-O(11)#1</td>
<td>153.37(15)</td>
</tr>
<tr>
<td>O(1)-Bi(1)-O(4)</td>
<td>70.05(16)</td>
<td>O(19)-Bi(1)-O(11)#1</td>
<td>71.45(15)</td>
</tr>
<tr>
<td>O(11)-Bi(1)-O(4)</td>
<td>136.87(16)</td>
<td>O(1)-Bi(1)-O(22)</td>
<td>76.27(18)</td>
</tr>
<tr>
<td>O(14)-Bi(1)-O(4)</td>
<td>79.85(18)</td>
<td>O(11)-Bi(1)-O(22)</td>
<td>118.71(15)</td>
</tr>
<tr>
<td>O(1)-Bi(1)-O(19)</td>
<td>75.33(17)</td>
<td>O(14)-Bi(1)-O(22)</td>
<td>155.75(18)</td>
</tr>
<tr>
<td>O(11)-Bi(1)-O(19)</td>
<td>72.12(15)</td>
<td>O(4)-Bi(1)-O(22)</td>
<td>78.95(16)</td>
</tr>
<tr>
<td>O(14)-Bi(1)-O(19)</td>
<td>141.51(16)</td>
<td>O(19)-Bi(1)-O(22)</td>
<td>48.23(14)</td>
</tr>
<tr>
<td>O(4)-Bi(1)-O(19)</td>
<td>122.17(16)</td>
<td>O(11)#1-Bi(1)-O(22)</td>
<td>100.84(15)</td>
</tr>
<tr>
<td>O(1)-Bi(1)-O(11)#1</td>
<td>136.19(16)</td>
<td>N(2)-O(1)-Bi(1)</td>
<td>115.4(4)</td>
</tr>
</tbody>
</table>
Crystals of \([\text{Bi}_6(\text{CH}_3\text{OH})_2(\eta^1\text{-NO}_3)_2(\eta^2\text{-NO}_3)_2(\text{H}_2\text{O})_2(\text{Sha}_{1\text{H}})_{12}]\)(\text{CH}_3\text{OH})_4(\text{H}_2\text{O})_4\) (3a) suitable for X-ray analysis were obtained by slow evaporation from methanol. The X-ray intensity data were measured at 100 K using an Oxford Cryosystems Cobra low temperature device. A specimen, of approximate dimensions 0.050 mm x 0.080 mm x 0.150 mm, was used for refinement. A total of 1731 frames were collected. The total exposure time was 4.81 hours. The complex crystallizes in the \(\text{P}\bar{1}\) space group with \(Z = 1\) for \(\text{C}_{90}\text{H}_{108}\text{Bi}_6\text{N}_{18}\text{O}_{66}\) and is completed by inversion symmetry. Crystal data and details of collection are summarised in Table 3.7.

**Table 3.7. Table of collection parameters for 3.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>(\text{C}<em>{90}\text{H}</em>{108}\text{Bi}<em>6\text{N}</em>{18}\text{O}_{66})</td>
</tr>
<tr>
<td>Formula weight</td>
<td>3751.82</td>
</tr>
<tr>
<td>Temperature</td>
<td>100 K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Triclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>(\text{P}\bar{1})</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>(a = 15.0432(6)\text{ Å} \quad \alpha = 112.5660(10)^\circ)</td>
</tr>
<tr>
<td></td>
<td>(b = 15.2366(5)\text{ Å} \quad \beta = 116.5850(10)^\circ)</td>
</tr>
<tr>
<td></td>
<td>(c = 16.5434(6)\text{ Å} \quad \gamma = 94.4850(10)^\circ)</td>
</tr>
<tr>
<td>Volume</td>
<td>2984.48(19) (\text{Å}^3)</td>
</tr>
<tr>
<td>(Z)</td>
<td>1</td>
</tr>
<tr>
<td>Density (calculated)</td>
<td>2.087 (\text{Mg/m}^3)</td>
</tr>
<tr>
<td>Absorption coefficient</td>
<td>8.926 (\text{mm}^{-1})</td>
</tr>
<tr>
<td>(F(000))</td>
<td>1800</td>
</tr>
<tr>
<td>Crystal size</td>
<td>0.150 x 0.080 x 0.050 (\text{mm}^3)</td>
</tr>
<tr>
<td>Theta range for data collection</td>
<td>1.518 to 30.135(^\circ).</td>
</tr>
<tr>
<td>Index ranges</td>
<td>-21 (\leq) (h) (\leq) 21, -21 (\leq) (k) (\leq) 21, -23 (\leq) (l) (\leq) 23</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>111802</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>17529 ([\text{R(int)} = 0.0443])</td>
</tr>
<tr>
<td>Completeness to theta (= 25.242^\circ)</td>
<td>100.0 %</td>
</tr>
<tr>
<td>Absorption correction</td>
<td>Numerical</td>
</tr>
<tr>
<td>Max. and min. transmission</td>
<td>0.7837 and 0.4747</td>
</tr>
<tr>
<td>Refinement method</td>
<td>Full-matrix least-squares on (F^2)</td>
</tr>
<tr>
<td>Data / restraints / parameters</td>
<td>17529 / 14 / 827</td>
</tr>
<tr>
<td>Goodness-of-fit on (F^2)</td>
<td>1.004</td>
</tr>
<tr>
<td>Final R indices ([I&gt;2\sigma(I)])</td>
<td>(R_1 = 0.0254, \text{wR}_2 = 0.0489)</td>
</tr>
<tr>
<td>(R) indices (all data)</td>
<td>(R_1 = 0.0411, \text{wR}_2 = 0.0533)</td>
</tr>
<tr>
<td>Largest diff. peak and hole</td>
<td>1.894 and -1.451 (\text{e.Å}^{-3})</td>
</tr>
</tbody>
</table>

The formula of 3a consists of the cationic complex \([\text{Bi}_6(\text{CH}_3\text{OH})_2(\eta^1\text{-NO}_3)_2(\eta^2\text{-NO}_3)_2(\text{H}_2\text{O})_2(\text{Sha}_{1\text{H}})_{12}]^2^+\) with two isolated nitrate counter ions, to balance the charge, as well as solvent molecules (four water and four methanol). The complex
itself consists of six bismuth centres, each chelated by two salicylhydroxamato ligands. One terminal bismuth atom, Bi2, is additionally bonded to a H₂O and methanol while the other terminal bismuth, Bi3, is additionally bound by a nitrate group in a monodentate fashion. Each central Bi atom is also bonded by one nitrate group, however in a bi-dentate mode. All Sha ligands in this structure bind bismuth in the previously observed bidentate bridging (O, μ-O’) mode, forming a stable five membered ring, while the deprotonated hydroxamic acid hydroxyl group also bridges to an adjacent bismuth. The solid state structure of 3a is shown in Figure 3.9.

Figure 3.9. The solid state structure of the 6 bismuth complex of [Bi₆(CH₃OH)₂(η¹-NO₃)₂(η²-NO₃)₂(H₂O)₂(Sha-H)₁₂]⁻(CH₃OH)⁺(H₂O)₄, 3a.

The crystal structure of 3a exhibits distorted dodecahedral coordination geometry with each bismuth atom having eight bonds associated. The 8 bismuth bonds are made up of 7 near interactions and one long distance interaction. Although the crystal structure is quite complex, there is a centre of symmetry present in the complex, where three different coordination environments represent the six bismuth
atoms in the lattice. These are identified in Figure 3.10. The polyhedral coordination environments are shown in Figure 3.11. A table of significant bond lengths and bond angles are found in Tables 3.8 and Table 3.9.

Figure 3.10. The three different bismuth coordination environments present in 3 are shown above. Observed in Bi1 are two bidentate hydroxamato ligands and one nitrato group (mono-dentate) bound to the bismuth centre. In Bi2 two bidentate hydroxamato ligands, one methanol and one water are bound to the bismuth centre. In Bi3 two bidentate hydroxamato ligands and one nitrato (bidentate) are bound to the bismuth centre.

Figure 3.11. Polyhedral coordination environment around 3a. Phenyl rings removed from ligands for clarity.

Table 3.8. A list of the significant bond lengths of 3.
<table>
<thead>
<tr>
<th>Atoms</th>
<th>Length (Å)</th>
<th>Atoms</th>
<th>Length (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bi(1)-O(10)</td>
<td>2.256(2)</td>
<td>Bi(2)-O(5)</td>
<td>2.604(3)</td>
</tr>
<tr>
<td>Bi(1)-O(22)</td>
<td>2.268(2)</td>
<td>Bi(2)-O(54)</td>
<td>2.639(2)</td>
</tr>
<tr>
<td>Bi(1)-O(11)</td>
<td>2.327(2)</td>
<td>O(2)-Bi(3)</td>
<td>2.515(3)</td>
</tr>
<tr>
<td>Bi(1)-O(21)</td>
<td>2.337(2)</td>
<td>Bi(3)-O(65)</td>
<td>2.226(2)</td>
</tr>
<tr>
<td>Bi(1)-O(32)</td>
<td>2.521(2)</td>
<td>Bi(3)-O(54)</td>
<td>2.329(2)</td>
</tr>
<tr>
<td>Bi(1)-O(21)#1</td>
<td>2.577(2)</td>
<td>Bi(3)-O(55)</td>
<td>2.378(3)</td>
</tr>
<tr>
<td>Bi(2)-O(32)</td>
<td>2.230(2)</td>
<td>Bi(3)-O(66)</td>
<td>2.380(2)</td>
</tr>
<tr>
<td>Bi(2)-O(33)</td>
<td>2.277(2)</td>
<td>Bi(3)-O(43)</td>
<td>2.427(2)</td>
</tr>
<tr>
<td>Bi(2)-O(44)</td>
<td>2.312(3)</td>
<td>Bi(3)-O(10)#1</td>
<td>2.721(2)</td>
</tr>
<tr>
<td>Bi(2)-O(43)</td>
<td>2.459(2)</td>
<td>O(10)-Bi(3)#1</td>
<td>2.721(2)</td>
</tr>
<tr>
<td>Bi(2)-O(6)</td>
<td>2.509(3)</td>
<td>O(21)-Bi(1)#1</td>
<td>2.577(2)</td>
</tr>
</tbody>
</table>

**Table 3.9.** A list of the significant bond angles of 3.

<table>
<thead>
<tr>
<th>Atoms</th>
<th>Angle (°)</th>
<th>Atoms</th>
<th>Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O(10)-Bi(1)-O(22)</td>
<td>96.40(9)</td>
<td>O(6)-Bi(2)-O(5)</td>
<td>97.44(10)</td>
</tr>
<tr>
<td>O(10)-Bi(1)-O(11)</td>
<td>70.28(8)</td>
<td>O(32)-Bi(2)-O(54)</td>
<td>71.88(8)</td>
</tr>
<tr>
<td>O(22)-Bi(1)-O(11)</td>
<td>77.70(9)</td>
<td>O(33)-Bi(2)-O(54)</td>
<td>135.91(8)</td>
</tr>
<tr>
<td>O(10)-Bi(1)-O(21)</td>
<td>76.79(8)</td>
<td>O(44)-Bi(2)-O(54)</td>
<td>121.71(8)</td>
</tr>
<tr>
<td>O(22)-Bi(1)-O(21)</td>
<td>68.86(8)</td>
<td>O(43)-Bi(2)-O(54)</td>
<td>56.16(8)</td>
</tr>
<tr>
<td>O(11)-Bi(1)-O(21)</td>
<td>129.38(9)</td>
<td>O(6)-Bi(2)-O(54)</td>
<td>139.93(8)</td>
</tr>
<tr>
<td>O(10)-Bi(1)-O(32)</td>
<td>139.98(8)</td>
<td>O(5)-Bi(2)-O(54)</td>
<td>75.89(8)</td>
</tr>
<tr>
<td>O(22)-Bi(1)-O(32)</td>
<td>78.25(8)</td>
<td>O(65)-Bi(3)-O(54)</td>
<td>77.18(8)</td>
</tr>
<tr>
<td>O(11)-Bi(1)-O(32)</td>
<td>69.83(8)</td>
<td>O(65)-Bi(3)-O(55)</td>
<td>79.12(9)</td>
</tr>
<tr>
<td>O(21)-Bi(1)-O(32)</td>
<td>133.86(8)</td>
<td>O(54)-Bi(3)-O(55)</td>
<td>68.28(8)</td>
</tr>
<tr>
<td>O(10)-Bi(1)-O(21)#1</td>
<td>87.17(8)</td>
<td>O(65)-Bi(3)-O(66)</td>
<td>69.55(8)</td>
</tr>
<tr>
<td>O(22)-Bi(1)-O(21)#1</td>
<td>129.35(8)</td>
<td>O(54)-Bi(3)-O(66)</td>
<td>130.97(8)</td>
</tr>
<tr>
<td>O(11)-Bi(1)-O(21)#1</td>
<td>147.56(8)</td>
<td>O(55)-Bi(3)-O(66)</td>
<td>134.66(8)</td>
</tr>
<tr>
<td>O(21)-Bi(1)-O(21)#1</td>
<td>62.90(10)</td>
<td>O(65)-Bi(3)-O(43)</td>
<td>81.05(8)</td>
</tr>
<tr>
<td>O(32)-Bi(1)-O(21)#1</td>
<td>126.99(8)</td>
<td>O(54)-Bi(3)-O(43)</td>
<td>60.70(8)</td>
</tr>
<tr>
<td>O(32)-Bi(2)-O(33)</td>
<td>71.02(8)</td>
<td>O(55)-Bi(3)-O(43)</td>
<td>128.09(8)</td>
</tr>
<tr>
<td>O(32)-Bi(2)-O(44)</td>
<td>87.69(9)</td>
<td>O(66)-Bi(3)-O(43)</td>
<td>79.03(8)</td>
</tr>
<tr>
<td>O(33)-Bi(2)-O(44)</td>
<td>79.54(9)</td>
<td>O(65)-Bi(3)-O(2)</td>
<td>76.84(9)</td>
</tr>
</tbody>
</table>
Complexes 2 and 3 are the first class of structurally defined bismuth hydroxamato nitrato complexes in the literature. They have shown that bismuth binds to hydroxamates in a bidentate fashion, with two possible coordination modes. They may bind via bidentate bridging (O, $\mu$-O’) fashion or in a non-bridging bidentate (O, O’) fashion.\textsuperscript{38}

Andrews published the first X-ray crystal structure of a Bi(III) hydroxamato complex; a unique 34 bismuth oxido-cluster, $[\text{Bi}_{134}\text{O}_{22}(\text{Bha-2H})_{22}(\text{Bha-1H})_{14}(\text{DMSO})_{6}]$, isolated on dissolution of a [Bi$_2$(Bha-2H)$_3$] complex in DMSO/toluene. This complex features both the doubly deprotonated benzohydroximate and singly deprotonated hydroxamate ligands.\textsuperscript{28, 29} The Bi(III) complexes described here consist of the Bi monodeprotonated hydroxamato complexes only.
6. Stability Testing

Bismuth carboxylates have a natural tendency to undergo hydrolysis to oxido clusters, as seen by Andrews et al.\textsuperscript{39} Therefore the stability of our novel Bi(III) hydroxamato nitrato complexes 2, 3 and 4 were investigated as solids, in solution and under acidic conditions.

The stability of the complexes 2, 3 and 4 as solids were assessed by performing \textsuperscript{1}H NMR analysis. Analysis was conducted on a sample stored at atmospheric temperature and pressure one, three and six months apart. The stability of these complexes in solution in MeOD were assessed by performing \textsuperscript{1}H NMR analysis on 2, 3 and 4. Data were collected regularly on the same sample over a period of one month. The \textsuperscript{1}H NMR spectra showed no variation in signals over the time period studied, which indicates good stability in the solid state and in solution in methanol.

It was proposed that in the low pH environment of the stomach, the Bi(III) hydroxamato nitrato complexes would dissociate, releasing hydroxamic acids with concomitant formation of BiOCl, Scheme 3.6.

\begin{center}
  \textbf{Scheme 3.6. Predicted hydrolysis of Bi hydroxamato complexes in acid.}
\end{center}

To assess the stability in acidic solution, the synthesised complexes were added to a 1 M solution of HCl, with stirring. After 20-30 minutes, the mixture was extracted into ethyl acetate, isolated, dried under vacuum and analysed using NMR spectroscopy, IR and MS. The analysis indicated that the corresponding hydroxamic acid ligand was recovered. Therefore at low pH, Bi(III) hydroxamato nitrato complexes are unstable, resulting in dissociation and liberation of the hydroxamic acid, which is then free to act as a potential urease inhibitors.\textsuperscript{40}

These findings support one of the fundamental concepts behind this research. It was predicted that when administered, these complexes would be activated in the acidic environment of the stomach and in turn exert their activity.
7. Biological Testing

Complex 2 and 3 are the first reported examples of Bi complexes specifically designed to inhibit the activity of the urease enzyme. The urease inhibitory activity of these complexes were tested against isolated Jack Bean urease. Complex 2 was brought forward for testing against *H. pylori*, given it is free of undesirable coordinated solvent molecules and therefore a suitable candidate as an anti-*H. pylori* agent. Complex 3, in contrast to 2, contains coordinated MeOH molecules, and so is not a suitable drug candidate. Given the solid-state structure of 4 was not solved, nor was it isolated in sufficient purity, complex 4 was not brought forward for biological anti-*H. pylori* testing.

**Urease Inhibitory Activity**

Complex 2 and 3 and their corresponding hydroxamic acid ligand, Bha and Sha, were tested to investigate their urease inhibition activity against Jack Bean urease, which shows a high level of homology with *H. pylori* urease.

This inhibition study was carried out at pH 6.8, as urease has an optimal activity at neutral or near neutral pH. Jack bean urease was pre-incubated with different concentrations of inhibitor (0.1, 1, and 10 mM) for 30 minutes. Following this incubation period, urea was added and the activity of the enzyme assessed by UV-vis spectroscopy. Changes in absorbance at 565 nm at 37 °C were measured, corresponding to activity of urease.

A summary of the urease inhibitory activity of these test complexes is shown in Figure 3.12. Complex 2 and Bha, at concentrations of 10 mM, resulted in a 96% and 83% decrease in activity of Jack Bean urease *in vitro*, respectively. Complex 3 completely inhibited the activity of urease at 10 mM, whereas Sha demonstrated slightly lower inhibition of 95% at the same concentration. Complex 3, at lower concentrations of 0.1 and 1 mM, showed good inhibitory activity of 80% and 94%, much greater than that of the free ligand at similar concentrations.

In contrast to 2 and 3, BSS showed poor inhibitory activity, 19%, even at the higher concentration range of 10 mM, Figure 3.12. From this it can be deduced that the urease inhibitory activity of 2 and 3 is primarily attributed to the inhibitory properties of their ligand and not due to the Bi(III) centres.
It is noteworthy that Sun et al. demonstrated both competitive and non-competitive inhibition of urease by Bi(III) in various bismuth complexes including RBC. Using NMR spectroscopic and site-directed mutagenesis studies, they indicate that Bi(III) binds to the highly conserved cysteine residue (Cys319) located at the entrance of the urease active site. Therefore bismuth can potentially also contribute to urease inhibition.

**In vitro anti-*H. pylori* Activity**

An evaluation of the *in vitro* anti-bacterial activity (*n* = 3) of 2, the corresponding free acid Bha and a bismuth standard, BSS, was carried out against three laboratory strains of *H. pylori*: 26695, J99 and 60190. The *H. pylori* strain 26695 was first isolated from a patient with gastritis and was used to develop an infection model in piglets. Strains J99 and 26695 were the first strains for which the full genome was sequenced. Strain 26695 has a *cag* pathogenicity island, which make this strain more virulent to the host.

The minimum inhibitory concentrations (MIC) of complex 2, its hydroxamic acid ligand Bha and BSS were determined using the agar dilution method, which is the method recommended by the Clinical and Laboratory Standards Institute (CLSI).
Agar plates were prepared, incorporating doubling dilutions of desired complex at a concentration range of 0.5-64 μg mL\(^{-1}\).

An MIC ≥ 16, μg mL\(^{-1}\) was determined for complex 2 against 26696, J99 and 60190, which were comparable to the MIC for BSS ≥ 16, μg mL\(^{-1}\) and less than the MIC for Bha ≥ 32 μg mL\(^{-1}\). Our findings, summarised in Table 3.10, are in agreement with other literary evidence for the MIC of BSS against *H. pylori*\(^{43}\).

<table>
<thead>
<tr>
<th><em>H. pylori</em> strain</th>
<th>2</th>
<th>Bha</th>
<th>BSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>26695</td>
<td>≥ 16</td>
<td>≥ 32</td>
<td>≥ 16</td>
</tr>
<tr>
<td>J99</td>
<td>≥ 16</td>
<td>≥ 32</td>
<td>≥ 16</td>
</tr>
<tr>
<td>60190</td>
<td>≥ 16</td>
<td>≥ 32</td>
<td>≥ 16</td>
</tr>
</tbody>
</table>

However, the Bi(III) hydroxamato/hydroximato complexes previously synthesised by Andrews *et al.* show far superior activity against similar strains of *H. pylori*. He has reported MICs in the range of 0.05-1.56 μg mL\(^{-1}\). These are remarkably low MIC values. Our results are in agreement with Andrews, in that, the bismuth complexes tested for anti-*H. pylori* activity all had higher activity than their free ligands.

One obvious reason for this increased activity of the Bi(III) complexes synthesised by Andrews *et al.* is the fact that some of the complexes formed possess a Bi to ligand ratio of 1:3, hence there is more ligand available to exert its activity at the active site. Another reason for the superior activity may be in relation to the solubility of the complexes in solution. The selection of ligands may improve solubility or lipophilicity hence having an effect on their up-take into cells and availability. In the majority of these complexes, only one ligand is present where as in our complexes it is observed that nitrates are also present. This may have an effect on the activity of the complexes also.
8. Conclusion

In conclusion, this chapter reports the synthesis and full characterisation of a novel class of Bi(III) hydroxamato nitrato complexes of type Bi(L-1H)2NO3 specifically designed to and demonstrated to inhibit urease, *H. pylori*’s Achilles heel.

In each case the products were obtained by the reaction of one equivalent of bismuth nitrate pentahydrate with two equivalents of hydroxamic acid. In this study, three different hydroxamic acids, Sha, Bha and 2-NH2-Pha were used. Both Sha and Bha were commercially available and 2-NH2-Pha was synthesised with high yield and purity as previously reported.

The X-ray crystal structures of two out of the three bismuth complexes were solved, with crystals being isolated after slow evaporation from methanol in both cases. The structure elucidation of 2, [Bi2(Bha-1H)2(η-Bha-1H)2(η2-NO3)2] revealed two hydroxamato coordination modes; bidentate bridging (O, µ-O’) and bidentate non-bridging (O, O’) while complex 3a, [Bi6(Sha-1H)12(CH3OH)2(η1-NO3)2(η2-NO3)(H2O)2](NO3)2 which was isolated in crystal form from methanol, comprises of only one coordination mode; bidentate bridging (O, µ-O’). These two complexes, although generated by the same reaction conditions, produce two different and interesting structures, further emphasising the diverse range of structural conformities that bismuth can generate. Complex 2 is a dinuclear bismuth complex, with distorted dodecahedral geometry, whereas, 3a comprises of a six bismuth cluster, and distorted dodecahedral coordination geometry.

These novel bismuth complexes were tested to assess their stability as solids, in solution and under acidic conditions. All the complexes were stable over the periods tested as solids and in solution. The complexes were shown to liberate their ligands in acidic medium as hypothesised.

Complex 2 and 3, along with their free ligands and BSS, were assessed for their urease inhibitory activity. The complexes were designed specifically for their ability to act as urease inhibitors, which could target *H. pylori*’s Achilles heel. All complexes tested displayed a level of urease inhibition. Complex 2, at 10 mM concentrations, shows a reduction in urease activity of 96%, slightly better than that of its free ligand which showed 83% inhibition. Complex 3 showed complete
inhibition of urease activity at 10 mM and 80% inhibition at 0.1 mM concentration. While BSS only displayed slight inhibition, even at high concentrations, suggesting that the inhibitory activity of the complexes originates from the hydroxamic acid moiety.

Complex 2 was the only suitable candidate for in vitro anti-\textit{H. pylori} testing and was assessed along with Bha and BSS, against three strains of \textit{H. pylori} (26695, J99 and 60190). 2 exhibits excellent anti-bacterial activity against all three strains of \textit{H. pylori} with MIC $\geq$ 16 $\mu$g mL$^{-1}$. It is twice as active when compared to its free ligand which has an MIC $\geq$ 32 $\mu$g mL$^{-1}$ and is equipotent with BSS.

9. Future direction

The results of this chapter shows good evidence that Bi(III) hydroxamate nitrato complexes are worthy candidates for further investigation as anti-\textit{H. pylori} agents. Further development of bismuth complexes as effective urease inhibitors should be undertaken and their potential activity against \textit{H. pylori} at low pH investigated.

To investigate the activity of these complexes at low pH firstly a protocol for the culture of these bacteria in a simulated acidic media, which mimics the environment of the stomach would have to be established. Following the successful growth of the bacteria in this simulated media, an investigation could be carried out to see if these complexes display heightened anti-\textit{H. pylori} activity at low pH. It is expected that our complex would have increased activity due \textit{H. pylori} being reliant on the activity of urease at low pH for survival and the ability of the complexes to readily release their hydroxamic acid as a potent urease inhibitor while also liberating bismuth to exert its own anti-bacterial activity, which it is known to possess.
References


34. Griffith D, Krot K, Comiskej J, Nolan KB, Marmion CJ. Monohydroxamic acids and bridging dihydroxamic acids as chelators to ruthenium(iii) and as nitric oxide donors: syntheses, speciation studies and nitric oxide releasing investigation. Dalton Transactions. 2008(1):137-47.
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Chapter 4

Synthesis of Bi(III) Hydroxamato Chlorido Complexes; Investigation of Anti-Bacterial Activity Beyond *H. pylori*.
1. Introduction

Bismuth preparations were used, prior to the discovery of antibiotics, as treatments for bacterial infections associated with wounds, cholera and gastroenteritis. The development of antibiotics in the 1940’s drastically reduced the use of Bi-based antibacterial medication. Significantly though, the rise in H. pylori resistance to conventional treatment has resulted in renewed interest in bismuth and the development of Bi-containing quadruple therapies which effectively overcome this resistance and are effective in the treatment of H. pylori. However these bismuth drugs are administered without an exact understanding of their structure, behaviour in biological environment or indeed their exact mechanisms of action.

