Development of quinoline and non-quinoline based organometallic complexes and their ligands conjugated to polyamine scaffolds as pharmacological agents

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May 2014

Development of quinoline and non-quinoline based organometallic complexes and their ligands conjugated to polyamine scaffolds as pharmacological agents

Thesis presented for the degree of

Doctor of Philosophy

by

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Department of Chemistry

University of Cape Town

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Co-Supervisor: Timothy J. Egan

2014

DECLARATION

I declare that **"Development of quinoline and non-quinoline based organometallic complexes and their ligands conjugated to polyamine scaffolds as pharmacological agents"** is my own work and has never been submitted for examination for any degree at any university. All sources of information are cited and fully referenced at the end of each chapter.

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Tameryn Stringer

May 2014

ACKNOWLEDGEMENTS

First and foremost, I would like to express my heartfelt gratitude and appreciation to my supervisors, Assoc. Professor Gregory Smith and Professor Timothy Egan for their invaluable advice, guidance and encouragement throughout this project.

I would also like to acknowledge Mr Pete Roberts and Mr Noel Hendricks for recording some of the NMR spectra. Mr Gianpiero Benincasa for microanalytical and mass spectral analyses. I would also like to thank Dr Marietjie Stander of the University of Stellenbosch for electro-spray ionisation mass spectral analyses. Sincere thanks to Prof Pete Smith, Dr Carmen de Kock, Dr Dale Taylor and Ms Jill Combrinck (UCT Department of Clinical Pharmacology) for antimalarial screenings. Thanks to Assoc. Professor Denver Hendricks and Ms Hajira Guzgay (UCT medical biochemistry) for testing the compounds for anticancer activity. Many thanks to Professor Kirkwood Land and his group (University of the Pacific) for the *T. vaginalis* screenings. Thanks to Andrew Burgoyne for screening of selected compounds against the KMST-6 cell line.

A special thanks goes to Preshen Govender, Dr Prinessa Chellan and Dr Emma Hager, for their friendship, encouragement and support over the past few years in and out of the laboratory. I would also like to thank the Organometallic Research group, Bioinorganic Research group, as well as Dr Jamy Feng and Mr Irwin Cassells for helpful discussions and assistance.

I would like to thank the Harry Crossley Foundation, National Research Foundation (NRF)/DAAD and the Ernst and Ethel Eriksen Trust for their financial support throughout my degree.

Thanks to all of my friends and family who have supported and encouraged me throughout my studies, especially my mom and grandmother. Special thanks to Jerome, for always being there for me and for all the encouragement.

ABSTRACT

Malaria remains a major global health problem and to date, hundreds of thousands of people die as a result of this disease every year. Malaria has been able to adapt and rebound despite various efforts made to combat the disease. The decrease in efficacy of many front-line drugs against malaria, due to increased resistance, prompts investigation into obtaining effective compounds that are able to overcome this resistance. This study investigated the synthesis, characterisation and biological evaluation of new quinoline as well as non-quinoline based compounds. Selected Rh(I) metal complexes thereof were also studied. The compounds were screened for antiplasmodial activity, in addition, their activity against WHCO1 oesophageal cancer cells and the parasite, *Trichomonas vaginalis* (*T vaginalis*) was also evaluated.

A series of new mono- and multimeric quinoline-containing and ferrocenyl thioureas conjugated to polyamine scaffolds were synthesised. In addition to this, new monomeric and dimeric salicylaldimine ligands containing a quinoline motif were also prepared. Coordination of these ligands with $[RhCl(COD)]_2$, yielded mononuclear and binuclear Rh(I) complexes. New ferrocenyl derivatives of some of the afore-mentioned were prepared. All of the compounds were characterised using an array of techniques including ^{1}H and $^{13}C\{^{1}H\}$ NMR spectroscopy, IR spectroscopy and mass spectrometry.

The thioureas were evaluated against the NF54 chloroquine-sensitive (CQS) and the Dd2 chloroquine-resistant (CQR) strains of *Plasmodium falciparum* (*P. falciparum*). The quinoline compounds showed enhanced activity compared to the ferrocenyl derivatives. The trimeric quinoline exhibited the highest activity in the CQS strain (IC_{50} = 0.09 μM). One of the biological targets of quinoline compounds is haemozoin. Studies were carried out in order to investigate the compounds' ability to inhibit synthetic haemozoin (β-haematin) formation. The trimeric and tetrameric quinolines were most effective at inhibiting β-haematin formation, perhaps due to an increased number of quinoline motifs. The thioureas were also screened against the G3 strain of *T. vaginalis*. This was conducted to gauge the efficacy of these compounds against other parasitic diseases as well. The ferrocene-containing thioureas exhibited slightly higher parasite growth inhibition compared to the quinolines. The quinolines were more cytotoxic when tested against WHCO1 compared to the ferrocenyl

derivatives. The tetrameric quinoline ligand exhibited the highest cytotoxicity (IC_{50} = 0.99 μM). The latter was further tested against KMST-6 human fibroblast and exhibited selectivity towards WHCO1 cancer cells.

The monomeric and dimeric salicylaldimine quinolines and their corresponding Rh(I) complexes were evaluated against the NF54 CQS and K1 CQR strains of *P. falciparum*. The monomeric systems (particularly the ligands) showed enhanced activity compared to the dimeric systems across both strains. The substituent on the salicylaldimine ring had no significant effect on antiplasmodial activity. While the monomeric systems showed potency in the sensitive strain, these compounds lost their efficacy in the resistant strain. The dimeric derivatives exhibited similar activity in each strain, giving RI values close to, or less than 1. The metal complexes exhibited similar activity profiles as their ligands. The dimeric ligands and complexes exhibited the highest β-haematin inhibition activity suggesting that size or lipophilicity plays a role in the inhibition of β-haematin formation. Generally, the metal complexes showed better activity against *T. vaginalis* compared to the ligands. Two dimeric complexes exhibited good activity giving IC_{50} values of 6.1 and 4.8 μM, respectively. Compounds containing the 5-Cl substituent showed good promise against this parasite. The dimeric derivatives exhibited increased cytotoxicity against the WHCO1 cell-line. The most potent being the 3-OMe binuclear Rh(I) complex (IC_{50} = 3.9 μM). This complex also showed selectivity towards WHCO1 cancer cells rather than human fibroblasts.

Ferrocenyl azines containing salicylaldimine motifs and their Rh(I) complexes were screened against the NF54 CQS and K1 CQR strains of *P. falciparum*. Three ferrocenyl quinolines were also tested. The ferrocenyl-containing salicylaldimines exhibited weak to moderate activity across both parasite strains. The monomeric ferrocenyl quinolines displayed good activity in the CQS strain, while the dimeric derivative exhibited low activity, possibly a consequence of its lipophilic nature. The ferrocenyl quinoline possessing the salicylaldimine moiety demonstrated higher activity than the non-ferrocenyl monomeric salicylaldimines. The quinoline-based ferrocenes displayed higher β-haematin inhibition activity compared to the non-quinoline compounds. All of the quinoline-based compounds exhibited cytotoxicity against the WHCO1 oesophageal cancer cell-line. With the exception of the ferrocenyl quinoline possessing the salicylaldimine motif, most of the compounds inhibited less than 50% parasite growth when tested against *T. vaginalis.*

PUBLICATIONS

Journal article

- ***Synthesis, characterisation, antiparasitic and cytotoxic evaluation of thioureas conjugated to polyamine scaffolds.***
  T. Stringer, D. Taylor, C. de Kock, H. Guzgay, A. Au, S. Hwan An, B. Sanchez, R. O'Connor, N. Patel, K. M. Land, P. J. Smith, D. T. Hendricks, T. J. Egan and G. S. Smith, *European Journal of Medicinal Chemistry*, 2013, **69**, 90.

Conferences and symposia

- **INORG 2013:** Durban, South Africa, Poster Presentation, **T. Stringer**, T. J. Egan and G. S. Smith – *Development of bioorganometallic antimalarials conjugated to polyamine scaffolds: Synthesis, characterisation and biological activity.*
- **Western Cape Organometallic Mini-Symposium 2012:** UCT, Cape Town, Oral Presentation, **T. Stringer**, T. J. Egan and G. S. Smith – *The development of conjugated organometallic polyamines as potential biological agents.*
- **H3D symposium 2012:** Cape Town, South Africa, Poster presentation, **T. Stringer**, T. J. Egan and G. S. Smith - *Development of antimalarials conjugated to polyamine scaffolds: Synthesis, characterisation and biological activity*.
- **XXV ICOMC 2012:** Lisbon, Portugal, Poster presentation, **T. Stringer**, T. J. Egan and G. S. Smith - *Development of organometallic antimalarials conjugated to polyamine scaffolds.*

ABBREVIATIONS

δ	chemical shift
2D	two-dimensional
ACT	artemisinin combination therapy
ATR	attenuated total reflectance
br	broad
Bu	butyl
ca.	approximately
COD	1,5-cyclooctadiene
COSY	correlation spectroscopy
Cp	cyclopentadiene
Cp*	1,2,3,4,5-pentamethylcyclopentadiene
CQ	chloroquine
CQDP	chloroquine diphosphate
CQR	chloroquine-resistant
CQS	chloroquine-sensitive
d	doublet
DAB-Am-4	*N*,*N*,*N*′,*N*′-tetrakis(3-aminopropyl)-1,4-butanediamine
DCM	dichloromethane
dd	doublet of doublets
DMSO	dimethylsulfoxide
DMSO-d_6	deuterated dimethylsulfoxide
EI	electron impact
EPR	enhanced permeability and retention
eq	equivalents

