SYNTHESIS, CHARACTERISATION AND EVALUATION OF NOVEL FERROCENE-THIAZOLE DERIVATIVES AS ANTIPLASMODIAL AGENTS

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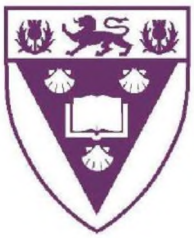

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ABSTRACT

Malaria is mosquito-transmitted disease which continues to pose threat to humanity, despite the efforts undertaken by the scientific community, government entities and international organizations. The major problem is that *Plasmodium* species have developed resistance against available drugs. In order to counter this problem, antimalarial drugs that are efficacious and with novel mode of action are of great necessity.

Thiazole derivatives, in particular aminomethylthiazole analogues, have been shown to exhibit promising antimalarial activity against *Plasmodium falciparum* strains. Previous studies reported the hit compound **MMV010539**, which showed good antimalarial activity against both K1 (CQ and multidrug resistant strains) and NF54 (CQ sensitive strain). In this study, **MMV010539** was deemed to be as an attractive compound to generate novel analogues by addition of ferrocenyl organometallic unit. The ferrocene based compounds have shown biological activity; and with ferroquine currently in clinical trials there has been increasing research into identifying new ferrocenyl-containing molecules as potential antimalarial agents.

Herein, thiazole ferrocene based molecules **3.22a-e** were synthesised in low to good yields. Their structural identities were confirmed using conventional spectroscopic techniques (^{1}H and ^{13}C NMR, FT-IR spectroscopy and mass spectrometry). The cell cytotoxicity assay of all final compounds confirmed that all ferrocene-thiazole blends **3.22a-e** were non-toxic against HeLa cell lines. However, the *in vitro* biological assay revealed that despite the absence of cell cytotoxicity these compounds poorly inhibited the growth of *Plasmodium falciparum* parasite. As the aim was to expand further the structure-activity relationship (SAR) of **MMV010539**, this study confirmed the previous findings that there is a limited structural modification that could be accommodated as indicated in **Figure 3.3** (Panel C). Moreover, the combination of ferrocenyl moiety and various alkylamines resulted in compounds with poor antiplasmodial potency, further suggesting that the free amine (Panel A, **Figure 3.3**) is important for activity.

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LIST OF ABBREVIATIONS

δ	Chemical shift
(MMV)	Medicines for Malaria Venture
µM	Micromolar
ACT	Artemisinin-based combination therapy
AIDs	Acquired immune deficiency syndrome
Amodiaquine	AQ
ATQ	Atovaquone
$CDCl_3$	Deuterated chloroform
CNS	Central nervous system
Cp	Cyclopentadienyl
CQ	Chloroquine
CQR	Chloroquine resistant
CQS	Chloroquine sensitive
d	Doublet
DCM	Dichloromethane
DDT	Dichoro diphenyltrichloroethane
DIPEA	Diisopropylethylamine
DMAP	Dimethylamine pyridine
DMF	Dimethylformamide
DMSO-d_6	Deuterated dimethylsulfoxide
EDCI.HCl	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
E°	Standard electrode potential
ESI-MS	Electron spray ionozation mass spectrometer
Et_2O	Diethylether
EtOAc	Ethyl acetate
EtOH	Ethanol
FCZ	Fluconazole

FT-IR	Fourier transform infrared spectroscopy
g	Gram
gp	Glycoprotein
h	Hour
Hb	Haemoglobin
HIV	Human immunodeficiency virus
HOBt	Hydroxybenzotriazole
HRMS	High resolution mass spectrometer
HTS	High throughput screening
IC_{50}	50% inhibitory concentration
IRS	Indoor residual spraying
J	Spin-spin coupling constant
LLINs	Long-lasting insecticidal nets
LogP	Logarithm of partition coefficient
LSM	Larval source management
m	Multiplet
m.p	Melting point
MDR	Multidrug resistance
MeCN	Acetonitrile
MeOH	Methanol
MFQ	Mefloquine
MHz	Megahertz
MIC	Minimum inhibitory concentration
mL	Milliliter
MTB	*Mycobacterial tuberculosis*
NCEs	New chemical entities
°C	Degree Celsius
P	*Plasmodium*
pH	Potential of hydrogen
PPh_3	Triphenylphosphine
ppm	Parts per million

PTLC	Preparative thin layer chromatography
QN	Quinine
RAGE	Receptor for advanced glycation end products
rt	Room temperature
s	Second
s	Singlet
SARS	Structure activity relationship studies
SCE	Saturated Calomel Electrode
SERM	Selective estrogen receptor modulators
t	Triplet
TB	Tuberculosis
TBTU	O-(Benzotriazol-1-yl)-*N,N,N′,N′*-Tetramethyluronium Tetrafluoroborate
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TOF MS	Time of flight mass spectrometer
UV	Ultraviolet
WHO	World Health Organization

DEDICATION

TO MY MOM

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Malaria: Burden and global distribution

Malaria is a disease caused by a protozoan parasite of the genus *Plasmodium* species. It is transmitted by a female *Anopheles* mosquito after the bite during feeding of human blood.[1, 2] This happens from dusk to dawn, a time of the day that is ideal for a next life cycle. The disease remains one of the major causes of deaths worldwide and puts the lives of 3.3 billion of world's population at risk.[3] According to WHO report in 2013, malaria was responsible for 627 000 malaria deaths worldwide in 2012. This number dropped to 584 000 malaria deaths in 2013.[4] The WHO malaria report 2015 estimated that there were 438 000 deaths in 2014. Of the estimated deaths, most occur in Sub-Saharan Africa (90%) and in children under 5 year of age (77%).[5] Despite the improving statistics with regards to mortality, the emergency of mutant drug resistance strains particularly to Artemisinin based therapies calls for urgent need for research to find novel candidates to control the advancing of this disease.[6]

1.2 The life cycle of *Plasmodium falciparum* parasite

Plasmodium falciparum is one of the five species of protozoan parasites that infect humans and the most dangerous, the others are: (*P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*). The life cycle of the malaria parasite is depicted in Figure 1.1.[2] It comprises the asexual and sexual phases. The lifespan of this organism requires both human and mosquito. Briefly, during the blood meal the *Anopheles* mosquito injects infectious sporozoites into the human (1). The sporozoites injected enter the blood stream and end up to the liver (2).[2] Over a period of two weeks, the sporozoites develop and divide to produce tens of thousands of the haploid form, known as merozoites (3). Some malaria parasite species (*p.ovale*) remain dormant for extended periods in the liver, which are potential to cause relapses when the illness is not well treated.[2] The active merozoites move to the bloodstream, where many processes take place: invasion of red blood cells happens, asexual replication, and release of newly formed merozoites in the red blood cells repeatedly over 1 – 3 days. The parasite reproduction results in thousands of parasite-

infected cells in the host, leading to illness and complications that can result in deaths if not treated.[2]

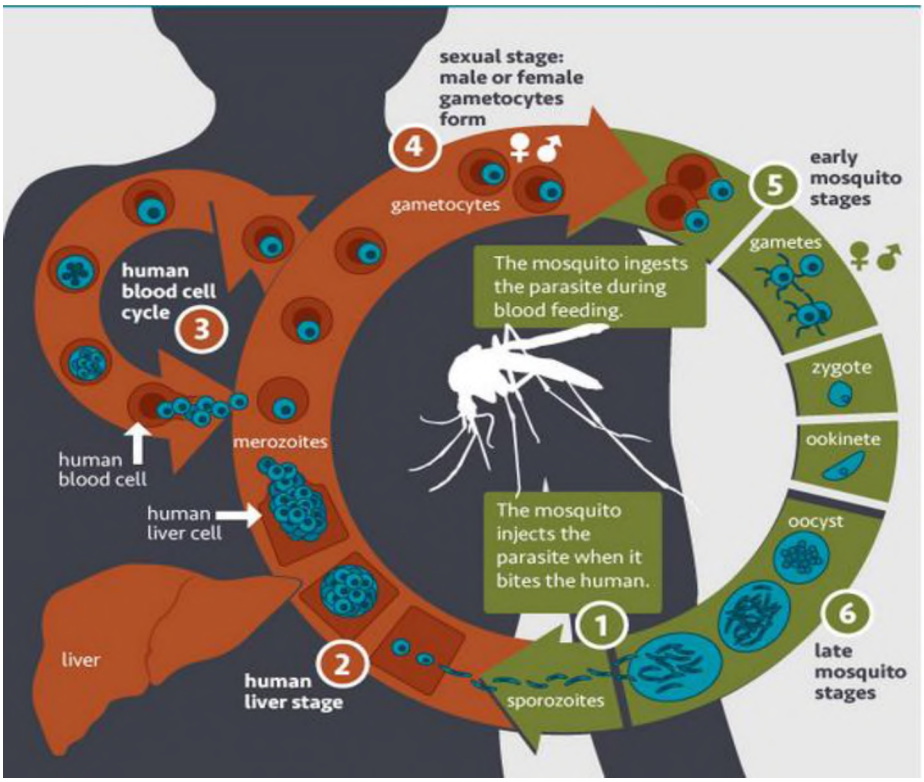


Figure 1.1: The malaria parasite life cycle.[2]

Some of the merozoite-infected blood cells leave the cycle of asexual multiplication. Instead of replicating, the merozoites in these cells develop into sexual forms of the parasite, called male and female gametocytes **(4)**.[2] During a next meal, the mosquito ingests gametocytes from human infected red blood cells. These cells burst in the midgut of the mosquito releasing the gametocytes, which develop further into mature gametes **(5)**. The fusion of male and female gametes form diploid zygotes, which develop into actively moving ookinetes that burrow into the mosquito midgut wall and form oocysts **(6)**.[2] Growth and division of each oocyst produce thousands of active haploid forms called sporozoites. After 8−15 days, the oocyst bursts and release sporozoites into the body cavity of the mosquito, from which they travel to and invade the mosquito salivary glands. The cycle re-starts when the mosquito takes a blood meal from a human, injecting the sporozoites from its salivary glands into the bloodstream.[2]

1.3 Measures for combating malaria

As mentioned earlier, malaria is transmitted through the bite of the responsible vector, the Anopheles mosquito. Control and prevention measures available to manage the spread of the parasites include, long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), and larval source management (LSM) to recite some.[7] Mosquito-nets contribute in the reversal of malaria spreading, with WHO and its partners putting emphasis in a wide distribution, mostly in countries where the disease is endemic. In 2014, the WHO reported the distribution of LLINs among the exposed population to curb the incidence of the disease. In 1997, countries with ongoing malaria transmission had ITNs distributed to residents free of charge, while 85 of those countries distributed ITNs or LLINs to all age groups (**Figure 1.2**).[2]

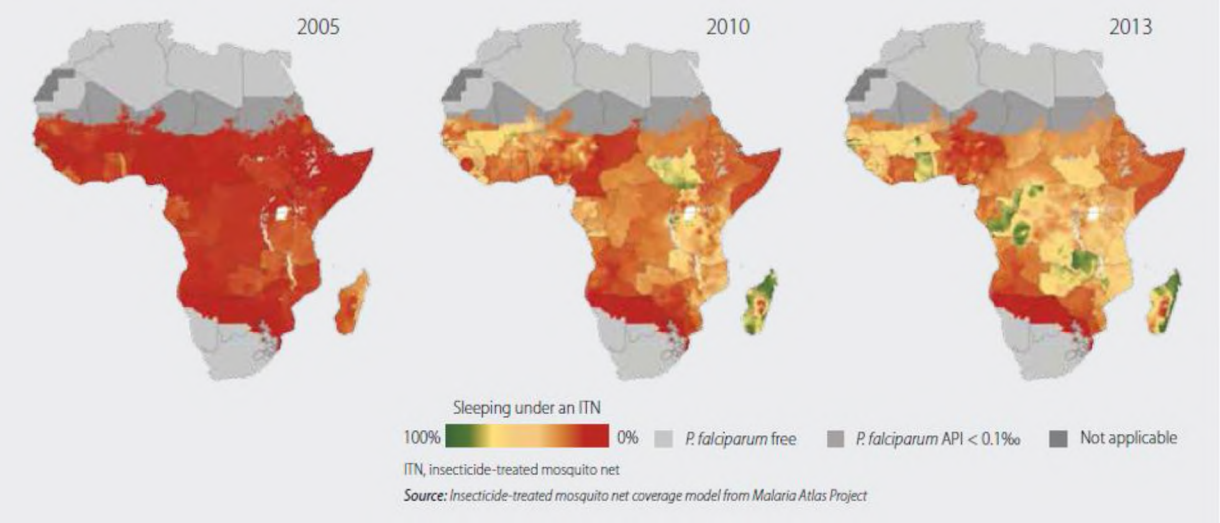


Figure 1.2*:* Distribution of a population sleeping under ITN, sub-Saharan Africa[2]

The development of resistance by parasite vector has jeopardized available control strategies and some insecticides are no longer efficient to kill the mosquitoes. Therefore, drugs that target the transmission and mosquito stages remain as important tools to prevent the infection of humans and treatment of the disease.[1] Despite efforts in malaria transmission control, infections continue to occur due to many factors. Apart from mosquito resistance to available insecticides, the banning of effective ones due to environmental impact contributed also to the spread of new cases of infections. Besides insecticide resistance, inefficiency, and lack of access to ITNs and IRS is also another cause of continuing transmission. Prophylaxis can contribute to combat the

ongoing transmission, but it is only effective in low transmission countries. This preventive measure is used by people traveling to malaria endemic areas for a short period. Different antimalarial drugs are used as prophylactic agents depending on their half-life and the desired effect. For instance, mefloquine (MFQ) as combination of atovaquone (ATQ) and proguanil hydrochloride are some of the drugs that can be used for chemoprevention purposes.

1.4 Chemotherapeutic and chemoprevention intervention

1.4.1 Malaria chemotherapy

Malaria remained a mystery until the discovery of the disease vector and the causative agent in the late 1890s.[8] In the 17th century, the first attempt to treat malaria came to light with indigenous people using *Cinchona* tree to treat malarial infections.[9] To date, chemotherapy remains an important tool to combat malaria infections in endemic tropical regions.[9]

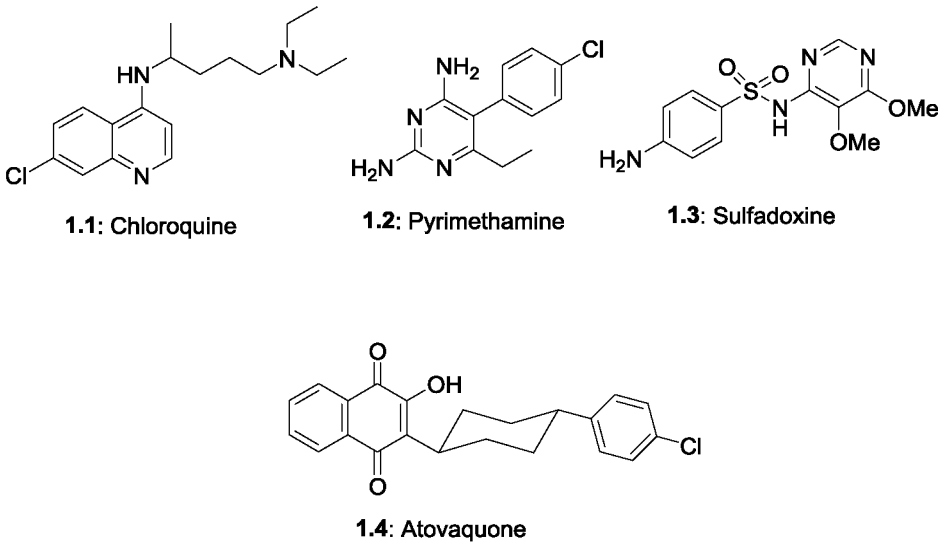


Figure 1.2: Chemical structure of current standard antimalarial drugs.[1]

Quinoline–containing antimalarial drugs, particularly chloroquine (CQ, **1.1**), were amongst the first effective drugs for treatment of malaria. Quinoline derivatives are mostly divided into three main classes: 4aminoquinolines, quinine methanols and 8–aminoquinolines. Both Chloroquine grouped in 4–aminoquinolines and other entities from the above mentioned groups are derived from quinine which is in turn extracted from the cinchona tree. Chloroquine(**Figure 1.1**) became an important drug due to its affordability in low and medium income countries[6]. The aminoquinoline derived antimalarial molecules are believed to act by interfering with haemozoin formation, thereby preventing haeme polymerization.[9] However, the emergence of resistance against this class of compounds overshadowed their success.