Bismuth has been demonstrated to have a particular affinity for histidine and cysteine rich residues of proteins and as such, bismuth can bind to bacterial proteins and enzymes. This interference and disruption has been shown to undoubtedly have a negative effect on bacterial survival and colonisation.

Given that Bi(III) complexes are components of effective regimens for the treatment of H. pylori, it is very surprising that there are so few reports in the literature on the investigation of the activity of bismuth complexes against bacteria beyond H. pylori. Significantly BSS was recently demonstrated to exhibit anti-bacterial activity against E. coli strain O157:H7 and a bismuth-2,3-dimercaptopropanol complex exhibited in vitro efficacy against biofilms formed by MRSA isolated from human chronic wounds. Furthermore it is noteworthy that there has been a dearth of research into the sterilising properties of Bi(III) complexes. Recent research into the use of bismuth as air and water purification agents have also shown bismuth oxyhalides to be effective in removal of biofouling in water systems.

Hydroxamic acids, of general formula RCONHOH, represent a very important family of bioligands. They are well-known metal chelators and metalloenzyme inhibitors that classically coordinate metal centres in a bidentate (O, O’) chelating fashion, coordinating through the carbonyl oxygen atom and the deprotonated hydroxyl group, to form very stable five membered hydroxamato chelates. Another common mode of binding is the bidentate bridging (O, µ-O’) mode of chelation.
Other hydroxamic acid-metal binding modes are possible though less observed, including mono-dentate coordination. Secondary coordinating groups, such as hydroxyl, amino groups or heterocyclic groups such as pyridine, offer the possibility to generate interesting hydroxamato complexes by acting as anchors which encourage (O$_2$-OH, O), (N$_{\text{NH}_2}$, O) or (N$_{\text{Py}}$, O) hydroxamato coordination to Bi(III). In turn hydroxamic acids have been employed to develop a diverse range of interesting complexes including metallacrowns, coordination polymers and cluster complexes.$^{12, 18}$

The synthesis of a novel class of Bi(III) nitrato hydroxamato complexes, [Bi$_2$(Bha-1H)$_2$(μ-Bha-1H)$_2$(η$_2$-NO$_3$)$_2$] (2), [Bi(NO$_3$)(Sha-1H)$_2$]$^n$ (3) and [Bi(2-NH$_2$-Pha-1H)$_2$(NO$_3$)]$_n$ (4), and investigation of their urease inhibitory activity and anti-bacterial activity against H. pylori was reported in chapter 3.$^{19}$

Herein, is reported the synthesis and characterisation of novel polymeric Bi(III) hydroxamato chlorido complexes of Sha, 2-NH$_2$-Pha and 2-Pyha. The X-ray crystal structure of the THF-solvated polymeric complex, [BiCl$_2$(μ-Sha-1H)(THF)].THF$_\infty$ was determined and the anti-bacterial activity of the THF-free complex, [BiCl$_2$(μ-Sha-1H)]$_n$, against a broad panel of bacteria was investigated.
2. Project rational

The reaction of bismuth chloride with hydroxamic acids such as Sha, 2-NH$_2$-Pha and 2-Pyha would generate interesting Bi(III) hydroxamato complexes. The availability of the hydroxyl, amino or pyridine nitrogen groups at the 2-position to act as anchors could encourage hydroxamato coordination to Bi(III) and possibly generate interesting novel binding modes and complexes.

Given the clinical success of bismuth complexes against the pathogen *H. pylori*, an investigation of the scope of the anti-bacterial properties of novel bismuth complexes against a range of non-*H. pylori* pathogenic and environmental Gram-negative and Gram-positive bacteria would support the hypothesis that Bi(III) complexes have an important role to play as anti-bacterial agents and/or sterilising agents.

Given hydroxamic acids effectively bind Bi(III), they were selected as ligands to generate structurally well-defined Bi(III) hydroxamato chlorido complexes for anti-bacterial testing.
3. Chapter Aims

- To design, synthesise and characterise a novel class of Bi(III) hydroxamato chlorido complexes.

- To elucidate the exact structure of these novel bismuth complexes by X-ray crystallography.

- To investigate the stability of the novel bismuth complexes as solids and in solution.

- To determine the anti-bacterial activity of these novel bismuth complexes against a broad panel of bacteria beyond *H. pylori.*
Judge and Bha were commercially available and used without further purification. 2-NH$_2$-Pha, (1) was synthesised and fully characterised previously (Chapter 3).

**Synthesis of 2-Pyha (5)**

As previously reported, synthesis of hydroxamic acids proceed via the reaction of hydroxylamine with an activated acyl carbonyl, in the form of an acid chloride, amide or ester for example. 2-Pyha, 5, was synthesised from the corresponding ethyl ester, ethyl-2-picolinate, as shown in the reaction scheme below, **Scheme 4.1.**

![Scheme 4.1. Synthesis of 2-Pyha, 5, from ethyl-2-picolinate.](image)

5 was obtained in good yield (80%) and excellent purity. This complex was characterised by EA, IR, MS, $^1$H and $^{13}$C NMR spectroscopy.

EA was consistent with the required formula of C$_6$H$_6$N$_2$O$_2$.

As discussed earlier, a feature of hydroxamic acids in general, is the frequency at which the $\nu$(C=O) appear in their IR spectra. Typically as a result of intermolecular bonding, one stretch is observed which encompasses the symmetric and asymmetric $\nu$(C=O) stretches. The $\nu$(C=O) generally occur between 1615 cm$^{-1}$ and 1660 cm$^{-1}$ and in 5 the $\nu$(C=O) at was observed at 1651 cm$^{-1}$.

$^1$H NMR analysis for this complex was performed in DMSO-$d_6$. This solvent allows for observation and identification of the hydroxamic N-H and O-H peaks, which occur at 9.10 ppm and 11.44 ppm respectively. Three signals appear in the aromatic region of this spectra from 8.60-7.53 ppm, corresponding to and integrating well for the four protons of this pyridine ring.

ESI-MS in the positive mode identification the full molecular ion ([M+H]$^+$) at 138.1 a.m.u.
Synthesis of Bismuth Hydroxamato chlorido Complexes

Reaction of hydroxamic acids (L) with BiCl₃, in anhydrous THF, gave novel complexes of type [BiCl₂(L-1H)]ₙ and [BiCl₃(L-1H)]ₓ, (x = 2 or 1) representing a novel class of Bi(III) hydroxamato chlorido complexes.

Synthesis of [BiCl₂(µ-Sha-1H)]ₙ  (6)

Reaction of Sha with BiCl₃, in anhydrous THF, under an inert atmosphere for 72 hours afforded the novel Bi(III) complex [BiCl₂(µ-Sha-1H)]ₙ, 6. This product was obtained in 77% yield and high purity, Scheme 4.2.

Scheme 4.2. Synthesis of [BiCl₂(µ-Sha-1H)]ₙ 6, a novel Bi(III) chlorido salicylhydroxamato complex.

[BiCl₂(µ-Sha-1H)]ₙ 6 was characterised by EA, IR, ¹H and ¹³C NMR, MS and X-ray crystallography.

Elemental analysis of 6 is consistent with the presence of one hydroxamic acid and two chloride atoms per Bi(III) centre.

¹H NMR spectroscopy was carried out in methanol-d₄ (MeOD). No signals associated with the hydroxamic acid protons (N–H and O-H) or the proton of the hydroxyl group in the ortho-position were observed, as expected, due to rapid exchange of protons with the solvent. In 6, three main aromatic signals are observed at 7.9-6.9 ppm, corresponding and integrating well for the five aromatic protons of the ligand. The most significant shift is that of an aromatic doublet, associated with protons closest to the hydroxamic acid group at the 6 position on the phenyl ring, from 7.63 ppm in the free ligand to 7.9 ppm in 6. The remaining two signals shift slightly in 6 compared to the free ligand, Figure 4.1.
Chapter 4

Figure 4.1. The stacked $^1$H NMR spectra of Sha (top) and complex 6 (bottom), and the expansion of the area of interest (insert), showing the shifts in the aromatic signals associated with coordination of Bi(III).

Six signals were observed in the $^{13}$C NMR spectrum of 6, five of which, at 156.4, 133.5, 129.6, 120.0 and 116.1 ppm, are associated with the aromatic carbons, with the signal at 120.0 ppm accounting for two aromatic carbons. A noteworthy shift in the signal associated with the carbonyl group which occurs at 163.8 ppm in 6, compared with the corresponding signals at 170.4 ppm in the ligand, was apparent.

The IR spectra of 6 exhibits a distinctive $\nu$(C=O) at 1599 cm$^{-1}$, displaying the characteristic shift associated with (O, O') hydroxamato coordination when compared to the corresponding $\nu$(C=O) of the uncoordinated ligand at 1619 cm$^{-1}$. This shift suggests bonding to the bismuth atom via formation of the thermodynamically favoured five membered bidentate chelate, via the carbonyl group and deprotonated hydroxyl group of the hydroxamic acids moiety. Similar shifts in Bi(III) hydroxamato complexes which feature bidentate (O, O') chelation and bidentate bridging (O, $\mu$-O') coordination modes have also previously been observed, as discussed in Chapter 3. The stretch at 1564 cm$^{-1}$ is assigned to the $\nu$(C-
N) of the hydroxamic acid, while a strong characteristic stretch at 1033 cm\(^{-1}\) identifies \(\nu(\text{Bi-Cl})\), as previously observed for bismuth chlorido complexes.\(^{24}\)

ESI-MS in the positive mode assists in identification of [BiCl\(_2\)(\(\mu\)-Sha\(-1\text{H}\))]\(_n\), 6. The full molecular ion ([M+H]\(^+\)) was not observed, but fragments corresponding to [BiCl(Sha\(-1\text{H}\))] and [Bi(Sha\(-1\text{H}\)]\(_2\)] at 396.1 and 512.9 a.m.u were evident.

Crystals suitable for X-ray diffraction were isolated upon standing of the filtrate after filtration of 6. The structure identified is a THF-solvated polymeric structure of [BiCl\(_2\)(\(\mu\)-Sha\(-1\text{H}\))(THF)].THF\(_\infty\), 6a. The X-ray crystal structure confirms a bidentate bridging (O, \(\mu\)-O\(^-\)) mode of coordination of the ligand. No coordination of the hydroxyl group at the 2-position of the phenyl ring partakes in bonding.

Following the identification of this crystal structure, which shows multiple bridging chlorido and hydroxamato ligand moieties, it was proposed that the solid structure of 6 is the THF-free complex as shown in Figure 4.2.

![Figure 4.2. Proposed structure of 6.](image)

**Synthesis of [BiCl\(_3\)(2-NH\(_2\)-Pha\(-1\text{H}\))]\(_n\) (7)**

Reaction of 2-NH\(_2\)-Pha with BiCl\(_3\), in anhydrous THF under an inert atmosphere for 72 hours afforded the novel Bi(III) complex [BiCl\(_3\)(2-NH\(_3\)-Pha\(-1\text{H}\))]\(_n\), 7. This product was obtained in 49% yield and high purity, Scheme 4.3.

![Scheme 4.3. Synthesis of [BiCl\(_3\)(2-NH\(_3\)-Pha\(-1\text{H}\))]\(_n\) 7, a novel Bi(III) 2-aminophenyl hydroxamato chlorido complex.](image)
[BiCl₂(2-NH₃-Pha)]ₙ 7 was characterised by EA, IR, ¹H and ¹³C NMR and MS.

Elemental analysis of 7 is consistent with the presence of one hydroxamato and three chlorido ligands per Bi(III) centre, which strongly suggests that the ligand is charge neutral.

The IR spectra of 7 exhibits distinctive ν(C=O) at 1614 cm⁻¹ displaying the characteristic shift of bidentate (O, O') hydroxamato coordination when compared to the corresponding ν(C=O) of the uncoordinated ligands at 1639 cm⁻¹. These findings are in accordance with earlier studies carried out by Deacon et al. They predict a bi-dentate chelating mode as observed for 6. The shift to lower wavenumber for the ν(C=O) suggests the formation of the thermodynamically preferred five membered chelate. A strong signal at 1541 cm⁻¹ represents the ν(C-N) of the hydroxamic acid, while a strong signal at 1092 cm⁻¹ is indicative of the ν(Bi-Cl). Significantly the amino ν(N-H), which are found at 3244 cm⁻¹ and 3194 cm⁻¹ in the spectrum of the free ligand are not evident in the IR spectrum of the corresponding Bi(III) complex 7, further suggesting a change in the environment of the amino group.

In the ¹H NMR spectra of 7 (Figure 4.3), carried out in MeOD, no signals associated with the hydroxamic acid protons (N–H and O–H) are observed. Significant shifts in all four of the aromatic signals related in the free ligand were observed. In 7, only three main aromatic signals are observed in the region of 8.5-7.5 ppm. Those signals correspond and integrate well for the four aromatic protons. The amino signal found at 4.59 ppm in the free ligand is not evident in the spectrum of the 7, suggesting there is a significant change in the environment of these protons.

Seven signals were observed in the ¹³C NMR spectrum of 7. Six signals at 134.6, 132.9, 128.4, 127.0, 123.6 and 123.3 ppm are associated with the 6 aromatic carbons. As was the case for complex 6, a shift in the signal associated with the carbonyl group at 165.6 ppm, compared with the corresponding signals at 171.9 ppm in the free 2-NH₂-Pha ligand, was evident.
Figure 4.3. The stacked $^1$H NMR spectra of 2-NH$_2$-Pha (top) and complex 7, (bottom) clearly showing the shifts and changes in the aromatic signals associated with the binding of bismuth.

ESI-MS in the positive mode assisted in characterising 7. The full molecular ion, ([M+H]$^+$), was observed for [BiCl$_3$(2-NH$_3$-Pha$_{-1H}$)$_2$] at 619.0 a.m.u, along with the molecular fragment associated with [BiCl$_2$(2-NH$_3$-pha$_{-1H}$)]$^+$ with mass peaks at 430.9 a.m.u. detected.

Given the elemental analysis indicates that the hydroxamato ligand in complex 7 is charge neutral, the typical ν(C=O) shift associated with (O, O’) chelating mode is observed in the IR spectrum and the loss of the amino signals in the $^1$H NMR spectrum and amino ν(N-H) in the IR, it is likely that the amino group at the 2-position is protonated while the hydroxamic acid functional group is mono-deprotonated, 2-NH$_3$-Pha$_{1H}$. The downfield shift in the aromatic signals relative to the free ligand signals, although mainly due to protonation of the amino group, may also in part be due to metal coordination. An X-ray crystal structure of an antimonial complex reported in Chapter 6 features 2-NH$_2$-Pha as a ligand where the hydroxamic acid group is mono-deprotonated (O, O’) coordinated to Sb(III) and the amino group protonated. It is predicted that this is also the case for complex 7 and the ligand is of the form 2-NH$_3$-Pha$_{-1H}$.
Recrystallization of 7 from various solvents did not yield the desired crystals suitable for X-ray crystallography analysis. The proposed polymeric structure for 7 \([\text{BiCl}_3(\mu-2\text{-NH}_3\text{-Pha})]_n\) is shown in Figure 4.4.

![Figure 4.4. Potential structure for 7; predicted product from EA analysis only (A) and predicted polymeric structure (B) based on X-ray crystallography analysis of complex 6a.]

**Synthesis of \([\text{BiCl}_3(2\text{-Py}^{1\text{H}}\text{ha}^{1\text{H}})_2]2\text{H}_2\text{O}]_n\) (8)**

Reaction of 2-Pyha with BiCl, in anhydrous THF under an inert atmosphere for 72 hours afforded the novel Bi(III) complex \([\text{BiCl}_3(2\text{-Py}^{1\text{H}}\text{ha}^{1\text{H}})]_n\), 8. This product was obtained in 50% yield and high purity, Scheme 4.4.

![Scheme 4.4. Synthesis of \([\text{BiCl}_3(2\text{-Py}^{1\text{H}}\text{ha}^{1\text{H}})_2]_n\), a novel Bi(III) 2-pyridine hydroxamato chlorido complex.]

\([\text{BiCl}_3(2\text{-Py}^{1\text{H}}\text{ha}^{1\text{H}})_2]_n\), 8, was characterised by EA, IR, \(^1\text{H}\) NMR spectroscopy and MS. The \(^1\text{H}\) NMR spectra of 8 does not show the hydroxamic acid protons (N–H and O–H), as it was carried out in MeOD. Slight shifts are observed in the four aromatic protons signals of this complex from 8.58-7.53 ppm in the free ligand to 8.64-7.59 ppm in the complex. The original four aromatic signals in the ligand are now displayed as three signals, as merging of signals at 8.05 ppm was observed as a result of bismuth binding, Figure 4.5.
Figure 4.5. Stacked $^1$H NMR spectra of 2-Pyha (top) and complex 8 (bottom). An expansion shows a shift in aromatic signals associated with Bi(III) binding.

The IR spectra of 8 follows the same trend as seen in complex 6 and 7. The intense $\nu$(C=O) in the free ligand, 2-Pyha, which occurs at 1651 cm$^{-1}$, is shifted to lower wavenumber in the corresponding Bi(III) complex to 1602 cm$^{-1}$, displaying the characteristic shift associated with (O, O') hydroxamato coordination. The signal at 1518 cm$^{-1}$ represents the $\nu$(C-N) of the hydroxamic acid, while a strong signal at 1042 cm$^{-1}$ is indicative of the $\nu$(Bi-Cl).

A summary of all the IR frequency stretches of the ligand and their complexes are shown below in Table 4.1 for purposes of comparison.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\nu$(C=O)</th>
<th>$\nu$(C-N)</th>
<th>$\nu$(Bi-Cl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sha</td>
<td>1619 cm$^{-1}$</td>
<td>1555 cm$^{-1}$</td>
<td>---</td>
</tr>
<tr>
<td>6</td>
<td>1599 cm$^{-1}$</td>
<td>1564 cm$^{-1}$</td>
<td>1033 cm$^{-1}$</td>
</tr>
<tr>
<td>2-NH$_2$-Pha (1)</td>
<td>1639 cm$^{-1}$</td>
<td>1559 cm$^{-1}$</td>
<td>---</td>
</tr>
<tr>
<td>7</td>
<td>1614 cm$^{-1}$</td>
<td>1541 cm$^{-1}$</td>
<td>1092 cm$^{-1}$</td>
</tr>
<tr>
<td>2-Pyha (5)</td>
<td>1651 cm$^{-1}$</td>
<td>1576 cm$^{-1}$</td>
<td>---</td>
</tr>
<tr>
<td>8</td>
<td>1602 cm$^{-1}$</td>
<td>1518 cm$^{-1}$</td>
<td>1042 cm$^{-1}$</td>
</tr>
</tbody>
</table>
ESI-MS in the positive mode assisted in characterising 8. The full molecular ion, ([M+H]⁺) was not detected though a molecular fragment was observed at 483.2 a.m.u attributed to [Bi(2-Pyha-H)₂] was observed.

The EA result for complex 8 indicates the presence of two hydroxamato ligands and three chlorido ligands per Bi(III) centre, suggesting that the two 2-pyridine hydroxamato ligands are charge neutral as was the case for 2-NH₂-Pha in 7. EA results for all complex in this chapter are shown in Table 4.2 for purposes of comparison.

Table 4.2. Summary of the EA results for complexes 6, 6a, 7 and 8.

<table>
<thead>
<tr>
<th>Complex</th>
<th>% C</th>
<th>% H</th>
<th>% N</th>
<th>% Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Calculated</td>
<td>19.46</td>
<td>1.40</td>
<td>3.24</td>
</tr>
<tr>
<td></td>
<td>Found</td>
<td>19.40</td>
<td>1.14</td>
<td>2.84</td>
</tr>
<tr>
<td>6a</td>
<td>Calculated</td>
<td>31.27</td>
<td>3.85</td>
<td>2.43</td>
</tr>
<tr>
<td></td>
<td>Found</td>
<td>30.85</td>
<td>3.63</td>
<td>2.20</td>
</tr>
<tr>
<td>7</td>
<td>Calculated</td>
<td>18.72</td>
<td>1.87</td>
<td>5.90</td>
</tr>
<tr>
<td></td>
<td>Found</td>
<td>18.84</td>
<td>1.72</td>
<td>5.92</td>
</tr>
<tr>
<td>8</td>
<td>Calculated</td>
<td>22.89</td>
<td>2.88</td>
<td>8.90</td>
</tr>
<tr>
<td></td>
<td>Found</td>
<td>23.05</td>
<td>1.88</td>
<td>8.58</td>
</tr>
</tbody>
</table>

Recrystallization of 8 from various solvent failed to yield a crystal for X-ray crystallographic analysis. The proposed structure for 8 is shown in Figure 4.6.

Figure 4.6. Potential structures of 8; predicted product from EA analysis alone (A) and predicted polymeric structure (B) based on X-ray crystallography analysis of complex 6a.
There are literature precedents where reaction of BiCl$_3$ with ligands produced polymeric complexes. Yim et al. for example reported that the reaction of BiCl$_3$ with a tridentate thioether, MeSi(CH$_2$SMe)$_3$, produced a polymeric complex of repeating units of Bi$_2$(MeSi(CH$_2$SMe)$_3$)$_2$Cl$_6$. Genge et al. identified and isolated polymeric structures upon reaction of BiCl$_3$ with dithioether ligands, [Bi$_4$Cl$_{12}$MeSCH$_2$CH$_2$CH$_2$SMe]$_n$H$_2$O.

In relation to the polymeric structure in this chapter, 6a, and the previously mentioned polymeric Bi thioether complexes, a common property is the ability of the chloride atoms to form bridges between adjacent bismuth atom centres, seemingly essential for polymer formation. The formation of bismuth-chloride bonds is a driving force for formation of these polymeric of structures. Bismuth typically exhibits coordination numbers of six to nine. This diverse coordination characteristic coupled with the bridging effect of chlorine allows for formation of polymeric complexes and intriguing bismuth structures.

**Attempted synthesis of [BiCl$_2$(Bha$\cdot$1H)$_n$]**

Reaction of Bha with BiCl$_3$, in anhydrous THF under an inert atmosphere for 72 hours did not result in the isolation of any product, even after numerous attempts. It was predicted that this reaction would proceed as per the previous reaction with BiCl$_3$ but this was not seen to be the case, **Scheme 4.5**.

![Scheme 4.5. Attempted Synthesis of [BiCl$_2$(Bha$\cdot$1H)$_n$].](image)

Clear crystals were isolated on standing of the filtrate at room temperature and analysed by X-ray crystallography. These crystals were identified as N-(benzoyloxy)benzamide, 9.

Hydroxamic acids have significant value as key intermediates for functional group transformation and O-Acyl/O-tosyl hydroxamates are key reactants in the Lossen rearrangement. Formation of O-acyl/O-tosyl hydroxamates through base catalysed reactions are the most common method of formation. O-benzoyl benzohydroxamic acid was isolated and structurally characterised in reaction of Bha with BiCl$_3$ in anhydrous THF.
5. Crystal Structure Studies

The X-ray crystal structures described in this section were determined for complex 6a by Dr Helge Müller Bunz, School of Chemistry, in University College Dublin, Dublin and for compound 9 by Dr Brendan Twamley, School of Chemistry, in Trinity College, Dublin.

\([\text{BiCl}_2(\mu-\text{Sha}_1\text{H})(\text{THF})].\text{THF}\infty\) (6a)

Single crystals of THF-solvated \([\text{BiCl}_2(\mu-\text{Sha}_1\text{H})(\text{THF})].\text{THF}\infty\) suitable for X-ray crystallography analysis were obtained upon standing of the filtrate post isolation of \([\text{BiCl}_2(\mu-\text{Sha}_1\text{H})]_n\), 6. The X-ray intensity data were measured at 100 K using an Oxford Cryosystems Cobra low temperature device. A specimen, of approximate dimensions 0.3408 x 0.1964 x 0.1525 mm was used for refinement. The solid state structure of 6a is shown in Figure 4.7. The complex crystallizes in the Pna2₁ space group. Crystal data and details of data collection are summarised in Table 4.3. The coordination sphere around each Bi(III) in 6a is completed by a coordinated THF molecule with each hydroxyl group at the 2-position on the aromatic ring of Sha hydrogen bonding to one uncoordinated THF molecule in the lattice, Figure 4.7.