ESI-MS	electrospray ionisation mass spectrometry
Et	ethyl
Fc	ferrocene
FQ	ferroquine
Hb	haemoglobin
HR	high resolution
HSQC	heteronuclear single quantum coherence
IC_{50}	50% inhibitory concentration
IR	infrared
J	coupling constant
m	multiplet
M	metronidazole
Me	methyl
M.p.	melting point
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
m/z	mass to charge ratio
nd	not determined
NMR	nuclear magnetic resonance
PAT	polyamine transporter
PAMAM	polyamido amine
PEG	polyethylene glycol
PfCRT	*Plasmodium falciparum* chloroquine-resistant transporter
PPI	poly(propyleneimine)
ppm	parts per million
RI	resistance index
RMSD	root mean square deviation

r.t.	room temperature
s	singlet
SD	standard deviation
SE	standard error
SI	selectivity index
sub	substituted
t	triplet
td	triplet of doublets
unsub	unsubstituted
WST1	4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate

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CHAPTER 1

Introduction

1.1. Malaria

Malaria is an infectious disease that affects humans and is considered to be the most common parasitic disease in the world.[1] According to the World Health Organisation (WHO), in 2010; 216 million cases of malaria were reported worldwide and 655 000 cases of malaria-related deaths were documented.[2] This disease is caused by a protozoan of the genus *Plasmodium*. There are five main species of *Plasmodium* that are recognised to infect human beings i.e. *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*. The latter is a malaria parasite of Old World monkeys but has been reported to infect humans in Malaysian Borneo.[3,4] Amongst these species of *Plasmodium*, the most deadly strain to humans is *P. falciparum*.[5]

Malaria is spread by *Anopheles* mosquitoes. When an infected mosquito feeds on a human host, it injects the parasite in the form of sporozoites, which are asexual, into the bloodstream of the human (Figure 1.1).[6] The sporozoites travel to the liver and divide asexually by a process known as schizogony to produce thousands of haploid forms of the parasite, called merozoites. These merozoites invade other liver cells and then enter the bloodstream where they invade erythrocytes (red blood cells). Once inside, the merozoites enlarge forming a uninucleate cell known as a ring trophozoite. The trophozoite's nucleus divides asexually to produce a schizont containing several nuclei. This then divides further to produce mononucleated merozoites. Eventually the erythrocyte ruptures and releases toxins into the host's body, which in turn leads to typical symptoms of malaria such as fever and chills. A sexual phase begins when some merozoites develop into gametocytes (cells that are able to produce both male and female gametes). These gametocytes are not able to produce gametes inside of their human hosts but do so once inside the gut of a mosquito. Zygotes develop within the intestinal walls of a mosquito and eventually develop into oocysts (a stage of the parasite that is produced when male and female gametes combine). A large number of sporozoites then develop due to repeated mitotic division occurring within the oocyst. Once formed, the sporozoites migrate to the salivary gland of the mosquito to continue the life cycle of the parasite.[6]

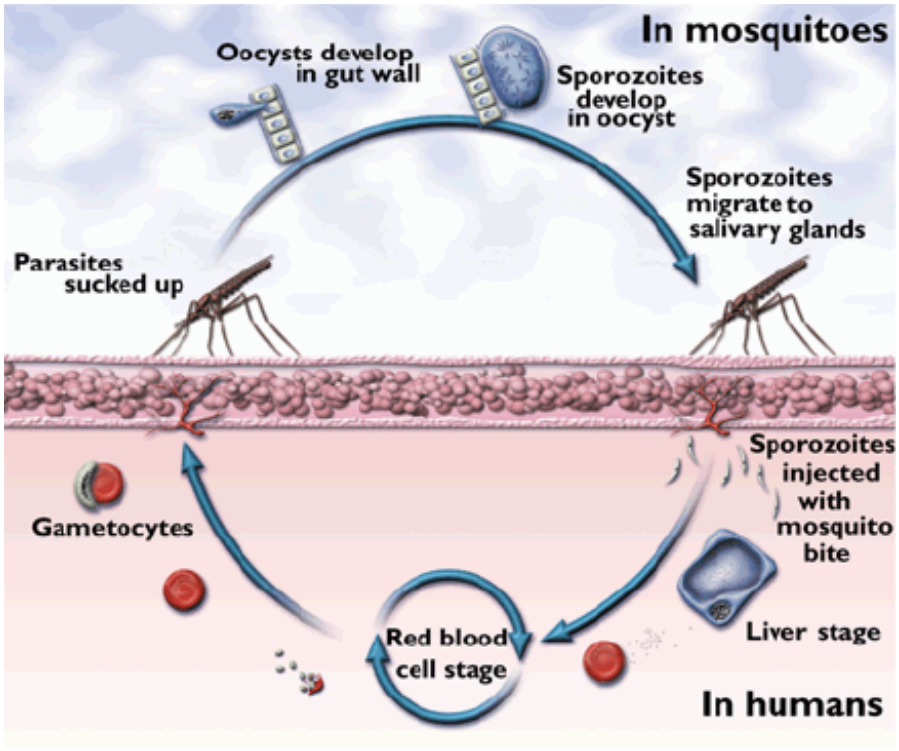
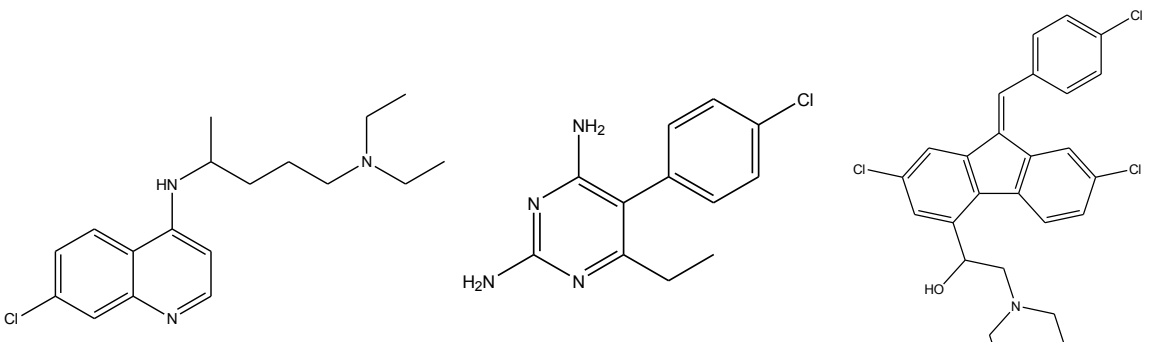


Figure 1.1: Life cycle of malaria parasite[6]

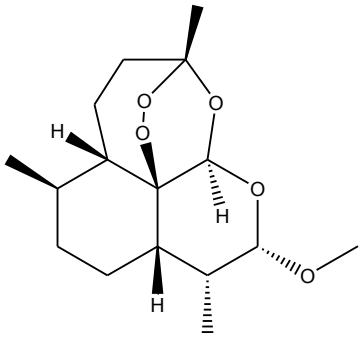
The spread of malaria is prevented by protection against mosquito bites by means of mosquito nets, mosquito repellent creams or insecticides. Preventative medicines are also used when visiting areas where malaria is prevalent.[7] There are many chemotherapeutic options for people who contract this disease, however, the management of malaria is impeded by increasing drug resistance or in some cases decreased efficacy of some very potent antimalarials including chloroquine (**1.1**, Figure 1.2), quinine, primaquine, amodiaquine, pyrimethamine (**1.2**, Figure 1.2), mefloquine and lumefantrine (**1.3**, Figure 1.2).[8]



1.1 **1.2** **1.3**

Figure 1.2: Potent compounds that have been used to combat malaria

Chloroquine (CQ) has been used successfully to treat this disease. It was a highly effective and safe drug to use. However, currently chloroquine-resistance is a major issue.[9] A common practice being implemented recently is artemisinin combination therapy (ACT). This involves the treatment of an infected individual with artemisinin or a derivative thereof in conjunction with another drug of known efficacy, which in turn delays the onset of drug resistance.[10] Lumefantrine and artemether (**1.4**, Figure 1.3) are currently used against multi-drug resistant strains of *Plasmodium falciparum*.