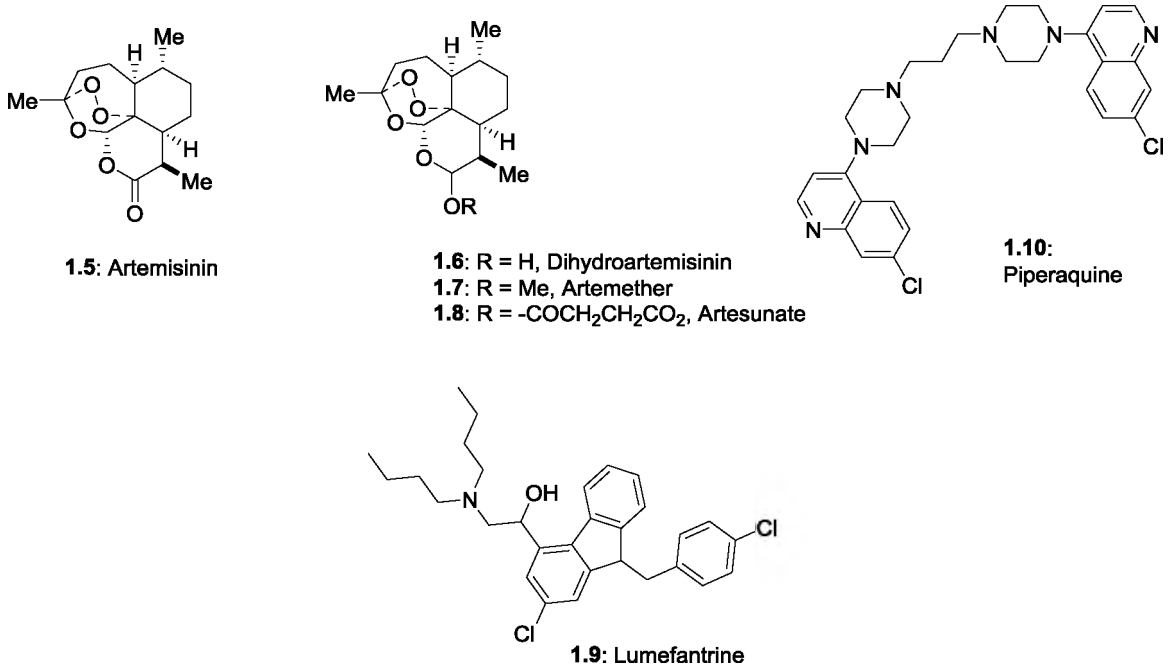


Figure 1.3: Chemical structure of artemisinins and partner drugs

Pyrimethamine is one of the antimalarial drugs that exert its action on the primary tissue phase or the pre-erythrocytic form of the most virulent malaria parasite. Primethamine/sulfadoxine combination also known as Fansidar is an effective cure for malaria.[1] In order to counteract the resistance of the malaria, the above mentioned combination can be recombined with artesunate (**1.8**).[1] The artemisinin-based combination therapy (ACTs) has completely replaced this combination, due to resistance and side effects associated with the former combination. Artemisinin (**1.5**), a sesquiterpene lactone extracted from a Chinese wormwood (*Artemisia*

annua) in 1970s that was once used to treat malaria and fever, is now recommended in the form of ACTs as a standard of care for uncomplicated malaria.[7] In addition, artemisinin (**1.5**) and its derivatives act by stopping the onset of malaria faster compared to other drugs, and are cleared from the host bloodstream in a short time ($t_{1/2}$ < 60 min). For this reason artemisinins (**1.5** – **1.8**) are used with other slow clearing drug (e.g. Lumefanrtine, **1.9** and Piperaquine **1.10**) to provide efficient treatment and prevent relapse.[1] Other combinations such as artesunate (**1.4**)/pyrimethamine (**1.2**)/sulfadoxine (**1.3**) are highly effective in some endemic parts, but in areas where there is resistance to a partner drug their use is negatively impacted.[6]

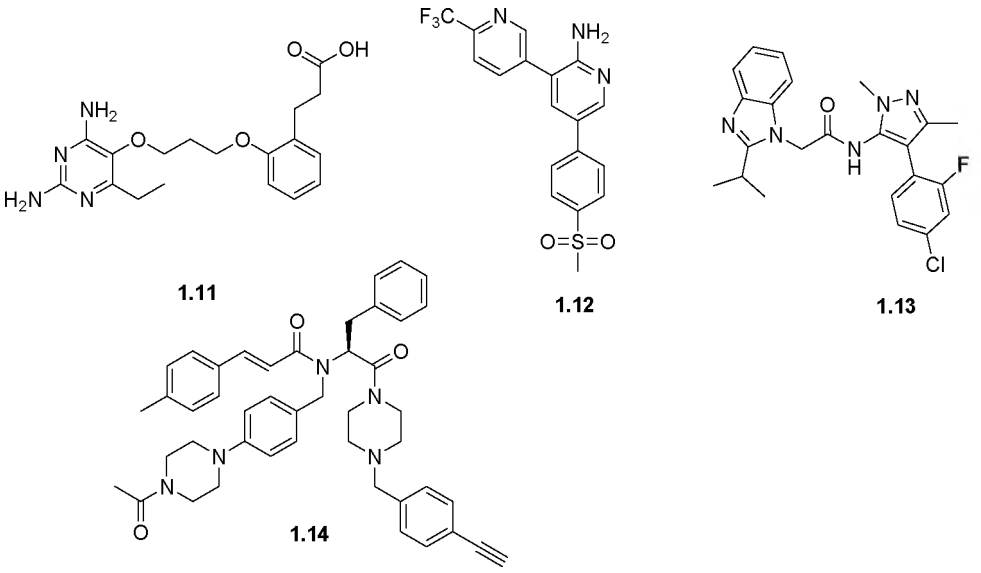


Figure 1.4: Current antimalarial drugs in clinical trials[10]

Despite the effectiveness of ACTs, reports of the drug resistance in Cambodia and Thailand have emerged.[11] Due to the parasite resistance towards available treatment regimen, more efforts are continuously being made in developing new potent drugs with novel mode of actions.[1] The last decade has seen a record number of promising compounds reaching the clinical trials (**Figure 1.5**). However, the battle is far from over considering the high failure rate in this process.[12] More drug candidates are needed to broaden the drug pipeline supply.

1.5 Application of metals in chemotherapy

1.5.1 Coordination complexes

The use of alternative elements in pharmaceutical products can be traced as far as discovery of arsenic containing drugs such as Salvarsan (**1.15**) and NeonSalavarsan (**1.16**) for treatment of syphilis (**Figure 5**).[13] However, the advent of Cisplatin (**1.17**) and its derivatives, for example Carboplatin (**1.18**), as anti-cancer metallodrugs sparked interests in utilizing metal-containing complexes for treatment of various ailments.[13] Despite their success, associated issues such as multidrug-resistance, toxicity and limited spectrum of activity are the driving forces to investigate alternative metallo-complexes for treatment of diseases such as cancer and malaria.[13] In recent years, there has been an increase in applications of metal complexes for treatment of diseases such as parasitic diseases.[13] In addition, coordination complexes with suitable ligands can be tailored to exhibit unique properties including their potential to be molecular probes to unravel the functioning of proteins, sensors, chemotherapeutics and diagnostic tools.[13]

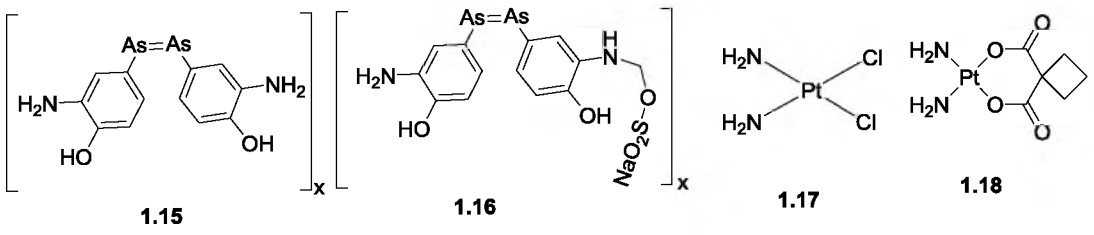


Figure 1.5: Structure of some pioneering work on organomettalics[13]

1.5.2 Organometallic compounds

Organometallic compounds are a class of compounds comprising at least one carbon metal bond. These compounds were first recognized in stoichiometric and catalytic processes, for instance in Ziegler-Natta catalysis.[13] Nowadays, organometallic compounds are gaining much attention in chemotherapeutic research and nanotechnology.[14] In chemotherapy, organometallics are serving as scaffolds in the design of new 'prodrug' molecules given the unusual reactivity of this class of compounds.[15] Organometallics can also be used in chemical sensors.[13] Atypical example is ferrocene (**1.19**) that can be used to detect glucose content in the blood and in the design of

biosensor probes due to its fascinating electrochemistry.[13] **Figure 1.7** below summarizes major applications of organometallic compounds.

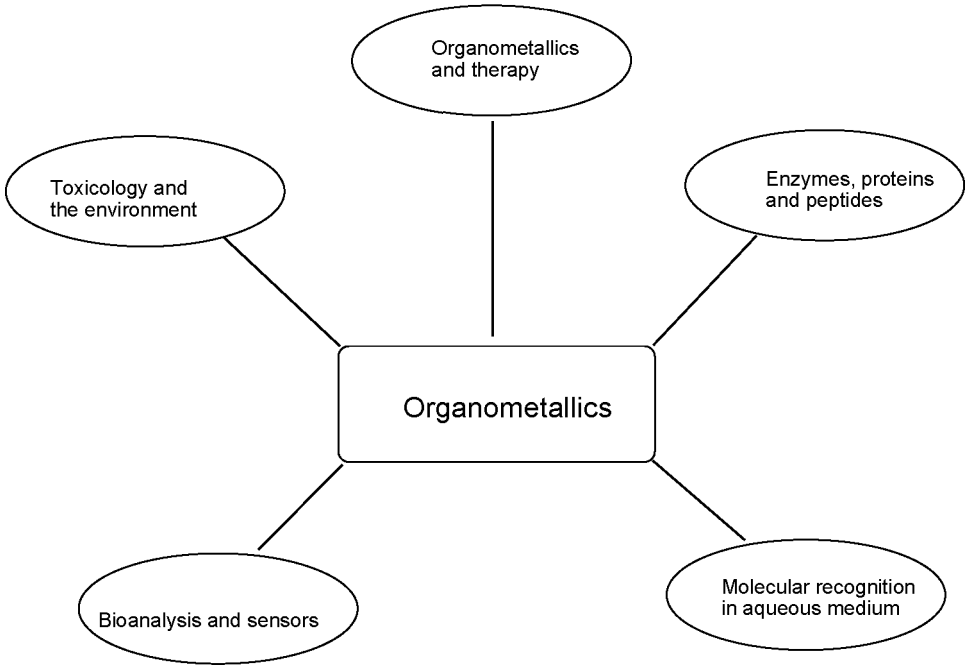


Figure 1.6: Major applications of organometallic compounds[13]

Organometallic compounds which have been extensively investigated for treatment of illness are by and large metallocenes.[13] A metallocene is a compound consisting of two cyclopentadienyl (Cp) rings bound to a metal center in the oxidation state (II).[16, 17]

1.19

Figure 1.7: Chemical structure of ferrocene

The literature survey shows that different metals have been used to design antimalarial drugs and these includes gold (Au), ruthenium (Ru) and gallium (Ga).

In terms of reactivity, ferrocene displays a similar chemical behavior as benzene, but some differences are to be noted. For example, ferrocene is more prone to electrophilic substitution faster than the benzene due to five electrons delocalized over five atoms.[17] It has been reported by *Nada et al.*[14] that the co-immobilization of ferrocene in conducting films increases the electronic conduction and hence the rates of charge transfer. In addition, ferrocenium incorporated into poly(3-methylthiophene) mediates the electron transfer reaction of the oxidation of some biological molecules.[21]

1.6 Medicinal chemistry of ferrocene and its derivatives

Ferrocene (**1.19**), which was discovered in the early 1951, is an archetype of metallocenes with iron atom sandwiched between two cyclopentadienyl rings.[18] The high lipophilic nature of ferrocene ($\log P_{octanol/water}$ = 3.28) and its electrochemical behavior [redox potential of the ferrocene/ferricinium couple, E° = +0.400 V vs. SCE (Saturated Calomel Electrode)] render it attractive for medicinal chemistry, drug delivery, membrane penetration and in electron transfer reactions.[19,20]

Iron is abundant in nature and crucial to living things including humans. This transition metal, which is commonly found in +2 and +3 oxidation states, is also utilized for a spectrum of application from making hard material like steel to the development of drugs molecules. The concept of including a ferrocenyl moiety inside an established antimalarial molecule was first applied during the mid-1990s, and currently this is an area of active research.[23] Biot *et al.* reported a series of ferrocene-chloroquine compounds. Ferrocene-penicillin derivatives have also been investigated and the derivatives explored proved to be active against tumor cells.[24]

1.6.1 Antitumour agents

Cancer is the biggest threat to the world's population. Following the success of platinum (Pt) complexes in treatment of cancer, several studies embarked on the anti-cancer activity of metallocenes.[13] While ferrocene by itself is not toxic, its ferrocenium ion has been found to exert

an antiproliferative effect against various cancer cell lines.[25-27] Considering that ferrocene can be easily functionalized, the ferrocene derivatives have been coupled with gold (**1.20**),[28] silver,[29] and other transition metal complexes.[30]

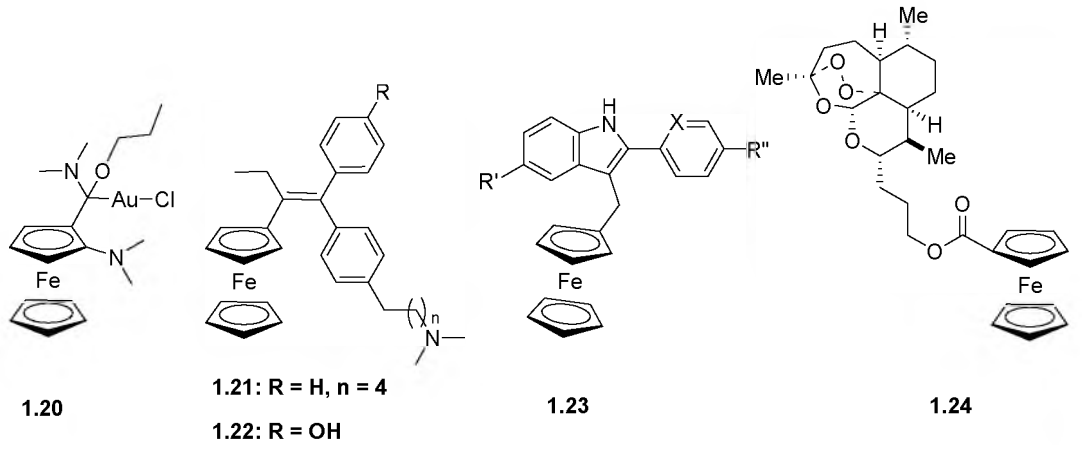


Figure 1.8: Structure of ferrocene derivatives with antitumor activity.[17, 31]

In the quest to synthesise a potent anticancer drug, the tamoxifen molecule, which was used as an anticancer drug, was modified by replacing the phenyl group with ferrocene to form ferrocifen (**1.21**, **Figure 1.9**).[32, 33] Ferrocifen (**1.21**) exhibited anticancer activity against selective estrogen receptor modulators (SERM) and non-selective estrogen receptor modulators.[31] The activity of ferrocifen (**1.21**) and its analogue hydroxyferrocifen (**1.22**) is derived from the anti-hormonal structure of the organic tamoxifen skeleton and the cytotoxic nature of ferrocene moiety.[33] Given the importance of various anticancer drugs in modulating microtubule assembly, several studies have been conducted to explore compounds that can stop cell proliferation through this pathway.[34]

Von Angerer and his group synthesised 2-phenylindole derivatives and evaluated this series against breast cancer cell lines.[35] It is believed that the synthesized compounds act by blocking the polymerization of α/β-tubulin dimers. However, the potent 2-phenylindole-3-carbaldehyde compound of this series obtained by replacing the 3-formyl group did not inhibit tubulin polymerization. Their capability to block the cycle prompted the study to include an organometallic moiety. The results showed that the ferrocene-indole hybrids (**1.23**) were more active than the parent organic molecules.[36] In a separate study, Reiter *et al.*[37] reported a series of ferrocene-artemisinin derivatives, which were investigated for the potential anti-tumor activity.

From the series, compound **1.24** was found to be the most active against CCRF−CEM cells with a 50% inhibition concentration (IC_{50}) of 0.25 ± (0.14) μm as well as against the CEM/ADR5000 cells (IC_{50} of 053 ± (0.22) μm.[37]

1.6.2 Antimicrobial agents

The multidrug resistance (MDR) has become the major setback to a number of clinical proven therapeutic agents rendering them ineffective. Thus, various strategies, including introducing organometallic units to develop alternatives potential drugs for cancer and tropical disease like malaria, have been applied to counter drug resistance.[38] In this regard, ferrocene was coupled with fluconazole (FCZ) by replacing phenyl moiety with ferrocene to obtain compound **1.25**, which was tested for potential anticandida activity.[39] Compared to FCZ which induces growth inhibition, its ferrocene analogue showed no effect on fungi growth inhibition except at high concentrations.[40] The strategy of switching to organometallic ferrocene in attempts to design bioactive compounds was also applied to penicillin. The replacement of the aromatic group in penicillin with ferrocenyl moiety led to the development of compounds **1.26** – **1.28** with considerable activity against *Staphylococcus aureas*. These compounds have been suggested to inhibit β-lactamase, an enzyme responsible for cross resistance. Other ferrocene carborane derivatives were also reported as antimicrobial candidates.[41]

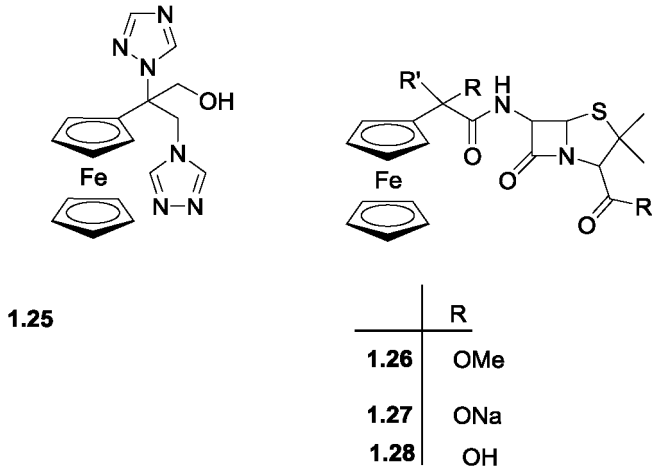
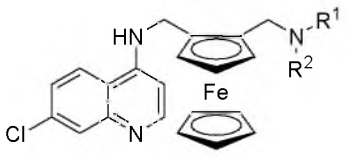


Figure 1.10: Structure of ferrocene derivatives with potential antimicrobial activity.[17, 31]

Thiacetazone, is a thiosemicarbazone-based drug that reached the market as an anti-TB drug. However, its efficacy has been compromised by resistance. In attempt to reverse this incidence, and shorten the treatment period, ferrocene thiosemicarbazone have also been synthesized and evaluated against TB strains, the results were quite promising.[42, 43] Thiosemicarbazone ligands designated HL3 showed moderate activity against M. tuberculosis virulent strains H37Rv with MIC values of 11.7 and 11.0 μM respectively. [42, 43]. In this study rifampicin, isoniazid, moxifloxacin and streptomycin were used as tuberculosis control drugs.