![Figure 4.7](image-url)  
**Figure 4.7.** Crystal structure of \([\text{BiCl}_2(\mu-\text{Sha}_1\text{H})(\text{THF})].\text{THF}\infty\), 6a featuring neighbouring environment of one Bi center with adjacent Bi atoms continuing the chain; thermal ellipsoids are drawn on the 50% probability level.
### Table 4.3. Table of collection parameters for 6a.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C_{15}H_{22}NO_{5}Cl_{2}Bi</td>
</tr>
<tr>
<td>Molecular formula</td>
<td>C_{11}H_{14}NO_{5}Cl_{2}Bi x C_{4}H_{8}O</td>
</tr>
<tr>
<td>Formula weight</td>
<td>576.22</td>
</tr>
<tr>
<td>Temperature</td>
<td>100 K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Orthorhombic</td>
</tr>
<tr>
<td>Space group</td>
<td>Pna2₁ (#33)</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>a = 16.6571(1) Å, α = 90°, b = 33.0779(3) Å, β = 90°, c = 20.4220(2) Å, γ = 90°</td>
</tr>
<tr>
<td>Volume</td>
<td>11252.15(16) Å³</td>
</tr>
<tr>
<td>Z</td>
<td>24</td>
</tr>
<tr>
<td>Density (calculated)</td>
<td>2.041 Mg/m³</td>
</tr>
<tr>
<td>Absorption coefficient</td>
<td>9.710 mm⁻¹</td>
</tr>
<tr>
<td>F(000)</td>
<td>6624</td>
</tr>
<tr>
<td>Crystal size</td>
<td>0.3408 x 0.1964 x 0.1525 mm³</td>
</tr>
<tr>
<td>Theta range for data collection</td>
<td>2.93 to 29.69°</td>
</tr>
<tr>
<td>Index ranges</td>
<td>-22 &lt;= h &lt;= 20, -43 &lt;= k &lt;= 45, -27 &lt;= l &lt;= 27</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>122294</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>27266 [R(int) = 0.0361]</td>
</tr>
<tr>
<td>Completeness to theta = 28.00°</td>
<td>99.0 %</td>
</tr>
<tr>
<td>Absorption correction</td>
<td>Analytical</td>
</tr>
<tr>
<td>Max. and min. transmission</td>
<td>0.319 and 0.102</td>
</tr>
<tr>
<td>Refinement method</td>
<td>Full–matrix least–squares on F²</td>
</tr>
<tr>
<td>Data / restraints / parameters</td>
<td>27266 / 23 / 1304 a)</td>
</tr>
<tr>
<td>Goodness–of–fit on F²</td>
<td>1.059</td>
</tr>
<tr>
<td>Final R indices [I&gt;2σ(I)]</td>
<td>R1 = 0.0342, wR2 = 0.0842</td>
</tr>
<tr>
<td>R indices (all data)</td>
<td>R1 = 0.0355, wR2 = 0.0859</td>
</tr>
<tr>
<td>Absolute structure parameter</td>
<td>0.325(3) b</td>
</tr>
<tr>
<td>Largest diff. peak and hole</td>
<td>1.999 and -1.650 e.Å⁻³</td>
</tr>
</tbody>
</table>
The structure of [BiCl₂(µ-Sha.₁H)(THF)].THFₙ consists of a charge neutral Bi(III) polymer where each Bi(III) centre has a coordination number of 7 and possesses distorted pentagonal bipyramidal geometry. Each Bi(III) centre is coordinated to three chlorido ligands, two of which are bridging with different but adjacent Bi(III) centres and a salicylhydroxamato ligand with a bidentate bridging (O, µ-O’) chelating mode, where the deprotonated hydroxyl oxygen bridges with one adjacent Bi(III) centre. The oxygen bridge is asymmetric. The bridging oxygen is significantly closer in length to the hydroxamato chelated Bi(III) centre (c. 2.25 Å) as compared to the adjacent Bi(III) centre (c. 2.8 Å). The extended polymeric structure is shown in Figure 4.8.

Figure 4.8.  ORTEP diagram of [BiCl₂(µ-Sha.₁H)(THF)].THFₙ, 6a, featuring repeating units of the chain. Uncoordinated THF molecules omitted for clarity.

As reported in Chapter 3, the Bi(III) nitrate salicylhydroxamato complex 3 has bismuth atoms that are bridged by salicylhydroxamato ligands with bond lengths in accordance with what is observed in 6a, (2.226-2.82 Å).¹⁹ The atomic numbering scheme and atom connectivity for [BiCl₂(µ-Sha.₁H)(THF)].THFₙ are shown above and a selection of bond lengths and angles are reported in Table 4.4 and Table 4.5 respectively.
Table 4.4. Selected bond lengths (Å) for 6a.

<table>
<thead>
<tr>
<th>Atoms</th>
<th>Dist (Å)</th>
<th>Atoms</th>
<th>Dist (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bi(1)–O(2)</td>
<td>2.237(4)</td>
<td>Cl(2)–Bi(2)</td>
<td>3.0059(15)</td>
</tr>
<tr>
<td>Bi(1)–O(1)</td>
<td>2.290(4)</td>
<td>O(2)–Bi(2)</td>
<td>2.750(4)</td>
</tr>
<tr>
<td>Bi(1)–Cl(1)</td>
<td>2.5190(17)</td>
<td>Bi(2)–O(5)</td>
<td>2.225(4)</td>
</tr>
<tr>
<td>Bi(1)–O(19)</td>
<td>2.641(5)</td>
<td>Bi(2)–O(4)</td>
<td>2.277(4)</td>
</tr>
<tr>
<td>Bi(1)–Cl(2)</td>
<td>2.7272(14)</td>
<td>Bi(2)–Cl(3)</td>
<td>2.5255(16)</td>
</tr>
<tr>
<td>Bi(1)–O(5)#1</td>
<td>2.797(4)</td>
<td>Bi(2)–O(20)</td>
<td>2.709(5)</td>
</tr>
<tr>
<td>Bi(1)–Cl(4)#1</td>
<td>3.0159(15)</td>
<td>Bi(2)–Cl(4)</td>
<td>2.7356(14)</td>
</tr>
</tbody>
</table>

Table 4.5. Selected bond angles (°) for 6a.

<table>
<thead>
<tr>
<th>Atoms</th>
<th>Angle (°)</th>
<th>Atoms</th>
<th>Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O(2)–Bi(1)–O(1)</td>
<td>70.88(14)</td>
<td>O(1)–Bi(1)–O(5)#1</td>
<td>138.31(13)</td>
</tr>
<tr>
<td>O(2)–Bi(1)–Cl(1)</td>
<td>92.25(11)</td>
<td>Cl(1)–Bi(1)–O(5)#1</td>
<td>94.21(9)</td>
</tr>
<tr>
<td>O(1)–Bi(1)–Cl(1)</td>
<td>87.62(13)</td>
<td>O(19)–Bi(1)–O(5)#1</td>
<td>99.76(13)</td>
</tr>
<tr>
<td>O(2)–Bi(1)–O(19)</td>
<td>77.15(14)</td>
<td>Cl(2)–Bi(1)–O(5)#1</td>
<td>75.99(9)</td>
</tr>
<tr>
<td>O(1)–Bi(1)–O(19)</td>
<td>79.86(17)</td>
<td>O(2)–Bi(1)–Cl(4)#1</td>
<td>144.89(10)</td>
</tr>
<tr>
<td>Cl(1)–Bi(1)–O(19)</td>
<td>165.68(11)</td>
<td>O(1)–Bi(1)–Cl(4)#1</td>
<td>75.49(10)</td>
</tr>
<tr>
<td>O(2)–Bi(1)–Cl(2)</td>
<td>74.64(10)</td>
<td>Cl(1)–Bi(1)–Cl(4)#1</td>
<td>96.19(5)</td>
</tr>
<tr>
<td>O(1)–Bi(1)–Cl(2)</td>
<td>145.51(10)</td>
<td>O(19)–Bi(1)–Cl(4)#1</td>
<td>87.44(12)</td>
</tr>
<tr>
<td>Cl(1)–Bi(1)–Cl(2)</td>
<td>94.01(5)</td>
<td>Cl(2)–Bi(1)–Cl(4)#1</td>
<td>138.18(4)</td>
</tr>
<tr>
<td>O(19)–Bi(1)–Cl(2)</td>
<td>92.43(13)</td>
<td>O(5)#1–Bi(1)–Cl(4)#1</td>
<td>62.90(9)</td>
</tr>
<tr>
<td>O(2)–Bi(1)–O(5)#1</td>
<td>150.28(13)</td>
<td>Bi(1)–Cl(2)–Bi(2)</td>
<td>98.78(4)</td>
</tr>
</tbody>
</table>

The Bi–Cl bond lengths of the complexes solved by Yim et al. are of the range 2.5-3.0 Å, and agree with what is observed in complex 6a. Asymmetric binding is observed in 6a, a characteristic which is also present in Bi₂[MeSi(CH₂SMe)_3]₂Cl₆.²⁶ Again Bi–Cl bond lengths of 6a are similar to those observed in the reported structure of [Bi₄Cl₁₂(MeSCH₂CH₂SMe)₄]₄·H₂O by Genge et al.²⁷

While our complex shows similarities in many ways to the polymeric bismuth thioether complexes, 6a is the first Bi(III) hydroxamate chlorido complex structurally characterised and hence represents a novel class of bismuth hydroxamic acid complexes.
N-(benzoyloxy)benzamide (9)

In a failed attempt to synthesise \([\text{BiCl}_2(\text{Bha}_{-1}\text{H})]\)_n, single crystals of N-(benzoyloxy)benzamide were isolated and an X-ray crystallography investigation undertaken. The structure of N-(benzoyloxy)benzamide, 9, is shown in Figure 4.9.

![Figure 4.9. N-(benzoyloxy)benzamide, compound 9.](image)

The X-ray intensity data were measured at 100 K using an Oxford Cryosystems Cobra low temperature device. A specimen, of approximate dimensions 0.37 x 0.11 x 0.09 mm was used for refinement. The solid state structure of 9 is shown in Figure 4.10. The complex crystallizes in the P2_12_12_1 space group. A total of 360 frames were collected and the total exposure time was 2.05 hours.

Figure 4.10. Molecular structure of 9 with atomic displacement parameters shown at 50% probability.

![Figure 4.11. Packing diagram of 9. Hydrogen atoms omitted for clarity. Dashed lines indicate strong hydrogen bonding.](image)
The formula of 9 consists of a charge neutral organic small molecule. The molecule display disorder, with each phenyl ring occupying two positions. The packing diagram of 9, showing hydrogen bonding, is shown in Figure 4.11. Crystal data and details of data collection are summarised in Table 4.6 while significant bond lengths and bond angles are displayed in Tables 4.7 and Table 4.8. The bond lengths and angles are in accordance with those of benzohydroxamic acid.

### Table 4.6. Crystal data and structure refinement for compound 9.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C_{14}H_{11}NO_{3}</td>
</tr>
<tr>
<td>Formula weight</td>
<td>241.24</td>
</tr>
<tr>
<td>Temperature</td>
<td>100 K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Orthorhombic</td>
</tr>
<tr>
<td>Space group</td>
<td>P2₁2₁2₁</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>a = 8.9401(5) Å, α = 90°</td>
</tr>
<tr>
<td></td>
<td>b = 9.1767(6) Å, β = 90°</td>
</tr>
<tr>
<td></td>
<td>c = 14.0341(8) Å, γ = 90°</td>
</tr>
<tr>
<td>Volume</td>
<td>1151.37(12) Å³</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
</tr>
<tr>
<td>Density (calculated)</td>
<td>1.392 Mg/m³</td>
</tr>
<tr>
<td>Absorption coefficient</td>
<td>0.099 mm⁻¹</td>
</tr>
<tr>
<td>F(000)</td>
<td>504</td>
</tr>
<tr>
<td>Crystal size</td>
<td>0.37 x 0.11 x 0.09 mm³</td>
</tr>
<tr>
<td>Theta range for data collection</td>
<td>2.903 to 30.502°</td>
</tr>
<tr>
<td>Index ranges</td>
<td>-6 ≤ h ≤ 12, -12 ≤ k ≤ 13, -19 ≤ l ≤ 20</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>9872</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>3504 [R(int) = 0.0372]</td>
</tr>
<tr>
<td>Completeness to theta</td>
<td>99.8 %</td>
</tr>
<tr>
<td>Absorption correction</td>
<td>Semi-empirical from equivalents</td>
</tr>
<tr>
<td>Max. and min. transmission</td>
<td>0.7461 and 0.7036</td>
</tr>
<tr>
<td>Refinement method</td>
<td>Full-matrix least-squares on F²</td>
</tr>
<tr>
<td>Data / restraints / parameters</td>
<td>3504 / 0 / 168</td>
</tr>
<tr>
<td>Goodness-of-fit on F²</td>
<td>1.041</td>
</tr>
<tr>
<td>Final R indices [I&gt;2σ(I)]</td>
<td>R1 = 0.0430, wR2 = 0.0945</td>
</tr>
<tr>
<td>R indices (all data)</td>
<td>R1 = 0.0608, wR2 = 0.1026</td>
</tr>
<tr>
<td>Absolute structure parameter</td>
<td>0.2(13)</td>
</tr>
<tr>
<td>Largest diff. peak and hole</td>
<td>0.382 and -0.253 e.Å⁻³</td>
</tr>
</tbody>
</table>
Table 4.7. A list of the significant bond lengths (Å) for 9*

<table>
<thead>
<tr>
<th>Atoms</th>
<th>Dist (Å)</th>
<th>Atoms</th>
<th>Dist (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O(8)-C(7)</td>
<td>1.234(2)</td>
<td>N(9)-C(7)</td>
<td>1.339(2)</td>
</tr>
<tr>
<td>O(10)-N(9)</td>
<td>1.400(2)</td>
<td>N(9)-H(9)</td>
<td>0.87(3)</td>
</tr>
<tr>
<td>O(10)-C(11)</td>
<td>1.382(2)</td>
<td>C(6)-C(7)</td>
<td>1.494(3)</td>
</tr>
<tr>
<td>O(12)-C(11)</td>
<td>1.192(2)</td>
<td>C(11)-C(13)</td>
<td>1.485(2)</td>
</tr>
</tbody>
</table>

*All aromatic C-H bonds are 0.9500 Å and all aromatic C-C bonds are 1.387 Å.

Table 4.8. A list of the significant bond angles (°) for 9*

<table>
<thead>
<tr>
<th>Atoms</th>
<th>Angle (°)</th>
<th>Atoms</th>
<th>Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(11)-O(10)-N(9)</td>
<td>113.06(14)</td>
<td>O(10)-C(11)-C(13)</td>
<td>110.31(16)</td>
</tr>
<tr>
<td>O(10)-N(9)-H(9)</td>
<td>116.2(19)</td>
<td>O(12)-C(11)-O(10)</td>
<td>123.13(17)</td>
</tr>
<tr>
<td>C(7)-N(9)-O(10)</td>
<td>117.10(15)</td>
<td>O(8)-C(7)-C(6)</td>
<td>122.13(16)</td>
</tr>
<tr>
<td>C(7)-N(9)-H(9)</td>
<td>126.7(19)</td>
<td>N(9)-C(7)-C(6)</td>
<td>115.81(15)</td>
</tr>
</tbody>
</table>

*All aromatic C-C-C bond angles are 120° and all aromatic C-C-H bond angles are also 120°.

Proposed Mechanism of Formation

The traditional method of synthesising N-(benzoyloxy)benzamide which is also known as O-benzoyl benzohydroxamic acid, an O-acyl hydroxamic acid, proceeds via the reaction of Bha with base such as sodium tert-butoxide, and further reaction with benzoyl chloride, Scheme 4.6.28

Scheme 4.6. General mechanism for N-(benzoyloxy)benzamide formation.

A proposed mechanism for the formation of 9 is shown in Scheme 4.7. Activation of the carbonyl bond by complexation to the bismuth centre allows for nucleophilic attack by the liberated chloride ion on the carbonyl carbon. This results in decomplexation, which in turn gives deprotonated hydroxylamine and benzoyl chloride. Nucleophilic attack of the Bha oxygen at the electrophilic carbonyl of the
benzoyl chloride as previously seen\textsuperscript{29}, could result in the formation of \(N\)-(benzoyloxy)benzamide.

![Scheme 4.7. Proposed mechanism for the synthesis of 9.](image)

**6. Stability Testing**

Bismuth complexes hydrolyse in aqueous solutions as discussed by Andrews et al.\textsuperscript{30} The stability of our novel Bi(III) hydroxamato chlorido complexes 6, 7 and 8 were investigated as solids and in solution.

The stability of the complexes as solids were assessed by performing \(^1\)H NMR analysis on fresh solid samples one, three and six months apart, while the stability of these complexes in solution of MeOD were assessed by performing \(^1\)H NMR analysis on 6, 7 and 8 regularly over a period of one month. The \(^1\)H NMR spectra showed no variation in signals over the time period studied, indicating good stability in the solid state and in solution in methanol.
7. Anti-bacterial Testing

Given that a solid state structure was not fully elucidated for complexes 7 and 8, the anti-bacterial testing of complex 6 alone was investigated against a range of bacteria. This work was carried out by Thayse Marques Passos and Lauren Fagan, in collaboration with Dr. Bríd Quilty, in Dublin University College, Dublin and is described below.

**LC$_{50}$ Evaluation**

The anti-bacterial activity of [BiCl$_2$(µ-Sha$_{1H}$)$_n$], ampicillin and bismuth subsalicylate (BSS) were investigated against six environmental bacterial strains; *E. coli DSM498, E. coli T37-1, P. putida DSM6125 (KT2440), P. putida CP1, P. fluorescens DSM50090 and B. subtilis DSM10*, and three biohazard Class II pathogenic strains; *E. coli DSM1103, E. aerogenes DSM30053 and S. aureus DSM799*. Ampicillin is a β-lactam broad spectrum antibiotic, which features on the WHO’s List of Essential Medicines and was chosen as a control for these tests.\(^{31}\)

LC$_{50}$ values, where LC$_{50}$ is the lethal concentration which inhibits the growth of 50% of viable cells present, were determined using the broth micro-dilution test \((n = 3)\).

Initial studies were carried out using agar dilution tests, indicated that the LC$_{50}$ values of 6 and BSS were in the range of 0-10 µg mL$^{-1}$, while ampicillin was previously determined to have varying activities across the 0-500 µg mL$^{-1}$ range. For this reason the concentrations used in the broth micro-dilution test for LC$_{50}$ were in the range of two fold dilutions from 0.3125-10 µg mL$^{-1}$ for complex 6 and BSS, and two fold dilutions ranging from 0.977-500 µg mL$^{-1}$ for ampicillin.\(^{32}\) Stock solutions of complex at required concentrations were initially dissolved in DMSO and then made up to have a final DMSO concentration of ≤ 1% which was observed to have no effect on bacterial growth.

Bacteria were incubated over-night in the presence of a range of concentrations of test complexes. The presence or absence of growth was determined by optical density measurements and the LC$_{50}$ values were determined as the concentration that caused a 50% reduction in cell growth. LC$_{50}$ values for each of the strains are shown below in Table 4.9.
Complex 6 and BSS exhibit similar anti-bacterial activity against all of the strains tested, although 6 is slightly more active than BSS against every strain tested apart from P. putida DSM 6125. One interesting point to note, is that the bismuth complexes do not display preferential activity for the Gram-positive (S. aureus and B. subtilis) over the Gram-negative bacteria or vice versa.

In contrast to the two bismuth complexes, ampicillin exhibits diverse activity against the panel of bacteria. As ampicillin is a broad spectrum antibiotic it is able to cross the cell membrane of both Gram-positive and Gram-negative bacteria. Ampicillin acts as an irreversible inhibitor of the enzyme transpeptidase, which is involved in cell wall synthesis and inhibition of this enzyme can lead to cell lysis. This antibiotic exhibits significantly superior activity (LC$_{50}$ ≤ 0.98 µg mL$^{-1}$) against all three strains of E. coli including the pathogenic strain, E. coli DSM 1103, as compared to the bismuth complexes (LC$_{50}$ 3.5-8.7 µg mL$^{-1}$). It is noteworthy though that both bismuth complexes exhibit similar activity as ampicillin against the remaining two pathogenic strains E. aerogenes DSM 30053 and S. aureus DSM 799.

The bismuth complexes have superior activity with respect to ampicillin in four out of the six environmental strains, two of which are resistant to ampicillin (P. fluorescens DSM50090 and B. subtilis DSM 10) with LC$_{50}$ ≥ 500 µg mL$^{-1}$.

Other studies have also investigated the activity of bismuth based complexes against bacteria beyond H. pylori. In these tests, BSS, BiOCl, Colloidal bismuth hydroxide

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### Table 4.9. LC$_{50}$ values for 6, BSS and Ampicillin against nine bacterial strains.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Strain</th>
<th>6</th>
<th>BSS</th>
<th>Ampicillin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>DSM 498</td>
<td>5.7 ± 0.04</td>
<td>8.7 ± 0.032</td>
<td>≤ 0.98</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>T37-1</td>
<td>3.5 ± 0.036</td>
<td>7.4 ± 0.018</td>
<td>≤ 0.98</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>DSM 1103</td>
<td>6 ± 0.026</td>
<td>6.7 ± 0.010</td>
<td>≤ 0.98</td>
</tr>
<tr>
<td><em>E. aerogenes</em></td>
<td>DSM 30053</td>
<td>6.9 ± 0.024</td>
<td>7 ± 0.010</td>
<td>7 ± 0.016</td>
</tr>
<tr>
<td><em>P. putida</em></td>
<td>DSM 6125</td>
<td>4.7 ± 0.039</td>
<td>4.1 ± 0.09</td>
<td>62.5 ± 0.02</td>
</tr>
<tr>
<td><em>P. putida</em></td>
<td>CP1</td>
<td>3.9 ± 0.022</td>
<td>6 ± 0.05</td>
<td>98 ± 0.019</td>
</tr>
<tr>
<td><em>P. fluorescens</em></td>
<td>DSM50090</td>
<td>2.3 ± 0.029</td>
<td>4.4 ± 0.03</td>
<td>&gt; 500</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>DSM 799</td>
<td>5.8 ± 0.03</td>
<td>6.8 ± 0.028</td>
<td>7.81 ± 0.012</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>DSM 10</td>
<td>2.5 ± 0.032</td>
<td>4.6 ± 0.03</td>
<td>&gt; 500</td>
</tr>
</tbody>
</table>
gel, bismuth nitrate and bismuth thiol complexes were tested for their inhibitory and growth reductive effect on a range of bacteria including *Staphylococci*, *Escherichia coli* and *Clostridium difficile*. The results obtained from these tests show that these complexes are also active against these bacteria. The activities displayed are comparable to our Bi(III) hydroxamato chlorido complex.\textsuperscript{33-35}

These studies, along with our assays show that there is the possibility to develop structurally well-characterised bismuth based complexes as anti-bacterial agents. A novel class of Bi(III) hydroxamato chlorido complex have been synthesised, which show good LC\textsubscript{50} values against a range of bacteria.
8. Conclusion

In conclusion, this chapter reports the synthesis and full characterisation of a novel class of Bi(III) hydroxamato chlorido complexes of type [BiCl₃(L-1H)ₓ]. When tested, these complexes demonstrated anti-bacterial activity against a range of bacteria, both pathogenic and environmental.

In each case, the products were obtained by the reaction of one equivalent of BiCl₃ with one equivalent of hydroxamic acid. Three different hydroxamic acids were used, Sha, 2-NH₂-Pha and 2-Pyha which formed novel Bi(III) hydroxamato chlorido complexes.

The Bi(III) hydroxamato chlorido complexes [BiCl₂(µ-Sha-1H)]ₙ, [BiCl₃(2-NH₃-Pha. 1H)]ₙ and [BiCl₃(2-Pyha-1H)]₂ₙ were successfully synthesised and fully characterised and are proposed to be polymeric given the propensity for Bi chlorido complexes to be polymeric.

The structure of the THF solvated [BiCl₂(µ-Sha-1H)]THF₇ was isolated from slow evaporation of the filtrate of 6 and solved by X-ray crystallography. This crystal features binding of the ligand to the bismuth centre via a bidentate bridging (O, µ-O’) chelation mode. The deprotonated hydroxyl oxygen bridges with one adjacent Bi(III) centre. There is no evidence to suggest that the secondary groups of Sha, 2-NH₂-Pha or 2-Pyha i.e. the hydroxyl group, amino group or pyridine nitrogen at the 2-position respectively, are involved in binding in any way. The only interaction that this side moiety has demonstrated is in 6a, where the 2-OH can hydrogen bond with an uncoordinated THF molecule in the crystal structure. Efforts to isolate and identify crystals of the other two product were unsuccessful. It is noteworthy that the amino group and the heterocyclic nitrogen of the 2-NH₂-Pha and 2-Pyha respectively, are likely to be protonated and as ligands in the reported complexes, charge neutral.

Stability tests were carried out on all the complexes as solids and in solution. It was observed that these complexes were stable as solids and in solution.

Given that full analysis and structural data was achieved for complex 6, an investigation of its anti-bacterial activity of was carried out. In conjunction to our
investigation, activity of BSS and ampicillin as a comparison were also investigated. The activities of these complexes were tested against nine bacterial strains. The results revealed that both bismuth complexes were equally as active and exhibited broad anti-bacterial activity, with noteworthy activity against two pathogenic strains, *E. aerogenes* DSM 30053 and *S. aureus* DSM 799. Furthermore both bismuth complexes exhibited appreciable activity against six environmental bacterial strains and, in four cases, far superior activity than ampicillin.

This anti-bacterial study suggest that bismuth complexes do have the potential to act as anti-bacterial agents alone or in combination with existing antibiotics for the treatment of bacteria beyond *H. pylori*. Given the bismuth complexes exhibited activity against pathogenic bacterial strains they have potential applications as luminal (gut) or surface acting agents (cathether, skin, wounds). Furthermore given bismuth complexes exhibit activity against environmental bacterial strains and knowing that orally delivered Bi(III) is non toxic, bismuth complexes immobilised on a polymer, for example, could potentially have applications in water sterilisation or disinfection.

### 9. Future Plans

There is an urgent need for effective and novel class of anti-bacterial agents given the last class to be brought to the clinic was over 40 years ago. The incorporation of bismuth into treatment regimens to treat bacterial infections beyond *H. Pylori* should be investigated thoroughly.
References


Chapter 5

Synthesis of Bi(III) and Ga(III) Retro Hydroxamato Complexes; A Comparison of Anti-Bacterial Activities Against *Escherichia coli* and *Staphylococcus aureus*. 
1. Introduction

Since the initial development and clinical use of antibiotics, there has been a steady and alarming increase in the levels of antibiotic resistant bacterial infections arising worldwide.\(^1\) The reduced efficacy of antibiotics coupled to the lack of new classes of antibiotics being developed, may result in antibiotic resistance infections rising to epidemic levels.