1.4

Figure 1.3: Artemether, an artemisinin derivative commonly used in combination therapy

1.2. Mode of action of quinoline compounds

Compounds containing quinoline moieties have been used extensively for the treatment of malaria over many years. Specifically, 4-aminoquinolines have been very successful in the treatment of malaria because of their high efficacy, low toxicity, efficiency as well as low cost preparation. To date, chloroquine (CQ) has been the most widely used defence against *P. falciparum,* it is cheap and practical for outpatient use. It is also very effective against *P.ovale, P. vivax* as well as *P. malariae*. Chloroquine is active against the blood stages of the parasite, mainly in the trophozoite and early schizont stages of the life-cycle. CQ is a diprotic weak base that when unprotonated, passess through the membrane of the infected cell as well as the parasite, to accumulate in the acidic digestive vacuole (Figure 1.4).[11-13] At this stage of the parasite's life-cycle, haemoglobin is ingested and transported to the digestive vacuole of the parasite.[14]

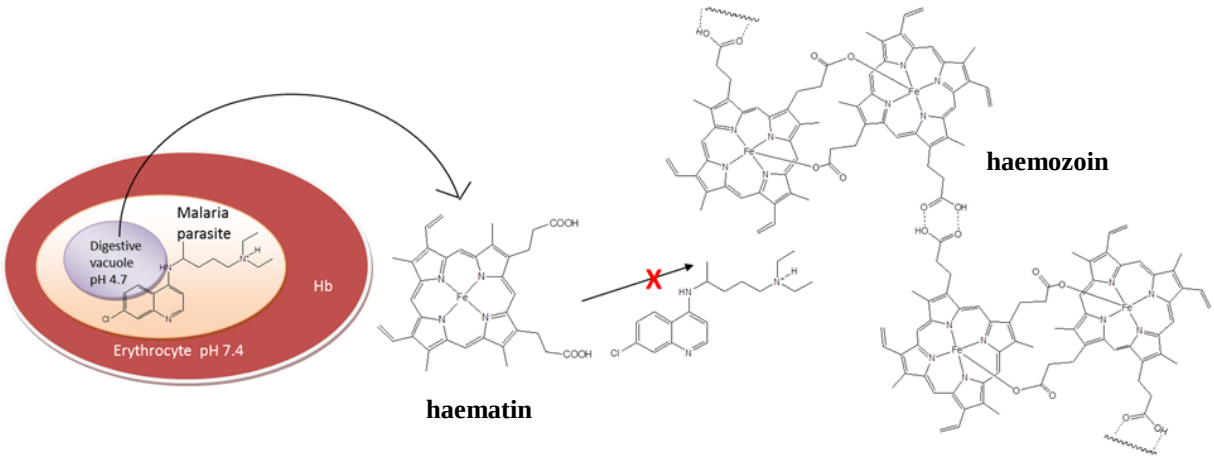


Figure 1.4: Possible mechanism of action of chloroquine and quinoline-based antimalarials

It is broadly accepted that many 4-aminoquinolines disturb the process of detoxification of free haem, which is generated upon haemoglobin (Hb) degradation.[15] A series of proteases found within the digestive vacuole of the parasite degrade haemoglobin into small peptides which are then displaced to the parasite's cytoplasm. The oxidised by-product of Hb degradation is ferriprotoporphyrin-IX (haematin), which is a haem moiety toxic to the parasite (Figure 1.4). The parasite therefore converts this compound into a less toxic form by biomineralisation of this moiety into haemozoin (malarial pigment). Chloroquine is believed to form a complex with the toxic haem and in turn inhibits crystallisation, causing a build-up of haematin. Haematin therefore appears to be the target of many 4-aminoquinolines. It has been suggested that haem may also be detoxified by two other mechanisms including degradation by hydrogen peroxide within the digestive vacuole and also by a glutathione-dependant degradation which occurs in the cytoplasm of the parasite.[16,17] However, studies conducted by measuring cellular haem fractions as well as Mössbauer spectroscopy suggests that about 95% of the free haem that is released due to haemoglobin degradation is found in haemozoin.[18-20] Recent evidence provided by Buller *et al.*[21] suggests haemozoin may itself be the target of quinoline-based drugs. The fastest growing crystal face of β-haematin (synthetic haemozoin) appears to be the binding site for quinoline-based drugs. This then leads to inhibition of haemozoin crystal growth.[21] Experimental support for this was provided by de Villiers *et al.*, whereby solvated single crystals of β-haematin were grown in the presence of antimalarial drugs.[22] These crystals were almost indistinguishable from haemozoin.

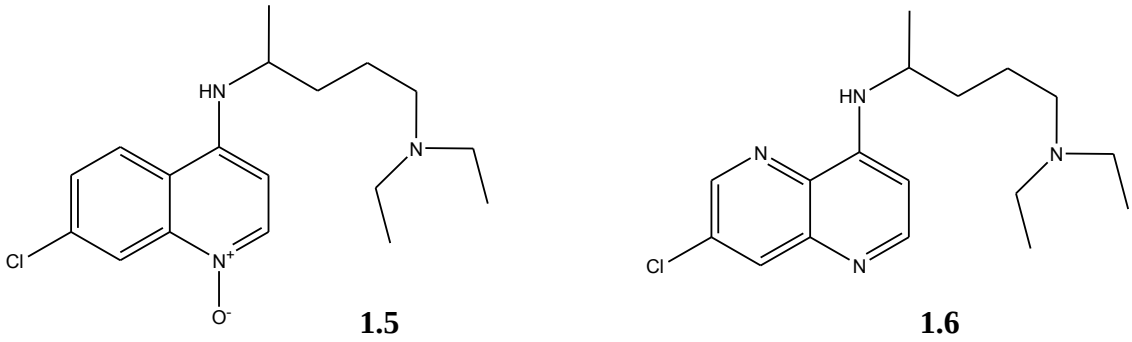
A decreased rate in β-haematin formation in the presence of the antimalarial drugs chloroquine and quinidine, was attributed to the assumption that drugs are adsorbed to areas on the fastest growing face of β-haematin.[22]

1.3. Quinolines as potential antimalarial chemotherapeutics

Quinoline compounds have been used extensively to treat malaria for decades. The first quinoline compound that was used as an antimalarial was quinine. It consists of an (8S, 9R) quinuclidine-methanol group.[23] Pamaquine was discovered in the 1920's and is an 8-aminoquinoline. This particular compound is active against erythrocytic stages of malaria.[24] Primaquine, an analogue of pamaquine, is known to be an effective treatment against *P. vivax* liver infections.[25,26] Chloroquine was envisaged from quinacrine, a 4-aminoacridine, by conversion of its core to a chloroquinoline resulted in this very important antimalarial drug.

Due to widespread resistance towards many of these compounds, there have been numerous attempts made to prepare drugs possessing superior antimalarial activity and that overcome this resistance. Generally, resistance occurs due to spontaneous mutations in the parasite and as a result drugs become less effective. Mutations include single-point and multiple mutations. For some drugs, a single-point mutation is sufficient to introduce resistance, while in other cases multiple mutations are required.[27] Chloroquine is able to accumulate to a greater extent inside of the digestive vacuole of sensitive parasite strains compared to resistant ones.[28-30] This is due to mutations in the chloroquine resistance transporter (PfCRT). These mutations cause chloroquine to be expelled from the inside of the digestive vacuole.[31]

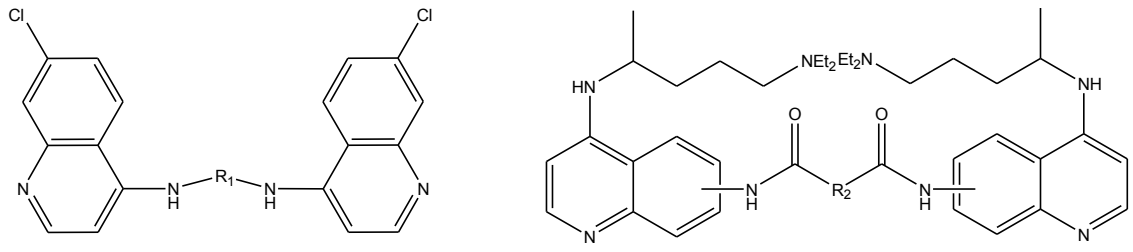
In order to overcome this resistance, various modifications of chloroquine have been explored; however, it is clear that the 4-amino-7-chloroquinoline moiety optimises the antimalarial activity of many of these compounds. In most cases, modification of the quinoline ring leads to decreased efficacy with the exception of chloroquine N-oxide (**1.5**, Figure 1.5) and 5-azachloroquine (**1.6**, Figure 1.5). These particular compounds were reported to have greater activity *in vivo* in comparison to chloroquine.[32]



1.5 **1.6**

Figure 1.5: Two chloroquine analogues displaying superior *in vivo* activity compared to CQ

In many cases incorporation of electron withdrawing and electron donating groups such as NH_2, OH, OCH_3, H, CH_3, NO_2 and CF_3 onto the aminoquinoline ring has resulted in a decrease in antimalarial activity of the parent compound.[33,34] Modification of the length (shortening or lengthening) of the aminoquinoline side chain has also resulted in a favourable effect on the antimalarial activity against chloroquine-resistant strains.[35,36] Bis-aminoquinolines are compounds containing two quinoline moieties linked by various organic linkers (aromatic and aliphatic). These quinoline derivatives were initially prepared based on the rationale that these bulky compounds may be retained more effectively by chloroquine resistant strains of *P. falciparum*. Many bisquinolines, such as those displayed in Figure 1.6 (**1.7** and **1.8**), exhibit activity against CQ-sensitive and resistant strains of *Plasmodium*. Many of these compounds are also cytotoxic.[37-40]



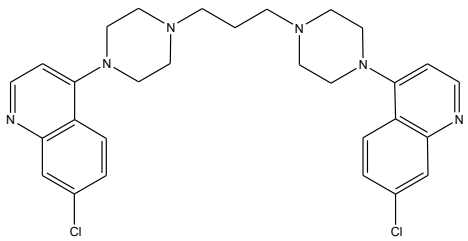
1.7 **1.8**

$R_1 = (CH_2)_n$; trans-l,2-cyclohexyl; $(CH_2)_3CH(CH)_3CH_2$; $(CH_2)_2O(CH_2)_2$; $(CH_2)_2NH(CH_2)_2$.