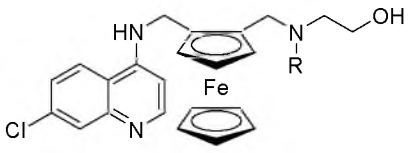
1.6.3 Antiviral agents

Viruses like other disease causative agents are manifesting resistance towards available treatments. A significant number of aminoquinoline based antimalarials like mefloquine (MQ) have been found to exhibit antiviral efficacy, and showed synergistic effects with HIV-protease inhibitors.[44] For example, Biot *et al.*[44] synthesised a series of aminoquinoline-ferrocenyl derivatives and evaluated resultant compounds against a panel of viral strains. Majority of these metallocenic derivatives showed antiviral activity against feline and human (SARS) coronavirus.[44] The activity of these molecules was linked to the inhibition of HIV surface envelope glycoprotein, gp120.[44] Another compound **1.34** was also found to interact with gp120 of the HIV.[45] Since aminoquinolines are basic in nature, it has been suggested that the basicity contributes to their antiviral activity by increasing the endosomal pH, thereby interfering with the production of gp120.[44]



1.29: $R^1 = R^2 = CH_3$

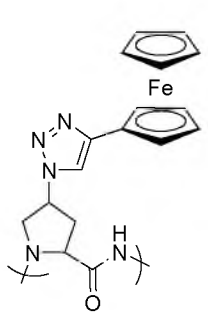
1.30: $R^1 = R^2 = CH_2CH_3$



1.31: R = H

1.32: $R = CH_3$

1.33: $R = CH_2CH_3$



1.34

Figure 1.11: Structure of ferrocene derivatives with potential antiviral activity.[17, 31, 45]

1.6.4 Anti-inflammatory agents

The principle of bioisosterism has been used for a long time to design drugs with improved bioactivity including related physicochemical properties.[46] Bioisosterism involves replacing one functional group on a drug molecule with another group with similar properties. In their research works Bruce *et al.*[46] investigated the analogues of tolmetin by replacing the pyrrole core with the organometallic ferrocene moiety to a series of tolmetin ferrocenic analogues **1.35 – 1.38** (**Figure 1.12**). However, the synthesised analogues were not active and others exhibited low anti-arthritic activity. In this study, it was suggested that the observed low activity could be due to poor bioisosterism of ferrocene.[46]

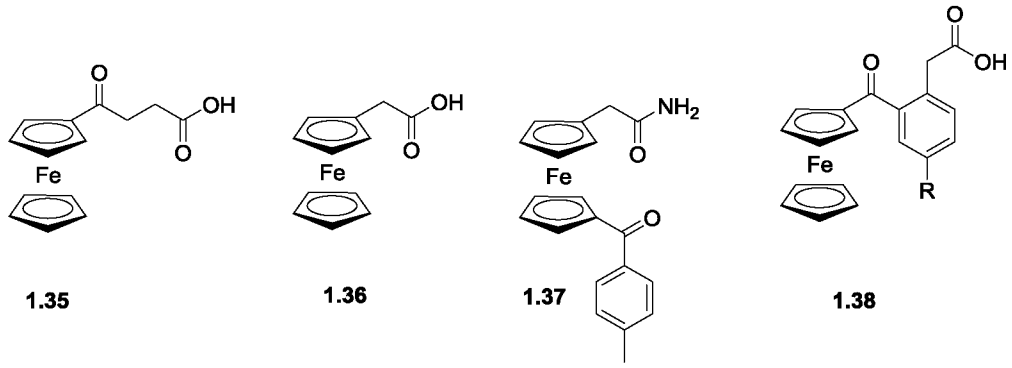


Figure 1.9: Tolmetin and ferrocene derivatives with potential anti-inflammatory activity.[17, 31]

1.6.5 Antiparasitic agents

The versatility of ferrocene when comes to biological activity, and their electrochemistry continue to attract the interest of medicinal chemists. Studies on the chloroquine-ferrocene derivative (Ferroquine, **1.29**) revealed that its activity may emanate from the parasite affinity towards iron.[47] Currently, there is ongoing research for potential antimalarial of ferrocene containing compounds.

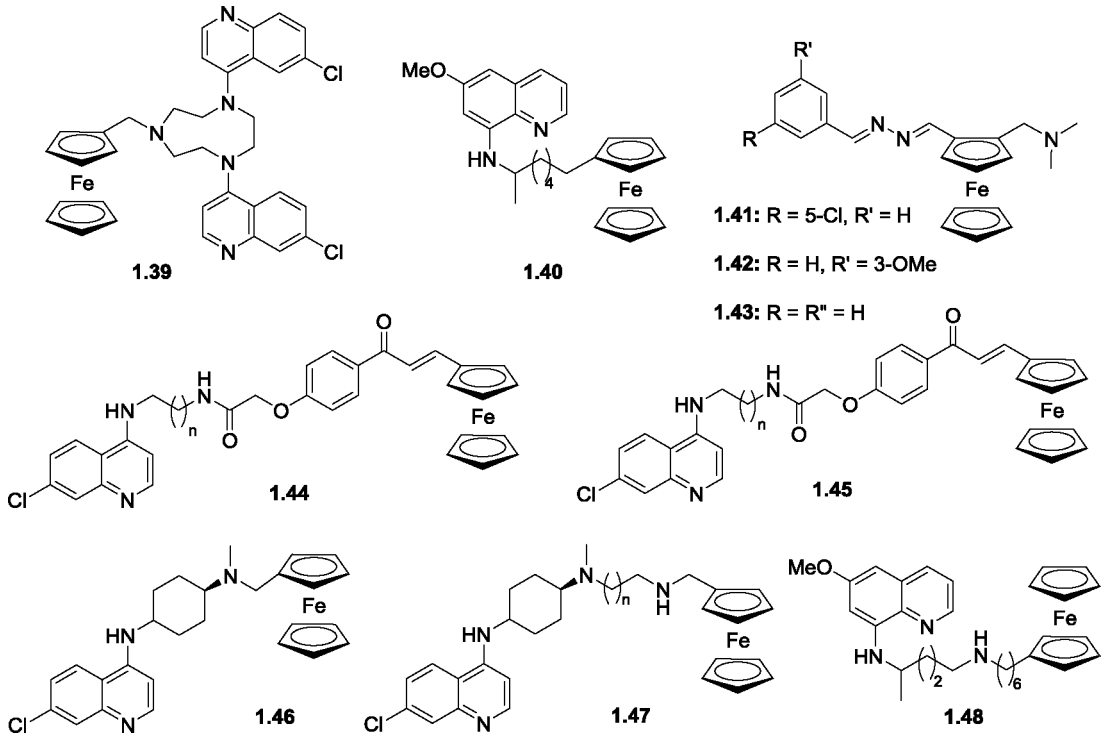


Figure 1.10: Structures of ferrocene derivatives with potential antimalarial activity.[17, 31, 48, 49]

Recently, Maguende and co-workers synthesised ferrocenoyl aminoquinolines derivatives (**1.39** and **1.40**), which were evaluated against F32 (chloroquine sensitive, CQS), FCB1 and K1 (chloroquine resistant, CQR) strains.[49] Thus far, different antiplasmodial compounds (**Figure 1.12**) have been coupled with ferrocene in a view of the identification of antimalarial compounds with enhanced activity and lack cross-resistance with current clinical drugs.[50] However, none of the synthesized derivatives exhibited activity against CQRs comparable to ferroquine (**1.29**). Another series of quinolones and imidazole coupled with ferrocene was evaluated against human *African Trypanosomiasis* (HAT).[49] Although different compounds in this series showed promising activity, there was no clear indication that ferrocene redox properties were responsible for their mechanism of action.[49] However, another research by Chibale and Co-authors synthesized ferroquine analogues which were evaluated for their antimalarial activity relative to methylene spacers and their ease of oxidation potency. In one series there was a correlation between antimalarial activity against D10 strains, chain length and ease of oxidation of ferrocenyl group. Note that the other series didn't show any clear correlation at all.[51]

1.7 Conclusion

Malaria is the biggest health burden to the society, apart from it being life threatening issue mostly in developing countries, it is also an impediment to economic development. More importantly, resistance to available drugs has given an impetus to develop drugs with a novel mode of action. The successful inclusion of ferrocene moiety into already existing drugs provide more drive to synthesise ferrocene derivatives with improved potency. The development of ferrocifen from tamoxifen is one example which has been a stimulus to ferrocene investigation. It is evident that from the above instance that inserting organometallic ferrocene unit into bioactive organic drugs could result in compounds with improved activity against various ailments. Despite the fact that only a few compounds that have reached clinical trials, there is an unlimited scope to design new ferrocene containing compounds for treatment of diseases like malaria, which affect millions of lives.

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CHAPTER TWO

THIAZOLE CONTAINING DRUGS IN TREATMENT OF VARIOUS DISEASES

2.1 Introduction

Thiazole is a common heterocyclic five membered ring, 1,3-azole usually referred to as thiazole (**2.1**). It is isomeric to 1,2-azole known as isothiazole (**2.2**). 1,3-Azole is a parent motif in substances exhibiting medicinal potency, dyes, insecticides and non-linear optical susceptibility.[1-3] In this chapter, a literature survey is presented and describes the occurrence of thiazole in natural systems as well as synthetic products. Different biological activities of this scaffold are discussed to illustrate its versatility. Structure activity relationship studies (SARs) of thiazole derivatives with antimalarial potency are briefly covered in this chapter.

2.2 Thiazole: A promising scaffold for development of potent drug candidates

Thiazole is commonly present in many natural products and synthetic compounds having a broad range of biological activities.[4,5] For example, thiazole containing vitamin B1 (thiamine, **2.3**) helps in the normal functioning of the central nervous system and serves as a precursor for the synthesis of acetylcholine, which is responsible for uncoding memories and human ability to concentrate.[6]

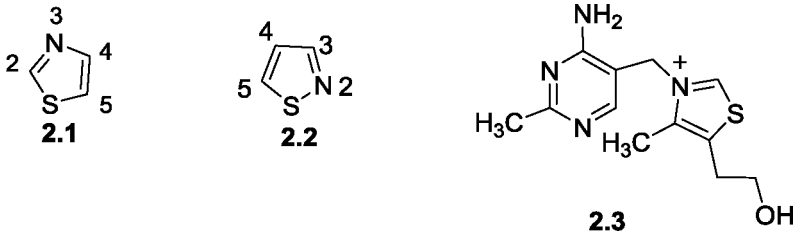


Figure 2.1: Chemical structures of thiazole ring (**2.1**), isothiazole (**2.2**) and thiamine (**2.3**)

2.3 Medicinal chemistry and biological activity of thiazole

In medical chemistry, thiazole motif is an important and widely utilized moiety to generate novel bioactive compounds. A number of thiazole containing compounds have proved to possess broad biological potency, including antifungal (**2.4**) activity,[8] antibacterial (**2.5**),[8, 9] anticancer (**2.6**) antiviral (**2.7**) and anti-inflammatory (**2.8**) potency (**Figure 2.2**).[5, 10] The biological activity of thiazole is thought to be associated with the C-S bond in the five membered ring, which has the low lying σ* orbitals of the C-S bond that allows the interactions with electron donors including but not limited to oxygen or nitrogen atoms.[11]

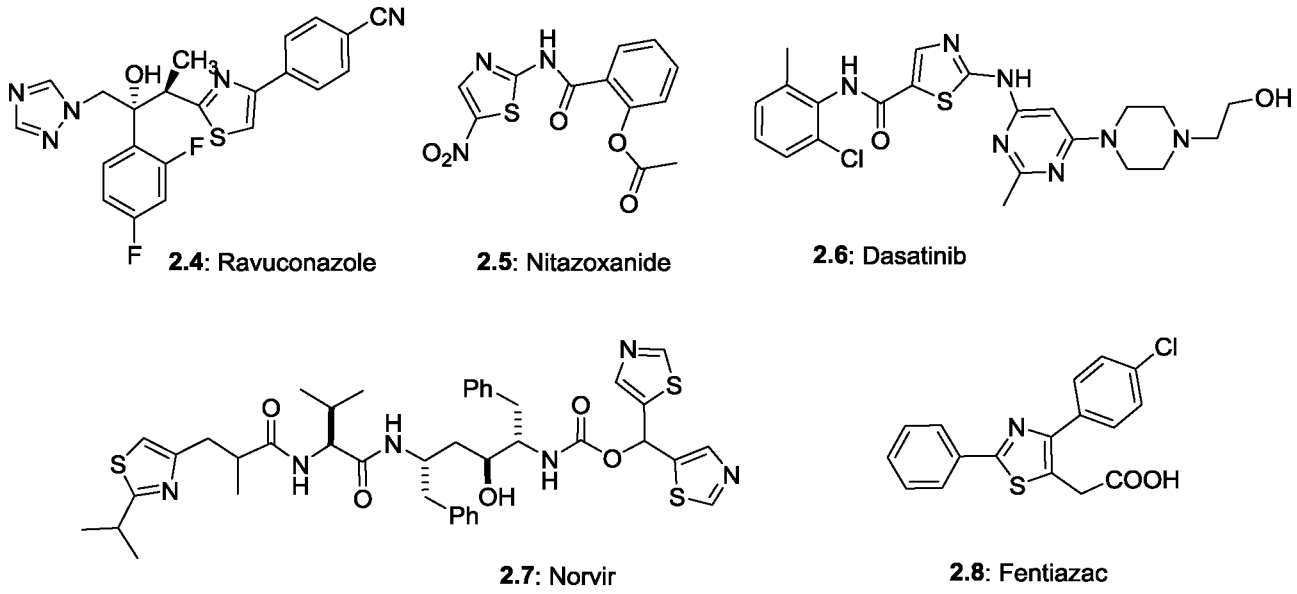


Figure 2.2: Structures of thiazole containing drugs

2.3.1 Anti-tumor activity

Several studies have illustrated the importance of thiazole scaffold in the design and development of some anticancer drugs.[12, 13] Different thiazole analogues have been designed and investigated for their potential antitumor properties.[14, 15] Biological activities of different derivatives of thiazole moiety showed improved potency and the study of these compounds continue to attract interest of researchers. In an attempts to identify compounds with anticancer potency, Shao *et al.* synthesised a series of ferrocenyl thiazoles-containing molecules.[16] Compounds **2.9** and **2.10** emerged as the most active compounds against human cancer cell lines with percentage inhibitions of 81.4 and 62.0 against HL-60 (Leukemia) cell line.[16]

In another investigation a tiazofurin analogues were synthesised and investigated for their activity also against a panel of human tumor cells.[17] The most potent analogue was found to be 100 fold more potent than the standard drug tiazofurin (**2.11**) against K562 cells.[17] Gulsory *et al.* synthesised a series of arylidenehydrazides, which were screened for their anticancer activities[15] against three cancer cell lines, i.e. NCI-H460 (lung), MCF-7 (breast) and SF-268 (CNS). Compound (**2.12**) was the most potent and also showed superior activity against the prostate cancer cell lines (PC-3, $\log_{10}$ GI_{50} value < -8).[15]

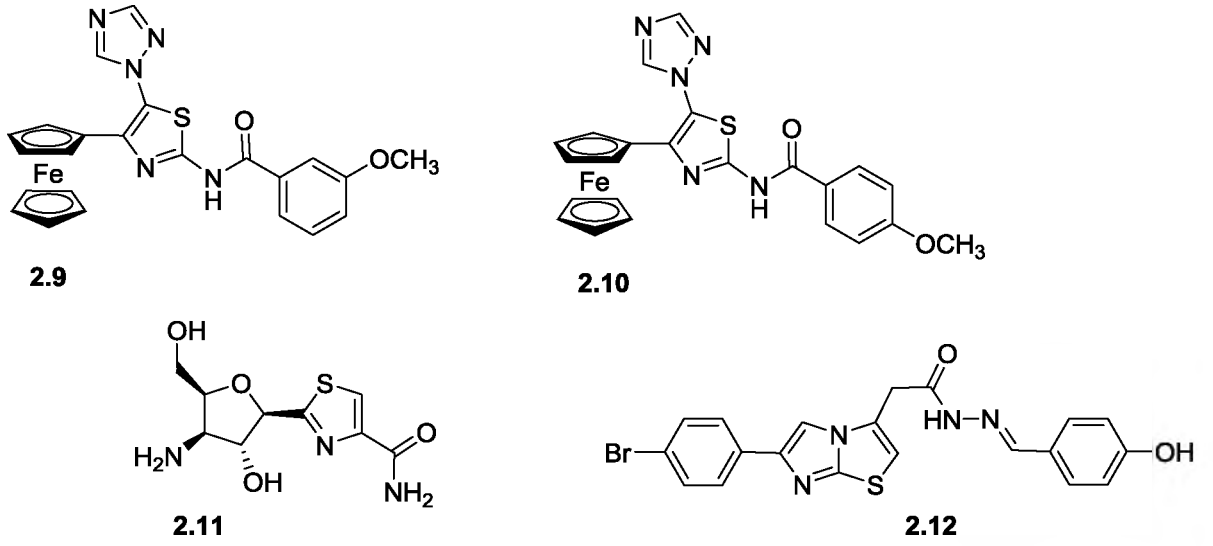


Figure 2.3: Chemical structures of some thiazole containing antitumor compounds. [4, 14]

2.3.2 Antiviral activity

As it has previously stated, viral infections present a threat to humans with alarming numbers of people affected being reported each year.[17] HIV is the leading viral infection that kill many people and has ravaged humanity for several decades. According to the WHO there are an estimated 36.9 million people infected with HIV globally.[18] More importantly, HIV-1 is showing resistance against clinical useful drugs for treatment of HIV/AIDs.[19] Thiazole containing molecules have been reported to show antiviral properties.[5] Norvir (**2.7**, **Figure 2.2**), is amongst many clinical approved anti-viral drugs, that contain the thiazole motif in their structure. Currently, there is growing interest in designing compounds bearing the thiazole motif within their structures.[9]

Compound **2.15** was evaluated for its inhibitory effect against HIV-1 in human MT-4 assay,[20] but it showed no marked anti-HIV at a concentration below their toxicity threshold, compounds **2.13** and **2.14** exhibited IC_{50} of 0.005 to 0.006 μM, respectively.[21]

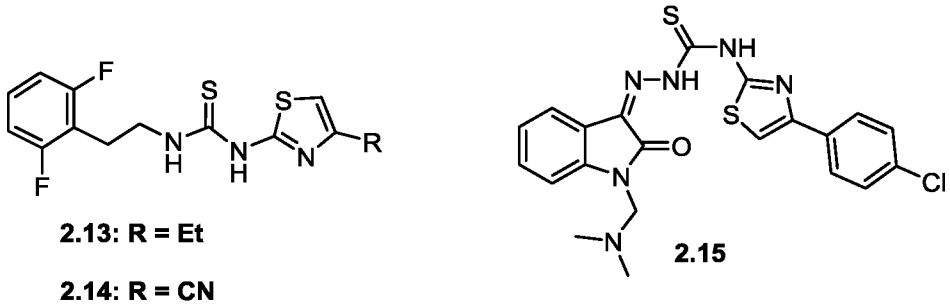
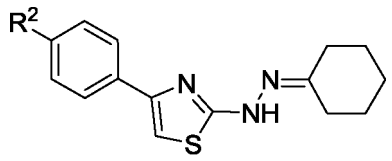


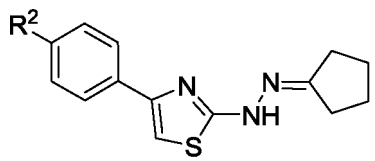
Figure 2.4: Structures of some antiviral thiazole containing compounds. [59, 70]