*Klebsiella pneumoniae* is a common intestinal bacteria and a major cause of hospital-acquired infections such as pneumonia, bloodstream infections, and infections in new-borns and intensive-care unit patients. Multi drug resistant *K. pneumoniae* has spread to all regions of the world and is becoming increasingly hard to treat. Likewise, resistance to *E. coli*, the main causative agent for urinary tract infections, is also widespread. In many countries the treatment of this bacteria is ineffective in more than half of patients. Resistance to first-line drugs for the treatment of *Staphylococcus aureus* is rife. MRSA are linked to a 64% increased risk of death in comparison to the non-resistance forms of the infection. MRSA resistance to Colistin, the so called “last antibiotic” (Figure 5.1), has been detected in several countries, making infections caused by these bacteria untreatable. It is estimated that by 2050, 10 million people could die every year due to ARB unless new and effective treatments are developed.\(^2\)

![Figure 5.1. Colistin, clinically used to treat MRSA, but resistance has been detected in several countries.](image-url)
As a result of the urgent requirement for new anti-bacterial treatments there has been a surge of interest in repositioning/repurposing of previous and current anti-bacterial treatments, investigating traditional medicines and therapies, and identification of novel anti-bacterial biomolecular targets which could be exploited in the effective eradication of resistant and non-resistant bacteria.³

Bismuth preparations had been used, prior to the discovery of antibiotics, as treatments for bacterial infections associated with wounds, cholera and gastroenteritis. The development of antibiotics in the 1940’s drastically reduced the use of Bi-based anti-bacterial medications until the discovery of the gastric pathogen *H. pylori* in the 1980s.⁴

Currently Bi-containing quadruple therapies for *H. pylori* represent a successful strategy for overcoming multidrug bacterial resistance.¹ As outlined in Chapter 3, bismuth based drugs such as Pepto-Bismol (BSS), De-Nol (CBS) and the more recently developed Pylorid (RBC) are administered orally, however without an exact understanding of their structure, behaviour in biological environment or indeed their mechanisms of action.¹

As discussed previously, bismuth has also been observed to have activity against bacteria in addition to *H. pylori*. Chapter 4 demonstrated the anti-bacterial activity of novel Bi(III) hydroxamato chlorido against a range of environmental and pathogenic bacteria. BSS also demonstrated activity against biofilms formed by MRSA isolated from human chronic wounds.⁵ Furthermore even though bismuth has been clinically used for decades, no *H. pylori* resistance to bismuth has been observed, which is significant.⁶

Polypeptide deformylase is a novel anti-bacterial biomolecular target. GlaxoSmithKline developed an important retro hydroxamic acid based PDF inhibitor, GSK-1322322 (GSK322), Figure 5.2.⁷⁻¹⁰ The mechanism of inhibition of GSK322 on PDF which is thought to be due to its binding to the iron centre is believed to be a unique inhibitory mechanism, as no cross-resistance with current treatments was observed against a range of Gram-positive or Gram-negative bacteria.¹¹
As outlined in Chapters 3 and 4, hydroxamic acids classically coordinate metal centres in a bidentate chelating fashion, coordinating through the carbonyl oxygen atom and the deprotonated hydroxyl group, to form very stable five-membered hydroxamato chelates. Retro hydroxamic acids are predicted to bind in a similar fashion.

Furthermore, in Chapters 3 and 4, methods for synthesising novel classes of bismuth hydroxamato nitrato and bismuth hydroxamato chlorido complexes were investigated and demonstrated to exhibit anti-bacterial activity against *H. pylori*, as well as a broad range of pathogenic and environmental strains of bacteria respectively. To the best of my knowledge, there are no examples of bismuth retro hydroxamates in the literature.

In addition to bismuth, gallium has attracted recent attention as a metal that possesses inherent anti-bacterial properties and ability to suppress microbial growth.

Gallium has been extensively used as a clinical diagnostic and therapeutic agent in cancer, autoimmune diseases and bone resorption. Gallium nitrate, gallium maltololate and less so gallium chloride have been employed and demonstrated to have good antineoplastic activity, while newly developed gallium citrate originally an anti-tumour agent has been repurposed and is under investigated for its activity against a variety of infections, Figure 5.3.
Figure 5.3. (A) Gallium nitrate, FDA approved for the treatment of cancer-associated hypercalcemia. Gallium chloride (B) and Gallium maltolate (C), have been developed to improve antimetabolite activity. Gallium citrate (D) a previous tumour imaging agent currently investigated for anti-infectious activity.

The pharmacological properties of Ga(III) are attributable to its similarity with Fe(III) at the level of nuclear radius (octahedral ionic radius is 0.620 Å for Ga\[^{3+}\] and 0.645 Å for Fe\[^{3+}\]), tetrahedral ionic radius is 0.47 Å for Ga\[^{3+}\] and 0.49 Å for Fe\[^{3+}\]), coordination chemistry and ionization potential (Ga\[^{3+}\] is 64 eV and Fe\[^{3+}\] is 54.8 eV).\(^{17}\) Therefore Ga(III) can substitute for Fe(III) in the prosthetic group of several enzymes.\(^{18}\) Transferrin (Tf) and lactoferrin (Lf) are important iron binding groups which are responsible for the transport of iron around the body and can form complexes with Ga(III) to deliver it to cells.\(^{19, 20}\) Ga(III) is then incorporated into essential proteins and enzymes in place of iron, but is unable to be utilised in the same manner due to its inability to be reduced to Ga(II), resulting in the inhibition of a number of vital cell survival functions.\(^{18}\)

Ga(III) retro hydroxamates have been identified previously. Gallium complexes of tris-hydroxamic acids, Scabichelin and Turgichelin (Figure 5.4), have been identified and the structural conformation predicted by computational modelling. The coordination mode of the Ga(III) retro hydroxamic acid complex was predicted as a bidentate (O, O’) chelation mode via the deprotonated hydroxyl group and the oxygen of the formyl carbonyl, similar to that of traditional hydroxamic acids.\(^{13}\)
Gallium formed complexes with Scabichelin and Turgichelin and analysis predicts a bidentate (O, O’) mode of chelation, displayed at one of the hydroxamic acid centres.

A proton dissociation study of GSK322 and a complex formation equilibrium study of GSK322 with Ga(III) were undertaken. An investigation of the reactivity of HFA and GSK322 with Bi(III) and Ga(III) will be discussed.

### 2. Project rational

GSK322 had shown excellent potential as an anti-bacterial agent, displaying a broad range of activity, even against drug resistant bacteria. However, the clinical trials were halted after toxic metabolites were predicted to be formed.

It was postulated that bismuth and gallium could potentially revive the interest in GSK322, by stabilising the hydroxamic acid group to limit the generation of unfavourable metabolites and side effects. Furthermore, given the inherent anti-bacterial activity of Bi and Ga, it was postulated that bismuth GSK322 complexes and/or gallium GSK322 complexes could target existing anti-bacterial resistance while preventing the initial development of resistance through a dual mechanism of action (i) inherent metal-induced anti-bacterial action and (ii) PDF inhibition.
3. Chapter Aims

- To investigate the reactivity of Bi(III) and Ga(III) with the retro hydroxamic acid, \(N\)-hydroxy-\(N\)-phenylformamide, HFA, (also known as \(N\)-formyl-\(N\)-phenyl hydroxylamine).

- To investigate the proton dissociation processes of GSK322 and the stability constants of Ga(III)-GSK322 complexes.

- To design, synthesise and characterise novel Bi(III) and Ga(III) GSK322 complexes.

- To determine the anti-bacterial activity of novel Bi(III) and Ga(III) complexes against strains of susceptible and resistant \(E.\ coli\) and \(S.\ aureus\).
4. Proton Dissociation of GSK322

The proton dissociation study on GSK322 was carried out by Dr. Éva A. Enyedy at the University of Szeged, Hungary. The results are outlined below.

Proton dissociation of GSK322 was followed by the combined use of pH-potentiometric, UV–vis spectrophotometric and $^1$H NMR titrations. A 30% (w/w) dimethyl sulfoxide DMSO/H$_2$O solvent mixture was used for the measurements due to poor GSK322 solubility in H$_2$O. pH-potentiometric titrations showed that the ligand is stable under an argon atmosphere in the pH range investigated, 2.0–12.5. Four functional groups, pyrimidinium NH$^+$, oxazine NH$^+$, hydroxamic acid (CONHOH) and hydrazinic NH, possess dissociable protons, Scheme 5.1.

![Scheme 5.1](image)

Scheme 5.1. Functional groups involved in the deprotonation steps of GSK322

Proton dissociation constants (pKa) for all these deprotonation steps were determined using the three methods outlined, and are in good agreement, Table 5.1. The low and high pK$_1$ and pK$_4$ values, respectively, hamper accuracy levels as the deprotonation steps take place in the ranges where the pH measurements become uncertain. Significantly the deprotonation processes do not overlap, facilitating the designation of each pKa; pK$_1$ (c 1.8) is associated with the pyrimidinium nitrogen, pK$_2$ (5.0) the oxazine NH$^+$ and pK$_3$ (c 8.9) and pK$_4$ (c 12.2) are attributed to the proton dissociation of the hydroxamic acid and the hydrazide groups, respectively, Scheme 5.1.
The pKₐ of the structurally similar acetohydroxamic acid (Aha) was also determined under the same conditions as a comparison. The value of 9.89±0.01 is markedly higher in the presence of DMSO than in pure water (pKₐ = 9.26 in H₂O), due to the deprotonation of a neutral functional group as expected with reference to the Born electrostatic solvent model.¹¹,²² The pK₃ of the hydroxamic acid group of GSK322 is one order of magnitude lower than pKₐ of the Aha, though possesses a similar lower pKₐ to that of formohydroxamic acid (pKₐ = 8.65 in H₂O) due to the presence of the electron donating methyl group in Aha.²³

The UV−vis spectra were not expected to significantly change as a result of deprotonation of the oxazine NH⁺ (pK₂: GSK+H → GSK) and the hydroxamic acid group (pK₃: GSK → GSK⁻H) moieties, since they are located relatively far from the chromophoric units. However significant characteristic spectral changes at pH < ~4 and pH > ~10 are observed, resulting from the deprotonation of the pyrimidinium NH⁺ (pK₄: GSK+2H → GSK+H) and the hydrazide NH (pK₄: GSK⁻H → GSK⁻2H) due to their proximity to the chromophoric pyrimidinium moiety, Figure 5.5.

![Image](image.png)

**Figure 5.5.** Individual calculated molar absorbance spectra of ligand species at various protonation states. Absorbance spectra of GSK+H, GSK and GSK⁻H species show strong similarities.

The localization of the negative charge on the deprotonated hydrazide moiety cannot be established due to the keto/enol tautomeric equilibrium, Scheme 5.2.
Scheme 5.2. The localisation of the negative charge on the hydrazine moiety is unknown; proposed to switch between the keto/enol tautomeric forms.

$^1$H NMR spectra recorded for GSK322 show that certain proton resonances are sensitive to stepwise proton dissociation steps, Figure 5.6. The chemical shift ($\delta$) of CH=O proton of the retro hydroxamic acid shifts considerable only for the third deprotonation step, pK$_3$, and remained unchanged during the other deprotonation steps.

![Figure 5.6](image)

Figure 5.6. $^1$H NMR spectra recorded for GSK322 at indicated pH values. The shifts of the CH=O and the CH$_3$ signals in response to pH is shown.
Furthermore the signal associated with the methyl group at the pyrimidine ring experiences a notable signal shift for the first deprotonation, Figure 5.6. Therefore pK₁, pK₂ and pK₃ values could be accurately determined based on the shift of the position of the CH=O and CH₃ protons, and the dissociation constants correspond well with those generated using pH-potentiometry and UV–vis spectrophotometry, Table 5.1. Representative concentration distribution curves calculated with the pKa values determined by pH-potentiometry are shown in Figure 5.7. The results of the UV–vis and ¹H NMR titrations confirm the sequence for the deprotonation steps in Scheme 5.1.

![Concentration distribution curves](image)

**Table 5.1.** Proton dissociation constants of GSK322 and Aha determined by pH-potentiometric, UV-vis spectrophotometric and ¹H NMR titrations.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Method</th>
<th>pK₁</th>
<th>pK₂</th>
<th>pK₃</th>
<th>pK₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSK322</td>
<td>pH-metry</td>
<td>1.78 ±0.09</td>
<td>5.02 ±0.03</td>
<td>8.89 ±0.02</td>
<td>12.2 ±0.1</td>
</tr>
<tr>
<td>GSK322</td>
<td>UV–vis</td>
<td>1.89 ±0.05</td>
<td>4.97 ±0.02</td>
<td>8.75 ±0.01</td>
<td>12.2 ±0.1</td>
</tr>
<tr>
<td>GSK322</td>
<td>¹H NMR</td>
<td>1.8 ±0.1</td>
<td>5.07 ±0.04</td>
<td>8.96 ±0.01</td>
<td>&gt;12</td>
</tr>
<tr>
<td>Aha</td>
<td>pH-metry</td>
<td>9.89 ±0.01⁴</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

⁴ pK₁ = 9.26 in neat water⁴
5. Complex Formation Studies

Complex formation equilibrium studies of GSK322 with Bi(III) were not undertaken due to the insolubility and instability of Bi precursor complexes such as Bi(NO$_3$)$_3$, BiCl$_3$ and Bi$_2$O$_3$, in aqueous solution or mixtures. For example, BiCl$_3$ readily hydrolyses in water to give the insoluble salt BiOCl and H$^+$. Complex formation equilibrium studies of GSK322 with Ga(III) were studied by pH-potentiometry and $^1$H NMR spectroscopy in 30% (w/w) DMSO/H$_2$O. Formation of mono, bis and tris complexes containing the ligand in mono or doubly deprotonated forms of GSK322 were found. The hard Lewis acid Ga(III) centre is most likely coordinated by the bidentate hydroxamato (O, O’) donor mode. Stability constants for Ga complexes formed with Aha were also determined under identical conditions for comparison.

In the mono-protonated GSK322 complexes, ([Ga(GSK$_{1H}$)]$^{2+}$, [Ga(GSK$_{1H}$)$_2$]$^+$, [Ga(GSK$_{1H}$)$_3$]), the non-coordinating hydrazinic nitrogen is most probably still protonated, whereas in the minor species, [Ga(GSK)]$^{3+}$, the oxazine nitrogen is most likely also protonated. Concentration distribution curves were calculated using the determined stability data for the Ga(III) complexes, Figure 5.8. Complex formation has already started at pH 2, although the complexes decompose at pH > 9. Comparing the fractions of the bound ligand, it can be concluded that GSK322 is a slightly stronger chelating agent for Ga(III) than Aha. In the presence of the 30% DMSO, the maximum of the bound ligand fraction is 69% (at pH 6.5) and 56% (at pH 5.8) for GSK322 and Aha, respectively at ~1 mM concentration of the ligand and at 1:3 metal-to-ligand ratio.

![Figure 5.8. Concentration distribution curves calculated for the Ga(III)–GSK322 complexes.](image-url)
The stability constants of these complexes were determined by pH-potentiometry studies and the results are shown below in Table 5.2.

### Table 5.2. Stability constants of Ga(III)-GSK322 and Ga(III)-Aha complexes.

<table>
<thead>
<tr>
<th></th>
<th>GSK322</th>
<th></th>
<th>Aha</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ga(GSK)]^{3+}</td>
<td>26.5 ±0.1</td>
<td>[Ga(Aha_{1H})]^{2+}</td>
<td>11.18 ±0.07</td>
</tr>
<tr>
<td>[Ga(GSK_{1H})]^{2+}</td>
<td>23.50 ±0.07</td>
<td>[Ga(Aha_{1H})]^{2+}</td>
<td>20.63 ±0.06</td>
</tr>
<tr>
<td>[Ga(GSK_{1H})]^{+}</td>
<td>44.80 ±0.06</td>
<td>[Ga(Aha_{1H})]^{3+}</td>
<td>29.51 ±0.07</td>
</tr>
<tr>
<td>[Ga(GSK_{1H})]_{3}</td>
<td>64.32 ±0.07</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In order to confirm the speciation model obtained for the Ga(III)–GSK322 system, $^1$H NMR spectra were recorded in the pH range from 2 to 12.5. Signals associated with the various Ga(III) complexes could not be distinguished. $^1$H NMR spectra indicated complex formation at pH between 2 and 9. Chemical shifts of the peaks assigned to the CH=O and CH$_3$ protons of the ligand bound to Ga(III) are strongly downfield and upfield shifted, respectively compared to free ligand, Figure 5.9. The chemical shifts of these protons show a pH-dependence up to pH ~5.3, and become constant due to the formation of the tris complex becoming the single species containing both Ga(III) and the ligand. However, the complete decomposition can be observed at pH > 9, in accordance with the findings of the pH-potentiometric titrations.

![Figure 5.9. $^1$H NMR spectra recorded for Ga(III)–GSK322 (1:3) system (top) and for the ligand alone (bottom) at pH 5.50 showing shifts in the CH=O and CH$_3$ protons.](image-url)


Chapter 5

6. Synthesis

Synthesis of Hydroxamic Acid

GSK322 was received from GlaxoSmithKline and used without further purification.

Synthesis of \(N\)-hydroxy-\(N\)-phenylformamide (HFA) \(\text{10}\)

As previously reported, synthesis of \(\text{HFA, 10}\), proceeds via the reaction of nitrosobenzene with glyoxylic acid monohydrate in glacial acetic acid at room temperature for 1 hour. The reaction scheme is outlined in Scheme 5.3.

\[
\begin{align*}
\text{\(\text{NO}\)} & \quad \text{\(\text{HCOOH}\)} \\
\text{Acetic Acid} \quad \text{rt, 1 h} & \quad \text{\(\text{HO} \text{-\text{C} = \text{N} - \text{Ph}}\)}
\end{align*}
\]

Scheme 5.3. Synthesis of \(\text{\(N\)-hydroxy-\(N\)-phenylformamide, 10, from nitrosobenzene and glyoxylic acid monohydrate.}\)

The retro hydroxamic acid was obtained in excellent yield (90%) and high purity. It was characterised by IR, EA, \(^1\text{H}\) and \(^{13}\text{C}\) NMR spectroscopy.

Elemental analysis of \(\text{10}\) is consistent with the formula of \(\text{C}_7\text{H}_7\text{NO}_2\).

In general, for IR spectra analysis of hydroxamic acids, the \(\text{\(\nu\)}(\text{C=O})\) is a significant and diagnostic feature. One stretch is typically observed, encompassing the symmetric and asymmetric \(\text{\(\nu\)}(\text{C=O})\), and generally occur between 1615 cm\(^{-1}\) and 1660 cm\(^{-1}\). The \(\text{\(\nu\)}(\text{C=O})\) of \(\text{10}\) is found at 1655 cm\(^{-1}\) and the \(\text{\(\nu\)}(\text{C-N})\) appears at 1559 cm\(^{-1}\).

\(^1\text{H}\) NMR analysis was performed in DMSO-\(d_6\). The \(^1\text{H}\) NMR shows signals in the region of 7.70-7.19 ppm which corresponded and integrated for the aromatic protons on \(\text{10}\). The signals of the formyl and hydroxyl group are split in a ratio of 2:3. This indicates that \(\text{10}\) is present in solution as rotamers, most likely with rotation around the N-CO bond as shown in Figure 5.10. The signals tend to equilibrate over time to a 1:1 ratio of rotamers.\(^{26}\)
Figure 5.10. Possible rotamer formation of 10.

Synthesis of BiPh$_3$ (11)

As previously reported,\textsuperscript{27} 11 is synthesised on reduction of the Bi(V) complex triphenylbismuth dichloride. This can be achieved by a variety of reducing agents such sodium hydrosulfite, liquid ammonia, LiAlH$_4$, NaBH$_4$, sodium sulphite or, as was used in this reaction, hydrazine hydrate. The electrolytic reduction is found to be a one-step, two electron process, with the chlorine atoms being released as chloride ions.\textsuperscript{28} Scheme 5.4.

Scheme 5.4. Reduction of Bi(V) to Bi(III), triphenylbismuth, 11.

Triphenylbismuth was synthesised in excellent yields (95\%). 11 was characterised by EA, $^1$H, $^{13}$C NMR spectroscopy and melting point analysis.

The EA results indicate the formation of the product, with three phenyl ligands identified per Bi(III) centre, and no chlorine present in the samples.

$^1$H NMR analysis was performed in DMSO-$d_6$. Three signals are observed in the aromatic region of this spectra. The doublet at 7.73 ppm accounts for the protons at the 2 and 6 positions on the phenyl ring, while the triplets at 7.39 ppm and 7.31 ppm corresponded and integrated well for the remaining three protons.

Given the basic structure of 11, melting point is an important analysis technique. The observed melting point was in the range of 75-77 °C which is in accordance with the predicted melting point for 11 at 75 °C.\textsuperscript{27}
Synthesis of Metal Hydroxamato Complexes

Reaction of hydroxamic acid (HFA) with Bi(III) and Ga(III) gave the novel complexes \([\text{Bi(HFA-\text{H})}_3]\) and \([\text{Ga(HFA-\text{H})}_3]\) respectively, representing a novel class of Bi(III) and Ga(III) retro hydroxamato complexes.

**Synthesis of \([\text{Bi(HFA-\text{H})}_3]\)  (12)**

HFA was reacted with BiPh\(_3\) under solvent free conditions at 90 °C. Stirring for one hour, followed by washing the product with toluene to remove any residual benzene, afforded the novel Bi(III) complex \([\text{Bi(HFA-\text{H})}_3]\), \textbf{12}. This product was obtained in 75% yield and high purity, Scheme 5.5.

\begin{equation}
\text{[Bi(HFA-\text{H})}_3] \rightarrow \text{[Bi(HFA-\text{H})}_3]_\text{12}
\end{equation}

\textbf{Scheme 5.5.} Synthesis of \([\text{Bi(HFA-\text{H})}_3]\) \textbf{12}, a novel Bi(III) retro hydroxamato complex.

\textbf{[Bi(HFA-\text{H})}_3] \textbf{12} was characterised by EA, IR, \(^1\text{H}\) and \(^{13}\text{C}\) NMR and MS.

Elemental analysis of \textbf{12} is consistent with the presence of three hydroxamic acid ligands per Bi(III) centre. This is in accordance work carried out by Andrews \textit{et al.} where they also observed complete substitution of the phenyl ligands when reacted with hydroxamic acids, for example in the formation of \([\text{Bi(Bha-\text{H})}_3]\) from reaction of Bha and BiPh\(_3\) under solvent free conditions.\textsuperscript{29}

\(^1\text{H}\) NMR spectroscopic analysis was carried out in DMSO-\textit{d}_6. The loss of the signal associated with the hydroxyl group is indicative of binding of the ligand to the metal centre. The signal associated with the formyl hydrogen is both shifted downfield and is resolved to a single signal, in contrast to the two rotamer signals which were present in the \(^1\text{H}\) NMR spectrum of \textbf{10}. In the free ligand the \(CH=O\) signals appear at 8.74/8.63 ppm, whereas, in \textbf{12} the formyl signal appears at 9.48 ppm, as a singlet. In \textbf{12}, three main aromatic signals are observed at 7.51-7.19 ppm, integrating for the
five aromatic protons on each ligand. The most significant shift is that of an aromatic doublet, associated with protons closest to the hydroxamic acid group at the 2 and 6 positions on the phenyl ring, from 7.7 ppm in the free ligand to 7.51 ppm in complex 12. The remaining two signals shift slightly in the complex compared to the free ligand as can be seen in Figure 5.11.

![Figure 5.11. The stacked $^1$H NMR spectra of 10 (top) and complex 12 (bottom), and the expansion of the area of interest (insert), clearly showing the shifts in the aromatic signals associated with coordination of Bi(III).](image)

Seven signals were observed in the $^{13}$C NMR spectrum of 12, six of which represent the aromatic carbons at 129.1, 128.4, 125.9, 119.2, 118.5 and 112.9. The carbonyl signal occurs at 152.1 ppm.

The IR spectra of 12 exhibits distinctive $\nu$(C=O) at 1607 cm$^{-1}$ displaying the characteristic shift associated with (O, O') hydroxamato metal coordination, when compared to the corresponding $\nu$(C=O) of the uncoordinated ligand at 1655 cm$^{-1}$. The shift to lower wavenumber of this stretch suggests bonding to the bismuth atom via the formation of the thermodynamically preferred five membered bidentate chelate, via the hydroxamato carbonyl group and deprotonated hydroxyl group.
This mode of binding has been observed for bismuth hydroxamato complexes previously in Chapter 3 and Chapter 4. The stretch at 1564 cm\(^{-1}\) is assigned to the \(\nu(C-N)\) of the hydroxamic acid.

ESI-MS in the positive mode assists in identification of [\(\text{Bi(HFA-1H})_3\)], 12. The full molecular ion ([M+Na]\(^+\)) was observed at 640.6 a.m.u, along with molecular fragment corresponding to [\(\text{Bi(HFA-1H})_2\)] at 481.4 a.m.u, further supporting the proposed structure of [\(\text{Bi(HFA-1H})_3\)] for 12.

Numerous efforts to isolate crystals suitable for X-ray diffraction were unsuccessful.

**Synthesis of [\(\text{Ga(HFA-1H})_3\)].H\(_2\)O  (13)**

Reaction of 10 with \(\text{Ga(NO}_3\)\(_3\)), in \(\text{H}_2\)\(_2\)O on adjusting the pH to 6.2, afforded the novel Ga(III) complex [\(\text{Ga(HFA-1H})_3\)]\(\cdot\)\(\text{H}_2\)\(_2\)O, 13. This product was obtained, in 49% yield and high purity. The complex formation equilibrium studies of GSK322 with Ga(III) and specifically the concentration distribution curves calculated for the Ga(III)–GSK322 indicate that at pH 6-7, the formation of a 1:3 complex of Ga(III):retro hydroxamate complexes is favoured. The reaction scheme and product formed are shown below, **Scheme 5.6**.

![Scheme 5.6. Synthesis of [\(\text{Ga(HFA-1H})_3\)]\(\cdot\)\(\text{H}_2\)\(_2\)O 13, a novel Ga(III) retro hydroxamato complex.](image)

13 was characterised by EA, IR, MS and \(^1\)H and \(^{13}\)C NMR spectroscopy. Recrystallization of 12 from various solvents did not yield single crystals suitable for X-ray crystallography analysis.
Elemental analysis of 13 is consistent with the presence of three hydroxamic acid ligands and one H$_2$O molecule per Ga(III) centre. EA results for 10, 12 and 13 are displayed in Table 5.3 for purposes of comparison.

Table 5.3. Summary of the EA results for 10, 12 and 13.