$R_2 = (CH_2)_n$, where n = 0, 2, 4, 6, 8.

Figure 1.6: Bisquinoline compounds displaying antimalarial activity as well as cytotoxicity

Many bisquinolines containing piperazine moieties also exhibit antiplasmodial activity. Bisquinolines such as piperaquine (**1.9**, Figure 1.7), hydroxypiperaquine (**1.10**, Figure 1.7), dichloroquinazine (**1.11**, Figure 1.7), 12,494RP (**1.12**, Figure 1.7) and 1,4-bis(7-chloro-4-quinolylamino)piperazine (**1.13**, Figure 1.7) are believed to act via the same mechanism as chloroquine.[41-43] Initially these drugs were identified as promising drug candidates but their activity was not comparable to chloroquine. More recently, these drugs have been re-evaluated and have shown activity against CQ-resistant strains of *P. falciparum*.[42]



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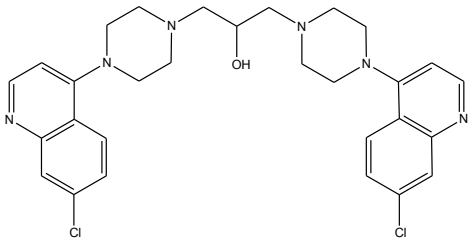

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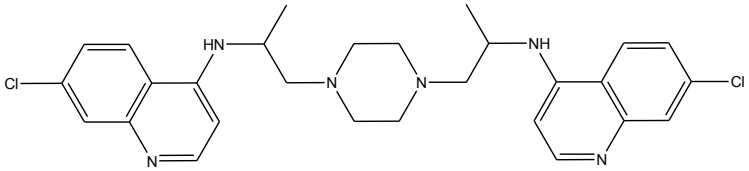

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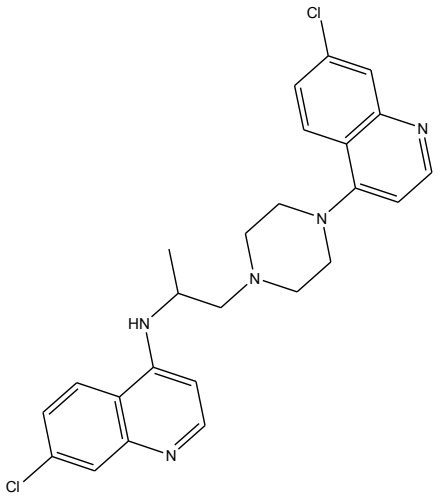

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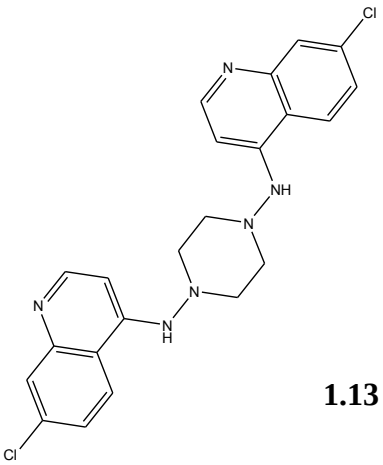
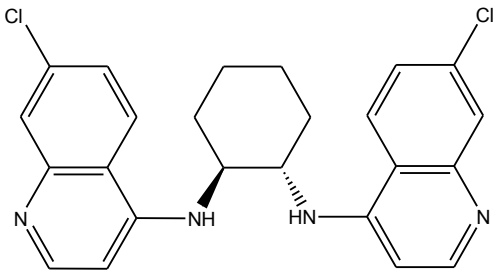


1.13

Figure 1.7: Bisquinolines possessing piperazinyl spacers

A bisquinoline displaying activity against CQ-sensitive strains of *P. falciparum* is *trans*-N^1,N^2-bis(7-chloroquinolin-4-yl)cyclohexane-1,2-diamine or WR 268,668. This racemic mixture was found to be two-hundred times more effective against a CQ-sensitive strain of *P. falciparum* than chloroquine, but only six times more potent against a CQ-resistant strain. When this particular compound was used in conjunction with a chloroquine efflux inhibitor i.e. desipramine, resistance to WR 268,668 decreased in the resistant strain. This suggests that the compound experiences the same mechanism of resistance as chloroquine.[44] Preparation and evaluation of the two enantiomeric forms of this compound showed that the *S,S* form also known as Ro 47-7737 (**1.14**, Figure 1.8) displays better activity against CQ-resistant parasites in comparison to the *R,R* form or the racemic mixture.[45] Ro 47-7737 was studied in more detail and proved to be potent against *P. berghei in vivo* and *P. vivax ex vivo*. This compound has been ruled out as a drug candidate however, due to its toxic nature.[46]

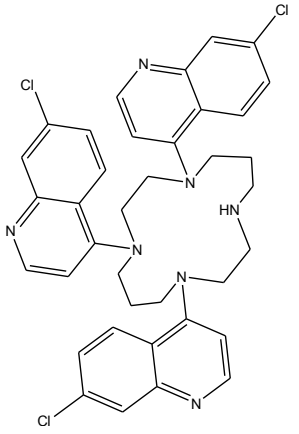


1.14

Figure 1.8: Ro 47-7737, a promising molecule suspended due to its toxicity

In order to evaluate the effect of increased bulkiness as well as rigidity on the antiplasmodial activity of certain compounds, a series of novel bis-, tris- (containing 3 quinoline moieties) and tetraquinolines (containing 4 quinoline moieties) were prepared and evaluated against various strains of *P. falciparum* (**1.15 - 1.17**, Figure 1.9). Compounds displaying greater rigidity than their linear derivatives did not show any significant activity compared to them, however, the rigid analogues did not exhibit any appreciable cytotoxicity. Some of the trisquinolines did not display any enhanced activity in comparison to their parent bisquinoline derivatives, some of the tetraquinolines however, were non-cytotoxic and displayed potent activity against CQ-resistant strains of *P. falciparum*.[47]

Quinoline compounds still remain one of the most important classes of compounds for malaria treatment. Not much research has been done with regards to compounds containing multiple quinoline units. As is observed for many bisquinolines, multimeric quinoline systems may be favourable for enhancement of antiplasmodial activity compared to CQ.



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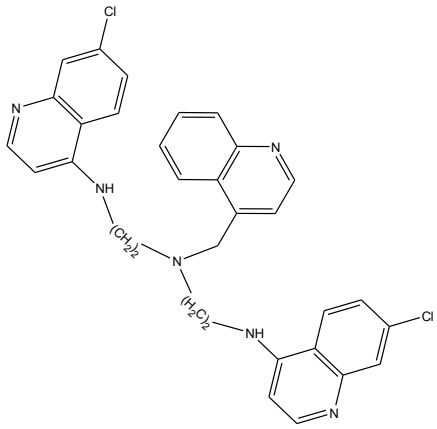

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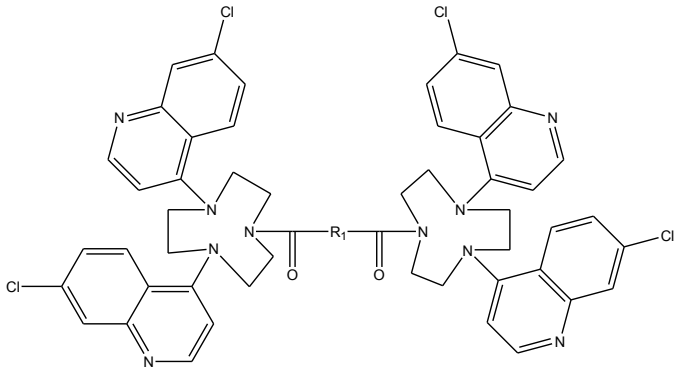


1.17

R_1 = CH_2-piperazine-CH_2; phenyl; CH_2-NH-CH_2; $(CH_2)_n$ (where n = 2, 3, 5, 7, 10).

Figure 1.9: Various tris- and tetraquinolines synthesised for biological evaluation

1.4. Metal complexes as antimalarials

The success of metal-containing compounds such as cisplatin and its analogues in cancer treatment has generated interest into the use of metal complexes in the field of medicinal chemistry. These compounds are used either because they contain chemical elements essential

for life, such as iron used to treat anaemia or they contain toxic elements such as platinum, used to treat various cancers.[48] Much emphasis has been placed on the use of inorganic compounds as part of cancer chemotherapy due to the success of many platinum-containing compounds. Based on this rationale, there have been examples of metal-containing compounds being prepared as potential antimalarial agents. Great success has been achieved by the modification of chloroquine by means of incorporating metal-containing segments.[49] This section highlights quinoline-containing metal complexes and their antiplasmodial activity, with the focus on ferrocene-containing and some transition metal-quinolines.