2.3.3 Antimicrobial activity

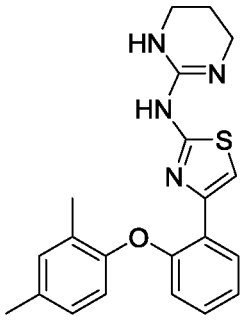
A number of researchers are putting much effort to combat the problem of multi-drug resistance. For example, Pandeya *et al.* reported a synthesis of a series of Schiff/Manich bases derived isatin containing thiazole compounds and evaluated this series against 28 pathogenic bacteria and 8 pathogenic fungi.[20] In addition to antiviral potency, compound **2.15** (**Figure 2.4**) exhibited enhanced potency against bacterial infections compared to the other members of the series. Moreover, compound **2.15** exhibited better activity against *Enterococcus faecalis* (Gram positi+ve) with MIC = 1.2 μg/mL and was more active compared to a standard drug sulphamethoxazole. Another series containing the thiazole scaffold was investigated for microbial inhibition and compounds **2.16** and **2.17** (**Figure 2.5**) showed promising antimicrobial activity. The results suggested that imine functional group conferred antibacterial and antifungal activities compared to cyclohexanimine derivatives. The thiazole core structure occurs in many biologically active molecules such as abafungin (**2.18**) and sulfathiazole (**2.19**), which are utilized for treatment of microbial and fungal infections, respectively.[4]



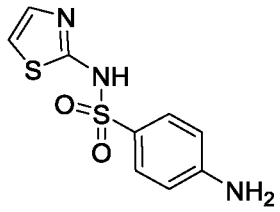
2.16: R^2= OCH_3 or Br



2.17 : R^2= OCH_3 or Br



2.18: Abafungin



2.19: Sulfathiazole

Figure 2.5: Structures of antimicrobial thiazole.[55, 59, 72]

2.3.4 CNS activity

Thiazole core is also found in many bioactive compounds and drugs targeted for the central nervous system.[23] Thiamine **(2.3, Figure 2.1)** is a natural occurring organo sulfur compound, consisting of aminopyrimidine coupled with a thiazole ring. It acts on many receptors, including adenosine, dopamine, serotonin and is very important to the organism, more specifically the nervous system.[7] Many thiazole derivatives have been synthesised and investigated for their potential as anti-Alzheimer agents (**Figure 2.6**). Studies conducted showed that thiazole derivatives **2.20** and **2.21** exhibit excellent binding affinity towards Aβ aggregates with K_d values of 0.006 and 0.13 nM, respectively.[7] Both **2.20** and **2.21** were found suitable to be used as high quality biomarkers for studying Aβ (1-40) and Aβ (1-42) aggregates of amyloidogenesis in Alzheimer's disease. In another study conducted on thiazole derivatives as anti-Alzheimer potent drugs, derivatives with alkylamino groups proved to exhibit promising inhibitory activity towards Aβ-RAGE binding.[24] Surprisingly, another compound **2.22** without the alkylamino group exhibited promising inhibitory activity on Aβ-RAGE binding with IC_{50} 1.21 μM.

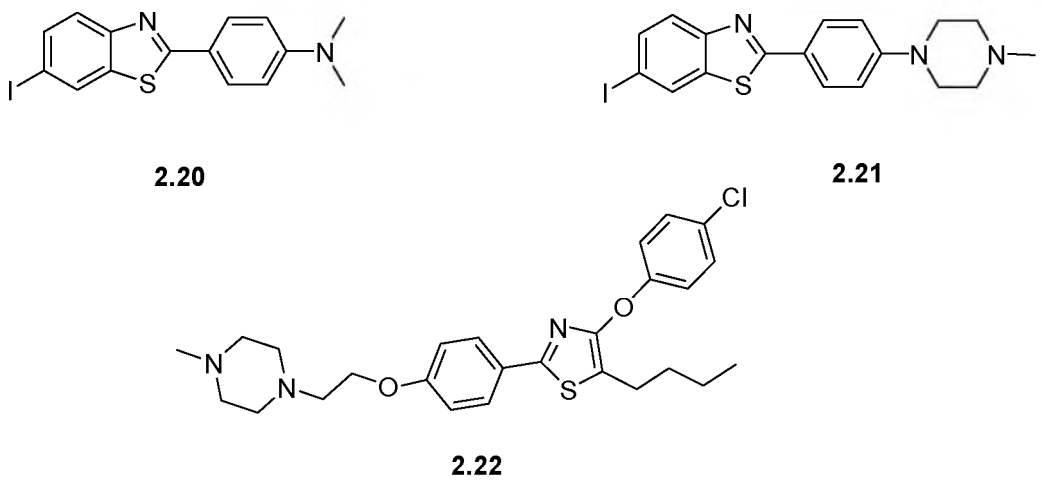


Figure 2.6: Anti-Alzheimer thiazole derivatives with efficacy.

2.3.5 Anti-Mycobaterial tuberculosis activity

The medicinal chemistry of aminomethylthiazoles is becoming fascinating given some of these compounds that are in clinical trials.[25] Due to microbial resistance towards anti-TB drugs the effort to find new lead compounds is underway, and high throughput screening (HTS) has been beneficial in generating versatile chemical entities,[25, 26] including the 2-aminomethylthiazoles.[26] For example, Mjambili *et al.* reported a series of 2-amino-4-(2pyridyl)thiazole derivatives as potential anti-*Mycobacterial tuberculosis* agents. Antimycobacterial results from the HTS indicated that compounds with 2-pyridyl substituents at position-4 of the thiazole moiety proved to be active.[26] The SAR was conducted on series **2.23** while investigating the role of the pyridyl position on the thiazole ring as well as the type of the linker used.[26] All compounds in this series were evaluated against the *M. tuberculosis* $H_{37}Rv$ strain with rifampicin and kanamycin used as positive controls. In addition these compounds were screened for their potential antiplasmodial activity against CQS *P. falciparum* NF54 with CQ used as a positive control.[26]

In order to assess the role of the linker, compound **2.23a** (urea), **2.23b** (amino), **2.23c** (acylthiourea) were synthesised.[6] Compound **2.23c** with an thiourea linker exhibited superior antimicrobial activity compared to **2.23a** and **2.23b**. Compound **2.23c** also exhibited encouraging antiplasmodial activity in addition to its antitubercular potency.[26] Mjambili and co-workers found that alteration on the position of substituent on phenyl, as well as changing the position of pyridyl around thiazole influenced the antiplasmodial and antimycobacterial activity of synthesised analogues.[26]

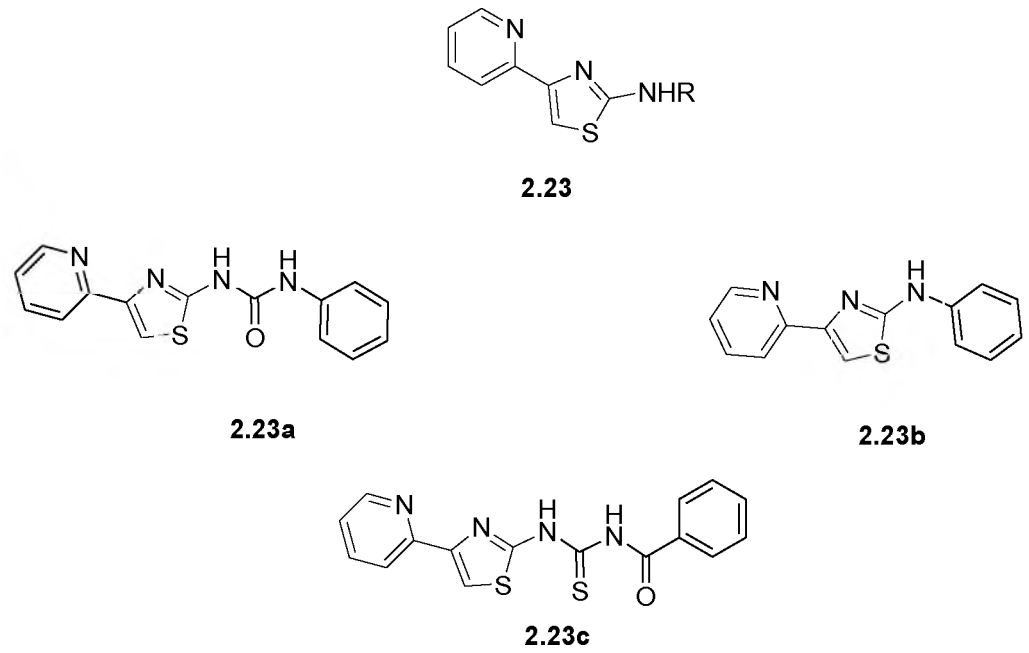


Figure 2.7: Structures of potential anti-mycobacterial agents with thiazole scaffold.

2.3.6 Anti-plasmodial activity

The emergency of CQ resistance has prompted the investigation of new compounds with novel mode of action. 4-Aminoquinolines such as quinine (QN) and amodiaquine (AQ) became successful, but the parasite resistance quickly arose soon after their discovery. In the quest of novel antimalarial agents, thiazole derived compounds have been shown to exhibit promising antiplasmodial activity against a selection of *P. falciparum* strains.[27, 28] In a high-throughput screening campaign of 3500 compounds from BioFocus library, approximately 170

aminomethylthiazole were found to be active against the malaria parasite without obvious cytotoxicity at 1.82 μm.[27]

From this screening campaign, compound **2.24** was identified as a hit compound. With the primary aim to explore the (SAR) around the hit **2.24**, three substructural units (thiazole, aminomethyl and pyrazole) were replaced with different aromatic and heteroaromatic groups. Most of the synthesised analogues exhibited excellent activity against K1 and NF54 strains with the aminomethyl group appeared to be critical for potency.[27] Compound **2.24** exhibited superior *in vitro* antiplasmodial activity with IC_{50} values of 0.08 μM against the CQR strain (K1)_ and 0.07 μM against the CQS strain (NF54), respectively. More importantly, compound **2.24** also showed good *in vivo* pharmacokinetic studies in *P. berghei* mouse model with terminal half-life of 3.7 h following IV administration. During the study it was noted that the maximum plasma concentration occurred at 195 min post dose and the oral bioavailability was estimated at 50%.[27]

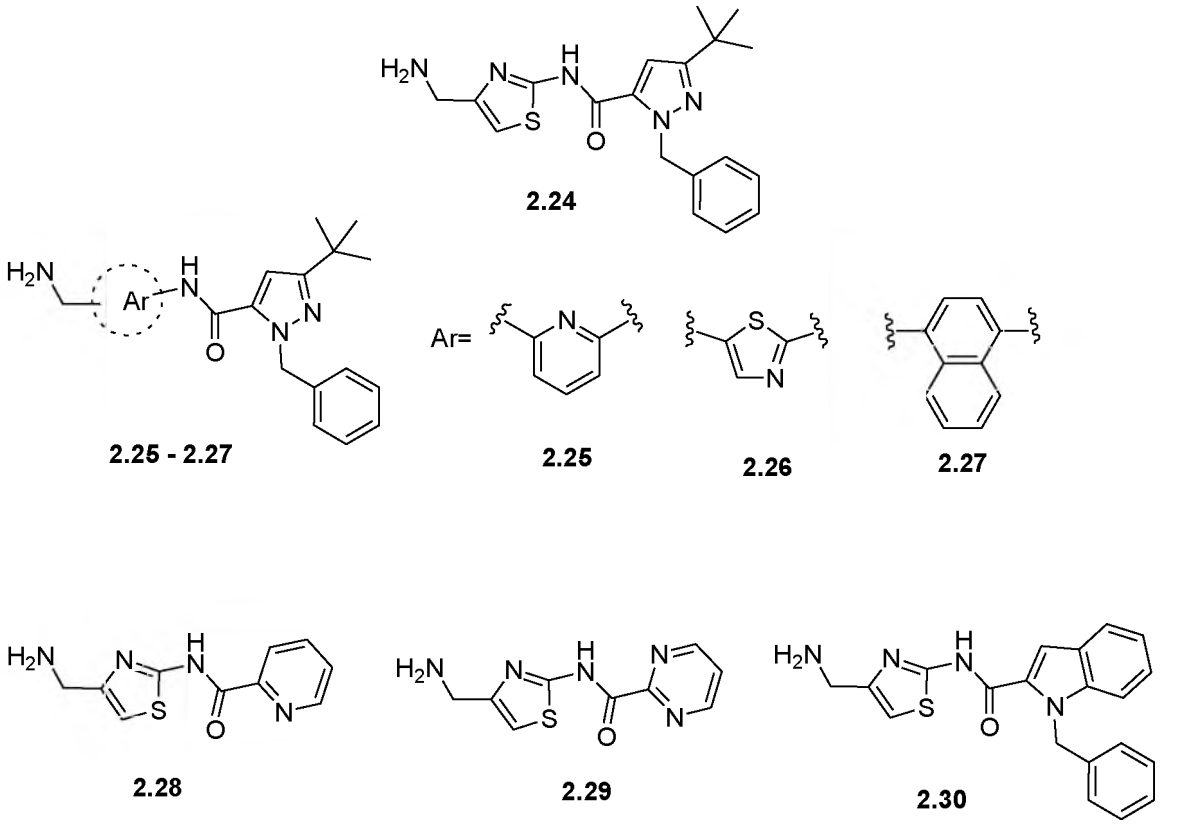


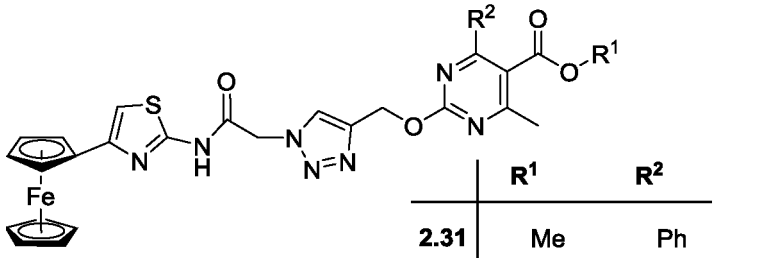
Figure 2.8: Chemical structures of aminomethylthiazoles with antimalarial potency.[27]

A recent study on the aminomethylthiazoles by Cheuka *et al.* revealed key parts of this scaffold that are required and important for antiplasmodial activity. The modification of the free amine (see **2.25** – **2.27**) resulted in compounds showing no activity. This observation emphasised the importance of unsubstituted aminomethyl functionality for potency.[27] It is also important to note that changing the position of sulfur and nitrogen (e.g. **2.26**) of the thiazole ring, which is equivalent to changing the position of aminomethyl group, resulted in strikingly lower activity.[27] The compound **2.24** was further investigated to gain insight in terms of required SAR by changing the pyrazole moiety (see also section 3.2 in **Chapter 3**). Compounds **2.25** – **2.27** showed reduced activity suggesting that the pyrazole moiety is crucial for activity.

Recent antiplasmodial activity of ferrocene-pyrimidine conjugates in which the two moieties are linked through the heterocyclic thiazole unit prompted us to investigate the effects of incorporating the ferrocene moiety into the aminomethylthiazole hit **2.24** in view of expanding further the SAR. As discussed previously (see **Chapter 1**), incorporation of ferrocene into known bioactive to enhance activity and equally address multi-drug resistance issues is a well-known and accepted strategy with a number of promising compounds feature in literature.

2.4 Ferrocene thiazole derivatives

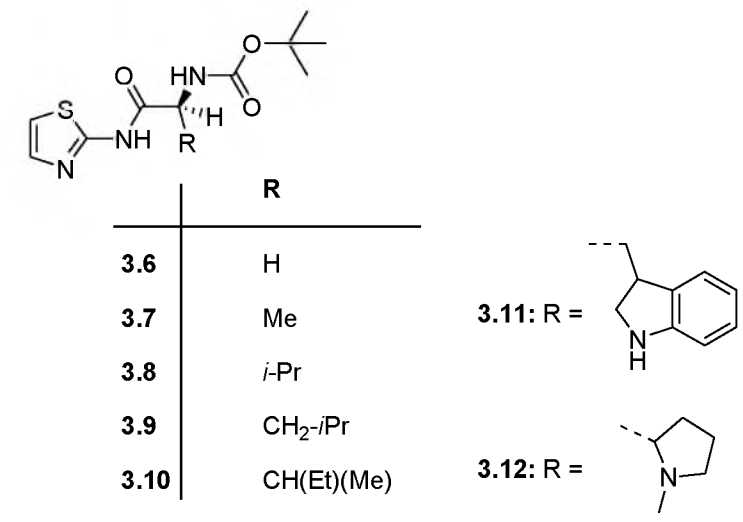
As stated previously, Ferroquine **(1.29)** is a successful ferrocene containing drug that has reached clinical trials for treatment of malaria.[28] To date, a number of ferrocene-based compounds have been explored for treatment of various ailments including malaria.[2] For example, Chopra *et al.*[3] recently reported a series of ferrocene pyrimidine hybrid compounds (**Figure 2.9**), where the thiazole moiety is incorporated as a linker between ferrocene and pyrimidine moieties.[3] The *in vitro* data of synthesised hybrids revealed that compounds with aromatic substituents at C-4 position of the pyrimidine core and isopropyl ester group at C-5 position (**2.33 – 2.35**) were the most active.[3] All compounds exhibited a single electron reversible oxidation behavior like ferrocene.[3]



	R^1	R^2
2.31	Me	Ph
2.32	Et	Ph
2.33	*i*-Pr	Ph
2.34	*i*-Pr	*p*-NO_2-Ph
2.35	Et	Me

Figure 2.9: Ferrocene-pyrimidine hybrids containing thiazole motif as a linker.

The reactivity of thiazole and its attributes as a good synthon for drug molecules have led to the synthesis of versatile drug candidates. Karade *et al.* developed a series of thiazole-derived amino acids (**Figure 2.10**) and evaluated these compounds against *P. falciparum* CQS and CQR strains.[4] Two of the synthesised (**2.41** and **2.42**) compounds from the series were active with the IC50 values ranging from 3.45 - 6.12 μM.[4] Molecular docking studies suggested that most active compounds bind to the active site of the plasmepsin-II, which is one of aspartic proteases involved in degradation of Hb in human red blood cells.[4]



	R
3.6	H
3.7	Me
3.8	*i*-Pr
3.9	CH_2-*i*Pr
3.10	CH(Et)(Me)

3.11: R =

3.12: R =

Figure 2.10: Structure of thiazole amino acids derivatives

With the advancing of Ferroquine (**1.29**, Figure **1.10**) and now in phase IIb clinical trial as a treatment for malaria, the organometallic ferrocenyl moiety has become an appealing motif for drug design to overcome multi-drug resistance, which continues to compromise existing effective drugs.[28] In this study, we considered the synthesis of a small series of ferrocene-thiazole derivatives and investigated their potential antiplasmodial activity against the *P. falciparum* parasite strains.