<table>
<thead>
<tr>
<th>Compound</th>
<th>% C</th>
<th>% H</th>
<th>% N</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculated</td>
<td>61.31</td>
<td>5.15</td>
<td>10.21</td>
</tr>
<tr>
<td>Found</td>
<td>61.09</td>
<td>5.05</td>
<td>10.21</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculated</td>
<td>40.86</td>
<td>2.94</td>
<td>6.81</td>
</tr>
<tr>
<td>Found</td>
<td>40.48</td>
<td>2.80</td>
<td>6.60</td>
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<tr>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculated</td>
<td>50.84</td>
<td>4.06</td>
<td>8.47</td>
</tr>
<tr>
<td>Found</td>
<td>50.75</td>
<td>3.14</td>
<td>8.11</td>
</tr>
</tbody>
</table>

The $^1$H NMR spectra of 13 was carried out in DMSO-$d_6$. The signal associated with the formyl hydrogen is both shifted downfield and is resolved to a single signal, at 9.15 ppm, in contrast to the two rotamer signals which were present in the $^1$H NMR spectrum of 10. This result is similar to what was observed in the analysis of 12. The aromatic signals also experience shifting from 7.70-719 ppm in 10 to 7.66-7.32 ppm in 13, as shown in Figure 5.12. The signals correspond and integrate well for the five aromatic protons in 13. The signals are far better resolved in both metal complexes, 12 and 13, compared to the free ligand. This is most likely due to the metal stabilising and restricting the rotation of the ligand and hence resolution is increased. The doublet and triplet splitting patterns can be clearly observed in the metal complex.
Figure 5.12. The stacked $^1$H NMR spectra of HFA (top) and complex 13, (bottom) showing the shifts in the aromatic region of signals associated with the binding of Gallium (insert).

Five signals were observed in the $^{13}$C NMR spectrum of 7. Four signals at 138.8, 129.3, 127.2 and 118.1 ppm are associated with the aromatic carbons and the signal at 153.6 ppm is associated with the carbonyl group.

The IR spectra of 13 exhibits distinctive $\nu$(C=O) at 1604 cm$^{-1}$ displaying the characteristic shift associated with a bi-dentate (O, O') hydroxamato coordination. This stretch is shifted when compared to the corresponding $\nu$(C=O) of the uncoordinated ligand at 1655 cm$^{-1}$, indicating the formation of a stable five membered ring upon chelation. The $\nu$(C-N) is found at 1566 cm$^{-1}$. For comparison all the IR frequency stretches of the ligand and complexes are shown in Table 5.4.

Table 5.4. **Overview of the stretches observed in IR spectroscopy.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\nu$ C=O</th>
<th>$\nu$ C-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1655 cm$^{-1}$</td>
<td>1590 cm$^{-1}$</td>
</tr>
<tr>
<td>12</td>
<td>1607 cm$^{-1}$</td>
<td>1564 cm$^{-1}$</td>
</tr>
<tr>
<td>13</td>
<td>1604 cm$^{-1}$</td>
<td>1566 cm$^{-1}$</td>
</tr>
</tbody>
</table>
**Attempted Synthesis of \([\text{Bi}(\text{GSK}-1\text{H})_3] \) (14)**

Given the successful reaction of HFA with BiPh₃, reaction of GSK322 with BiPh₃, to yield \([\text{Bi}(\text{GSK}-1\text{H})_3] \) (14), was attempted using both solvent free and solvated reaction conditions, as Scheme 5.7.

![Scheme 5.7. Attempted synthesis of \([\text{Bi}(\text{GSK}-1\text{H})_3] \) 14, by solvent free and solvated conditions.](image)

This reaction was carried out in accordance with the work of Andrews et al. A solvent free reaction of GSK with BiPh₃ in a 3:1 ratio did not prove successful in the generation of the required product. In the solvated reaction procedure, method (ii), the reaction mixtures were refluxed in EtOH and the resulting clear solution was allowed stir for 8 hours. No product was isolated upon work up of this reaction however. Therefore, the successful synthesis of \([\text{Bi}(\text{GSK}-1\text{H})_3] \) by the reaction conditions outlined above was not achieved. There was some evidence of product formation as evidence by a fragment in the MS of the reaction carried out in EtOH, with a signal at 714.4 a.m.u representing \([\text{Bi}(\text{GSK}-1\text{H})\text{Na}]^t\). However the fragment intensity was very low and the over-riding signal was that of the ligand itself. A possible reason for the non-formation of the product in comparison to HFA, may be as a result of stabilisation ability of HFA in comparison to GSK322. The HFA anion is likely stabilised through the electron withdrawing inductive effect of the phenyl group.

**Attempted Synthesis of \([\text{Ga}(\text{GSK}-1\text{H})_3] \) (15)**

Reaction of GSK322 with Ga(NO₃)₃, in 30% (w/w) DMSO/H₂O at 25 °C was carried out in an attempt to generate the novel Ga(III) complex \([\text{Ga}(\text{GSK}-1\text{H})_3] \), 15. These reaction conditions were used to directly mimic the potentiometric study conditions, in which \([\text{Ga}(\text{GSK}-1\text{H})_3] \) was observed to be the main constituent of the reaction when the pH was maintained between pH 6-7. Upon addition of the reagents, the pH was adjusted to pH 6.2, and the reaction stirred for a further 24 hours, Scheme 5.8.
Scheme 5.8. Attempted synthesis of $[\text{Ga(GSK-111)}_3]$ 15.

No solid was recovered from this reaction even after numerous attempts. The use of DMSO in this reaction and the relative solubility’s of the material in DMSO, can make the isolation of complexes very difficult. In the complex formation equilibrium studies of GSK322 with Ga(III), the required product is observed, however in solution only. The identification of a better solvent system which allows for easier isolation of complexes would help in the generation of desired product, but options are limited due to the poor solubility of GSK322.
7. Biological Testing

Given the successful synthesis and analysis of HFA complexes 12 and 13, their antibacterial activity was tested against two susceptible and two resistant strains of bacteria. This work was carried at the Clinical Microbiology Laboratory in RCSI Education and Research Centre, Smurfit Building, Beaumont Hospital, Dublin 9 under the supervision of Dr. Deirdre Fitzgerald-Hughes.

Anti-bacterial Activity Determination

The anti-bacterial activity of 10, 12, 13, GSK322 and ciprofloxacin, a commercially available fluoroquinoline (Figure 5.13), were investigated against two susceptible strains of bacteria; *E. coli* 25922 and *S. aureus* 25923, along with two resistant strains of bacteria; MRSA 43300 and ESBL *E. coli* CL2, a clinical isolate recovered from a patient with a urinary tract infection. This CL2 *E. coli* strain is resistant to penicillin and third generation cephalosporins. The WHO has recommended that the use of ciprofloxacin be closely monitored and prescribed only in essential cases due to the possibility of generation of resistance to this drug.34

![Figure 5.13. Structure of ciprofloxacin.](image)

Minimum inhibitory concentration (MIC), is the concentration which inhibits the growth of bacteria, was determined using the broth micro-dilution assay in accordance with the CLSI guidelines.35

Initially all complexes were dissolved in DMSO and then made up to have a final DMSO concentration of \( \leq 2\% \) which was observed to have no effect on bacterial growth, as per previous assessment.36 Stock solutions of test complexes were prepared fresh before each assay. The concentrations used in the MIC broth micro-dilution assay were in the range of two fold dilutions ranging from 1.56-200 \( \mu\text{M} \) for all complexes.
Bacteria, were exposed to a range of concentrations of each complex and were incubated over-night. The presence or absence of growth was determined by visual inspection of the wells after centrifuging at 3000 x g for 1 minute. The MIC is the lowest concentration at which no growth of bacteria was observed. MIC results are shown below in Table 5.5.

Table 5.5. MIC (µM) \((n = 3)\) for 10, 12, 13, GSK322 and Ciprofloxacin.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Strain</th>
<th>10</th>
<th>12</th>
<th>13</th>
<th>Ciprofloxacin</th>
<th>GSK322</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>25922</td>
<td>&gt; 200</td>
<td>100</td>
<td>&gt; 200</td>
<td>3.125</td>
<td>25</td>
</tr>
<tr>
<td>E. coli</td>
<td>ESBL Cl2</td>
<td>&gt; 200</td>
<td>100</td>
<td>&gt; 200</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>S. aureus</td>
<td>25923</td>
<td>&gt; 200</td>
<td>100</td>
<td>&gt; 200</td>
<td>3.125</td>
<td>6.25</td>
</tr>
<tr>
<td>S. aureus</td>
<td>43300</td>
<td>&gt; 200</td>
<td>50</td>
<td>&gt; 200</td>
<td>25</td>
<td>6.25</td>
</tr>
</tbody>
</table>

The results of the MIC test highlight that 10, the free ligand for complex 12 and 13, does not show any activity against any of the strains at the highest concentration ranges investigated. This ligand was chosen only as a mimic for the binding site of the GSK322 compound, and was not expected to have any anti-bacterial activity as is observed. It was used to investigate the ability of Bi(III) and Ga(III) to bind retro hydroxamic acids.

The bismuth complex, 12, shows activity against all four strains tested. 12 has an MIC of 100 µM against the susceptible and resistant strains of E. coli. The E. coli resistant strain comprises of β-lactamase enzymes responsible for the degradation of β-lactam based antibiotics. Our complexes are free of β-lactamase moieties, and for that reason do not show any difference in activity between the two strains.

The MIC of 12 against S. aureus 25923 is 100 µM whereas against the MRSA strain it has an MIC of 50 µM. This heightened activity of bismuth against MRSA over other bacterial strains has also been identified in bismuth doped implants in dental procedures, where inhibition growth zones of bismuth nitrate against MRSA (46 mm diameter) was far superior to the inhibition against Actinobacillus actinomycetemcomitans and Staphylococcus mutans (26 mm diameter). Further metalloproteomic investigations are required to investigate if the molecular targets are different between the strains.
It is noteworthy that 12 is observed to have activity against both Gram-negative and Gram-positive bacteria. These initial indications would further suggest that bismuth can be utilised as a broad spectrum anti-bacterial agent in combination with antibiotics.

Complex 13, the gallium derivative of 12 does not show any activity against any of the strains at the concentration range tested. The primary structural difference between 12 and 13 is the identity of the metal, which therefore must contribute to the differences in anti-bacterial activity determined.

The anti-bacterial activity of gallium requires up-take and incorporation into cell metabolic processes where it exerts its anti-bacterial activity. Bismuth may be much more readily available than gallium, as some studies have previously demonstrated that Bi(III) hydroxamate complexes hydrolyse in aqueous solution, liberating the ligand and generating bismuth-oxido clusters, thought to be the anti-bacterial moiety. Gallium may not hydrolyse as easily, and hence might not be as readily available for metabolic incorporation.

The PDF inhibitor, GSK322, shows good activity against all strains of bacteria tested, with an MIC values of 6.25 µM observed against both S. aureus strains. These figures are in accordance with previous anti-bacterial assays of GSK322 on S. aureus and MRSA strains. Given that GSK322 is designed as a post translational inhibitor, there is no difference in its inhibitory effects in the growth of both S. aureus strains. This complex targets a different biological pathway than traditional anti-bacterial complexes. The low MIC values of GSK against Gram-positive bacteria can be attributed to the fact it may easily cross the cell membrane, as was observed with a structurally similar peptide deformylase inhibitor, Actinonin. Figure 5.14. Actinonin was observed to be easily transported into the cell cytoplasm due to its hydrophilic character.

![Actinonin](image_url)

Figure 5.14. Actinonin, a structurally similar PDF inhibitor of GSK322.
The anti-bacterial of activity of GSK322 against *E. coli* has not been reported previously in the literature. GSK322 demonstrates activity against both *E. coli* strains but has a higher activity against the non-resistant strain, with an MIC of 25 µM compared to an MIC of 100 µM against the ESBL strain.

Given these results, our hypothesis to bind GSK322 to either bismuth or gallium could prove very fruitful in the establishment of a new treatment for a broad range of bacteria. From the $^1$H NMR complex formation studies with Gallium, it is known that Ga(III)-GSK322 complexes can be formed with relatively high stabilities in solution but isolation of suitable product from these reactions was not achieved. Bi(III)-GSK322 complexes could also potentially form interesting anti-bacterial agents. Ritcher *et al.* demonstrated that gallium complexes with Deferiprone significantly decreased bacterial load and increased survival of *S. aureus* infected *C. elegans*. Therefore it can be predicted that complexion of GSK322 with bismuth or gallium may have a similar positive anti-bacterial effect.

Ciprofloxacin, which inhibits DNA gyrase, preventing its normal function of releasing tightly packed DNA in preparation for replication, has an MIC value of 3.125 µM against the susceptible *E. coli* and *S. aureus* strain. Ciprofloxacin exhibits a slightly higher MIC of 25 µM against ESBL *E. coli* and MRSA. As ciprofloxacin is a broad spectrum antibiotic it is able to cross the cell membrane of both Gram-positive and Gram-negative bacteria.
8. Conclusion

In conclusion, this chapter reports the findings of the proton dissociation study of a novel peptide deformylase inhibitor, GSK322. pH-potentiometric, UV–vis spectrophotometric and $^1$H NMR titrations were carried out by Dr. Éva A. Enyedy and collaborators in University of Szeged, Hungary, to calculate and verify the pKa of four functional groups in GSK322, namely the pyrimidinium NH$,^+$, oxazine NH$,^+$, hydroxamic acid (CONHOH) and hydrazinic NH groups. The pKa’s were calculated independently using each method and the results are in close agreement.

The complex formation study was carried out between GaCl$_3$ and GSK322. In this study pH-potentiometry and $^1$H NMR spectroscopy were used to analyse the different complexes formed when Ga(III) is reacted with GSK322 at a pH range 2-12. Formation of mono, bis and tris complexes, ([Ga(GSK$_{1H}$)$_2$]$^2^+$, [Ga(GSK$_{1H}$)$_2$]$^+$, [Ga(GSK$_{1H}$)$_3$] and [Ga(GSK)]$^3^+$) containing the ligand in mono-deprotonated form was found with [Ga(GSK$_{1H}$)$_3$] predominating at pH 6-7. The complexes completely decompose at pH > 9.

Reaction of bismuth and gallium with retro hydroxamic acid, HFA (10) produced the novel Bi(III) and Ga(III) hydroxamato complexes [Bi(HFA$_{1H}$)$_3$] and [Ga(HFA$_{1H}$)$_3$].H$_2$O. No structural evidence was attained in the form of a crystal structure but it is predicted that these complexes follow a similar binding pattern to conventional hydroxamic acids, chelating in a (O, O$'$) bidentate fashion through the carbonyl oxygen and the deprotonated hydroxyl group.

Attempts to synthesise [Bi(GSK$_{1H}$)$_3$] and [Ga(GSK$_{1H}$)$_3$] were not successful.

In vitro investigation of its MIC of 10, 12, 13, GSK322 and ciprofloxacin against four bacterial strains was carried out. Two Gram-negative E. coli strains, incorporating an ESBL resistant clinical strain and two Gram-positive strains, one of which was an MRSA strain were used for testing. The results revealed that 10 and 13 were not active against the strains. The bismuth complex 12 showed variable levels of activity against the four strains with the best activity shown against the MRSA strain.
GSK322 showed good activity against all four strains of bacteria tested. It displayed the best activity against the Gram-positive bacteria, due in part to its ability to cross the cell membrane. However, none of the complexes which were tested are as active as the anti-bacterial agent ciprofloxacin, which showed superior activity against all strains in comparison to the other agents tested, suggesting that these complexes have a potential to be administered in combination with traditional medicine as a means of preventing the occurrence or prevalence of anti-bacterial resistance. As no resistance to bismuth based therapies for *H. pylori* treatment have arisen to date, this bodes well for the use of bismuth as a treatment to prevent resistance in other bacterial diseases.

9. Future Plans

Further investigation into formation and isolation of GSK322 complexes with bismuth or gallium could result in the generation of potentially useful anti-bacterial agents for the treatment of resistant bacterial infections. The activity of Bi(HFA·1H)₃ in combination with GSK using the checkerboard method should be investigated. Also the ability of novel Bi and Ga hydroxamic acid complexes to inhibit biofilm formation should be investigated.

Furthermore, alternative metal GSK322 complexes could stabilise the hydroxamic acid functional group and reduce associated side effects, which has prevented its progression in clinical trials to date.
References


36. T Wadhani KD, D Patel, D Lawani, P Bahaley, P Joshi, V Kothari. Effect of various solvents on bacterial growth in context of determining MIC of various antimicrobials. The Internet Journal of Microbiology. 2008;Volume 7 (Number 1.).


Chapter 6

Novel Class of Sb(III) Hydroxamato and Sb(III) Hydroxamato/Hydroximato Compounds;
An Investigation of their Anti-Leishmanial Activity.
1. Introduction

Neglected tropical diseases (NTDs) affect over 1 billion people world-wide and represent a major financial and health challenge for developing countries. Surprisingly, there is a distinct lack of safe, effective and affordable treatments for these diseases, principally due to under-investment by global pharmaceutical companies in developing new drugs.¹

Leishmaniasis is an NTD caused by trypanosomatid protozoans of the genus *Leishmania*, which are transmitted by the bite of infected female phlebotomine sandfly. There are three main forms of leishmaniasis; visceral (kala-azar), cutaneous and mucocutaneous, where visceral is the lethal form if not treated abruptly.²-⁴ Leishmaniasis prevails in subtropical and tropical regions and is endemic in 98 countries, putting more than 350 million people at risk of infection. Approximately 1.3 million new cases of leishmaniasis and 30,000 leishmaniasis associated deaths are recorded each year.²-⁴ Significantly the number of incidence of leishmaniasis is increasing due to failing preventative and therapeutic measures, human migration, global warming and resistance, as highlighted in the introductory Chapter 1.³

Antimonial based drugs have been used to treat leishmaniasis since 1910 with trivalent antimony potassium tartrate, APT. Currently the pentavalent antimonial compounds, sodium stibogluconate (Pentostam) and meglumine antimoniate (Glucantime), are used in many subtropical countries as first choice anti-leishmanial drugs due to their favourable therapeutic indices and relatively low toxicity, Figure 6.1.⁵

![Figure 6.1. Examples of antimonial based drugs used to treat leishmaniasis; (A) Sodium Stibogluconate, (B) Antimony Potassium Tartrate, APT and (C) Meglumine Antimoniate.](image-url)
Though in use for over 100 years, the exact mode of action of antimony-based antileishmanial compounds, has not been fully elucidated. Nonetheless evidence suggests that Sb(V) is reduced to Sb(III) \textit{in vivo} and Sb(III) targets the trypanothione/trypanothione reductase system, which is found only in parasitic protozoa such as \textit{Leishmania} and \textit{Trypanosomes}. The trypanothione/trypanothione reductase system, similar to the glutathione/glutathione reductase system protects against oxidative stress and is essential to the survival of \textit{Leishmania}.\textsuperscript{5,6}

Current drug treatments in addition to the antimonials include repurposed drugs such as paromomycin (antibiotic), miltefosine (anticancer) and amphotericin B (antifungal), Figure 6.2. However these are not fit for purpose due to toxicity, lack of efficacy, uncomfortable parenteral administration and growing resistance.\textsuperscript{3} Significantly, \textit{Leishmania} are recognised to possess remarkable genomic plasticity and in turn rapidly evolve towards drug-resistant phenotypes.\textsuperscript{2} The Bihar state in India, for example, has seen the efficacy of antimonial therapies fall from over 85% to under 50%, due to the presence of drug resistant strains.\textsuperscript{7} It is therefore vital that new, safe, effective and affordable treatment options are developed against leishmaniasis to overcome the increasing problem of resistance.

Figure 6.2. Repurposed drug candidates now used in the treatment of leishmaniasis. (A) Paromomycin, (B) Miltefosin and (C) Amphotericin B.

Histone deacetylase (HDAC) enzymes regulate chromatin structure, transcription and gene expression by catalysing the removal of acetyl groups from histone lysine
residues, Figure 6.3. They also deacetylate non-histone protein targets which play important roles in tumour progression, cell cycle control, apoptosis, angiogenesis and metastasis. Currently 18 HDAC’s are recognised and categorised amongst 4 classes. Class I, II and IV have a zinc dependent active site and can be inhibited by zinc chelating ligands including hydroxamic acid derivatives.

Figure 6.3. HDAC enzymes catalyse the removal of acetyl groups from the histone lysine residue and results in more tightly packed chromatin.

The *Leishmania* genome contains four genes which code for class I/II HDAC homologues and three genes which code for Class III homologues. HDAC proteins are recognised as being crucial to the growth of the parasite.

Class III HDAC inhibitors have been investigated for their anti-Leishmanial activity. Sirtinol, for example, was reported to inhibit axenic amastigote multiplication by promoting apoptosis-like cell death but did not affect the *in vitro* growth of the promastigote form. In addition SAHA, an FDA approved anticancer drug and class I, II and IV inhibitor, though less active than amphotericin B for example, was demonstrated to exhibit appreciable activity against *L. donovani* promastigotes, Figure 6.4.

Figure 6.4 Examples of HDAC inhibitors; Sirtinol (left) and SAHA (right).
2. Project rational

*Leishmania amazonensis* and *Leishmania chagasi* are two of the main causative agents of leishmaniasis, a condition that affects millions of people worldwide, especially in developing countries. There is an urgent need for novel, efficient and cost-effective treatments for these diseases, given the growing resistance and side-effects of current therapies.

Given (i) combination therapies are routinely administered with a view to reducing toxicity and tackling resistance and (ii) repurposing strategies are becoming increasingly common, antimony could be potentially used in the repurposing of FDA approved histone deacetylase inhibitors (HDACi), such as SAHA which belongs to a class of anticancer agents.9

“Repurposing” the histone deacetylase inhibitor (HDACi) SAHA and developing Sb SAHA compounds will potentially provide a compound which exhibits dual activity; (i) Sb associated activity and (ii) HDAC inhibition, hence allowing the use of lower concentrations of antimony. The use of a lower concentration of antimony will in turn reduce antimony associated toxicity. It is hypothesised that the HDACi will potentially tackle resistance, through increasing efficacy and providing an additional anti-leishmanial mechanisms of action, i.e. HDAC inhibition.
3. Chapter Aims

- To design, synthesise and characterise a novel class of Sb(III) hydroxamato and/or hydroximato chlorido compounds.

- To elucidate the exact structure of novel Sb compounds by X-ray crystallography.

- To investigate the stability of the novel Sb compounds both as solids and in solution.

- To determine the anti-parasitic activity of novel Sb compounds against *Leishmania amazonensis* and *Leishmania chagasi* promastigotes.

- Assess the toxicity of novel Sb compounds on murine macrophages and determine the selectivity index of the Sb(III) compounds.
4. Synthesis

Synthesis of Hydroxamic Acids

Both Bha and Sha were commercially available and used without further purification. 2-NH$_2$-Pha (1) and 2-Phya (5) were synthesised, purified and analysed as previously discussed in Chapter 3 and Chapter 4 respectively.

Synthesis of Suberoylanilide Hydroxamic Acid (SAHA) (18)

As previously reported, synthesis of SAHA proceed via suberoyl anhydride, 16, which was formed by refluxing suberic acid in acetic anhydride. 16 was then reacted with aniline to produce suberanilic acid, 17, in 62% yield. This acid intermediate, 17, was in turn converted into SAHA, 18, by a one-step reaction with ethyl chloroformate/triethylamine and hydroxylamine. Activation of the carboxylic acid \textit{in situ} on reaction with ethyl chloroformate, gives the corresponding mixed anhydride, which is then converted to 18 upon reaction with hydroxylamine, Scheme 6.1.

Scheme 6.1. Synthesis of SAHA, 18, from suberic acid.
The hydroxamic acid was obtained in poor yield overall (39%) but excellent purity and was characterised by IR, EA, MS, $^1$H and $^{13}$C NMR spectroscopy and is in agreement with previously reported analytical data.

**Synthesis of SAHA derivatives**

The synthesis of SAHA derivatives, namely its 2- and 3-aminophenyl analogues, Figure 6.5, was attempted to investigate if the secondary amino group would improve the bioavailability properties of the compound by making it more water soluble.

![Figure 6.5](A) 3-NH$_2$-SAHA and (B) 2-NH$_2$-SAHA derivatives.

**Attempted Synthesis 3-NH$_2$-SAHA** (22)

Attempted synthesis of 22 proceed via Scheme 6.2. Methyl 8-((3-nitrophenyl)amino)-8-oxooctanoate, 19, was synthesised via nucleophilic addition of 3-nitroaniline to methyl 8-chloro-8-oxooctanoate. Base hydrolysis of the ester 19, produced acid 20. Direct formation of the benzyl protected hydroxamic acid, 21, proceeds via the formation of the mixed anhydride in situ, following reaction of 20 with ethyl chloroformate. The formation of 21 was confirmed by elemental analysis, including X-ray crystallography. The final step in this process required simultaneous deprotection of the benzyl group and reduction of the nitro group in the meta position of the phenyl ring. Typically nitro groups are readily reduced to amino groups and O-benzyl protected hydroxamic acids are easily deprotected on reduction using H$_2$ and Pd/C. Numerous different methods were investigated. Orthogonal methods of deprotection and reduction were also attempted but none were successful in generating 22 in sufficient yield or purity.
Scheme 6.2. Attempted Synthesis of 3-NH$_2$-SAHA.

21 is a novel compound and was characterised by EA, IR, MS, $^1$H and $^{13}$C NMR spectroscopy and X-ray crystallography.

$^1$H NMR analysis for this compound was performed in DMSO-$d_6$. A significant and diagnostic feature of compound 21 is the presence of the benzyl protecting group. DMSO-$d_6$ allows for observation and identification of the hydroxamic N-H and the amide N-H which occur at 10.95 ppm and 10.38 ppm. Four signals appear in the aromatic region of 21 whereas only three signals were evident in 20. Three of these signals which occur in the region of 8.65-7.55 ppm correspond to and integrate well for the four protons of the meta nitro-substituted phenyl ring. The other strong signal which occurs at 7.36 ppm integrates and corresponds to the five aromatic protons of the benzyl group protecting. The presence of the protecting group is further confirmed by the singlet at 4.76 ppm which integrates well for the two CH$_2$ protons of the benzyl protecting group.
Crystals suitable for single crystal X-ray diffraction were obtained of 21 from recrystallization in ethyl acetate.