1.4.1. Transition metal quinoline complexes

Transition metals have often been used to enhance the activity of existing drugs. In many cases this has led to compounds with promising activities, but there have also been cases where metal complexes have not shown superior activity. Metal complexes of primaquine and amodiaquine have been prepared, but these complexes did not show increased activity compared to the parent organic compounds. Some of the compounds synthesised included Cr(III), Fe(III), Cu(II), Rh(III), Pd(II) and Au(III) quinoline complexes.[50] It was later proposed that metal complexes of chloroquine may result in enhanced activity. The first organometallic complex of chloroquine was reported by Sanchez-Delgado *et al*.[51] [RhCl(COD)(CQ)] (COD = 1,5-cyclooctadiene) (**1.18**, Figure 1.10) was evaluated *in vitro* against *Plasmodium berghei* and its activity was comparable to that of chloroquine diphosphate (CQDP). The compound was also tested *in vivo* and was found to decrease parasitemia by 73%.[51] In the same study, $[RuCl_2(CQ)]_2$ (**1.19**, Figure 1.10) was also synthesised and was found to be 5 times more active than CQDP. This compound was also tested against two CQ-resistant strains of *P. falciparum* and was 2-5 times more active than the parent compound.[51]

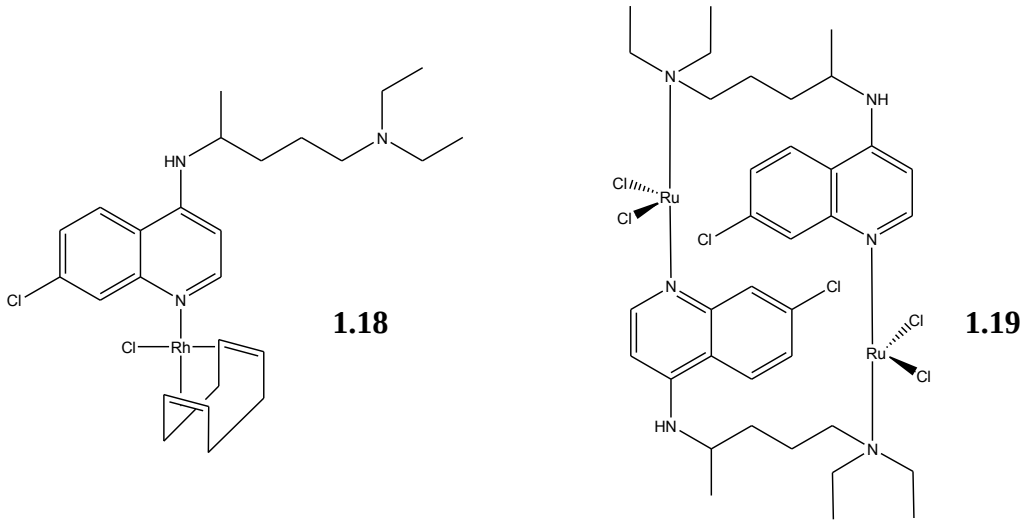


Figure 1.10: The first metal complexes of CQ

Based on the promising activity of the rhodium and ruthenium complexes of chloroquine, Ru(II)-arene complexes were synthesised and evaluated for their antiplasmodial activity.[52] The $[RuCl_2(\eta^6\text{-arene})(CQ)]$ (arene = *p*-cymene or benzene) compounds were active *in vitro* against four CQ-sensitive and three CQ-resistant strains of *P. falciparum*. Specifically, these compounds exhibited enhanced activity against resistant strains. It is believed that these compounds rapidly hydrolyse in aqueous media forming ruthenium(II) aqua compounds (**1.20** – **1.23**, Figure 1.11) which are believed to be the active species.[52]

Coordination of chloroquine to $[Au(PPh_3)]^+$ resulted in the active complex, $[Au(PPh_3)(CQ)]PF_6$ (**1.24**, Figure 1.12).[53] This complex was evaluated against the blood stage of two CQ-resistant strains of *P. falciparum* (FcB1 and FcB2) and displayed activity in the low nanomolar range. This particular compound also displayed *in vitro* and *in vivo* activity against *P. berghei*.[53] Similar Au(I) complexes (**1.24**, Figure 1.12) of the general formula [Au(CQ)(X)]Y (where X = PMe_3 or PEt_3 and Y = PF_6^- or NO_3^-) were also prepared and evaluated against several *P. falciparum* strains.[54] The nature of the anion did not appear to affect the activity of the complexes, although these complexes presented lower activity in comparison to the corresponding triphenylphosphine derivative previously mentioned. Au(III) complexes $[AuCl_2(CQ)_2]Cl$ (**1.25**, Figure 1.12) and [AuCl(CQ)(SR)(Et_2O)]Cl (where SR = 1-thio-β-D-tetraacetateglucose) were found to be moderately active, however, no structure-activity correlations were discernible for these complexes.[54]

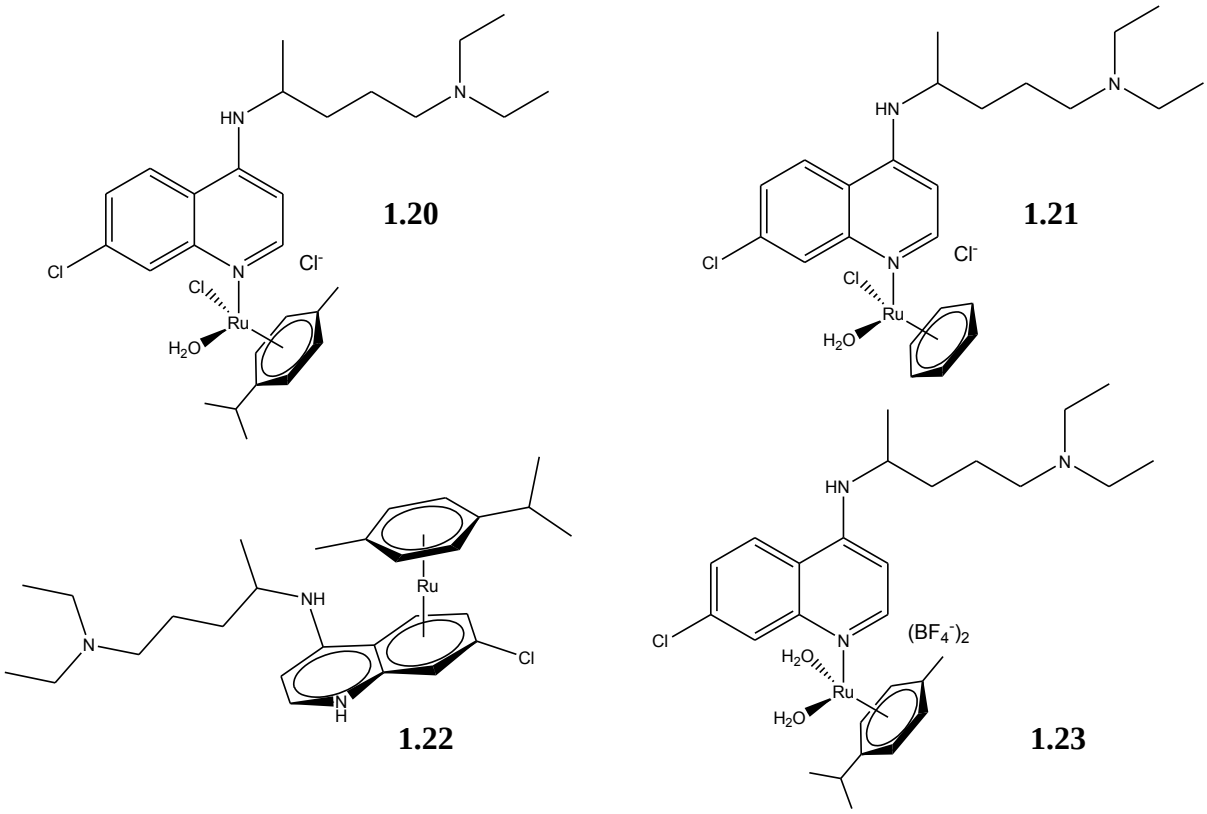
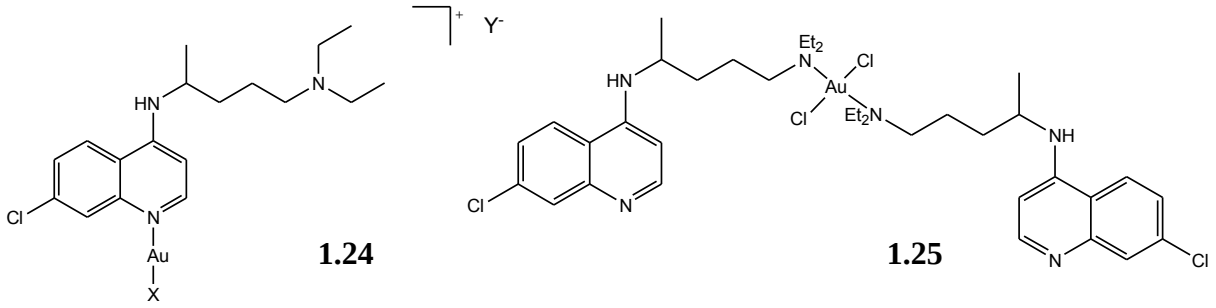


Figure 1.11: Ru(II) arene complexes of chloroquine with promising activity against CQ resistant strains of *P. falciparum*.