2.5 Aims and objectives of this study

Objective

The main objective of this research was to investigate the incorporation of ferrocene into 2-aminomethylthiazole containing compounds (see **Figure 3.4** in **Chapter 3**) in view of expanding the SAR.

Specific aims

- Synthesis and characterisation of designed ferrocene-thiazole derivatives as bioactive small molecule.
- Pharmacologically evaluation *(in vitro)* of synthesised compounds against the *P. falciparum* parasite strains.

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CHAPTER 3

SYNTHESIS, CHARACTERISATION AND *IN VITRO* BIOLOGICAL EVALUATION OF FERROCENE-BASED THIAZOLE DERIVATIVES

3.1 Rationale for design and synthesis of ferrocene-thiazole derivatives

The high throughput (HTS) screening campaign on BioFocus DPI SoftFocus library of 35, 000 compounds in collaboration with Medicines for Malaria Venture (MMV) and H3-D led to the identification of a number of thiazole-containing hits. Through optimization this campaign led to 170 hits, which included aminomethylthiazole pyrazole carboxamides such as compound (**MMV010539**, **Figure 3.3**).[6] As it has been previously discussed, this hit compound showed good activity against the *P. falciparum* strains.[6] More importantly, **MMV010539** exhibited low cytotoxicity and at least >10-fold selectivity against the murine L-6 mammalian cells. **Figure 3.3** illustrates the preliminary SAR around **MMV010539** where analogues appeared to be equally active against both *P. falciparum* strains.[6]

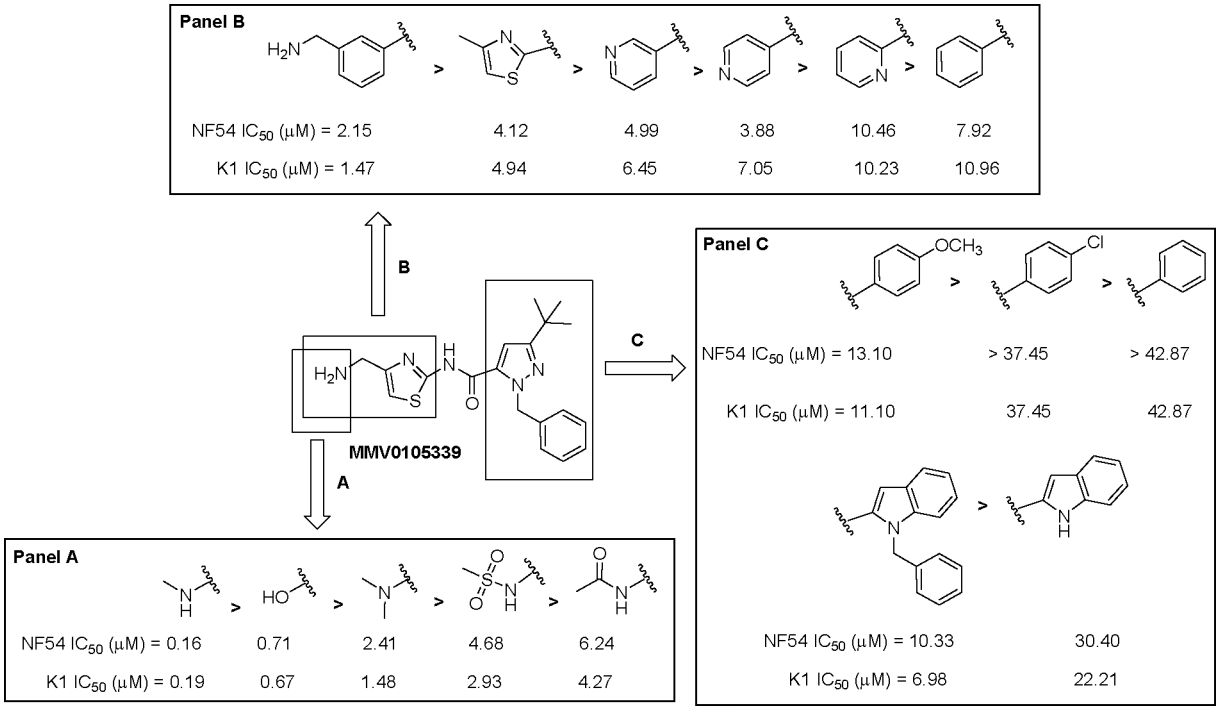


Figure 3.1: Preliminary SAR studies on **MMV0105339**.[6]

Briefly, functionalisation or replacement of the primary amine (**Panel A**) offered analogues with reduced antiplasmodial activity, suggesting that the free aminomethyl group is required for potency. Replacing the thiazole with various aromatic and heteroaromatic systems (**Panel B**) resulted with compounds which displayed moderate to poor activity. Similarly, derivatives with aromatic and heteroaromatic groups replacing the pyrazole moiety (**Panel C**) performed poorly against both strains of the parasite in comparison to **MMV010539**.[6] On separate study, Cheuka *et al.*[7] explored the SAR of aminomethylthiazole pyrazole carboxamide (see section **2.3.6**) derivatives with the primary objective to uncover other aromatic and heteroaromatic moieties that may be tolerated in place of both thiazole and pyrazole units.[7] However, the changes made on **MMV010539** resulted with compounds that displayed moderate to poor activity, suggesting that there is a limited structural variation that could be accommodated around **MMV010539**.[3]

The biological significance of ferrocene against malaria infections prompted us to explore the SAR of aminomethylthiazole pyrazole carboxamides by incorporating the ferrocenyl moiety in place of the pyrazole core. We reasoned that replacement of the pyrazole core with bioactive organometallic ferrocenyl unit and functionalisation of aminomethylthiazole would lead to derivatives with enhanced activity. **Figure 3.4** below illustrates the design of target ferrocenic derivatives which bears thiazole scaffold including the proposed structural features for modification.

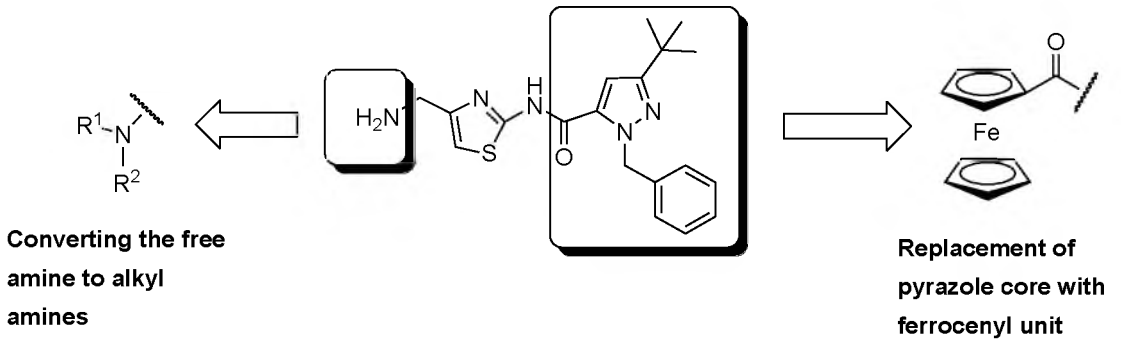
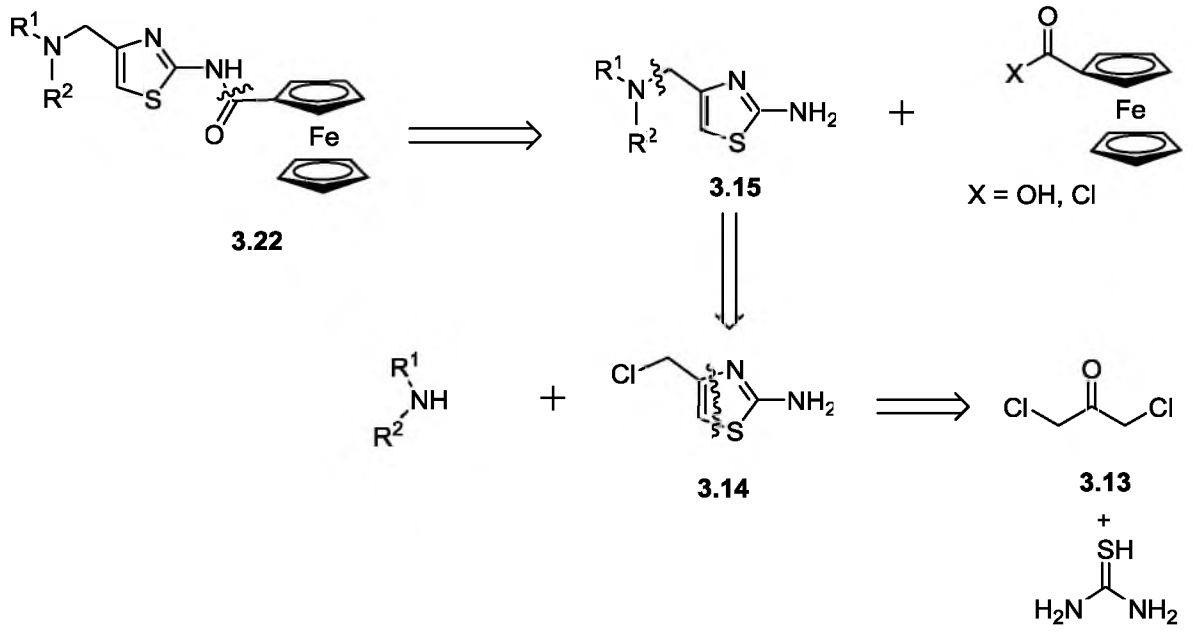


Figure 3.2: Proposed structural modification of compound **MMV010539**.[6]

3.2 Results and discussions

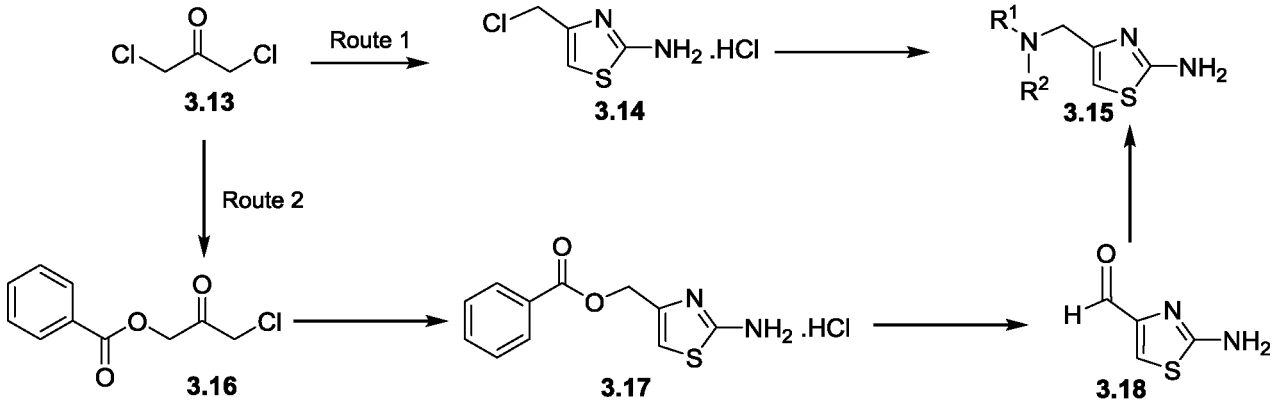
3.2.1 Retrosynthetic analysis of target compounds and proposed synthetic routes

The Scheme **3.1** illustrates the retrosynthetic analysis of target compounds. The amide bond disconnection leads to the amine intermediates and the carboxylic acid or acid chloride starting material. The C-N cleavage of the amine intermediates allowed the access of the thiazole salt and the starting amine. C-N and C-S cleavage of the thiazole salt led back to the formation of the starting thiourea and 1,3-dichloroacetone (**3.31**).



Scheme 3.1: Retrosynthesis of thiazole ferrocenyl molecules (**3.22a-e**)

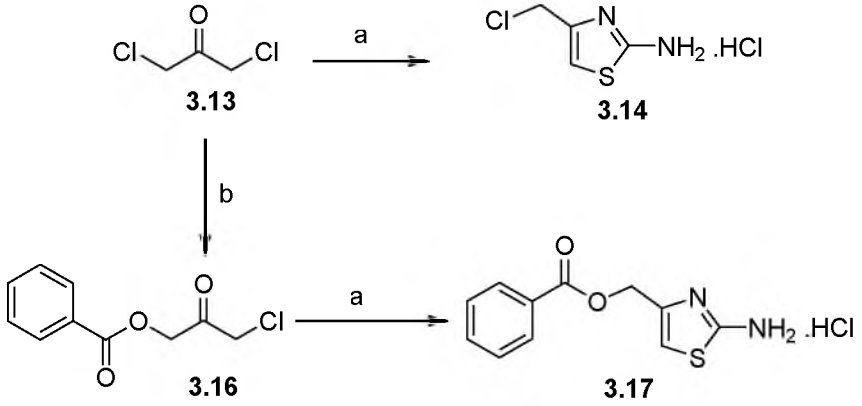
Regarding the synthesis of required thiazole derivatives, we envisaged to access the target intermediates *via* two synthetic routes. Following successful synthesis of **3.14** (route 1), it was thought that nucleophilic displacement of methylene chloro substituent of **3.14** with various amines would lead to desired aminomethyl derivatives **3.15**. Alternatively, the series **3.15** could be accessed through reductive amination reaction of the aldehyde **3.18**, which is generated in two steps from thiazole **3.17**, with various amines.



Scheme 3.2: Proposed synthetic routes

3.2.2 Preparation of starting thiazoles hydrochloride (3.14 and 3.17)

A number of methods have been employed to synthesise thiazole derivatives and these include Hartzsch, Gabriel and Cook-Heilborn syntheses.[14] Hartzsch's synthesis is extensively used for synthesis of thiazole and its derivatives. It involves the condensation and cyclization of haloketones with thioamides. Herein, the starting compound 4-chloromethylthiazole-2-amine **3.14** was synthesised using the above mentioned synthetic reaction.[9] **Scheme 3.3** illustrates the synthesis and reaction conditions for thiazole compounds.



Scheme 3.3: Synthesis of various thiazole analogues*: Reagents and conditions:* a) Thiourea, EtOH, 24 h; b) Benzoic acid, Na_2CO_3, 16 h.

Equimolar amounts of 1,3-dichloroacetone (**3.13**) and thiourea (**Scheme 3.3**) were allowed to stir at room temperature in ethanol overnight to access the key starting compound **3.14**.[9] Compound **3.14** was obtained in its pure form as a white crystalline solid in 70% yield. Using of acetone as a solvent led to poor yields (30%), while ethanol increased the yield to 70%. The ^{1}H NMR spectrum of compound **3.14** depicted in **Figure 3.5** showed a singlet peak at δ 7.00 ppm corresponding to the proton (H-2) on the thiazole ring. The methylene protons signal also appeared as a singlet with a chemical shift at δ 4.69 ppm, while the amine protons resonated at δ 9.48 ppm as a broad peak. The ^{13}C NMR corroborated the ^{1}H NMR data, and showed four signals which were consistent with skeletal structure of compound **3.14**.

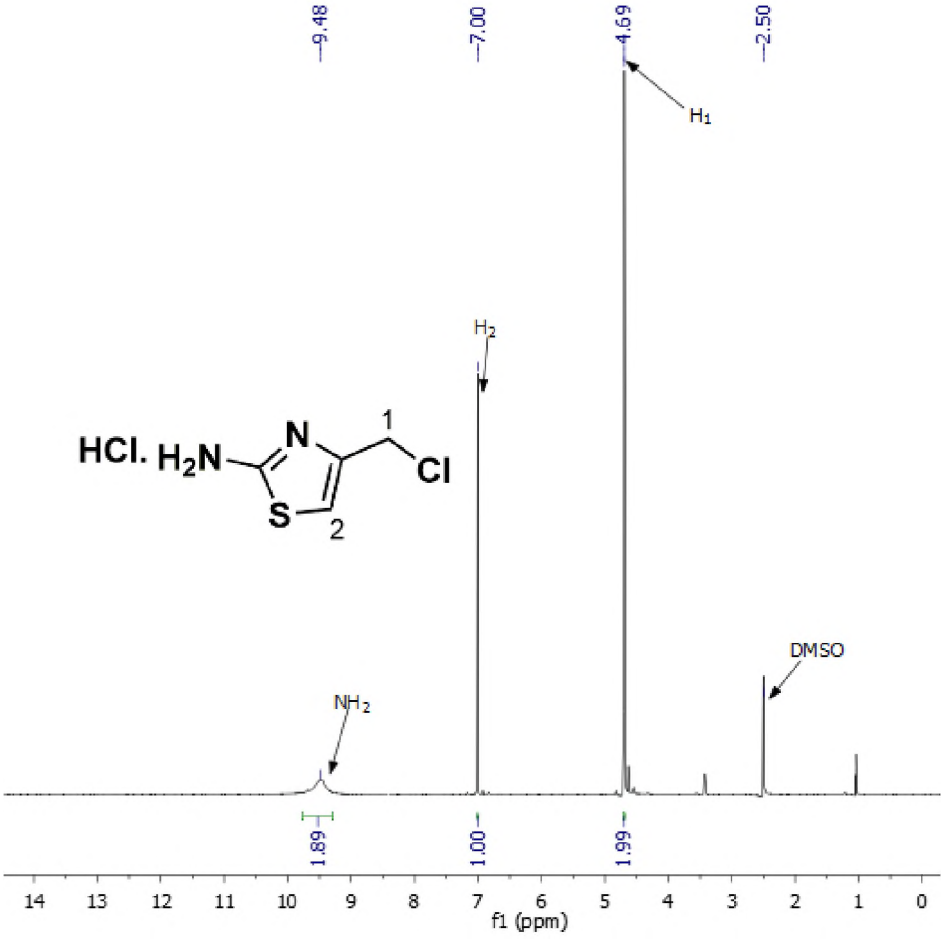


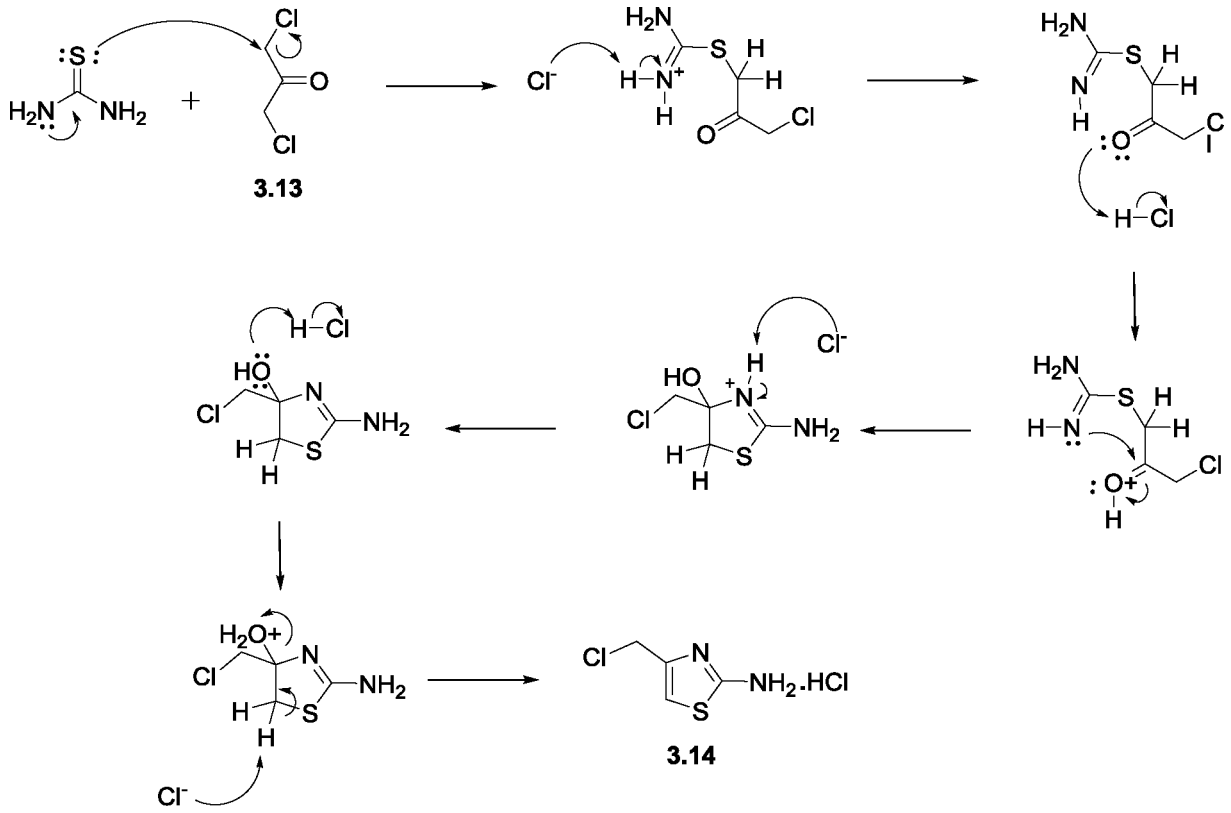
Figure 3.3: ^{1}H NMR of 4-chloromethylthiazol-2-amine