**Attempted Synthesis of 2-NH₂-SAHA** (25)

Attempted synthesis of 2-NH₂-SAHA proceed via **Scheme 6.3**. Methyl 8-((2-nitrophenyl)amino)-8-oxooctanoate, 23, was synthesised from nucleophilic addition of 2-nitroaniline to methyl 8-chloro-8-oxooctanoate. Reduction of the *ortho* substituted nitro group on the phenyl ring by Pd/C and hydrazine afforded methyl 8-((2-aminophenyl)amino)-8-oxooctanoate, 24. Direct conversion of 24 to the hydroxamic acid was attempted as per synthesis of compound 1. This was not successful. Other attempts to convert the ester to 25 were not successful, for example, BOC protection of the amino group followed by attempted hydrolysis of the ester to the acid, and subsequent formation of the hydroxamic acid or the benzyl protected hydroxamic acid. The synthesis of 25 was not achieved.

![Scheme 6.3 Attempted synthesis of 2-NH₂-SAHA, 25.](image)

24 is a novel compound and was characterised by EA, IR, MS, $^1$H and $^{13}$C NMR spectroscopy and X-ray crystallography.

$^1$H NMR analysis for this compound was performed in DMSO-$d_6$. A significant and diagnostic feature of compound 24 is the presence of the methyl ester group. The singlet which occurs at 3.58 ppm integrates and corresponds to the three protons of the methyl group. DMSO-$d_6$ allows for observation and identification of the amide
N-H and the amino protons which occur at 9.13 ppm and 4.82 ppm respectively. Four signals appear in the aromatic region of 24 at 7.15-6.51 ppm corresponding and integrating well for the four protons of the ortho-substituted phenyl ring. Four signals at 2.32-1.29 ppm, integrate and correspond well for the twelve methylene protons of 24.

Crystals suitable for single crystal X-ray diffraction were obtained of 24 from recrystallization in ethyl acetate.
Synthesis of Antimony Hydroxamato Chlorido Compounds

Reaction of hydroxamic acids (L) with SbCl₃, in EtOH, gave novel compounds of type Sb(L⁻⁻H⁻⁻⁻⁻⁻⁻)₂Cl or Sb(L⁻⁻⁻⁻⁻⁻¹H⁻⁻⁻⁻⁻⁻)Cl₂, representing a novel class of Sb(III) hydroxamato chlorido compounds.

Synthesis of [Sb(Bha⁻⁻⁻⁻⁻⁻¹H⁻⁻⁻⁻⁻⁻)₂Cl]  (26)

Reaction of Bha with SbCl₃, in absolute ethanol at room temperature for 3 days afforded the novel Sb(III) compound [Sb(Bha⁻⁻⁻⁻⁻⁻¹H⁻⁻⁻⁻⁻⁻)₂Cl], 26. This product was obtained in 40% yield and high purity, Scheme 6.4.

Scheme 6.4 Synthesis of [Sb(Bha⁻⁻⁻⁻⁻⁻¹H⁻⁻⁻⁻⁻⁻)₂Cl] 26, a novel Sb(III) benzohydroxamato chlorido compound.

[Sb(Bha⁻⁻⁻⁻⁻⁻¹H⁻⁻⁻⁻⁻⁻)₂Cl] was characterised by EA, IR, ¹H and ¹³C NMR, MS and X-ray crystallography.

EA of 26 is consistent with the presence of two hydroxamato and one chlorido ligand per Sb(III) centre.

The ¹H NMR spectra of 26 was carried out in D₂O. No signals associated with the hydroxamic acid protons (N–H and O–H) are observed, as expected, due to the rapid exchange of the protons with the deuterated solvent. In 26, three main aromatic signals are observed at 7.69-7.48 ppm, corresponding to and integrating well for the five aromatic protons of the ligand. The most significant shift is that of the signal at 7.65 ppm in the free ligand to 7.68 ppm in 26, corresponding to the protons in the 2 and 6 position, Figure 6.6.
Three signals were observed in the $^{13}$C NMR spectrum of 26, associated with the aromatic carbons, with the signal at 126.9 ppm and 128.8 ppm accounting for two aromatic carbons. No signal associated with the carbonyl group was observed.

The IR spectra of 26 exhibit distinctive $\nu$(C=O) at 1592 cm$^{-1}$ displaying the characteristic shift associated with bidentate (O, O') hydroxamato coordination when compared to the corresponding $\nu$(C=O) of the uncoordinated ligands at 1614 cm$^{-1}$.

The shift to lower wavenumber for the $\nu$(C=O) suggests bonding to the Sb(III) centre via the formation of the thermodynamically preferred five-membered bidentate chelate, via the hydroxamato carbonyl group and deprotonated hydroxyl group. A stretch at 1553 cm$^{-1}$ is associated to the $\nu$(C-N) of the hydroxamic acid.

ESI-MS in the positive mode assisted in the identification of [Sb(Bha$_{-1}$H)$_2$Cl]. The full molecular ion, ([M+H]$^+$), was not observed but a molecular fragment associated with [Sb(Bha$_{-1}$H)$_2$]$^+$ was evident at 393.5/395.5 a.m.u displaying the characteristic isotopic splitting pattern associated with antimonial compounds given antimony has two stable isotopes; $^{121}$Sb and $^{123}$Sb.
Crystals suitable for single crystal X-ray diffraction were obtained of 26 from slow evaporation of the filtrate, confirming 26 to be the mononuclear Sb(III) compound with two bidentate (O, O') coordinated Bha ligands and a single chlorido ligand.

**Synthesis of [Sb$_2$(µ-Cl)$_2$(Cl)$_2$(2-Pyha$_{-1H}$)$_2$] (27)**

Reaction of 2-Pyha (5) with SbCl$_3$, in absolute ethanol at room temperature for 10 minutes afforded the novel Sb(III) compound [Sb$_2$(µ-Cl)$_2$(Cl)$_2$(2-Pyha$_{-1H}$)$_2$], 27. This product was obtained in 32% yield and high purity, Scheme 6.5.

![Scheme 6.5. Synthesis of [Sb$_2$(µ-Cl)$_2$(Cl)$_2$(2-Pyha$_{-1H}$)$_2$] 27, a novel Sb(III) 2-pyridinehydroxamato chlorido compound.](image)

[Sb$_2$(µ-Cl)$_2$(Cl)$_2$(2-Pyha$_{-1H}$)$_2$] was characterised by EA, IR, $^1$H and $^{13}$C NMR, MS and X-ray crystallography.

EA of 27 is consistent with the presence of one hydroxamato ligand and two chlorido ligands per Sb(III) centre.

The $^1$H NMR spectra of 27 was carried out in D$_2$O, and subsequently no signals associated with the hydroxamic acid protons (N–H and O–H) are observed. Four main aromatic signals are observed in 27 at 8.87-7.94 ppm, corresponding to and integrating well for the four aromatic protons of the ligand. All signals shift considerably upon binding of the ligand to Sb(III) when compared to the free ligand as shown in Figure 6.7.

The IR spectra of 27 exhibits distinctive v(C=O) at 1603 cm$^{-1}$ displaying the characteristic shift associated with (O, O') hydroxamato coordination when compared to the corresponding v(C=O) of the uncoordinated ligands at 1651 cm$^{-1}$. This shift signifies the formation of the thermodynamically preferred five membered bidentate chelate, via the hydroxamato carbonyl group and deprotonated hydroxyl...
group. A signal at 1548 cm\(^{-1}\) is associated with the presence of the \(\nu(\text{C-N})\) of the hydroxamic acid.

![Image of NMR spectra](image)

**Figure 6.7.** The stacked \(^1\text{H}\) NMR spectra of 2-Pyha (top) and compound 27 (bottom), showing shifts in the aromatic signals associated with coordination of Sb(III).

Two signals were observed in the \(^{13}\text{C}\) NMR spectrum of 27, representing the aromatic carbons.

ESI-MS in the positive mode assisted in the identification of \([\text{Sb}_2(\mu-\text{Cl})_2(\text{Cl})_2(2-\text{Pyha}.1\text{H})_2]\). The full molecular ion again was not evident. A molecular fragment at 395.3/397.3 a.m.u associated with \([\text{Sb}(2-\text{Pyha}-.1\text{H})_2]^+\) was evident displaying the characteristic isotopic pattern of antimony.

Crystals suitable for single crystal X-ray diffraction were obtained of 21 from recrystallization from methanol, confirming the compound structure to be a dinuclear Sb(III) compound that features the bidentate (O, O’) coordination of 2-Pyha, one non bridging chlorido ligand and one bridging chlorido ligand, which bridges to the adjacent Sb(III) centre.
Synthesis of \([\text{Sb}(2\text{-NH}_2\text{-Pha-H})(2\text{-NH}_3\text{-Pha-H})]\)\(_2\)Cl\(_2\)  (28)

Reaction of 2-NH\(_2\)-Pha (1) with SbCl\(_3\), in absolute ethanol at room temperature for 30 minutes and subsequently at reflux for 1 hour afforded the novel Sb(III) compound \([\text{Sb}(2\text{-NH}_2\text{-Pha-H})(2\text{-NH}_3\text{-Pha-H})]\)\(_2\)Cl\(_2\), 28. This product was obtained in 38% yield and high purity, Scheme 6.6.

![Scheme 6.6 Synthesis of \([\text{Sb}(2\text{-NH}_2\text{-Pha-H})(2\text{-NH}_3\text{-Pha-H})]\)\(_2\)Cl\(_2\), 28 a novel Sb(III) 2-aminohydroxamato chlorido compound.](image)

Elemental analysis of 28 is consistent with the presence of two hydroxamato ligands and one chlorido ligand per Sb(III) centre.

The \(^1\)H NMR spectra of 28 was carried out in D\(_2\)O and no signals associated with the hydroxamic acid protons (N–H and O–H) or the amines are observed, as expected. The four aromatic protons are represented by two peaks in the region of 7.7-7.4 ppm.

There are three signals evident in the free acid which then are shifted downfield in the compound as shown in Figure 6.8.

The IR spectra of 28 exhibits distinctive \(\nu(C=O)\) at 1607 cm\(^{-1}\) displaying the characteristic shift associated with (O, O') hydroxamato coordination when compared to the corresponding \(\nu(C=O)\) of the uncoordinated ligand at 1639 cm\(^{-1}\).\(^{14}\)

This shift signifies the formation of the thermodynamically preferred five membered bidentate chelate, via the hydroxamato carbonyl group and deprotonated hydroxyl group. A signal at 1557 cm\(^{-1}\) represents \(\nu(C-N)\) of the hydroxamic acid.
Figure 6.8. The stacked $^1$H NMR spectra of 2-NH$_2$-Pha (top) and compound 28 (bottom), showing the shifts in the aromatic signals associated with coordination of Sb(III).

Four signals were observed in the $^{13}$C NMR spectrum of 28, representing the six aromatic carbons.

ESI-MS in the positive mode assisted in the identification of 28. The full molecular ion was not evident. Molecular fragments at 422.8/424.8 a.m.u associated with [Sb(2-NH$_2$-Pha-H(2-NH$_3$-Pha-HH))]$^{2+}$ was evident.

Crystals suitable for single crystal X-ray diffraction were obtained of 28 from recrystallization from ethanol, confirming the compound structure to be the mononuclear Sb(III) compound that features one neutral hydroxamato ligand, where the amino group is protonated and the hydroxamic acid group monodeprotonated. Both hydroxamic acids feature the bidentate (O, O') coordination mode.
Synthesis of \([\text{SbCl(Sha-1H)}_2]\) \((29)\)

Reaction of Sha with SbCl₃, in absolute ethanol at room temperature for 3 days afforded the novel Sb(III) compound \([\text{SbCl(Sha-1H)}_2]\), \(29\). This product was obtained in 26% yield but high purity, **Scheme 6.7**.

![Scheme 6.7. Synthesis of \([\text{SbCl(Sha-1H)}_2]\), 29 a novel Sb(III) salicylhydroxamato chlorido compound.](image)

Elemental analysis of \(29\) is consistent with the presence of two salicylhydroxamato ligands and one chlorido ligand per Sb(III) centre.

The \(^1\text{H}\) NMR spectra of \(29\) was carried out in D₂O. Three signals present in the aromatic region 7.7-6.9 ppm of compound \(29\) correspond and integrate well for the four aromatic protons. These signals are shifted in relation to the free ligand as shown in **Figure 6.9**.

![Figure 6.9. The stacked \(^1\text{H}\) NMR spectra of Sha (top) and compound 29 (bottom), and the expansion of the area of interest (insert), showing the shifts in the aromatic signals associated with coordination of Sb(III).](image)
Five signals were observed in the $^{13}$C NMR spectrum of 29, associated with the six aromatic carbons. The signal associated with the carbonyl group was evident at 155.9 ppm is shifted in comparison to carbonyl signal of the free ligand Sha which occurs at 170.0 ppm.

The IR spectra of 29 exhibits distinctive $\nu$(C=O) at 1602 cm$^{-1}$ displaying the characteristic shift associated with (O, O') hydroxamato coordination when compared to the corresponding $\nu$(C=O) of the uncoordinated ligand at 1619 cm$^{-1}$. The shift to lower wavenumber for the carbonyl suggests bonding to the Sb(III) centre via the formation of the thermodynamically preferred five membered bidentate chelate, via the hydroxamato carbonyl group and deprotonated hydroxyl group. The signal at 1566 cm$^{-1}$ represents the $\nu$(C-N).

ESI-MS in the positive mode assisted in the identification of [SbCl(Sha$_{1H}$)$_2$]. The full molecular ion is not observed but the molecular fragment associated with [Sb(Sha$_{1H}$)$_2^+$], occurring at 424.6/426.5 a.m.u was evident.

Crystals suitable for single crystal X-ray diffraction of 29 were not obtained.

**Attempted Synthesis of [SbCl(SAHA$_{1H}$)$_2$]** (30)

Numerous reactions of SAHA with SbCl$_3$ with a view to synthesising the target Sb HDACi compound, [SbCl(SAHA$_{1H}$)$_2$] 30, failed to produce a product sample of sufficient purity, though the reaction did occur, **Scheme 6.8**.

![Scheme 6.8. Attempted Synthesis of [SbCl(SAHA$_{1H}$)$_2$].](attachment:Scheme_6.8.png)

Elemental analysis of microcrystals of 30 is consistent with the presence of two hydroxamato ligands and one chlorido ligand per Sb(III) centre.
The $^1$H NMR spectra of 30 was carried out in MeOD. The signals associated with the hydroxamic acid protons (N–H and O–H) are not observed, as expected, due to the rapid exchange with the solvent. In 30, three main aromatic signals are observed at 7.6-7.0 ppm, corresponding to and integrating well for the five aromatic protons of the ligand. The most significant shift in 30 is in the region of the aliphatic signals at 2.5-1.0 ppm when compared to the signals in the free ligand as seen in Figure 6.10.

![Figure 6.10](image)

Figure 6.10. The stacked $^1$H NMR spectra of SAHA (top) and compound 30 (bottom), and the expansion of the area of interest (insert), showing the shifts in the aliphatic signals associated with coordination of Sb(III).

The IR spectra of 30 exhibits distinctive $\nu$(C=O) at 1596 cm$^{-1}$ displaying the characteristic shift associated with (O, O') hydroxamato coordination when compared to the corresponding $\nu$(C=O) of the uncoordinated ligand at 1654 cm$^{-1}$.$^{14}$ The shift to lower wavenumber for the carbonyl suggests bonding to the antimony atom via the formation of the thermodynamically preferred five membered bidentate chelate, via the hydroxamato carbonyl group and deprotonated hydroxyl group. A strong signal at 1530 cm$^{-1}$ is associated with the presence of the $\nu$(C-N) of the hydroxamic acid.
ESI-MS in the negative mode assisted in the identification of [SbCl(SAHA$_{1H}$)$_2$]. The full molecular ion, ([M-H]$^-$), was not observed but a molecular fragment associated with [Sb(SAHA$_{2H}$)$_2$] was evident at 645.1/647.1 a.m.u displaying the characteristic isotopic splitting pattern of antimony.

Micro-crystals suitable for single crystal X-ray diffraction were obtained of 30 but were unsuitable for X-ray crystallography.

Synthesis of Antimony Hydroxamato/Hydroximato Compounds

Synthesis of [Sb(SAHA$_{1H}$)(SAHA$_{2H}$)] (31)

Reaction of SAHA (18) with Sb(OEt$_3$)$_3$, in hexane at room temperature for 30 hours afforded the novel Sb(III) compound [Sb(SAHA$_{1H}$)(SAHA$_{2H}$)], 31. This product was obtained in 64% yield and high purity, Scheme 6.9.

Scheme 6.9. Synthesis of [Sb(SAHA$_{1H}$)(SAHA$_{2H}$)] 31, a novel Sb(III) SAHA hydroxamato/hydroximato compound.

31 was characterised by EA, IR, MS and $^1$H and $^{13}$C NMR spectroscopy.

EA of 31 is consistent with the presence of one hydroxamato ligand and one hydroximato ligand bound to each Sb(III) centre.

The elemental analysis of all the Sb(III) compounds in this chapter are shown below in Table 6.1, for purposes of comparison.
### Table 6.1. Summary of the EA results for compounds 26-31

<table>
<thead>
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<th>Compound</th>
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<th>% H</th>
<th>% N</th>
<th>% Cl</th>
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<td>51.52</td>
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The $^1$H NMR spectra of 31 was carried out in MeOD. Three main aromatic signals are observed in 31 at 7.0-7.6 ppm, corresponding and integrating well for the five aromatic protons of the ligand. These signals shift only slightly in comparison to the free ligand. No significant difference is noticed in the diagnostic peak in the aliphatic region of the compound as seen in Figure 6.11.
Figure 6.11. The stacked $^1$H NMR spectra of 18 (top) and compound 31 (bottom), and the expansion of the area of interest (insert), showing the slight shifts in the aliphatic signals arrangement associated with coordination of Sb(III).

The IR spectra of 31 exhibits three distinctive $\nu$(C=O) at 1658 cm$^{-1}$, 1628 cm$^{-1}$ and 1598 cm$^{-1}$ indicating the presence of two different ligands in the structure. The presence of the $\nu$(C=O) at 1658 cm$^{-1}$ in 31 is associated with the amide group of both ligands. The characteristic hydroxamic acid $\nu$(C=O) occurs at 1598 cm$^{-1}$ and displays the characteristic shift associated with (O, O') monodeprotonated hydroxamato coordination when compared to the corresponding $\nu$(C=O) of the uncoordinated ligand at 1619 cm$^{-1}$. A similar shift is also observed in 30, further confirming the existence of a SAHA Sb(III) compound in 30. The $\nu$(C=O) at 1628 cm$^{-1}$ is attributed therefore to the doubly deprotonated hydroximato SAHA ligand. A strong signal at 1545 cm$^{-1}$ is associated with the presence of the $\nu$(C-N).

A list of all the IR stretches of the Sb(III) compounds in this chapter are shown below in Table 6.2, for comparison reasons.
Table 6.2. Overview of the hydroxamic acid associated stretches observed in IR spectroscopy

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<th>$\nu\text{ C-O}$</th>
<th>$\nu\text{ C-N}$</th>
</tr>
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<tbody>
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<td>Bha</td>
<td>1645 cm$^{-1}$</td>
<td>1556 cm$^{-1}$</td>
</tr>
<tr>
<td>26</td>
<td>1592 cm$^{-1}$</td>
<td>1553 cm$^{-1}$</td>
</tr>
<tr>
<td>2-Pyha</td>
<td>1651 cm$^{-1}$</td>
<td>1567 cm$^{-1}$</td>
</tr>
<tr>
<td>27</td>
<td>1603 cm$^{-1}$</td>
<td>1548 cm$^{-1}$</td>
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<tr>
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<td>1639 cm$^{-1}$</td>
<td>1559 cm$^{-1}$</td>
</tr>
<tr>
<td>28</td>
<td>1607 cm$^{-1}$</td>
<td>1558 cm$^{-1}$</td>
</tr>
<tr>
<td>Sha</td>
<td>1619 cm$^{-1}$</td>
<td>1555 cm$^{-1}$</td>
</tr>
<tr>
<td>29</td>
<td>1602 cm$^{-1}$</td>
<td>1566 cm$^{-1}$</td>
</tr>
<tr>
<td>SAHA</td>
<td>1654 cm$^{-1}$</td>
<td>1545 cm$^{-1}$</td>
</tr>
<tr>
<td>30</td>
<td>1596 cm$^{-1}$</td>
<td>1530 cm$^{-1}$</td>
</tr>
<tr>
<td>31</td>
<td>1598/1628 cm$^{-1}$</td>
<td>1545 cm$^{-1}$</td>
</tr>
</tbody>
</table>

ESI-MS in the negative mode assisted in the identification of [Sb(SAHA-1H)(SAHA-2H)] with a signal at 645.3/647.3 a.m.u displaying the characteristic splitting pattern of antimony.

Attempts to obtain crystals suitable for single crystal X-ray diffraction of 31 were not successful.
5. Crystal Structure Studies

The X-ray crystal structures described in this section were determined by Dr. Brendan Twamley, School of Chemistry, in Trinity College, Dublin.

Organic Crystal Structures

\(\text{N}^1\)-(benzyloxy)-\text{N}^8\)-(3-nitrophenyl)octanediamide \hspace{1em} (21)

Clear colourless crystals of 21 suitable for X-ray analysis were obtained by slow evaporation of an ethyl acetate solution. The X-ray intensity data were measured at 105 K using an Oxford Cryosystems Cobra low temperature device and the structures solved using Bruker APEX Software Package and refined with XL in Olex2. A specimen, of approximate dimensions 0.31 mm x 0.08 mm x 0.07 mm was used for refinement. The solid state structure of 21 is shown in Figure 6.12. The compound crystallizes in the \(\text{P}1\) space group. Crystal data and details of data collection are summarised in Table 6.3.

Figure 6.12. Solid state structure of 21, with partial atom labelling for clarity. Atomic displacement shown at 50% probability.

Table 6.3. Crystal data and structure refinement for 21.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>(\text{C}<em>{21}\text{H}</em>{25}\text{N}<em>{3}\text{O}</em>{5})</td>
</tr>
<tr>
<td>Formula weight</td>
<td>399.44</td>
</tr>
<tr>
<td>Temperature</td>
<td>105 K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Triclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>(\text{P}1)</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>(a = 6.4325(4)) Å</td>
</tr>
<tr>
<td></td>
<td>(\alpha = 81.219(2)^\circ)</td>
</tr>
<tr>
<td></td>
<td>(b = 9.3343(6)) Å</td>
</tr>
<tr>
<td></td>
<td>(\beta = 80.208(2)^\circ)</td>
</tr>
<tr>
<td></td>
<td>(c = 16.6717(10)) Å</td>
</tr>
<tr>
<td></td>
<td>(\gamma = 88.163(2)^\circ)</td>
</tr>
<tr>
<td>Volume</td>
<td>974.85(11) Å(^3)</td>
</tr>
<tr>
<td>(Z)</td>
<td>2</td>
</tr>
<tr>
<td>Density (calculated)</td>
<td>1.361 Mg/m(^3)</td>
</tr>
<tr>
<td>Absorption coefficient</td>
<td>0.098 mm(^{-1})</td>
</tr>
</tbody>
</table>
The formula of 21 consists of a charge neutral compound. Tables of significant bond lengths and bond angles are shown below in Tables 6.4 and Table 6.5.

Table 6.4. A list of the significant bond lengths of 21*.

<table>
<thead>
<tr>
<th>Atoms</th>
<th>Dist (Å)</th>
<th>Atoms</th>
<th>Dist (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O(8)-N(9)</td>
<td>1.399(2)</td>
<td>N(9)-C(10)</td>
<td>1.348(2)</td>
</tr>
<tr>
<td>O(8)-C(7)</td>
<td>1.455(2)</td>
<td>N(9)-H(9)</td>
<td>0.933(10)</td>
</tr>
<tr>
<td>O(11)-C(10)</td>
<td>1.229(2)</td>
<td>N(20)-C(18)</td>
<td>1.361(2)</td>
</tr>
<tr>
<td>O(19)-C(18)</td>
<td>1.230(2)</td>
<td>N(20)-C(21)</td>
<td>1.406(2)</td>
</tr>
<tr>
<td>O(28)-N(27)</td>
<td>1.229(2)</td>
<td>N(20)-H(20)</td>
<td>0.934(10)</td>
</tr>
<tr>
<td>O(29)-N(27)</td>
<td>1.226(2)</td>
<td>N(27)-C(23)</td>
<td>1.475(2)</td>
</tr>
</tbody>
</table>

* Aromatic C-H bonds are 0.9500 Å. Aromatic C-C bonds are 1.385-1.375 Å. Methylene C-C bonds are 1.518 Å. Methylene C-H bonds are 0.9900 Å.

Table 6.5. A list of the significant bond angles of 21.

<table>
<thead>
<tr>
<th>Atoms</th>
<th>Angle (°)</th>
<th>Atoms</th>
<th>Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N(9)-O(8)-C(7)</td>
<td>108.80(14)</td>
<td>C(18)-N(20)-H(20)</td>
<td>116.6(14)</td>
</tr>
<tr>
<td>O(8)-N(9)-H(9)</td>
<td>112.6(14)</td>
<td>C(21)-N(20)-H(20)</td>
<td>114.7(14)</td>
</tr>
<tr>
<td>C(10)-N(9)-O(8)</td>
<td>119.39(16)</td>
<td>O(28)-N(27)-C(23)</td>
<td>118.22(16)</td>
</tr>
<tr>
<td>C(10)-N(9)-H(9)</td>
<td>121.1(14)</td>
<td>O(29)-N(27)-O(28)</td>
<td>123.58(17)</td>
</tr>
<tr>
<td>C(18)-N(20)-C(21)</td>
<td>128.64(16)</td>
<td>O(29)-N(27)-C(23)</td>
<td>118.20(16)</td>
</tr>
</tbody>
</table>

* All aromatic C-C-C bond angles are 120° and all aromatic C-C-H bond angles are also 120°.
Methyl 8-((2-aminophenyl)amino)-8-oxooctanoate (24)

Clear colourless plate like crystals of 24 suitable for X-ray analysis were obtained by slow evaporation of an ethyl acetate solution. The X-ray intensity data were measured at 105 K using an Oxford Cryosystems Cobra low temperature device and the structure was solved with the XT structure solution program using Intrinsic Phasing and refined with the XL refinement package using Least Squares minimisation with Olex2. A specimen, of approximate dimensions 0.12 mm x 0.25 mm x 0.39 mm was used for refinement. The solid state structure of 24 is shown in Figure 6.13. The compound crystallizes in the P2₁/n space group. Crystal data and details of data collection are summarised in Table 6.6.