X = PPh_3, Y = PF_6^-; X = PPh_3, Y = NO_3^-, X = PMe_3, Y = PF_6^-; X = PEt_3, Y = PF_6^-

Figure 1.12: Examples of gold complexes possessing antimalarial activity

Iridium-chloroquine complexes i.e. [IrCl(COD)(CQ)] (**1.26**, Figure 1.13) and [Ir_2Cl_6(CQ)] (**1.27**, Figure 1.13) were evaluated *in vitro* against *P. berghei*.[55] Complex **1.26** showed activity comparable to CQDP, while complex **1.27** showed slightly enhanced activity compared to CQDP.[55] Platinum-containing chloroquine, *trans*-[$PtCl_2(CQ)_2$] (**1.28**, Figure 1.13), has been tested and found to exhibit good antiplasmodial activity.[56]

Organometallic rhenium half-sandwich quinolines were evaluated against two strains of *P. falciparum* (3D7 and W2).[57] The complexes that were evaluated included two cyrhetrenylimine (**1.29**, Figure 1.14) and two cyrhetrenylamine (**1.30**, Figure 1.14) complexes. In addition to this, organic derivatives were also evaluated for comparison. *In vitro*, the cyrhetrenylimine complexes displayed enhanced activity in comparison to the corresponding amines against the 3D7 strain. The organic derivatives appeared to exhibit better activity than some of the complexes. The low activity of these compounds may be a consequence of their stability and lipophilicity.[57]

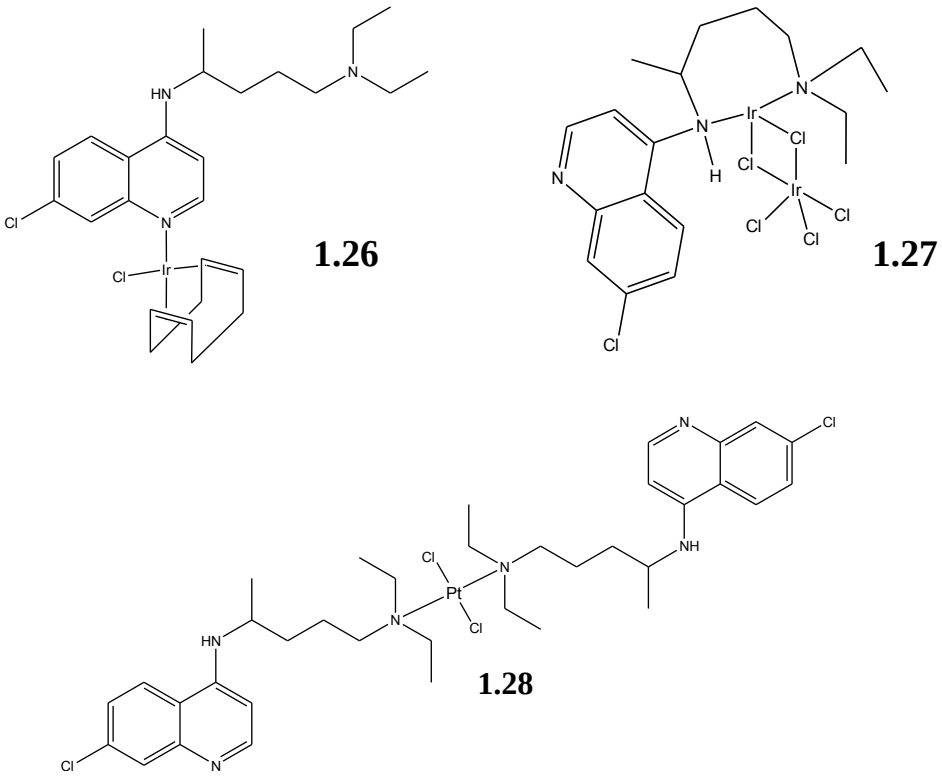
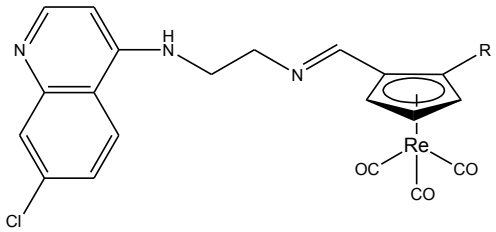
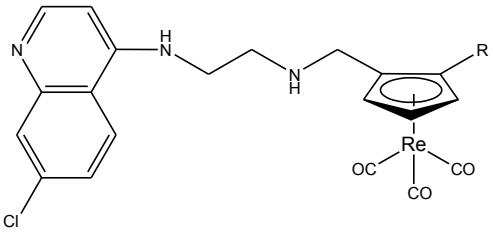


Figure 1.13: Proposed structures of iridium and platinum quinoline complexes.



1.29

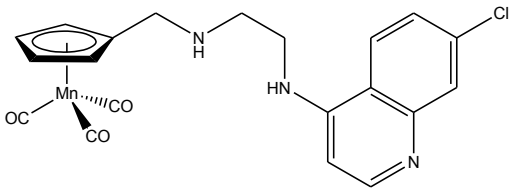


1.30

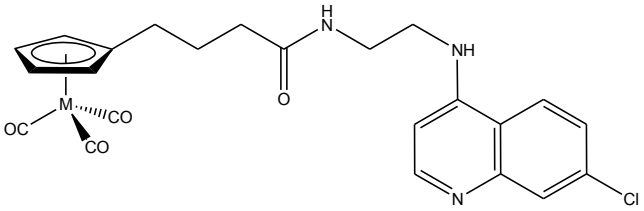
R = H or R = CH_2OH

Figure 1.14: Cyclopentadienyl rhenium quinoline complexes evaluated *in vitro* for antiplasmodial activity.

Cymantrene and cyrhetrene quinoline compounds (**1.31** and **1.32**, Figure 1.15) were evaluated against CQ-sensitive and CQ-resistant strains of *P. falciparum* for antiplasmodial activity. The cymantrene complex possessing an amine linker (**1.31**) exhibited good activity against the CQ-sensitive strain but no activity against the resistant strain. The cymantrene and cyrhetrene compounds (**1.32**) containing amide linkers were active against both strains of the parasite.[58]



1.31

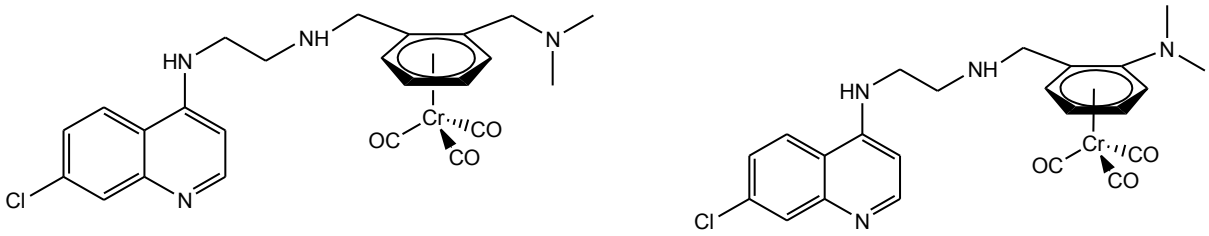


1.32

M = Mn or Re

Figure 1.15: Cymantrene and cyrhetrene quinolines

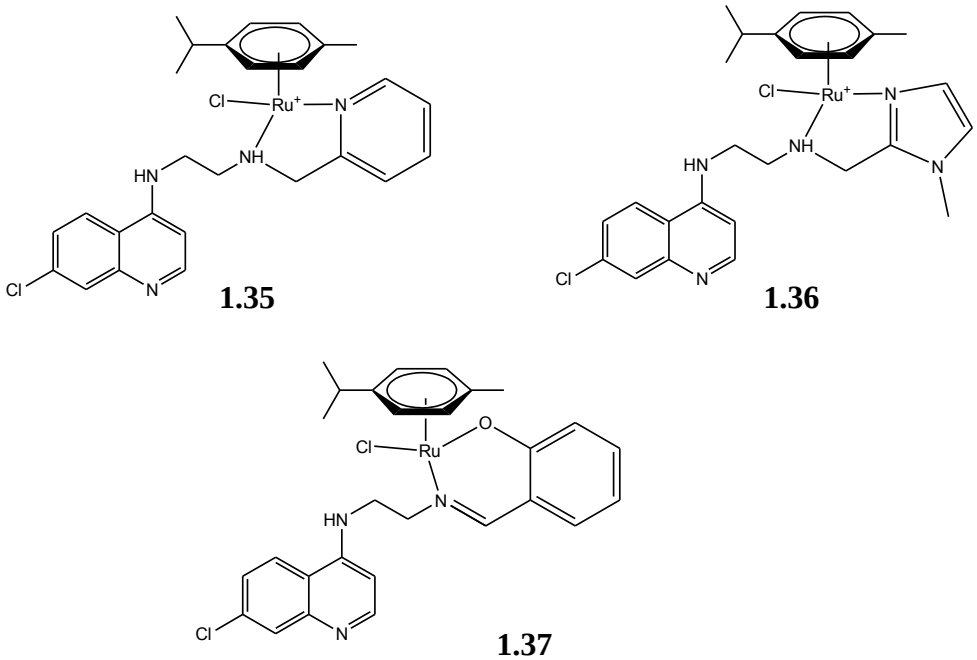
Tricarbonylchromium arene quinoline complexes were also tested against CQ-sensitive and resistant strains of *Plasmodium falciparum*. The two complexes, (**1.33** and **1.34**, Figure 1.16) were screened, showing high *in vitro* activity against both strains of *P. falciparum*. Complex **1.33** was active against the CQ-resistant parasite strain and was two times more active than a corresponding organic derivative (IC_{50} values of 33.9 nM versus 63.1 nM).[59]



1.33 **1.34**

Figure 1.16: Chromium complexes of a 4-amino-7-chloroquinoline derivative.