The treatment of 1,3-dichloroacetone (**3.13**) with benzoic acid in DMF at room temperature afforded 1-benzyloxy-3-chloropropane-2-one (**3.16**) in 32% yield following extraction and recrystallisation in hexane. Compound **3.16** was characterised using ^{1}H and ^{13}C NMR, which both confirmed the identity of **3.16**. The ^{1}H NMR displayed five protons in aromatic region corresponding to aromatic protons, and the methylene protons which appeared as singlets between δ 4.25 ppm.

Similarly, compound **3.16** was reacted with thiourea in EtOH as a solvent to achieve the thiazole analogue **3.17** in 56% yield. Formation of compound **3.17** was confirmed by both ^{1}H and ^{13}C NMR spectroscopic techniques, which showed the appearance of a broad peak at δ 9.38 ppm that was attributed to the amino group on the thiazole ring. A chemical shift signal at δ 7.03 ppm indicated the characteristic thiazole proton. A singlet peak at δ 5.02 ppm integrating for two protons was attributed to methylene protons. Aromatic protons integrating for five protons appeared as a triplet at 7.70, a pair of multiplet at 7.54 and another pair of doublet at 8.01 ppm.

3.2.2.1 Proposed mechanism for the formation of key compound 3.14

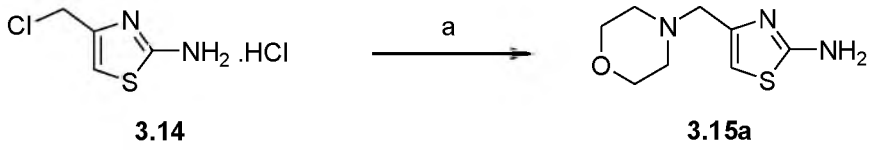
The reaction mechanism for thiazole ring formation **(Scheme 3.4)** generally involves the condensation of haloketones and thioamides or thioureas. Briefly, the reaction starts with the nucleophilic attack of sulphur atom of thioamide on the α-carbon of the α-haloketone resulting to the formation of thioketone intermediate. The dehydration of the intermediate generates the corresponding thiazole. The first step of the reaction mechanism in the thiazole ring synthesis consist of S-Alkylation and elimination of hydrogen chloride, in the next step the nitrogen lone pair attack the carbonyl carbon followed by proton transfer from nitrogen atom to oxygen. Eventually there is a second proton transfer, subsequently followed by elimination of water leading to the thiazole moiety. This process may also involve the use of α-diazoketone along with thiourea derivatives.[8]



Scheme 3.4: Mechanistic detail of formation of thiazole.[8]

3.2.3 Preparation of aminomethylthiazole derivatives

Generally, the reaction of an alkyl halide with primary or secondary amines is a commonly used approach for the preparation of secondary or tertiary amines.[9,10,11] The nucleophilic displacements of halides as leaving groups often resulted in the formation of tertiary amine. **Scheme 3.5** below shows a nucleophilic displacement reaction to access compound **3.15a**.



Scheme 3.5: *Reaction conditions* a) Morpholine, EtOH, 48 h.[12]

Different amines were selected to be coupled with the thiazole unit to form desired aminomethylthiazole derivatives. Some of these alkyl amine moieties have featured in medicinal chemistry or drug discovery and are widely use to modified drug molecules including several bioactive compounds that are promising as antimalarial agents.[13, 14] Initially, we reacted **3.14** with morpholine in DMF as a solvent and no product could be identified after refluxing for 16 h. The TLC showed mainly the presence of unreacted starting materials. Similarly, there was no evidence of formation of the product when the reaction was conducted in THF as a solvent (**Table 3.1**). Replacing of DMF and THF with EtOH as a solvent led to the isolation of desired compound **3.15a** after 48 h at room temperature. When this reaction was run under reflux in ethanol, there was a spot showing the product formation accompanied with multiple other spots, which made it complex to isolate the desired product. The product obtained using ethanol at room temperature was purified by recrystallisation using DCM:MeOH (10:1) to obtained light yellow crystals in 40% yield. In CH_3CN as a solvent compound **3.15a** was obtained in slightly high yield (52%) than in EtOH, but at elevated temperature for 3 – 12 h along with K_2CO_3 as a base.

Table 3.1: Optimizing reactions condition for the formation of aminomethylthiazole **3.15a.**

Entry	Solvent	Time (h)	Temperature	Yield (%)
1	DMF	16	Reflux	-
2	THF	16	Reflux	-
3	EtOH	48	rt	40
4	CH_3CN	3-12	reflux	52

Compound **3.15a** was characterised by ^1H and ^{13}C NMR spectroscopic techniques. The ^1H spectrum revealed two characteristic triplets due to CH_2 protons from morpholine at δ 3.72 and 2.46 ppm, respectively. The methylene protons on the carbon linking thiazole to morpholine appeared as a singlet at δ 3.36 ppm.

The chemical shift due to thiazole ring proton resonated at δ 6.30 ppm. The ^{13}C NMR spectrum (**Figure 3.6**) of **3.15a** exhibited a required number of carbons which was consistent with the proposed chemical structure of **3.15a**. The analysis using Electron Spray Ionization (ESI) mass spectroscopy revealed the molecular ion peak at *m/z* 185 that appeared consistent with fragmentation pattern of this compound.

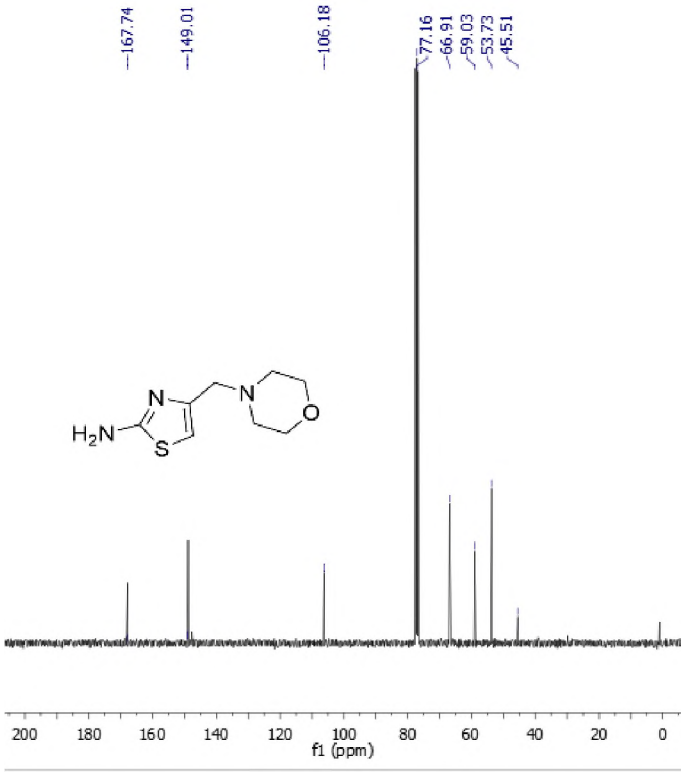
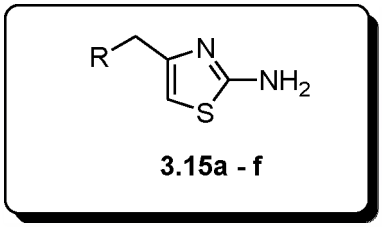


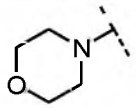
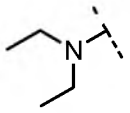
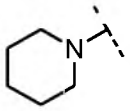
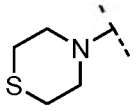
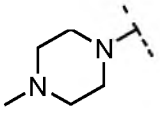
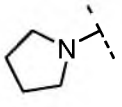
Figure 3.4: The ^{13}C NMR of 4-(morpholinomethyl)thiazol-2-amine, **3.15a**

Except **3.15f**, which was obtained by refluxing in CH_3CN for 6 h, the rest of other aminomethyl derivatives (**3.15b** – **e**) were accessed following similar reaction conditions in **Scheme 3.5.** The procedure using ethanol as a solvent was adopted to synthesise these derivatives as other solvents often resulted in no or low yields. **Table 3.2** below summarised the yields of isolated aminomethyl derivatives **3.15a** – **f**.

Table 3.2: Yields of isolated aminomethylthiazole derivatives **3.15a – f**.



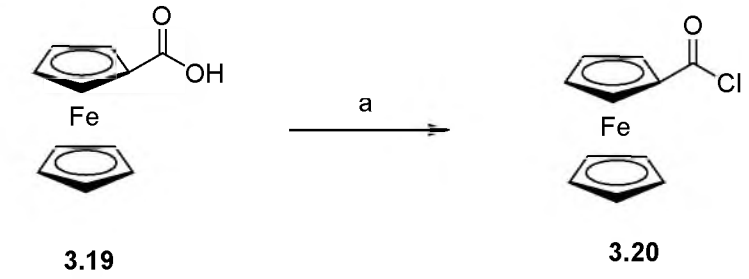
3.15a - f

Entry	Product	R	Yield (%)
1	**3.15a**		40
2	**3.15b**		50
3	**3.15c**		60
4	**3.15d**		70
5	**3.15e**		65
6	**3.15f**		90

The synthesised derivatives **3.15b-f** were characterised by common spectroscopic tools. The methylene protons in all structures resonated as singlets in the range δ 3.30 - 4.22 ppm. These protons are shielded compared to the key starting compound **3.14** where the same methylene protons appeared slightly downfield at around δ 4.6 ppm. In all structures the protons of the thiazole core resonated between δ 6.24 and 7.36 ppm. The ^{13}C NMR spectrum of each molecule corroborated the ^{1}H NMR data and showed required number of carbon signals, which were consistent with the proposed chemical structures of target compounds **3.15b – f**. In all cases the ESI mass spectrometry analysis indicated molecular ion peaks or fragments that were agreeing with molecular structures of target molecules.

3.3 Synthesis of ferrocene aminomethylthiazole derivatives

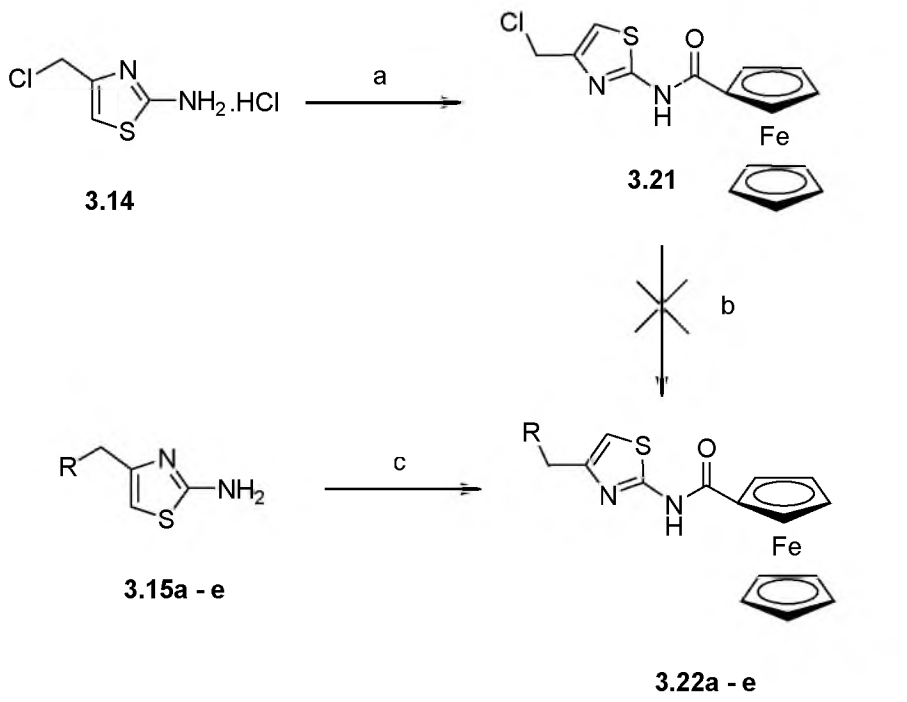
The synthesis of ferrocenic-thiazole coupled compounds **3.22a** – **e** firstly commenced with activation of ferrocene carboxylic acid **3.19** (**Scheme 3.6**). Nadia and co-workers undertook the *in situ* activation of ferrocene carboxylic acid using oxalyl chloride under nitrogen atmosphere.[15] Thus, ferrocene carboxylic acid was treated with oxalyl chloride for an hour. The excess oxalyl chloride was removed under vacuum and the residue partitioned between diethyl ether and pentane to obtain **3.20** as a dark red crystalline solid, which was used immediately. The FT-IR spectroscopic analysis revealed the prominent band due to C-Cl which appeared at 1748 cm^{-1}.[15]



3.19 **3.20**

Scheme 3.6: *Reagents and conditions:* a) Oxalyl chloride, dry DCM, r.t., 1 h.

In this research, we explored the formation of amide using conventional amidation procedure.[16] We started off the synthesis of target compounds by treating compound **3.14** with ferrocene carboxylic acid **3.19** in presence of DIPEA in DCM at 0 °C to form a red solid residue, which was subjected to silica gel column chromatography purification to give compound **3.21** in 30% yield (**Scheme 3.7**). Compound **3.21** was characterised using common spectroscopic techniques



Scheme 3.7: *Reagents and conditions*: a) Ferrocenoyl chloride, DIPEA, DCM, r.t., 12 h; b) i) NaN_3, DMF, 80 °C, 26h; ii) H_2O, PPh_3, r.t., 24h; c) Ferrocene carboxylic acid, DMAP, TEA, EDCI.HCl, THF, 0 °C then at r.t., 12h.

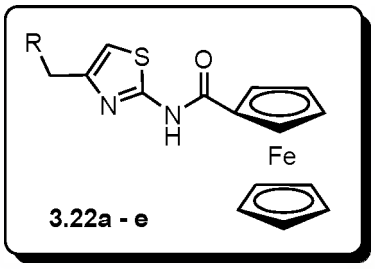
The literature survey uncovered the importance of the unsubstituted amino methylthiazole group for antimalarial activity.[6, 7] In an attempt to generate an aminomethyl functionality at the 4-position of thiazole using azide precursor yielded no results, as the initial preparation of the azide using sodium azide in DMF resulted in multiple spots (TLC) including starting materials. These spots proved to be difficult to separate as in many attempts co-eluted.

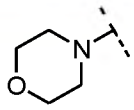
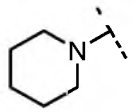
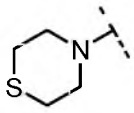
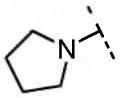
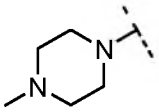
The ^{1}H NMR spectrum indicated unidentifiable complex mixture of products. The mass spectroscopy analysis of the reaction product showed no evidence of molecular ion or fragment appeared to be consistent with desired ferrocenic thiazole with an azide functionality. Then it was decided that instead of forming **3.21** followed by unsuccessful nucleophilic displacement reaction that aminomethylthiazoles **3.15a-f** be reacted with either **3.19** or **3.20** to give target compounds **3.22a-e**.

Except **3.22e** which was obtained in 40%, treatment of **3.15a-d** and **3.15f** with ferrocene carboxylic acid chloride **3.20** freshly prepared resulted in isolation of starting materials (TLC) with no evidence of formation of desired products. It was thought that perhaps during the reaction **3.20** may have reverted back (i.e. hydrolysis) to **3.19**, which is thought to be less reactive when combined with a slightly unreactive amine of aminomethylthiazole derivatives. Alternatively, in a one pot reaction compound **3.20** was treated with aminomethylthiazoles and yielded undesirable results and the TLC showed a complex mixture of spots, which were proved difficult to separate. Then, we opted to treat ferrocene carboxylic acid with aminomethylthiazoles **3.15a-f** using coupling agents such as HOBt and EDCI in DIPEA. These conditions amounted to no desired products achieved despite varying reaction conditions and solvents.