Figure 6.13. Solid state structure of 24, with partial atom labelling for clarity. Atomic displacement shown at 50% probability.
Table 6.6. Table of collection parameters for 24.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C_{15}H_{22}N_{2}O_{3}</td>
</tr>
<tr>
<td>Formula weight</td>
<td>278.34</td>
</tr>
<tr>
<td>Temperature</td>
<td>105 K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Monoclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>P2_1/n</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>a = 11.7759(3) Å, α = 90°</td>
</tr>
<tr>
<td></td>
<td>b = 4.90770(10) Å, β = 92.1329(14)°</td>
</tr>
<tr>
<td></td>
<td>c = 25.338(7) Å, γ = 90°</td>
</tr>
<tr>
<td>Volume</td>
<td>1463.09(6) Å³</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
</tr>
<tr>
<td>Density (calculated)</td>
<td>1.264 Mg/m³</td>
</tr>
<tr>
<td>Absorption coefficient</td>
<td>0.088 mm⁻¹</td>
</tr>
<tr>
<td>F(000)</td>
<td>600</td>
</tr>
<tr>
<td>Crystal size</td>
<td>0.39 x 0.25 x 0.12 mm³</td>
</tr>
<tr>
<td>Theta range for data collection</td>
<td>2.918 to 26.380°</td>
</tr>
<tr>
<td>Index ranges</td>
<td>-14≤h≤14, -6≤k≤6, -31≤l≤31</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>22671</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>2970 [R(int) = 0.0343]</td>
</tr>
<tr>
<td>Completeness to theta = 25.242°</td>
<td>99.8 %</td>
</tr>
<tr>
<td>Absorption correction</td>
<td>Semi-empirical from equivalents</td>
</tr>
<tr>
<td>Max. and min. transmission</td>
<td>0.7454 and 0.6782</td>
</tr>
<tr>
<td>Refinement method</td>
<td>Full-matrix least-squares on F²</td>
</tr>
<tr>
<td>Data / restraints / parameters</td>
<td>2970 / 0 / 193</td>
</tr>
<tr>
<td>Goodness-of-fit on F²</td>
<td>1.059</td>
</tr>
<tr>
<td>Final R indices [I&gt;2σ(I)]</td>
<td>R1 = 0.0414, wR2 = 0.0973</td>
</tr>
<tr>
<td>R indices (all data)</td>
<td>R1 = 0.0524, wR2 = 0.1035</td>
</tr>
<tr>
<td>Largest diff. peak and hole</td>
<td>0.215 and -0.218 e.Å⁻³</td>
</tr>
</tbody>
</table>

Tables of important bond length and bond angles are shown below, Table 6.7 and Table 6.8.

Table 6.7  A list of the significant bond lengths of 24°.

<table>
<thead>
<tr>
<th>Atoms</th>
<th>Dist (Å)</th>
<th>Atoms</th>
<th>Dist (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O(10)-C(9)</td>
<td>1.2324(18)</td>
<td>N(1)-H(1B)</td>
<td>0.88(2)</td>
</tr>
<tr>
<td>O(18)-C(17)</td>
<td>1.2039(18)</td>
<td>N(1)-C(2)</td>
<td>1.3900(19)</td>
</tr>
<tr>
<td>O(19)-C(17)</td>
<td>1.3390(18)</td>
<td>N(8)-H(8)</td>
<td>0.842(18)</td>
</tr>
<tr>
<td>O(19)-C(20)</td>
<td>1.4471(19)</td>
<td>N(8)-C(7)</td>
<td>1.4250(18)</td>
</tr>
<tr>
<td>N(1)-H(1A)</td>
<td>0.873(19)</td>
<td>N(8)-C(9)</td>
<td>1.3484(18)</td>
</tr>
</tbody>
</table>

Aromatic C-H bonds are 0.9500 Å. Aromatic C-C bonds are 1.385-1.402 Å. Methylene C-C bonds are 1.520 Å. Methylene C-H bonds are 0.9900
Table 6.8. A list of the significant bond angles of 24°.

<table>
<thead>
<tr>
<th>Atoms</th>
<th>Angle (°)</th>
<th>Atoms</th>
<th>Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(17)-O(19)-C(20)</td>
<td>116.29(13)</td>
<td>C(2)-N(1)-H(1A)</td>
<td>116.6(12)</td>
</tr>
<tr>
<td>H(1A)-N(1)-H(1B)</td>
<td>111.6(17)</td>
<td>C(2)-N(1)-H(1B)</td>
<td>112.4(12)</td>
</tr>
<tr>
<td>C(2)-N(1)-H(1A)</td>
<td>116.6(12)</td>
<td>C(7)-N(8)-H(8)</td>
<td>116.2(11)</td>
</tr>
<tr>
<td>C(2)-N(1)-H(1B)</td>
<td>112.4(12)</td>
<td>C(9)-N(8)-H(8)</td>
<td>117.9(11)</td>
</tr>
<tr>
<td>C(17)-O(19)-C(20)</td>
<td>116.29(13)</td>
<td>C(9)-N(8)-C(7)</td>
<td>125.91(12)</td>
</tr>
<tr>
<td>H(1A)-N(1)-H(1B)</td>
<td>111.6(17)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*All aromatic C-C-C bond angles are 120° and all aromatic C-C-H bond angles are also 120°.

Crystal Structures of Sb(III) Chlorido Hydroxamato Compounds

[Sb(Bha\textsubscript{1H})\textsubscript{2}Cl] \hspace{1cm} (26)

Crystals of 26 suitable for X-ray analysis were obtained by slow evaporation of the filtrate. The X-ray intensity data were measured at 100 K using an Oxford Cryosystems Cobra low temperature device. A specimen, of approximate dimensions 0.5 mm x 0.1 mm x 0.08 mm, was used for refinement. The solid state structure of 26 is shown in Figure 6.14. The compound crystallizes in the P2\textsubscript{1}/c space group. Crystal data and details of data collection are summarised in Table 6.9.

Figure 6.14. Solid state structure of the asymmetric unit of 26, with partial atom labelling for clarity. Atomic displacement shown at 50% probability.
Compound 26 is a di-benzohydroxamate monohalide substituted Sb(III) compound, as seen in Figure 6.14. The compound is charge neutral, with each Sb centre chelated by two ligands in a (O, O’) bi-dentate fashion, as predicted from IR data, via the carbonyl oxygen atom and the deprotonated hydroxamic acid hydroxyl group, (Sb1-O1, Sb1-O4, Sb1-O11 and Sb1-O14). A chloride completes the binding arrangement, (Sb1-Cl1).

Table 6.9. Table of collection parameters for 26.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C14H12ClN2O4Sb</td>
</tr>
<tr>
<td>Formula weight</td>
<td>429.46</td>
</tr>
<tr>
<td>Temperature</td>
<td>100 K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Monoclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>P21/c</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>a = 10.9598(4) Å, b = 4.9157(2) Å, c = 27.3577(11) Å</td>
</tr>
<tr>
<td></td>
<td>α = 90°, β = 6.0053(16)°, γ = 90°</td>
</tr>
<tr>
<td>Volume</td>
<td>1465.81(10) Å</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
</tr>
<tr>
<td>Density (calculated)</td>
<td>1.946 Mg/m³</td>
</tr>
<tr>
<td>Absorption coefficient</td>
<td>2.084 mm⁻³</td>
</tr>
<tr>
<td>F(000)</td>
<td>840</td>
</tr>
<tr>
<td>Crystal size</td>
<td>0.5 x 0.1 x 0.08 mm³</td>
</tr>
<tr>
<td>Theta range for data collection</td>
<td>2.995 to 33.195°</td>
</tr>
<tr>
<td>Index ranges</td>
<td>-16≤h≤16, -7≤k≤7, -38≤l≤42</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>50221</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>5612 [R(int) = 0.0243]</td>
</tr>
<tr>
<td>Completeness to theta = 25.242°</td>
<td>99.8 %</td>
</tr>
<tr>
<td>Absorption correction</td>
<td>Semi-empirical from equivalents</td>
</tr>
<tr>
<td>Max. and min. transmission</td>
<td>0.7465 and 0.5659</td>
</tr>
<tr>
<td>Refinement method</td>
<td>Full-matrix least-squares on F²</td>
</tr>
<tr>
<td>Data / restraints / parameters</td>
<td>5612 / 2 / 205</td>
</tr>
<tr>
<td>Goodness-of-fit on F²</td>
<td>1.214</td>
</tr>
<tr>
<td>Final R indices [I&gt;2σ(I)]</td>
<td>R1 = 0.0196, wR2 = 0.0445</td>
</tr>
<tr>
<td>R indices (all data)</td>
<td>R1 = 0.0214, wR2 = 0.0450</td>
</tr>
<tr>
<td>Largest diff. peak and hole</td>
<td>0.656 and -0.866 e.Å⁻³</td>
</tr>
</tbody>
</table>

This antimony molecule can also form a dimer as seen in Figure 6.15, via intermolecular contacts between the antimony and an oxygen of an adjacent ligand, (Sb1-·-O11), completing the Sb atom distorted octahedral coordination. In the case of dimerization, then one of the ligand experiences bidentate bridging (O, µ-O’) coordination. The dimers are stabilized by intermolecular hydrogen bonding (N12-·-Cl1 and N2-·-O4) as shown in Figure 6.16. An ORTEP view of the compound
is shown below along with some of significant bond lengths and bond angles in Tables 6.10 and Table 6.11.

**Figure 6.15.** Symmetry generated dimer of 26 with long Sb1-O11' bonds displayed. Partial atom labelling shown for clarity purpose and atomic displacement shown at 50% probability.

**Table 6.10.** A list of the significant bond lengths of 26.

<table>
<thead>
<tr>
<th>Atoms</th>
<th>Dist (Å)</th>
<th>Atoms</th>
<th>Dist (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sb(1)-Cl(1)</td>
<td>2.4964(3)</td>
<td>Sb(1)-O(11)</td>
<td>2.0766(10)</td>
</tr>
<tr>
<td>Sb(1)-O(1)</td>
<td>2.0416(10)</td>
<td>Sb(1)-O(14)</td>
<td>2.2568(10)</td>
</tr>
<tr>
<td>Sb(1)-O(4)</td>
<td>2.5679(10)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6.11. A list of the significant bond angles of 26.

<table>
<thead>
<tr>
<th>Atoms</th>
<th>Angle (°)</th>
<th>Atoms</th>
<th>Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl(1)-Sb(1)-O(4)</td>
<td>80.62(2)</td>
<td>O(11)-Sb(1)-O(14)</td>
<td>72.98(4)</td>
</tr>
<tr>
<td>O(1)-Sb(1)-Cl(1)</td>
<td>88.02(3)</td>
<td>O(14)-Sb(1)-Cl(1)</td>
<td>149.25(3)</td>
</tr>
<tr>
<td>O(1)-Sb(1)-O(4)</td>
<td>68.92(4)</td>
<td>O(14)-Sb(1)-O(4)</td>
<td>115.57(4)</td>
</tr>
<tr>
<td>O(1)-Sb(1)-O(11)</td>
<td>86.72(4)</td>
<td>N(2)-O(1)-Sb(1)</td>
<td>118.50(8)</td>
</tr>
<tr>
<td>O(1)-Sb(1)-O(14)</td>
<td>75.32(4)</td>
<td>C(3)-O(4)-Sb(1)</td>
<td>105.69(8)</td>
</tr>
<tr>
<td>O(11)-Sb(1)-Cl(1)</td>
<td>80.54(3)</td>
<td>N(12)-O(11)-Sb(1)</td>
<td>114.40(7)</td>
</tr>
<tr>
<td>O(11)-Sb(1)-O(4)</td>
<td>149.54(4)</td>
<td>C(13)-O(14)-Sb(1)</td>
<td>112.49(9)</td>
</tr>
</tbody>
</table>

Figure 6.16. Intermolecular Hydrogen bonding pattern of 26 viewed normal to the a-axis.
Crystals of 27 suitable for X-ray analysis were obtained by slow evaporation from methanol. Crystal data and details of collection are summarised in Table 6.12. The X-ray intensity data were measured at 100 K using an Oxford Cryosystems Cobra low temperature device. A specimen, of approximate dimensions 0.34 mm x 0.3 mm x 0.04 mm, was used for refinement. The solid state structure of 27 is shown in Figure 6.17. The compound crystallizes in the Pī space group and is completed by inversion symmetry.

Table 6.12. Table of collection parameters for 27.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C₁₂H₁₀Cl₄N₄O₅Sb₂</td>
</tr>
<tr>
<td>Formula weigh</td>
<td>675.54</td>
</tr>
<tr>
<td>Temperature</td>
<td>100 K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Triclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>Pī</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>a = 6.4349(2) Å, α = 81.4760(10)°</td>
</tr>
<tr>
<td></td>
<td>b = 7.7809(2) Å, β = 81.5970(10)°</td>
</tr>
<tr>
<td></td>
<td>c = 9.6588(3) Å, γ = 86.7140(10)°</td>
</tr>
<tr>
<td>Volume</td>
<td>472.82(2) Å³</td>
</tr>
<tr>
<td>Z</td>
<td>1</td>
</tr>
<tr>
<td>Density (calculated)</td>
<td>2.372 Mg/m³</td>
</tr>
<tr>
<td>Absorption coefficient</td>
<td>3.455 mm⁻¹</td>
</tr>
<tr>
<td>F(000)</td>
<td>320</td>
</tr>
<tr>
<td>Crystal size</td>
<td>0.34 x 0.3 x 0.04 mm³</td>
</tr>
<tr>
<td>Theta range for data collection</td>
<td>3.169 to 36.540°</td>
</tr>
<tr>
<td>Index ranges</td>
<td>-10≤h≤9, -13≤k≤12, -16≤l≤16</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>21969</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>4643 [R(int) = 0.0308]</td>
</tr>
<tr>
<td>Completeness to theta = 25.242°</td>
<td>99.9 %</td>
</tr>
<tr>
<td>Absorption correction</td>
<td>Semi-empirical from equivalents</td>
</tr>
<tr>
<td>Max. and min. transmission</td>
<td>0.7471 and 0.5582</td>
</tr>
<tr>
<td>Refinement method</td>
<td>Full-matrix least-squares on F²</td>
</tr>
<tr>
<td>Data / restraints / parameters</td>
<td>4643 / 3 / 129</td>
</tr>
<tr>
<td>Goodness-of-fit on F²</td>
<td>1.142</td>
</tr>
<tr>
<td>Final R indices [I&gt;2σ(I)]</td>
<td>R1 = 0.0206, wR2 = 0.0461</td>
</tr>
<tr>
<td>R indices (all data)</td>
<td>R1 = 0.0237, wR2 = 0.0470</td>
</tr>
<tr>
<td>Largest diff. peak and hole</td>
<td>0.736 and -0.932 e.Å⁻³</td>
</tr>
</tbody>
</table>
Figure 6.17.  Symmetry generated disordered structure of 27 with partial atom labelling for clarity.

The structure consists of a di-nuclear Sb(III) dimer, with each antimony coordinated to one monodeprotonated hydroxamate and two chloride ions, one of which was bridging. The formula of 27 consists of the charge neutral compound. The hydroxamato ligand and chlorine atom display disorder with each pyridine structure occupying two positions at 71:29% occupancy and each chlorine occupying 58:42% occupancy, as shown in Figure 6.17, above. The core of the compound demonstrates that the ligand binds via a bi-dentate fashion, as predicted from IR data, via Sb-O bonds (Sb1-O1, Sb1-O4). The Sb-Cl bonds are asymmetric (Sb1-Cl1, Sb1-Cl2). The full coordination sphere around the Sb atom is completed by intermolecular Sb···Cl asymmetric interactions (Sb1···Cl2a) forming a dimer. Complete octahedral geometry is displayed in Figure 6.18. Significant bond lengths and bond angles are shown in Tables 6.13 and Table 6.14.

Table 6.13.  A list of the significant bond lengths of 27.

<table>
<thead>
<tr>
<th>Atoms</th>
<th>Dist (Å)</th>
<th>Atoms</th>
<th>Dist (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sb(1)-Cl(1B)</td>
<td>2.462(8)</td>
<td>Sb(1)-Cl(1A)</td>
<td>2.499(7)</td>
</tr>
<tr>
<td>Sb(1)-Cl(2B)</td>
<td>2.701(14)</td>
<td>Sb(1)-Cl(2A)</td>
<td>2.631(9)</td>
</tr>
<tr>
<td>Sb(1)-O(1)</td>
<td>2.0306(10)</td>
<td>Cl(2B)-Sb(1)#1</td>
<td>2.931(17)</td>
</tr>
<tr>
<td>Sb(1)-O(4)</td>
<td>2.1119(11)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6.14. A list of the significant bond angles of 27.

<table>
<thead>
<tr>
<th>Atoms</th>
<th>Angle (°)</th>
<th>Atoms</th>
<th>Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl(1B)-Sb(1)-Cl(2B)</td>
<td>93.2(4)</td>
<td>O(4)-Sb(1)-Cl(2B)</td>
<td>151.1(3)</td>
</tr>
<tr>
<td>O(1)-Sb(1)-Cl(1B)</td>
<td>91.37(18)</td>
<td>O(4)-Sb(1)-Cl(1A)</td>
<td>90.33(18)</td>
</tr>
<tr>
<td>O(1)-Sb(1)-Cl(2B)</td>
<td>74.5(3)</td>
<td>O(4)-Sb(1)-Cl(2A)</td>
<td>152.8(2)</td>
</tr>
<tr>
<td>O(1)-Sb(1)-O(4)</td>
<td>76.58(4)</td>
<td>Cl(1A)-Sb(1)-Cl(2A)</td>
<td>87.7(4)</td>
</tr>
<tr>
<td>O(1)-Sb(1)-Cl(1A)</td>
<td>87.9(3)</td>
<td>Sb(1)-Cl(2B)-Sb(1)#1</td>
<td>95.2(4)</td>
</tr>
<tr>
<td>O(1)-Sb(1)-Cl(2A)</td>
<td>76.3(2)</td>
<td>N(2)-O(1)-Sb(1)</td>
<td>116.34(8)</td>
</tr>
<tr>
<td>O(4)-Sb(1)-Cl(1B)</td>
<td>88.57(17)</td>
<td>C(3)-O(4)-Sb(1)</td>
<td>110.52(9)</td>
</tr>
</tbody>
</table>

Figure 6.18. Completed octahedral symmetry around the Sb centre in 27. Dashed lines indicate intermolecular interactions. Only the symmetry unique molecule is partially labelled and only the major disordered moiety shown.
[Sb(2-NH₂-Phₐ-H)(2-NH₃-Phₐ-H)]Cl₂ (28)

Crystals of 28 suitable for X-ray analysis were obtained by slow evaporation from an ethanol solution. Crystal data and details of collection are summarised in Table 6.15. The X-ray intensity data were measured at 99.92 K using an Oxford Cryosystems Cobra low temperature device. A specimen, of approximate dimensions 0.34 mm x 0.17 mm x 0.11 mm, was used for refinement. The solid state structure of 28 is shown in Figure 6.19. The compound crystallizes in the Pī space group.

Table 6.15. Table of collection parameters for 28.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C₁₅H₂₂Cl₂N₄₀₆.₅Sb</td>
</tr>
<tr>
<td>Formula weight</td>
<td>555.01</td>
</tr>
<tr>
<td>Temperature</td>
<td>99.92 K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Triclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>Pī</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>a = 9.1379(3) Å</td>
</tr>
<tr>
<td></td>
<td>b = 10.1593(4) Å</td>
</tr>
<tr>
<td></td>
<td>c = 11.1638(4) Å</td>
</tr>
<tr>
<td></td>
<td>α = 82.915(2)°</td>
</tr>
<tr>
<td></td>
<td>β = 84.2630(10)°</td>
</tr>
<tr>
<td></td>
<td>γ = 86.2730(10)°</td>
</tr>
<tr>
<td>Volume</td>
<td>1021.87(6) Å</td>
</tr>
<tr>
<td>Z</td>
<td>2</td>
</tr>
<tr>
<td>Density (calculated)</td>
<td>1.804 Mg/m³</td>
</tr>
<tr>
<td>Absorption coefficient</td>
<td>1.654 mm⁻¹</td>
</tr>
<tr>
<td>F(000)</td>
<td>554</td>
</tr>
<tr>
<td>Crystal size</td>
<td>0.34 x 0.17 x 0.11 mm³</td>
</tr>
<tr>
<td>Theta range for data collection</td>
<td>2.574 to 31.670°</td>
</tr>
<tr>
<td>Index ranges</td>
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</tr>
<tr>
<td>Reflections collected</td>
<td>34395</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>6864 [R(int) = 0.0275]</td>
</tr>
<tr>
<td>Completeness to theta</td>
<td>99.9 %</td>
</tr>
<tr>
<td>Absorption correction</td>
<td>Semi-empirical from equivalents</td>
</tr>
<tr>
<td>Max. and min. transmission</td>
<td>0.7462 and 0.6549</td>
</tr>
<tr>
<td>Refinement method</td>
<td>Full-matrix least-squares on F²</td>
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<tr>
<td>Data / restraints / parameters</td>
<td>6864 / 0 / 310</td>
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<td>Goodness-of-fit on F²</td>
<td>1.083</td>
</tr>
<tr>
<td>Final R indices [I&gt;2σ(I)]</td>
<td>R1 = 0.0208, wR2 = 0.0419</td>
</tr>
<tr>
<td>R indices (all data)</td>
<td>R1 = 0.0259, wR2 = 0.0434</td>
</tr>
<tr>
<td>Largest diff. peak and hole</td>
<td>0.476 and -0.590 e.Å⁻³</td>
</tr>
</tbody>
</table>
Figure 6.19. The ORTEP solid state structure of 28 with atomic displacement parameters shown at 50% probability. Hydrogen atoms omitted for clarity.

The formula of 28 consists of a charge neutral compound with a solvent EtOH and two H$_2$O molecules. The hydroxamato ligands displays disordered arrangement with the phenyl rings occupying two positions. The antimony metal is chelated by two monodeprotonated ligands in a bidentate fashion, (Sb1-O9, Sb1-O10, Sb1-O20 and Sb1-O22). Intermolecular Sb···Cl and water molecules complete the coordination around the metal (Sb1···Cl2), giving a distorted octahedron geometry. Hydrogen bonding is substantial in this compound, with the coordinated water molecule linking two compounds as well as stabilizing the un-associated chloride atom. This chloride is also hydrogen bound to both the NH$_3^+$ and NH$_2$ groups (N1···Cl1 and N12···Cl1). Further stabilization is provided by the half occupied ethanol and lattice water molecule, Figure 6.20. Significant bond lengths and bond angles are shown in Tables 6.16 and Table 6.17.
Figure 6.20. Packing diagram of 28. Hydrogen atoms omitted for clarity. Dashed lines indicate strong hydrogen bonding.

Table 6.16. A list of the significant bond lengths of 28.

<table>
<thead>
<tr>
<th>Atoms</th>
<th>Dist (Å)</th>
<th>Atoms</th>
<th>Dist (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sb(1)-O(9)</td>
<td>2.1826(9)</td>
<td>Sb(1)···O(1s)</td>
<td>3.04974(8)</td>
</tr>
<tr>
<td>Sb(1)-O(11)</td>
<td>2.0562(10)</td>
<td>O(1s)···Cl(2)</td>
<td>3.1551(12)</td>
</tr>
<tr>
<td>Sb(1)-O(20)</td>
<td>2.1584(9)</td>
<td>O(1s)···Cl(1)</td>
<td>3.0603(12)</td>
</tr>
<tr>
<td>Sb(1)-O(22)</td>
<td>2.0413(10)</td>
<td>N(1)···Cl(1)</td>
<td>3.1436(12)</td>
</tr>
<tr>
<td>Sb(1)···Cl(2)</td>
<td>3.20364(9)</td>
<td>N(12)···Cl(1)</td>
<td>3.5326(13)</td>
</tr>
</tbody>
</table>

Table 6.17. A list of the significant bond angles of 28.

<table>
<thead>
<tr>
<th>Angle Atoms</th>
<th>Angle (°)</th>
<th>Angle Atoms</th>
<th>Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(8)-O(9)-Sb(1)</td>
<td>112.75(8)</td>
<td>O(11)-Sb(1)-O(20)</td>
<td>80.45(4)</td>
</tr>
<tr>
<td>N(10)-O(11)-Sb(1)</td>
<td>113.66(8)</td>
<td>O(20)-Sb(1)-O(9)</td>
<td>145.77(4)</td>
</tr>
<tr>
<td>C(19)-O(20)-Sb(1)</td>
<td>113.14(8)</td>
<td>O(22)-Sb(1)-O(9)</td>
<td>80.13(4)</td>
</tr>
<tr>
<td>N(21)-O(22)-Sb(1)</td>
<td>113.27(7)</td>
<td>O(22)-Sb(1)-O(11)</td>
<td>90.35(4)</td>
</tr>
<tr>
<td>O(11)-Sb(1)-O(9)</td>
<td>75.49(4)</td>
<td>O(22)-Sb(1)-O(20)</td>
<td>76.00(4)</td>
</tr>
</tbody>
</table>
In the literature there is only one reference to antimonial hydroxamato compounds, Sb(V) compounds of formula \([\text{Sb(Bha}_{1\text{H}})(\text{OMe})\text{Ph}_3]\) and \([\text{Sb(Bpha}_{1\text{H}})\text{Ph}_3\text{Cl}]\), Figure 6.21.\(^{16}\) The Sb(V) centres in these compounds are also octahedrally coordinated with much less distortion than seen in 26-28. The room temperature hydroxyl and carbonyl Sb-O bond lengths of these compounds are in adherence with what was observed in 26-28.