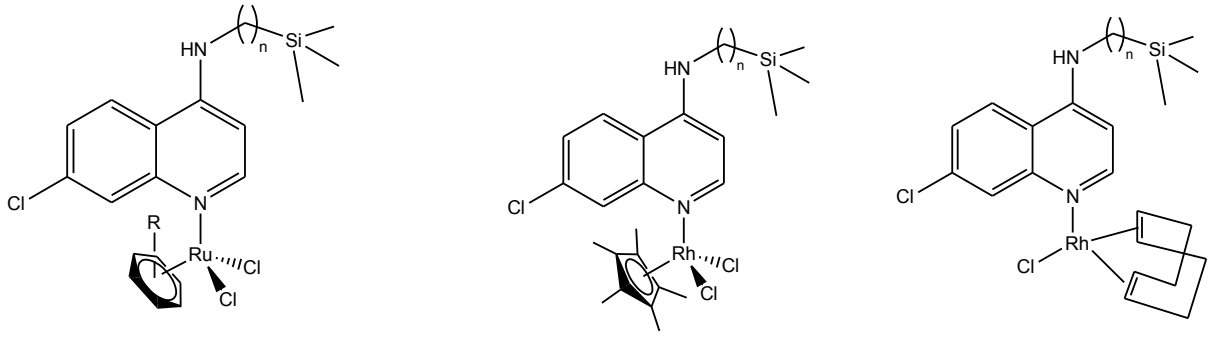
Ruthenium *p*-cymene quinoline complexes with bidentate coordination have been studied against the D10 (CQ-sensitive) and Dd2 (CQ-resistant) strains of *P. falciparum*. Compounds **1.35** and **1.36** (Figure 1.17) were less active than their corresponding ligands, while complex **1.37** was reasonably active.[60] New silicon-containing quinoline ligands and their corresponding Ru(II), Rh(I) and Rh(III) complexes (**1.38 – 1.40**, Figure 1.18) were prepared and tested against NF54 (CQ-sensitive) and Dd2 (CQ-resistant) *P. falciparum*.[61] In most cases the complexes showed better activity compared to the ligands. Generally the compounds were less active in the CQ-resistant strain than in the sensitive strain. This suggests that the compounds may experience cross-resistance similar to that of chloroquine.[61]



1.35 **1.36**

1.37

Figure 1.17: Ru(II) p-cymene quinoline complexes evaluated against the D10 and Dd2 strains of *P. falciparum*.

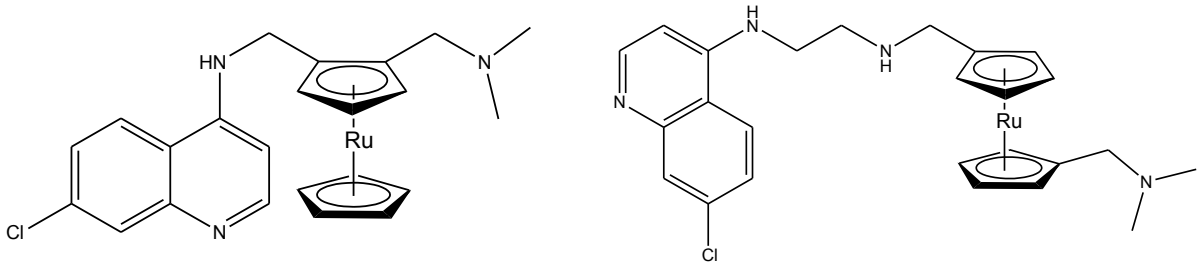


1.38 **1.39** **1.40**

n = 1 or 3; arene = *p*-cymene, benzene, η^6-$C_6H_5OCH_2CH_2OH$

Figure 1.18: Organosilicon Ru(II), Rh(I) and Rh(III) quinolines

A series of ruthenocene-chloroquine complexes (**1.41** and **1.42**, Figure 1.19) were also prepared and evaluated against CQ-resistant (K1) and CQ-sensitive (D10) strains of *P. falciparum*.[62] All compounds exhibited good efficacy against both parasitic strains. Activity exhibited against the CQ-resistant strain suggests that the metallocene aids in overcoming any resistance experienced by the chloroquine motif. The activity of the ruthenocene compounds were compared to analogous Fe derivatives and were found to exhibit comparable activity.[62]



1.41 **1.42**

Figure 1.19: Examples of ruthenocene 4-amino-7-chloroquinolines exhibiting good antiplasmodial activity

1.4.2. Ferrocene-quinoline compounds as antimalarials

Ferrocene (Fc) is an organometallic sandwich compound that was first prepared in 1951. This particular compound (and its derivatives) is used as a fuel additive, in catalysis and more recently, due to its stability in aqueous and aerobic media, lipophilicity and low toxicity, has been used in medicinal chemistry.

Incorporation of ferrocene as part of the chemical structures of active antimalarial drugs such as artemisinin (**1.43**, Figure 1.20),[63,64] atovaquone (**1.44**, Figure 1.20),[65] mefloquine (**1.45**, Figure 1.20) and quinine (**1.46**, Figure 1.20),[66] did not significantly improve the antimalarial activity of the parent compounds. In most cases the parent organic compounds displayed better activity in comparison to the ferrocenyl derivatives.

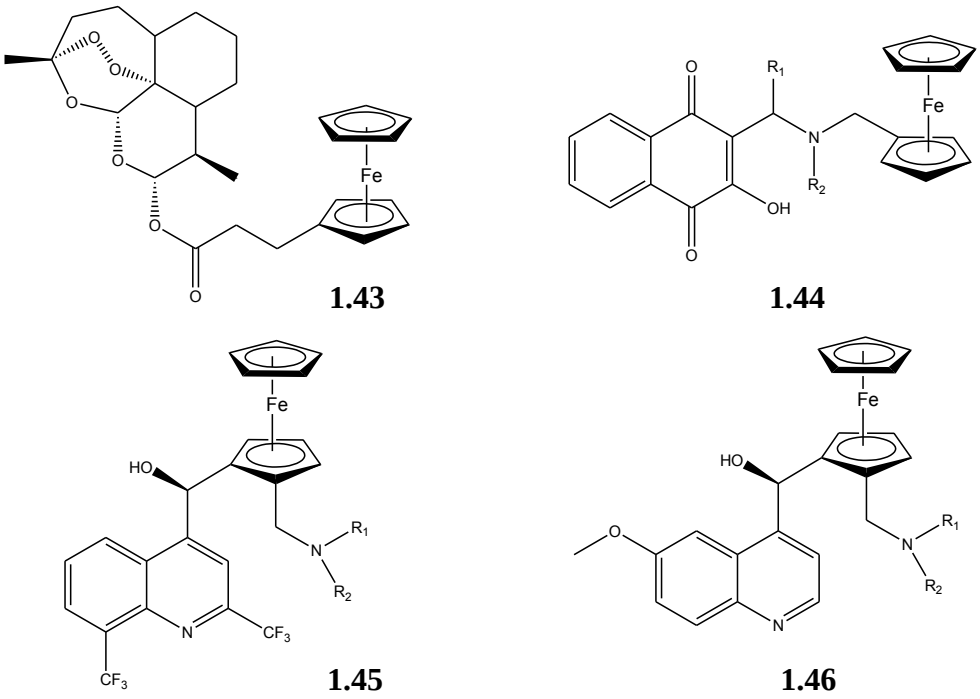
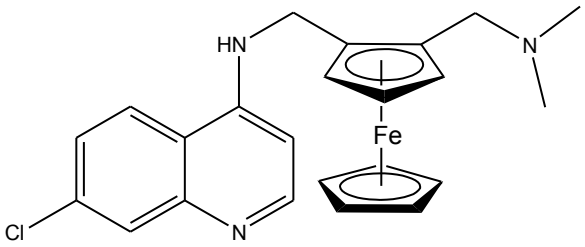


Figure 1.20: Ferrocene derivatives of established antimalarial agents.