To our delight the amide formation did occur when **3.19** was reacted with **3.15a** in presence of TBTU and DIPEA in dry DCM overnight. However, the product was recovered in low yield following purification of the crude by silica gel column chromatography.[1] Among the known approaches for amide synthesis, one involves the generation *in situ* of a reactive intermediate by addition of the activating reagent to the reaction mixture.[17] The recovery of product in low yield prompted us to carry out the reaction of **3.19** with other aminomethylthiazoles (**3.15c**, **3.15d** and **3.15f**) using DMAP, TEA and EDCI in dried THF. Using these reaction conditions the desired compounds **3.22b, 3.22c** and **3.22e** did form and were recovered in the yield ranging between 35 and 60%. Despite numerous attempts, the reaction of **3.19** and **3.20** with aminomethylthiazole derivative **3.15b** yielded no desired product. The desired ferrocene-based aminomethylthiazole compounds and isolated yields are summarised in **Table 3.3**.

Table 3.3: Yields of isolated aminomethylthiazole derivatives **3.22a – e**.

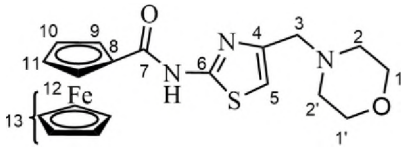


Entry	Product	R	Yield (%)
1	**3.22a**		30
3	**3.22b**		40
4	**3.22c**		60
5	**3.22d**		35
6	**3.22e**		40

All target compounds were analysed using common spectroscopic analytical techniques. For example, ^{1}H NMR spectra of compound **3.22a** displayed chemical shifts in the range δ 4.37 - 4.81 ppm, which were consistent with characteristic ferrocenyl protons. The chemical shifts corresponding to morpholine protons appeared in the range δ 1.45 - 3.75 ppm.

In all targeted molecules the unsubstituted cyclopentenyl ring of the ferrocene appeared as a singlet peak in the range between δ 4.20 - 4.38 ppm. The methylene protons in all compounds showed chemical shifts at δ 3.02 - 3.36 ppm. Overall, the ^{13}C NMR of all compounds **3.22a-e** supported the ^{1}H NMR data with each spectrum revealing a required number of carbons consistent with the presence of ferrocene unit and aminomethylthiazole motifs.

Table 3.4: 1H NMR data of compound **3.22a**



3.22a

δ/ppm	Multiplicity	Assignment
2.44-2.48	m	H_2
3.39	s	H_3
3.7-3.76	m	H_1
4.21	s	H_{13}
4.38-4.42	m	$H_{10/11}$
4.71-4.74	m	$H_{9/12}$
8.00	s	H_5

The positive-ion of EI-MS or ESI-MS spectra of all compounds showed molecular ion peaks at *m/z* 413.26 (**3.22a**), 431.50 (**3.22b**), 427.30 (**3.22c**), 396.02 (**3.22d**) and 425.11 (**3.22e**). The

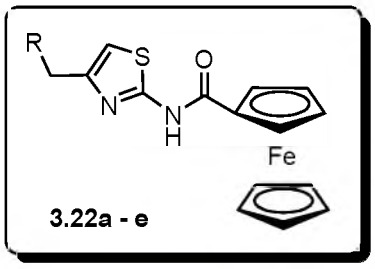
fragmentation signals observed served as a further confirmation that these compounds were successfully synthesised.

3.4 Biological results and discussion

3.4.1 *In vitro* antiplasmodial activity of 3.22a – e

The biological activities of prepared compounds were evaluated *in vitro* against *P. falciparum* CQS strain (3D7). The CQ was included as a positive control. The cytotoxicity evaluation of all compounds was conducted using HeLa cell line and emetine was used as a control drug. All *in vitro* assay tests were conducted in collaboration with the Centre for Chemico- and Biochemical Research at Rhodes University. The biological results are summarised in **Table 3.5**. From the data presented (**Table 3.5**), it is evident that ferrocene-thiazole derivatives **3.22a – e** were not active with IC_{50} values above 50 μM against *P. falciparum* strain 3D7. The results obtained nullify our hypothesis that the presence of organometallic ferrocenyl unit would resulted in new thiazole containing compounds with enhanced antiplasmodial activity. Consistent with previous observation,[7] this data suggested that modification of **MMV010539** by replacing the pyrazoline core with ferrocenyl unit is detrimental for activity of this class of compounds. In addition, conversion of the free amine to alkyl amines might be the cause of the loss of activity. Furthermore, this corroborated previous studies that in spite of known effects of incorporating organo-metallic fragments into organic scaffolds, there is a limited number of specific groups that are permitted in aminomethylthiazole **MMV010539** pharmacophore.[7] The modification of alkyl amines on the other side of the molecule didn't show any improved activity at all, this lead to the conclusion that the SAR of the lead compound is limited.

Table 3.5: In vitro antiplasmodial and cytotoxicity activities of compounds **3.22a – f**.



Product	IC_{50} (μM)	
	3D7	Cytotoxicity
3.22a	108.6	> 100
3.22b	51.9	> 100
3.22c	108.8	> 100
3.22d	68.8	> 100
3.22e	86.6	> 100
Emetine	-	0.034
Chloroquine	0.002	-

The cell toxicity assay showed that all synthesised compounds lacked any significant toxicity effects against HeLa (human cervix adenocarcinoma) cell line. **Figure 3.8** illustrates the cytotoxicity effects of compounds **3.22c** and **3.22e.** Overall, the data suggests that incorporating the ferrocenyl unit resulted in new compounds void of any toxic side effects.

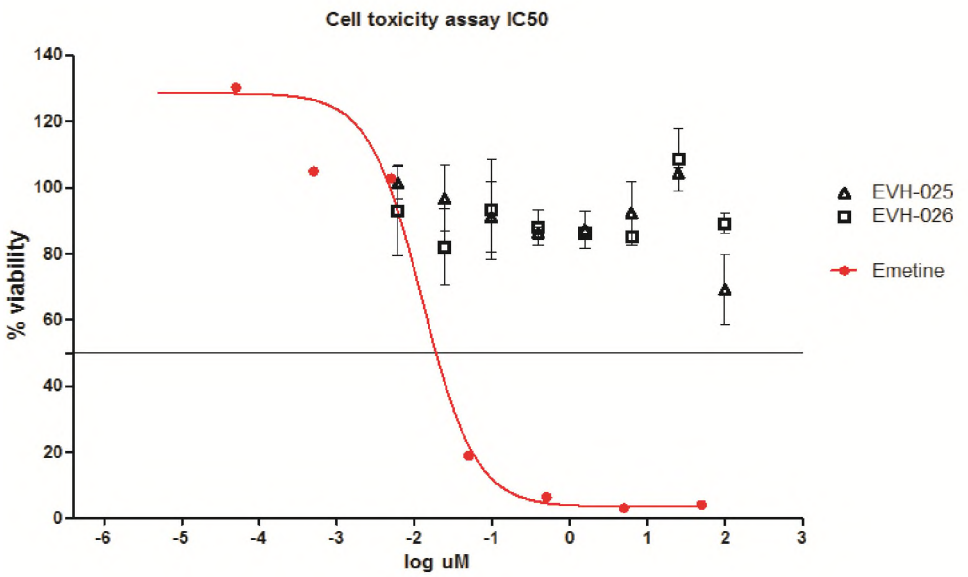


Figure 3.5: Cell toxicity assays.

3.5 Conclusions

In conclusion, a series of thiazole amine intermediates (**3.15a** – **f**) was achieved starting from the key compound **3.14**. With the aim to explore the SAR of the lead compound **MMV010539**, ferrocene-thiazole based compounds **3.22a-e** were synthesised through amidation, and all target compounds were successfully characterised using various analytical and spectroscopic techniques such as ^{1}H and ^{13}C NMR, mass spectrometry, FT-IR spectroscopy and melting point measurements. All intermediates and target molecules were obtained in low to high yields. Note that the conventional amide synthesis with some modification enabled the formation of desired amides. In general, the cytotoxicity assay showed that the final compounds were not toxic against Hela cell lines at maximum tested concentration (100 µM). More importantly, antimalarial assay results of target compounds did not show any promising activity against *P. falciparum* strain 3D7 and this suggested that there is limited structural modification that is allowed on the lead compound **MMV010539**.[7]

3.6 References

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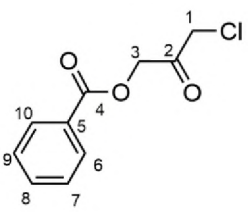
CHAPTER FOUR

EXPERIMENTAL PROCEDURE

4.1 General Procedure

All commercially available chemicals and reagents were purchased from Sigma-Aldrich and Merck, and were used without further purification or drying unless otherwise stated in the procedure. All reactions were carried out in oven-dried glassware (90 °C). The reactions were monitored by thin layer chromatography (TLC) and visualised under ultra violet (UV) light (254 nm), or subsequently stained in an iodine tank. Preparative thin layer chromatography (PTLC) was prepared and activated as prescribed by the supplier (Merck). Silica gel 60 (particle size 0.040-0.063 mm) was used as the stationary phase for flash and column chromatography. NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ at room temperature on Bruker Fourier 300 MHz, Bruker Fourier 400 MHz or 600 MHz Avance II spectrometer. Chemical shifts are reported relative to TMS on delta (δ) scale in parts per million (ppm). The splitting of proton resonances in the reported ^1H NMR spectra are defined as s = singlet, d = doublet, t = triplet, or m = multiplet, b = broad. Coupling constant values, *J* values are reported in Hz. Electron Spray Ionization mass spectrometer (ESI-MS) in the positive and negative ionization mode was used to record low resolution mass spectra. High resolution mass spectrometry data were recorded on Waters Snapt G2-TOF MS, University of Stellenbosch, and Ion's mass-to charge ratio was determined by time measurement. Infrared (IR) spectra were recorded on a Perkin Elmer FT-IR 400 instrument, absorption bands are reported as s = strong, m = medium, b = broad or w = weak. The melting points (m.p) were determined on Reichert 281313 melting point apparatus.

4.2 Synthesis of 3-chloro-2-oxopropyl benzoate, 3.16

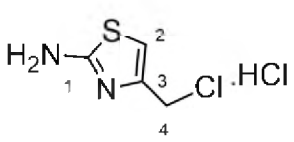


Dichloroacetone (1.0 g, 7.9 mmol) was added in one portion to a solution of $NaHCO_3$ (0.40 g, 4.8 mmol) and benzoic acid (0.48 g, 3.9 mmol) in dry DMF (15 mL) at 0 °C. The reaction was stirred at this temperature for 3 h, then at room temperature for a further 12 h. The reaction mixture was diluted with water and extracted with petroleum ether-ethyl acetate (95:5). The organic phase was washed with water and brine, successfully dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure to give a solid residue, which was recrystallized in hexane to give a white solid (0.48 g, 32%);[1] rf in EtOAc:Hexane (8:2) 0.6; M.p. 87 - 90 °C, lit.[1] 89 - 92 °C. δ_H (300 MHz, $CDCl_3$): 4.25 (2H, s, $\mathbf{H_1}$), 5.14 (2H, s, $\mathbf{H_3}$), 7.46−7.50 (2H, m, $\mathbf{H_{7,9}}$), 7.60−7.63 (1H, m, $\mathbf{H_8}$), 8.08−8.10 (2H, m, $\mathbf{H_{6,10}}$); δ_C (75 MHz, $CDCl_3$): 46.3 ($\mathbf{C_1}$), 67.4.($\mathbf{C_3}$), 129.1 ($\mathbf{C_{7,9}}$), 130.2 ($\mathbf{C_8}$), 130.4 ($\mathbf{C_{6,10}}$), 134.2 ($\mathbf{C_5}$), 165.8 ($\mathbf{C_4}$), 197.0 ($\mathbf{C_2}$).

4.3 General procedure for the synthesis of 3.14 and 3.17

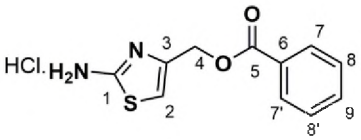
Thiourea (7.4 mmol) was treated with to a solution of 1,3-dichloropropanone or 1-benzoyloxy-3-chloropropan-2-one (7.4 mmol) in absolute ethanol (25 mL). The mixture was allowed to stir at room temperature for 24 h, then kept at 4 °C for 12 h. The crystalline solid was collected by filtration and recrystallised from ethanol to yield the desired hydrochloride salt.[1]

4.3.1 4-Chloromethylthiazol-2-amine hydrochloride, 3.14



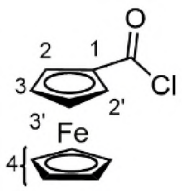
White solid (0.96 g, 70%); rf in EtOAc:Hexane (8:2) 0.3; M.p. 137 - 141 °C lit.[92] 141 - 143 °C. δ_H (300 MHz, DMSO-d_6): 4.52 (2H, s, $\mathbf{H_4}$), 6.89 (1H, s, $\mathbf{H_2}$), 9.53 (2H, br s, $N\mathbf{H_2}$); δ_C (75 MHz, DMSO-d_6): 37.4 ($\mathbf{C_4}$), 108.4 ($\mathbf{C_2}$), 137.3 ($\mathbf{C_3}$), 172.7 ($\mathbf{C{=}N}$).

4.3.2 (2-Aminothiazol-4-yl)methyl benzoate, 3.17



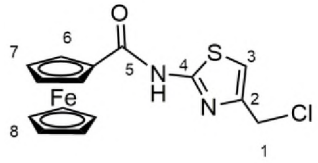
Yellow solid (0.96 g, 56%); rf in EtOAc:Hexane (8:2) 0.8; δ_H (300 MHz, DMSO-d_6): 5.20 (s, 2H, **H4**), 7.03 (s, 1H, **H2**), 7.54 (2H, m, J = 8.0 Hz, **H8,8'**), 7.70 (1H, t, J = 8.00 Hz, **H9**), 8.01 (2H, dd, J = 8.0 Hz, **H7,7'**), 9.30 (2H, br s, **NH2**). δ_C (75 MHz, DMSO-d_6): 58.8 (**C4**), 107.6 (**C2**), 128.9 (**C8,8'**), 129.0 (**C7,7'**), 129.4 (**C9**), 133.8 (**C6**), 135.3 (**C3**), 165.2 (**C5**), 170.1 (**C1**).

4.4 Synthesis of ferrocenoyl chloride, 3.20



Oxalyl chloride (0.97 mL, 11.2 mmol) was added to a stirred solution of ferrocene carboxylic acid (2.00 g, 5.6 mmol) in dry CH_2Cl_2 (10 mL) under argon atmosphere at room temperature. After 20 min the excess of oxalyl chloride and DCM were removed *in vacuo* and the solid residue was dissolved in Et_2O/pentane, filtered and concentrated *in vacuo* to give acid chloride **3.20** as dark red solid (1.14 g, 50%);[3] rf in EtOAc:$CHCl_3$ (1:1) 0.5; ν_{max} (cm^{-1}): 1650-1723s ν(C=O), 1748 ν(C-Cl); δ_H (300 MHz, $CDCl_3$): 4.26 (s, 5H, **H4**), 4.47 (2H, m, J = 4.47 Hz, **H3,3'**), 4.85 (2H, m, J = 4.47 Hz, **H2,2'**); δ_C (75 MHz, $CDCl_3$): 69.8 (**C1**), 70.3 (**C4**), 70.7 (**C3,3'**), 72.1 (**C2,2'**), 177.4 (C=O).

4.5 Synthesis of compound, 3.21



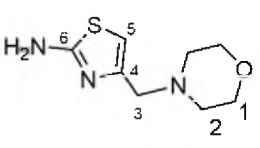
Freshly prepared ferrocenoyl chloride (0.250 g, 1.00 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and then added to a solution of 2-amino-4-chloromethylthiazole hydrochloride (0.250 g, 1.19 mmol) and diisopropylethylamine (0.6 mL) in dry CH_2Cl_2 (5 mL) at 0 °C. The resulting mixture was allowed to stir at room temperature overnight. After the consumption of starting material, as indicated by TLC, the reaction was quenched with water. The organic phase was separated and the aqueous phase extracted three times with dichloromethane. The combined organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a red solid residue which was purified by column chromatography (ethyl acetate: hexane,7:3) to give a dark red solid (0.14 g, 30%); rf in EtOAc: hexane (1:1) 0.4;

M.p. 134 − 140 °C; δ_H (300 MHz, $CDCl_3$): 3.49 (2H, s, **H_1**), 5.12 (1H, b, **NH**), 4.25 (5H, s, **H_8**), 4.50 (2H, t, J = 6.00 Hz, **$H_{7,7'}$**), 4.86 (2H, t, J = 6.00 Hz, **$H_{6,6'}$**), 6.53 (1H, s, **H_3**); *m/z* (ESI) 360.20 (40%, M^+); (Found: M^+, 360.20. $C_{15}H_{13}N_2ClFeSO$ requires 359.98).

4.6 General procedure for synthesis of 3.15a, 3.15b, 3.15c, and 3.15e

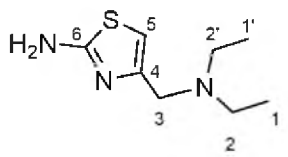
4-chloromethylthiazol-2-amine (0.158 g, 0.85 mmol) was dissolved in ethanol (5 mL) followed by addition of an appropriate amine (1.5 − 3.6 mmol) at room temperature. The reaction mixture was stirred at room temperature for 48 h. The reaction progress was monitored using TLC, and upon completion ethanol was removed to give a semi-solid residue, which was purified by column chromatography ($CHCl_3$:MeOH, 9:1) or recrystallised in CH_2Cl_2:MeOH (10:1) to give desired compounds as solids or oils.

4.6.1 4-(Morpholinomethyl)thiazol-2-amine, 3.15a



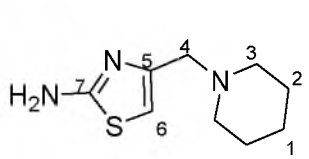
Light yellow crystals (0.12 g, 75%); δ_H (300 MHz, $CDCl_3$): 2.46 (4H, t, J = 9.0 Hz, **H_2**), 3.36 (2H, s, **H_3**), 3.72 (4H, t, J = 9.0 Hz, **H_1**), 5.20 (2H, br s, **NH_2**), 6.30 (1H, s, **H_5**); δ_C (75 MHz, $CDCl_3$): 53.9 (**C_2**), 59.1 (**C_3**), 67.0 (**C_1**), 106.2 (**C_5**), 148.5 (**C_4**), 168.0 (**C_6**). *m/z* (ESI) 185.50 (30%, M^+); (Found: M^+, 185.50. $C_8H_{13}N_3OS$ requires 185.09).