![Figure 6.21](Image)

**Figure 6.21** Previously synthesised Sb(V) compounds of \([\text{Sb(Bha}_{1\text{H}})(\text{OMe})\text{Ph}_3]\) and \([\text{Sb(Bpha}_{1\text{H}})\text{Ph}_3\text{Cl}]\).

The crystal structures of these compounds have been elucidated and the bond angles and bond distances are in accordance with our solved structures. Interestingly these compounds were developed as potential anti-cancer agents.

### 6. Stability Testing

The stability of the compounds 26, 27, 28, 29 and 31 as solids were assessed by performing \(^1\text{H}\) NMR analysis on the same solid samples one, three and six months apart.

The stability of these compounds in solution in D\(_2\)O were assessed by performing \(^1\text{H}\) NMR analysis on all the compounds. Data was collected regularly over a period of one month. The \(^1\text{H}\) NMR spectra showed no variation in signals over the time period studied, which indicates good stability in the solid state and in solution in D\(_2\)O.
7. Biological Testing

All the compounds in this chapter are the first reported example of a Sb(III) hydroxamato chlorido compound specifically designed to inhibit the survival of *Leishmania* promastigotes. This class of compound may provide novel and effective means of treating current resistant strains of the parasite. This work was carried out by the group of Prof Dos Santos. Two strains, *Leishmania amazonensis* (MHOM/BR/PH8) and *Leishmania chagasi* (NCL/UFRJ/IOC-L 3241 strain) were chosen to test the activity of our compounds. Compounds 26, 27, 28, 29 and 31 along with their free hydroxamic acids and a standard antimonial compound, Antimony Potassium Tartrate (APT), were tested for their multiplication inhibition ability (*n*=3) and their IC$_{50}$, the concentration at which 50% growth inhibition was achieved.

**Multiplication Inhibition Assay**

The activity of compounds were evaluated upon the growth rate of *L. amazonensis* and *L. chagasi* promastigote forms by incubation in Schneider’s insect medium with 10% FBS at 28°C. Briefly, promastigotes were counted using a Neubauer chamber and resuspended in fresh medium to a final concentration of $5 \times 10^5$ viable promastigotes per mL. The viability was assessed by mobility and lack of staining after challenging with Trypan blue. Compounds 26, 27, 28, 29 and 31 along with their free hydroxamic acids and a standard antimonial compound, APT, were investigated for their effect on the growth of promastigotes in order to establish the differences in susceptibility among the two species. Each compound was added to the culture at final concentrations in the 5-100 µM range and the APT was added to the culture at final concentrations in the 5-60 µM. After 24, 48, 72 and 96 h of incubation at 28 °C, the number of viable, motile promastigotes was quantified. The IC$_{50}$ was determined as the minimum drug concentration that caused a 50% reduction in survival/viability. This IC$_{50}$ was calculated by linear regression analysis by plotting the number of viable promastigotes versus log drug concentration using Origin Pro 7.5 computer software. The IC$_{50}$ values are shown in Table 6.18.

All compounds tested inhibited growth of *L. amazonensis*. All antimony compounds displayed better or equal growth inhibition of *L. amazonensis* than their corresponding ligand. Regarding activity against a specific strain, compounds 28 and
29 had better observed activity against *L. chagasi* than against *L. amazonesis*, a trend which is mirrored in the free ligand. Compound 26, 27 and 31 along with their ligands (except 2-Pyha) are more active against *L. amazonesis*.

In relation to inhibition of *L. chagasi* proliferation, all the antimonial compounds and free ligand show similar activities for inhibition of growth in the range 15-70 µM. In relation to the standard antimonial, APT, all our compounds and ligand show comparable activity.

### Table 6.18. IC₅₀ value of the compounds.

<table>
<thead>
<tr>
<th>Compounds</th>
<th><em>L. amazonensis</em> IC₅₀ (µM)</th>
<th><em>L. chagasi</em> IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bha</td>
<td>25.9</td>
<td>32.2</td>
</tr>
<tr>
<td>26</td>
<td>9.5</td>
<td>15.3</td>
</tr>
<tr>
<td>2-Pyha</td>
<td>71.5</td>
<td>70.8</td>
</tr>
<tr>
<td>27</td>
<td>33.2</td>
<td>43.1</td>
</tr>
<tr>
<td>2-NH2-Pha</td>
<td>57.3</td>
<td>51.3</td>
</tr>
<tr>
<td>28</td>
<td>35.1</td>
<td>34.2</td>
</tr>
<tr>
<td>Sha</td>
<td>60.3</td>
<td>55.3</td>
</tr>
<tr>
<td>29</td>
<td>22.9</td>
<td>19.3</td>
</tr>
<tr>
<td>SAHA</td>
<td>36.0</td>
<td>49.1</td>
</tr>
<tr>
<td>31</td>
<td>18.1</td>
<td>24.3</td>
</tr>
<tr>
<td>APT</td>
<td>19.8</td>
<td>25.1</td>
</tr>
</tbody>
</table>

Compound 26 and its related ligand, Bha, display the best activity against both strains of *Leishmania*, showing superior activity with an IC₅₀ of half that (9.5 µM) of APT (19.8 µM) against *L. amazonesis* and display far superior activity against *L. chagasi* with an IC₅₀ of 15.3 µM compared to that of APT of 25.1 µM. Compounds 27, 28, 29 and 31 possess reasonably good IC₅₀ values (18-35 µM) in comparison to APT against both strains.

31 was specifically chosen to provide dual functionality against strains of *Leishmania*. It possesses an antimonial moiety which has well-known antileishmanial properties and also possesses an HDACi moiety, SAHA, which has been
shown to be an effective inhibitor of HDAC proteins, of which classes I and II are present in *Leishmania* parasites.

The ligand which displayed the best activity of the five tested is Bha. This was not expected to be the case. It has been anticipated that the SAHA ligand would display the best activity as it is a well-known, FDA approved HDACi. However Bha is observed to have slightly better activity than SAHA. This could be due to a number of reasons. Solubility of compounds is known to have an impact on activity, in addition to uptake and stability of the compounds in physiological conditions.

Another possible reason for the heightened activity of Bha is that it may be acting on a secondary target. There is evidence in the literature that Bha is an effective ribonucleotide reductase (RNR) inhibitor.\(^\text{17}\) RNR catalyses the reduction of ribonucleotides to deoxyribonucleotides, the first and rate-limiting step for *de novo* synthesis of 2-deoxyribonucleoside 5-triphosphates. RNR is found in all living organisms as well as in some dsDNA viruses. Thus, RNR is a potential antimicrobial drug target in a wide variety of organisms and have been at the centre of development of effective anti-leishmanial treatments, as well as the basis for novel antibiotics against *Mycobacterium tuberculosis* and anti-virals. Hydroxamic acid based compounds are effective metal chelators and since a ferric iron centre plays a key role in RNR activity, the metal-chelating capacity of Bha could explain its ability to inhibit RNR. It is therefore possible that Bha is inhibiting RNR and in turn compound \(^\text{26}\), could be exhibiting dual anti-leishmanial functionality, via the Sb(III) moiety and via Bha associated RNR inhibition.\(^\text{18}\)

To my knowledge, there are no previously reported Sb(III) hydroxamate/hydroximate compounds designed as potential anti-leishmanial agents. There are examples of Sb carboxylates, Bi thiocarboxylates, Bi thiketonates and Cu(II)/Zn(II) aminopyridyl anti-leishmanial compounds in literature, Figure 6.22. These compounds are structurally similar to our compound, and tend to coordinate in a similar manner through the bidentate fashion. No exact comparison can be made between these studies but it is possible to get an indication of how our compounds compare to recent work.
The activity shown by our ligands are comparable to that of the ligands used by Andrews et al. The thioketones and thiocarboxylic acids have an IC\textsubscript{50} value in the region of 50–100 µM, whereas our free ligands show IC\textsubscript{50} activity in the 25-75 µM range. In contrast, the activity shown by carboxylic acids is negligible, even at concentrations of 500 µg mL\textsuperscript{-1}. This suggests an important role for the hydroxamic acid group on observed toxicity.

Bi(III) thioketonates and Bi(III) thiocarboxylates are more active against \textit{L. major} promastigotes than our antimony compounds. Bi(III) thiocarboxylates have IC\textsubscript{50} values in the range 0.39–5 µM and Bi(III) thioketonates exhibiting lesser activity.\textsuperscript{19}

In relation to the antimony carboxylates, our compounds were as active against \textit{L. major}. Binding strength of the compounds can have an effect on the activities of these compounds. If the metal-ligand interaction is too strong then there is the inability of the ligand to release the metal for action. Further studies on this stability constants would be valuable.\textsuperscript{20, 21}

**Mammalian Cell Associated Toxicity**

In vitro cytotoxicity experiments were undertaken to establish if \textsuperscript{26, 27, 28, 29} and \textsuperscript{31} along with their free hydroxamic acids and a standard antimonial compound, Antimony Potassium Tartrate (APT), were toxic to the RAW 264.7 (murine macrophage) cell line. Stock solutions of compounds were prepared fresh in the appropriate culture medium and diluted to the various working concentrations of 2-500 µM.

Macrophage toxicity was determined by the MTT assay, a colorimetric based test for assessing cell metabolic activity. It is based on the ability of succinic dehydrogenase enzyme in the cell to reduce the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-
diphenyltetrazolium bromide (MTT) to its insoluble formazan, which has a purple colour, Scheme 6.10. From the assay, \( CC_{50} \) which is defined as the 50% cytotoxicity inhibitory concentration was determined by linear regression analysis.

![Scheme 6.10](image)

Scheme 6.10. Scheme demonstrating the conversion of MTT into insoluble formazan by dehydrogenase enzymes found in metabolically active cells.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>( CC_{50} ) values (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bha</td>
<td>486</td>
</tr>
<tr>
<td>28</td>
<td>4.8</td>
</tr>
<tr>
<td>2-Pyha</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>27</td>
<td>121</td>
</tr>
<tr>
<td>2-NH2-Pha</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>28</td>
<td>275</td>
</tr>
<tr>
<td>Sha</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>29</td>
<td>46.3</td>
</tr>
<tr>
<td>SAHA</td>
<td>22.6</td>
</tr>
<tr>
<td>31</td>
<td>5.5</td>
</tr>
<tr>
<td>APT</td>
<td>11.7</td>
</tr>
</tbody>
</table>

Table 6.19  Cytotoxicity (\( n = 3 \)) of compounds on RAW macrophages.

As expected, all the antimonials exhibited cytotoxicity on the cell line tested. It is also interesting that the pentavalent, clinically used antimonial exhibit cytotoxicity also. The full list of \( CC_{50} \) results are reported in Table 6.19. Four out of the five free ligands tested were demonstrated to be relatively non-toxic to the cell line with a \( CC_{50} \geq 486 \) µM. Compounds 27, 28, 29 and SAHA were far less toxic in comparison to the standard used, APT. Only compounds 26 and 31 displayed higher levels of toxicity than APT.
Selectivity Index

The selectivity index (SI) of our compounds, their ligands and APT were assessed for *Leishmania amazonensis* and *Leishmania chagasi* and the results shown in Table 6.20. For the purpose of calculating SI, CC$_{50}$ values greater than 500 were taken as being 500. The SI of the compounds was calculated as follows$^{22}$:

\[ \text{SI} = \frac{\text{CC}_{50} (\mu M)}{\text{IC}_{50} (\mu M)} \]

Bha has the highest SI for both strains of *Leishmania*. The analogues of this ligand, also showed relatively good SI. Given the low IC$_{50}$ value of Bha, coupled with the high SI, derivatisation at the 2 position on the phenyl ring does not confer increased activity. Given it is now know that the moieties at this position do not partake in binding of a metal centre, derivatisation at other positions may prove useful in increasing the activity, selectivity and bioavailability of these compounds.

With regards the SI of the Sb(III) compounds, 27-29 display a relatively good SI for the two *Leishmania* strains, perhaps due to the initial high SI of the free ligand. Compound 26, 31, SAHA and APT exhibit poor SI of < 0.6. The fact that SAHA has a poor SI is peculiar, as it was expected it to have a good cytotoxicity against the parasites while being relatively non-toxic to the human macrophages as it is an FDA approved HDACi.

### Table 6.20  Shown below are the IC$_{50}$, CC$_{50}$ (µM) ($n = 3$) and SI values.

<table>
<thead>
<tr>
<th>Compounds</th>
<th><em>L. amazonensis</em></th>
<th><em>L. chagasi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC$_{50}$</td>
<td>IC$_{50}$</td>
</tr>
<tr>
<td>Bha</td>
<td>486</td>
<td>25.9</td>
</tr>
<tr>
<td>26</td>
<td>4.8</td>
<td>9.5</td>
</tr>
<tr>
<td>2-Pyha</td>
<td>&gt; 500</td>
<td>71.5</td>
</tr>
<tr>
<td>27</td>
<td>121</td>
<td>33.2</td>
</tr>
<tr>
<td>2-NH$_2$-Pha</td>
<td>&gt; 500</td>
<td>57.3</td>
</tr>
<tr>
<td>28</td>
<td>275</td>
<td>35.1</td>
</tr>
<tr>
<td>Sha</td>
<td>&gt; 500</td>
<td>60.3</td>
</tr>
<tr>
<td>29</td>
<td>46.3</td>
<td>22.9</td>
</tr>
<tr>
<td>SAHA</td>
<td>22.6</td>
<td>36.0</td>
</tr>
<tr>
<td>31</td>
<td>5.5</td>
<td>18.1</td>
</tr>
<tr>
<td>APT</td>
<td>11.7</td>
<td>19.8</td>
</tr>
</tbody>
</table>
8. Conclusion

In conclusion, this chapter reports the synthesis and full characterisation of a novel class of Sb(III) hydroxamato chlorido compounds of general type \([\text{Sb}(\text{L}^{-1H})_2\text{Cl}]\) and \([\text{Sb}(\text{L}^{-1H})\text{Cl}_2]\) designed to and demonstrated to inhibit proliferation of \textit{Leishmania} protozoa. A novel Sb hydroxamate/hydroximate compound was also synthesised.

All the products were obtained by the reaction of one equivalent of antimony precursor with two equivalents of hydroxamic acids. Five different hydroxamic acids were used in the generation of these compound, Bha, 2-Pyha, 2-NH\textsubscript{2}-Pha, Sha and SAHA. Both Sha and Bha were commercially available and 2-NH\textsubscript{2}-Pha, 2-Pyha and SAHA (18) were synthesised as previously reported.

The X-ray crystal structures of three out of the five bismuth compounds were solved, with crystals being isolated after slow evaporation from the filtrate, methanol or ethanol. The structure elucidation of 26, \([\text{Sb}(\text{Bha}^{-1H})_2\text{Cl}]\) revealed two hydroxamato ligands coordinated via a bidentate non-bridging (O, O’) mode. There was however the possibility of a dimer forming with long range interactions of adjacent Sb atoms and hydroxamic hydroxyl oxygen atoms.

Compound 27, the dinuclear Sb compound \([\text{Sb}_2(\mu-\text{Cl})(\text{Cl})_2(2-\text{Pyha}^{-1H})_2]\) was synthesised featuring only one coordination mode of the hydroxamic acid; bidentate non-bridging (O, O’). This compound displayed two different chlorido binding modes, bridging and non-bridging.

The structure of 28, \([\text{Sb}(2-\text{NH}_2-\text{Pha}^{-1H})(2-\text{NH}_3-\text{Pha}^{-1H})\text{Cl}_2]\) possesses a charge neutral 2-aminophenyl hydroxamic acid ligand containing a positively charged amino group and a monodeprotonated hydroxamic acid group.

These three compounds, although generated by the same reaction conditions, are distinctly different and interesting structures.

The stabilities of all the compound were tested as solids and in solution in D\textsubscript{2}O. In both cases, the compounds were observed to be stable over the period at which they were tested.
Compound 26, 27, 28, 29 and 31 were assessed for their anti-proliferative activity against two strains of *Leishmania, L. amazonensis* and *L. chagasi*. Each compound and their free ligand were tested at a concentrations range of 5-100 µM and the antimonial standard, APT, was tested in the concentration range of 5-60 µM.

All compound were observed to show superior activity than their free ligands against *L. amazonensis*, with the exception of compound 28 which showed equal potency with its 2-NH2-Pha ligand. In relation to *L. chagasi*, both the ligand and the compounds displayed similar levels of activity. All compounds showed activities similar to those observed for APT.

Compound 26 and 31, which possess Bha and SAHA ligands respectively, displayed superior IC$_{50}$ values in comparison to APT for both strains of *Leishmania*, and should be investigated further to assess their potential as ant-leishmanial agents.

Mammalian Cell Associated Toxicity was assessed for these compounds against RAW 264.7 (murine macrophage) cell lines by the MTT assay method. Using this method the CC$_{50}$ was determined by linear regression. All antimonial based drugs showed toxicity towards this cell line but compounds 27, 28 and 29 showed promising IC$_{50}$ values in comparison to the clinically used antimonial drug, and also had a better selectivity profile, which were calculated using the IC$_{50}$ and CC$_{50}$ values.
Chapter 6

9. Future Plans

Further development and investigation of the potential of compound 26 and 31 as anti-leishmanial agents should be carried out. Derivatisation of these compounds may increase activities, and bioavailability while reducing associated toxicity and increasing the selectivity. Examination into the reason for the heightened activity of Bha may also provide a novel route of treatment. The Sb(III) compound of Bha was the most active out of all the test compounds. If, as tentatively proposed, the Bha is acting at the RNR target, an investigation into other RNR inhibitors as ligands for Sb(III) compounds may prove successful in the development of novel and effective treatments of leishmaniasis.

An attempt to derivatise SAHA as 3-NH₂-SAHA and 2-NH₂-SAHA, ultimately failed, as the required product was not obtained. In an ongoing attempt to improve the bioavailability of this compound, other derivatisations could be effective, which may lead to an increased parasitic toxicity profile and may also help reduce the mammalian cytotoxicity.

It was also noted that recently, in an effort to increase the efficacy of the drug while also circumventing the problems with resistance and cytotoxicity, antimonial drugs are being administered in their Sb(V) form. This is the prodrug form of the compounds, which are reduced *in vivo* to their toxic Sb(III) form. It is believed that the mechanism of action involves reduction of the Sb(V) species to Sb(III) inside the parasite cells promoted by for example trypanothione which works in conjunction with trypanothione reductase (TR) at a cellular pH of ca. 5.²³ Lower doses of compound can be administered in this way hence the problematic side effects often associated with Sb treatment can be reduced. Investigations into the oxidation of the reported Sb(III) compounds to their corresponding Sb(V) analogues could be carried out in an attempt to increase their activities and reduce their cytotoxicity profile, hence increasing also the SI.

Further investigations into the toxicity of the Sb(III) compounds against human dermal fibroblasts and kidney cell lines may also provide important information on toxicity.²⁴
References


Chapter 6

Thesis Conclusion

This thesis was focused on the development of metallohydroxamates as novel antibacterial and anti-leishmanial agents. These metallohydroxamates comprised of hydroxamic acids bound to different metals including bismuth, gallium and antimony.

The first results chapter investigated the synthesis and anti-\textit{H. pylori} activity of novel Bi(III) hydroxamato nitrato complexes, of general formula \([\text{Bi}(L-1\text{H})_2\text{NO}_3]\), against a range of \textit{H. pylori} strains. These complexes were designed to exhibit a dual mode of anti-\textit{H. pylori} action. On being hydrolysed in low pH environments, the liberated hydroxamic acid can inhibit urease, an enzyme which is essential to the survival of the bacteria in the stomach, while the resultant bismuth complex can exhibit the accepted gastroprotective and antimicrobial properties associated with Bi. There is an urgent need for structurally well-defined active anti-\textit{H. pylori} complexes, given the structures of the bismuth complexes in use today are unknown. The Bi(III) complexes developed showed good urease inhibitory activity and were effective in the inhibition of \textit{H. pylori} growth \textit{in vitro}. These bismuth complexes are therefore a novel class of potential anti-\textit{H. pylori} agents.

In addition, it was demonstrated in the second results chapter that bismuth complexes possess anti-bacterial properties beyond just the Gram-negative \textit{H. pylori}. A novel class of polymeric Bi(III) hydroxamato chlorido complexes were developed and demonstrated to be active against both Gram-negative and Gram-positive environmental and pathogenic bacteria. There is an urgent need for effective and novel class of anti-bacterial agents given the last class to be brought to the clinic was over 40 years ago. The incorporation of bismuth into treatment regimens to reactivate or enhance the activity of failing antibiotics and in turn effectively treat bacterial infections should be investigated.

In the third results chapter, an investigation into the use of bismuth and gallium hydroxamate complexes as anti-bacterial agents against antibiotic resistant strains of \textit{E. coli} and \textit{S. aureus} was undertaken. A proton dissociation and gallium complexation study was carried out on a novel retro PDF inhibitor, GSK322. Ga(III) and Bi(III) complexes with the retro hydroxamic acid HFA were successfully
synthesised and their activity, along with the activity of GSK322 assessed against four bacterial strains investigated; two Gram-negative E. coli strains, incorporating an ESBL resistant clinical strain and two Gram-positive strains, one of which was an MRSA strain. The results from this work indicate that these complexes have a potential to be administered in combination with traditional medicine as a means of preventing the occurrence or prevalence of anti-bacterial resistance. As no resistance to bismuth based therapies for H. pylori treatment have arisen to date, this bodes well for the use of bismuth as a treatment to prevent resistance in other bacterial diseases.

Bismuth and gallium GSK complexes were not synthesised. Alternative metallo complexes of GSK should be developed and investigated for their anti-bacterial activity.

It is apparent that bismuth chemistry is not as well established or predictable as for example the chemistry of iron or platinum. In turn X-ray crystal structures of Bi complexes described in Chapters 3 and 4, highlight the difficulty in predicting the exact structures of Bi(III) complexes and in turn the importance of elucidating the solid state structures of representative members of novel classes of Bi complexes.

In the final chapter, Sb(III) hydroxamato chlorido and Sb(III) hydroximato/hydroxamato chlorido compounds were assessed for their activity as anti-leishmanial agents. These novel compounds were synthesised and structurally characterised. They were then tested for their anti-leishmanial activity against two strains of Leishmania, and the results showed that all antimony based compounds were active against the strains with good IC_{50} values, comparable to those of the antimonial standard APT. The compounds were also assessed for their macrophage cytotoxicity and in turn their SI was calculated. The Sb Bha compound, [Sb(Bha-1H)_{2}Cl], exhibited the best activity and noteworthy better activity than the Sb HDAC inhibitor compound [Sb(SAHA-1H)(SAHA-2H)]. The results of this work suggest that the use of antimonial hydroxamate compounds could potentially be used in combination with other anti-leishmanial treatments to prevent the development of resistance and decrease the associated toxicity through the use of lower doses of antimonial drugs.
This thesis supports and builds on previous work in relation to the important and versatile role hydroxamic acids play as bioligands. Furthermore it highlights the role metal complexes can continue to play as therapeutic agents. Significantly metallohydroxamates exhibit noteworthy anti-bacterial activity and anti-leishmanial activity and merit further development.
Appendix:

NMR ($^1$H, $^{13}$C), IR, MS and Crystal Structure data of isolated compounds
1. Organic Compounds

2-NH$_2$-Pha (1)
2-Pyha (5)
Appendix

$N$-hydroxy-$N$-phenylformamide (HFA)  (10)
Appendix

Oxonane-2,9-dione (16)
8-oxo-8-(phenylamino)octanoic acid (17)
Suberanilohydroxamic acid (SAHA) (18)
Methyl 8-((3-nitrophenyl)amino)-8-oxooctanoate (19)
8-((3-nitrophenyl)amino)-8-oxooctanoic acid (20)
$N^1$-(benzyloxy)-$N^8$-(3-nitrophenyl)octanediameide (21)
Methyl 8-((2-nitrophenyl)amino)-8-oxooctanoate  (23)
Methyl 8-((2-aminophenyl)amino)-8-oxooctanoate (24)
2. Inorganic Compounds

\[ \text{[Bi}_2\text{(Bha-H)}_2(\mu\text{-Bha-H})_2(\eta^2\text{-NO}_3)_2] } \quad (2) \]
[Bi(NO$_3$)(Sha-1H)$_2$]$_n$  (3)
\[ [\text{Bi}_6(\text{CH}_3\text{OH})_2(\eta^1-\text{NO}_3)_2(\eta^2-\text{NO}_3)_2\text{H}_2\text{O}_2(\text{Sha-1H})_{12}] \] (3a)
[Bi(2-NH$_2$-Pha.H)$_2$(NO$_3$)$_n$]$_n$ \hfill (4)
\[ \text{[BiCl}_2(\mu-\text{Sha}1\text{H})]_n \] (6)
Appendix

\[ [\text{BiCl}_2(\mu-\text{Sha}-1\text{H})(\text{THF})].\text{THF}_\infty \, \ (6a) \]

![Diagram and spectrum image]
$[\text{BiCl}_3(2-\text{NH}_3-\text{Pha-1H}) \text{]}_n \quad (7)$
[BiCl₃(2-Py⁺₁Ha⁻₁H)₂.₂H₂O]ₙ  (8)
BiPh₃ (11)
Appendix

\[ \text{[Bi(HFA-HH)₃]} \quad (12) \]
Appendix

\[ \text{[Ga(HFA-1H)\textsubscript{3}]H}_2\text{O} \quad (13) \]
Appendix

[Sb(Bha$_{111}$)$_2$Cl]  (26)
[Sb₂(μ-Cl)₂(Cl)₂(2-Pyha·1H)₂] (27)
Appendix
[Sb(2-NH$_2$-Pha$_{1H}$)(2-NH$_3$-Pha$_{1H}$)]Cl$_2$ (28)
[SbCl(Sha\textsubscript{1H})\textsubscript{2}] (29)
Attempted Synthesis of $[\text{SbCl(SAHA}_{\text{1H}})_2]$ (30)
\[ \text{[Sb(SAHA}_{1\text{H}})(\text{SAHA}_{2\text{H}})] } \quad (31) \]