4.6.2 4-((Diethylamino)methyl)thiazol-2-amine, 3.15b



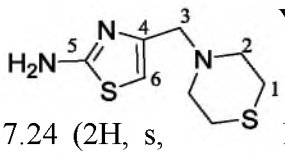
Brown solid (0.078 g, 50%); δ_H (300 MHz, DMSO): 1.13 (6H, t, J_{HH} = 6.0 Hz, **H_1**), 3.38 (4H, q, J_{HH} = 6.0 Hz, **H_2**), 4.22 (2H, s, **H_3**), 5.12 (2H, s, NH), 7.36 (1H, s, **H_5**); δ_C (75 MHz, DMSO): 15.3 (**C_1**), 46.4 (**C_2**), 65.3 (**C_3**), 103.2 (**C_5**), 149.7 (**C_4**), 168.8 (**C_6**). *m/z* (ESI) 170.20 (26%, $[M-H]^+$); (Found: M^+, 170.20. $C_7H_{12}N_3S$ requires 171.08).

4.6.3 4-(Piperidin-1-ylmethylthiazol-2-amine, 3.15c



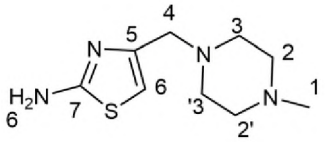
Yellow solid (0.14 g, 60%); rf in EtOAc:MeOH (9:1) 0.6; δ_H (600 MHz, $CDCl_3$): 1.41 − 1.56 (6H, m, $\mathbf{H_{1,2,2'}}$), 3.05 (4H, t, J = 11.4 Hz, $\mathbf{H_{3,3'}}$), 3.33 (2H, s, $\mathbf{H_4}$), 5.33 (2H, s, NH_2), 6.28 (1H, s, $\mathbf{H_6}$); δ_C (150 MHz, $CDCl_3$): 25.7 ($\mathbf{C_1}$), 45.0 ($\mathbf{C_2}$), 54.5 ($\mathbf{C_3}$), 59.0 ($\mathbf{C_4}$), 105.6 ($\mathbf{C_6}$), 149.2 ($\mathbf{C_5}$), 167.9 ($\mathbf{C_7}$).

4.6.4 4-(Thiomorphinomethyl)thiazole-2-amine, 3.15d



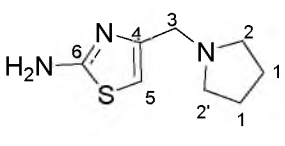
Yellow solid (0.12 g, 70%); δ_H (300 MHz, $CDCl_3$): 2.71 (4H, t, J = 12.0 Hz, $\mathbf{H_1}$), 2.94 (4H, t, J = 12.0 Hz, $\mathbf{H_2}$), 3.38 (2H, s, $\mathbf{H_3}$), 6.29 (1H, s, $\mathbf{H_6}$), 7.24 (2H, s, NH_2); δ_C (75 MHz, $CDCl_3$): 24.1 ($\mathbf{C_1}$), 45.1 ($\mathbf{C_2}$), 54.3 ($\mathbf{C_3}$), 105.8 ($\mathbf{C_6}$), 147.4 ($\mathbf{C_4}$), 167.5 ($\mathbf{C_5}$); *m/z* (ESI) 218.10 (50%, M^+); (Found: M^+, 218.10. $C_8H_{13}N_3S_2$ requires 218.00).

4.6.5 4-((4-Methylpiperazin-1-yl)methyl)thiazol-2-amine, 3.15e



Red brown dark oil (0.32 g, 65 %); rf in EtOAc: MeOH (9:1) 0.3; δ_H (300 MHz, DMSO-d_6): 2.31 (3H, s, $\mathbf{H_1}$), 2.64 (4H, t, J = 8.0 Hz, $\mathbf{H_{2,2'}}$), 3.13 (4H, t, J = 8.0 Hz, $\mathbf{H_{3,3'}}$), 3.81 (2H, s, $\mathbf{H_4}$), 5.61 (2H, s, $\mathbf{NH_2}$), 6.24 (1H, s, $\mathbf{H_6}$); δ_C (75 MHz, DMSO-d_6): 17.8 ($\mathbf{C_1}$), 43.2 ($\mathbf{C_{3,3'}}$), 51.7 ($\mathbf{C_{2,2'}}$), 57.4 ($\mathbf{C_4}$), 104.7 ($\mathbf{C_6}$), 147.3 ($\mathbf{C_5}$), 162.6 ($\mathbf{C_7}$).

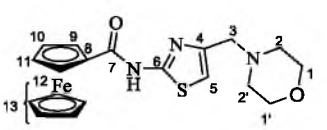
4.7 Synthesis of 4-(pyrrolidin-1-ylmethyl)thiazol-2-amine, 3.15f



To the solution of 4-chloromethylthiazol-2-aminehydrochloride (0.23 g, 1.24 mmol) and potassium carbonate (0.5 g, 3.6 mmol) in MeCN (15 mL) was added pyrrolidine (0.3 mL, 3.6 mmol). The reaction mixture was allowed to reflux for 6 h while monitoring it with TLC. Upon completion the mixture was poured into ice cold water (20 mL) and extracted with EtOAc (40 mL). The organic layer was dried using $MgSO_4$, filtered and the solvent removed under reduced pressure. The residue was

purified by flash chromatography using $CHCl_3$:MeOH (9:1) as eluent to afford the desired product as a dark red solid (0.2 g, 90%); rf in $CHCl_3$:MeOH (9:1) 0.4; δ_H (300 MHz, $CDCl_3$): 1.76 (4H, t, J = 12.0 Hz, $\mathbf{H_{1,1'}}$), 2.55 (4H, t, J = 12.0 Hz, $\mathbf{H_{2,2'}}$), 3.48 (2H, s, $\mathbf{H_3}$), 4.97 (2H, s, NH_2), 6.28 (1H, s, $\mathbf{H_5}$); δ_C (75 MHz, $CDCl_3$): 23.1 ($\mathbf{C_{1,1'}}$), 53.9 ($\mathbf{C_{2,2'}}$), 55.8 ($\mathbf{C_3}$), 104.5 ($\mathbf{C_5}$), 150.2 ($\mathbf{C_4}$), 167.2 ($\mathbf{C_6}$). *m/z* (ESI) 184.0 (65%, M^+); (Found: M^+, 184.0. $C_8H_{13}N_3S$ requires 184.0).

4.8 Synthesis of compound 3.22a



3.22a

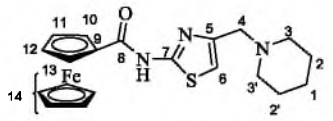
To a stirred solution of ferrocene carboxylic acid (0.12 g, 0.52 mmol) in dried DCM (5 mL) was added TBTU (0.11 g, 10.3 mmol) and the reaction mixture was allowed to stir at room temperature for 15 min. Then DIPEA (0.10 mL, 0.6 mmol) and 4-morpholinomethyl)thiazol-2-amine (0.18 g, 0.9 mmol) were added and the reaction was left to stir overnight. TLC showed the formation of the product, and the reaction mixture was poured into a saturated solution of $NaHCO_3$ and thoroughly extracted with ethyl acetate, washed with brine, dried over $MgSO_4$. After filtration, the solvent was concentrated under reduced pressure to give a dark red residue. The crude mixture was subjected to column chromatography (EtOAc:Hexane, 1:1) to give the desired product as a dark red solid (0.064 g, 30%); rf in EtOAc: Hexane (9:1) 0.15; M.p. 132 − 136 °C; ν_{max}/cm^{-1}(IR-FT) 3075s ν (N-H), 1612s ν (C=O); δ_H (600 MHz, $CDCl_3$): 2.44−2.48 (4H, m $\mathbf{H_2}$), 3.39 (2H, s, $\mathbf{H_3}$), 3.71− 3.76 (4H, m, $\mathbf{H_1}$), 4.23 (5H, s, $\mathbf{H_{13}}$), 4.70 − 4.74 (2H, m, $\mathbf{H_{9/12}}$), 4.38 – 4.42 (2H, m, $\mathbf{H_{10/11}}$), 8.00 (1H, s, $\mathbf{H_5}$), 9.15 (2H, br s, N**H**); δ_C (150 MHz, $CDCl_3$): 35.5 ($\mathbf{C_1}$), 55.7 ($\mathbf{C_3}$), 56.0 ($\mathbf{C_2}$), 70.3 ($\mathbf{C_{13}}$), 70.4 ($\mathbf{C_{10/11}}$), 70.7 ($\mathbf{C_{9/12}}$), 71.1 ($\mathbf{C_8}$), 106.2 ($\mathbf{C_5}$), 148.7 ($\mathbf{C_4}$), 156.5 ($\mathbf{C_6}$), 174.7 ($\mathbf{C_7}$); *m/z* (ESI) 413.2679 (4.5%, M^+); (Found: M^+, 413.2679, $C_{19}H_{21}N_3FeO_2S$ requires 413.0737).

4.9 General procedure for synthesis of 3.22b, 3.22c and 3.22e

A mixture of ferrocene carboxylic acid (0.43 mmol), aminomethylthiazoles (0.65mmol) and DMAP (5 mg, catalyst) was dissolved in anhydrous THF (5 mL) and treated with TEA (0.158 mL, 0.115 mmol). The reaction mixture was cooled at 0 °C and treated with EDCI.HCl (0.112 g,

0.005 mmol). The resulting reaction mixture was allowed to stir at room temperature for 12 h, and upon completion (TLC) the reaction mixture was diluted with water (10 mL) followed by $NaHCO_3$ (15 mL). The aqueous mixture was extracted with EtOAc (3 × 10 mL). Organic layers were combined, dried over $NaSO_4$, filtered and concentrated under reduced pressure to give solid products. The compounds were purified by recrystallisation using ethanol.

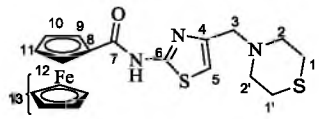
4.9.1 Compound 3.22b



3.22b

Red brown solid (67 mg, 40%); rf in EtOAc: MeOH (9.5:0.5) 0.48; M.p. 113 – 117 °C; ν_{max}/cm^{-1}(IR-FT) 3077s (N-H), 1667s (C=O); δ_H (400 MHz, $CDCl_3$): 1.42 - 1.88 (6H, m, **$H_{2,2'}$**), 2.22-2.41 (2H, m, **H_1**), 3.36 (2H, s, **H_4**), 3.64 (4H, m, **$H_{3,3'}$**), 4.23 (5H, s, **H_{14}**), 4.28 (2H, m, **$H_{11/12}$**), 4.54 (2H, b, **$H_{10/13}$**), 6.30 (1H, s, **H_6**); δ_c (100 MHz, $CDCl_3$): 24.8 (**C_1**), 25.8 (**$C_{2/2'}$**), 54.6 (**$C_{3/3'}$**), 59.3 (**C_4**), 69.0 (**C_{14}**), 69.9 (**$C_{11/12}$**)70.4 (**$C_{10/13}$**), 79.5 (**C_9**), 106.2 (**C_6**), 150.1 (**C_5**), 167.3 (**C_7**), 169.3 (**C_8**); *m/z* (ESI) 431.5 (100%, $[M + Na]^+$); (Found $[M + Na]^+$, 431.5, $C_{20}H_{23}N_3FeOS$ requires 432.0).

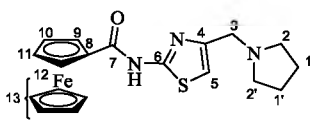
4.9.2 Compound 3.22c



3.22c

Dark red solid (0.060 g, 60%); rf in EtOAc:Hexane (1:1) 0.3; M.p. 124 – 126 °C; ν_{max}/cm^{-1}(IR-FT) 3170s, 3116s ν (N-H), 1586s (C=O); δ_H (60 MHz, $CDCl_3$): 2.62– 2.65 (4H, m, **$H_{1,1'}$**), 3.00 (2H, s, **H_3**), 3.96 (4H, t, *J* = 6.0 Hz, **$H_{2,2'}$**), 4.20 (5H, s, **H_{13}**), 4.30– 4.32 (2H, m, **$H_{10/11}$**) 4.52 – 4.53 (2H, m, **$H_{9/12}$**), 6.50 (1H, s, **H_5**); δ_C (150 MHz, $CDCl_3$): 27.8 (**$C_{1,1'}$**), 39.3 (**C_3**), 55.2 (**C_2**), 69.3 (**C_{13}**), 70.0(**$C_{10/11}$**), 70.3 (**$C_{9/12}$**), 70.5 (**C_8**), 106.5 (**C_5**), 148.7 (**C_4**), 156.5 (**C_6**), 170.0 (**C_7**); *m/z* (ESI) 427.30 (29%, M^+); (Found: M^+, 427.30, $C_{19}H_{21}N_3FeOS_2$ requires 427.05).

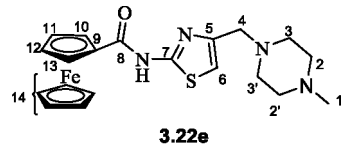
4.9.3 Compound 3.22d



3.22d

Dark red solid (50 mg, 35%); rf in EtOAc:Hexane (6:1) 0.4; M.p. 104-108 °C; ν_{max}/cm^{-1} (IR-FT),3170s, 3116s ν (N-H), 1586s ν (C=O); δ_H (600 MHz, $CDCl_3$): 1.25 −1.35 (4H, m, $\mathbf{H_{1,1'}}$), 2.14−2.16(4H, m, $\mathbf{H_{2,2'}}$), 3.0(2H, s, $\mathbf{H_3}$), 4.22 (5H, s, $\mathbf{H_{13}}$), 4.81 (2H, m, $H_{9/12}$), 4.37 (2H, $\mathbf{H_{10/11}}$), 4.95 (N**H**), 6.56 (1H, s, $\mathbf{H_5}$), δ_C (150 MHz, $CDCl_3$): 22.7 ($\mathbf{C_{1,1'}}$), 29.8 ($\mathbf{C_{2,2'}}$), 60.3 ($\mathbf{C_3}$), 69.7 ($\mathbf{C_{10}}$), 70.1 ($C_{10,11}$), 71.2 ($\mathbf{C_{9,12}}$), 71.46 (C_8)106.4 ($\mathbf{C_5}$), 143.5 ($\mathbf{C_4}$), 169.8 ($\mathbf{C_6}$), 171.9 ($\mathbf{C_7}$); *m/z* (ESI)*:* 396.0 (57%, $[M + H]^+$), (Found: $[M + H]^+$, 396.0, $C_{19}H_{21}N_3FeOS$ requires 396.0).

4.10 Synthesis of compound 3.22e



3.22e

A solution of freshly prepared ferrocenoyl chloride (0.10 g, 0.4 mmol) in DCM was added to 4-((4-methylpiperazin-1-yl)methyl)thiazol-2-amine (0.11 g, 0.52 mmol) and DIPEA (0.60 mL) in 10 mL of CH_2Cl_2 at room temperature. The reaction mixture was allowed to stir overnight at room temperature. The reaction progress was monitored using TLC, which showed the appearance of a new spot. The reaction was quenched with water, the organic layer was separated and the aqueous phase further extracted three times with DCM. The combined organic layers were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure to obtain a crude product. The crude was purified through flash chromatography using ethyl acetate and hexane (1:1) to give a dark red solid (0.069 g, 40%); M.p. 116 − 120 °C; ν/cm^{-1}(IR-FT) 3104 ν (NH), 1760 ν (C=O); δ_H (600 MHz, $CDCl_3$): 1.22 (CH_3, s, $\mathbf{H_1}$), 2.84−2.87 (4H, br, $\mathbf{H_2}$), 2.91−2.94 (4H, br, $\mathbf{H_3}$), 3.59 (2H, s, $\mathbf{H_4}$), 4.36 (5H, s, $\mathbf{H_{14}}$), 4.53−4.56 (2H, m, $\mathbf{H_{11/12}}$), 4.87−4.90 (4H, m, $\mathbf{H_{10/13}}$), 5.30 (1H, br s, N**H**), 6.33 (1H, s, $\mathbf{H_6}$); δ_C (150 MHz, $CDCl_3$): 31.0 ($\mathbf{C_1}$), 35.9 ($\mathbf{C_3}$), 39.1 ($\mathbf{C_2}$), 69.4 ($\mathbf{C_4}$), 70.3 ($\mathbf{C_{14}}$), 70.9 ($C_{11/12}$), 71.34 ($\mathbf{C_{10/13}}$),72.8 ($\mathbf{C_9}$), 106.0 ($\mathbf{C_6}$), 147.2 ($\mathbf{C_5}$), 162.6 ($\mathbf{C_7}$), 167.8 ($\mathbf{C_8}$); *m/z* (ESI) 425.1108 (100%, M^+); (Found: M^+, 425.1108, $C_{20}H_{24}N_4FeOS$ requires 425.1054).

4.11 Growth inhibition assays

4.11.1 *Plasmodium falciparum* growth inhibition assay

P. falciparum (3D7 strain) parasites were maintained in medium composed of RPMI 1640 supplemented with 2 mM L-glutamine, 25 mM Hepes, 5% (w/v) Albumax II, 20 mM glucose, 0.65 mM hypoxanthine, 60 µg/mL gentamicin sulfate and 2-4% (v/v) human red blood cells, in an atmosphere of 5% O_2, 5% CO_2, 90% N_2. For the growth inhibition assays, parasite cultures were adjusted to 2% parasitaemia and 1% haematocrit (final) and incubated with 3-fold serial dilutions of compounds in 96-well plates (200 µL culture/well; two wells per compound dilution) for 48 hours. Following the incubation, parasite levels in the wells were determined by colorimetric determination of parasite lactate dehydrogenase activity.[4] The Abs_{620} values in experimental wells were converted to percentage parasite viability relative to wells containing untreated parasite cultures and IC_{50} values derived from graphs of % parasite viability vs. log (compound concentration) using the non-linear regression function of GraphPad Prism v.5.02.

4.11.2 HeLa cell growth inhibition assay

HeLa cells were plated in 96-well plates at $2x10^4$ cell per well in 150 µL culture medium composed of DMEM supplemented with 5 mM L-glutamine, 10% (v/v) fetal bovine serum and antibiotics (penicillin/streptopmycin/amphotericin B).[5] After an overnight incubation in a 5% CO_2 humidified incubator, 3-fold serial dilutions of compounds were added to the cultures (duplicate wells; 200 µL final culture volume) and incubation continued for an additional 24 hours. Cell viability in individual wells was assessed by adding 20 µL per well resazurin toxicology reagent (Sigma-Aldrich) and measuring fluorescence intensity (exc. 560 nm, em. 590 nm) in a Spectramax M3 plate reader after an incubation of 2-4 hours. Fluorescence readings in experimental wells were converted to % cell viability relative to control wells containing untreated cells and used to derive IC_{50} values from dose-response plots of % cell viability vs. log (compound concentration) using the non-linear regression function of GraphPad Prism v.5.02.

4.12 References

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