In the case when ferrocene is covalently linked to a 7-chloroquinoline moiety, an important and potent antimalarial agent was produced, ferroquine (FQ) (**1.47**, Figure 1.21).[67] This

particular compound displays 22-times greater antimalarial activity than chloroquine against CQ resistant strains of *P. falciparum* when studied *in vitro*. *In vivo* this compound displayed potent activity in mice infected with *P. yoeli*, *P. berghei* and *P. vinkei vinkei*.[67,68] Due to its ability to overcome CQ resistance, ferroquine has undergone phase IIb clinical trials and is being developed by Sanofi-aventis.[69] This particular compound is extremely active against CQ sensitive and resistant strains of *P. falciparum*, suggesting that FQ may possess a distinct mechanism of action to that of CQ or there is an absence of interaction of this compound with resistance mechanisms.[64] It was found that ferroquine targets the digestive vacuole of the parasite by different transport mechanisms to that of chloroquine.[71,72] *In vitro* pharmacodynamic data supports the use of ferroquine as an antimalarial, however, further trials and evaluations are still required.[73]

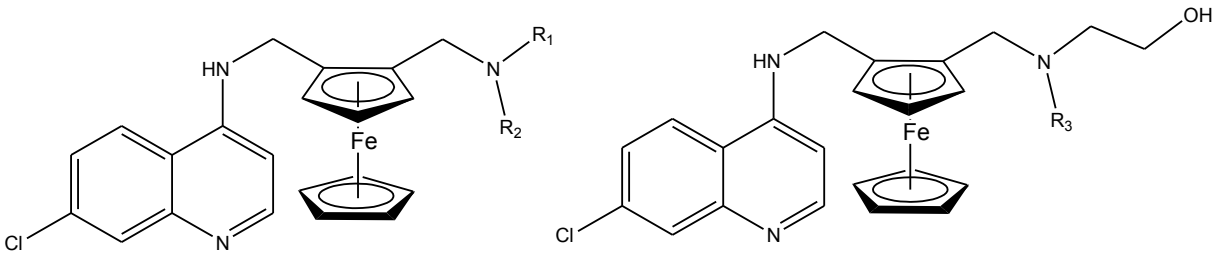


1.47

Figure 1.21: Ferroquine, a potent antimalarial.

Many structural derivatives of ferroquine have been prepared and tested to assess whether certain modifications may affect the activity of this particular compound. Modification of the basic tertiary amino group adjacent to the ferrocenyl moiety (**1.48**, Figure 1.22) by introduction of various alkyl substituents such as H, C_2H_5, CH_3 and $FcCH_2$, resulted in comparable activity to FQ and good activity against CQ resistant strains (Dd2 and W2). Contradictory to this, it was found that incorporation of a second ferrocenyl moiety onto the terminal nitrogen atom decreased the efficacy of the compounds.[74] Introduction of secondary amines as the basic amino group does not alter the activity in comparison to the parent compound, but does exhibit better activity than chloroquine against the Dd2 CQ sensitive strain.[75] Hydroxyferroquines or ferroquine derivatives possessing hydroxyl moieties on the terminal nitrogen atoms (**1.49**, Figure 1.22) have been shown to exhibit lower cytotoxicity in

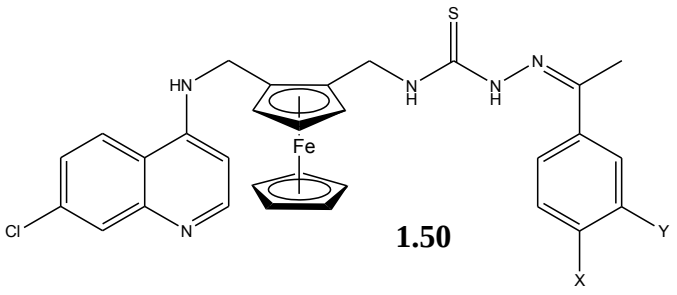
comparison to ferroquine. In addition to this, these compounds display much better *in vitro* activity against *P. falciparum* than chloroquine.[76]



$R_1 = R_2 = C_2H_5$ or $R_1 = C_2H_5$ and $R_2 = CH_3$; R_1 = H and $R_2 = CH_3$ or C_2H_5 or ^{t}Bu; R_3 = H, CH_3, C_2H_5

Figure 1.22: Ferrocene derivatives that display comparable antimalarial activity to FQ

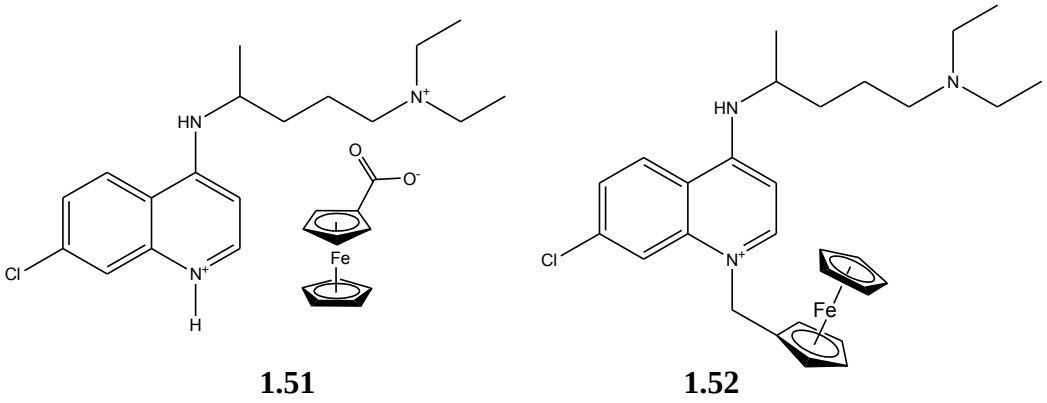
Incorporation of thiosemicarbazones (TSCs) onto the side chain of ferroquine has also been investigated. Thiosemicarbazones are another class of compounds that possess antimalarial activity. TSCs possessing the 2-acetylpyridine moiety have exhibited good antimalarial activity in *P. berghei* in mice.[77-79] Thiosemicarbazones are chelating ligands that are able to produce reactive oxygen species. This is believed to be the mode of action responsible for their potent activity.[80] In order to investigate the effect of combining thiosemicarbazones and ferroquine, a chimeric ligand approach was employed to investigate the effect on the antimalarial activity. The chimeras (**1.50**, Figure 1.23) were found to be active against four strains of *P. falciparum*. The presence of the aminoquinoline motif appears to be the major reason for the parasite activity and the ferrocenyl moiety is required to retain the activity.[81]



X = Cl or Br; Y = Cl or H

Figure 1.23: Thiosemicarbazone ferrocenyl conjugates

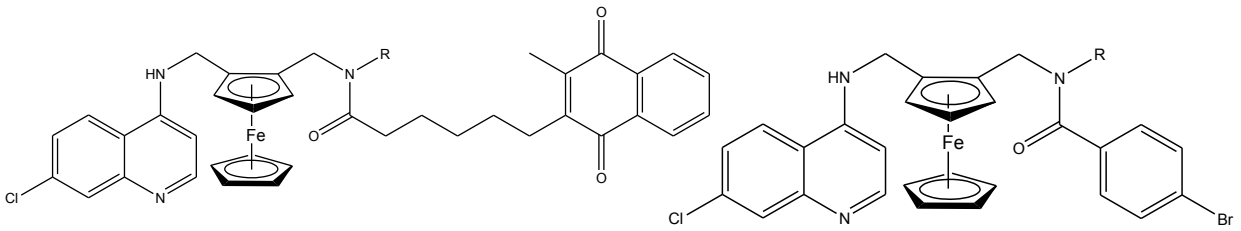
Many other ferrocene-containing conjugates have been prepared and their antimalarial activity tested. Association of chloroquine with a ferrocenecarboxylic acid via an electrostatic interaction (**1.51**, Figure 1.24) resulted in this compound displaying low activity against CQ-sensitive and CQ-resistant strains *in vitro*. It is believed that the low activity is as a result of an antagonistic effect between the chloroquine and the carboxylic acid derivative.[82] Condensation of a ferrocenylmethyl moiety onto the nitrogen atom of the quinoline ring of chloroquine afforded a quaternary ammonium salt (**1.52**, Figure 1.24). This particular compound was inactive against the CQ-sensitive (HB3) and the CQ-resistant (Dd2) strains of *P. falciparum*.[83]



1.51 **1.52**

Figure 1.24: Charged ferrocenyl complexes evaluated for their antimalarial activity

Ferrocenic dual molecules (**1.53** and **1.54**, Figure 1.25) consisting of a ferroquine analogue conjugated with either a glutathione reductase inhibitor or a glutathione depletor by means of an amide bond were evaluated for their antimalarial activity against NF54 (CQ-sensitive) and K1 (CQ-resistant) strains of *P. falciparum*. A slight decrease in the antimalarial activity of the dual molecules was observed in comparison to the corresponding ferroquine analogues. The decrease in activity may be due to cleavage of the amide bond as well as the side chain once metabolised in the food vacuole of the parasite.[84]

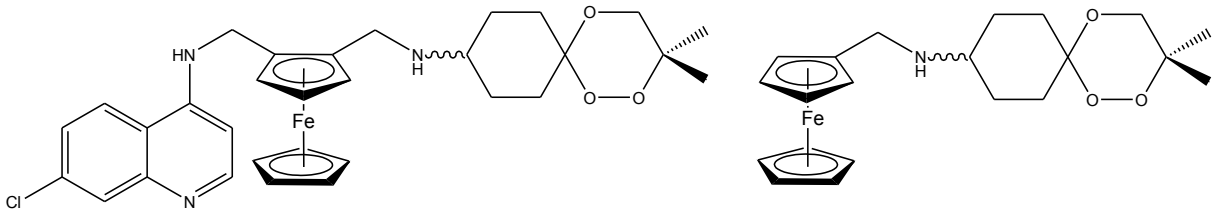


1.53 **1.54**

R = Me, Et, Pr, ^{i}Pr, Bu, ^{i}Bu

Figure 1.25: Ferroquine conjugates of glutathione reductase inhibitors and glutathione depletors

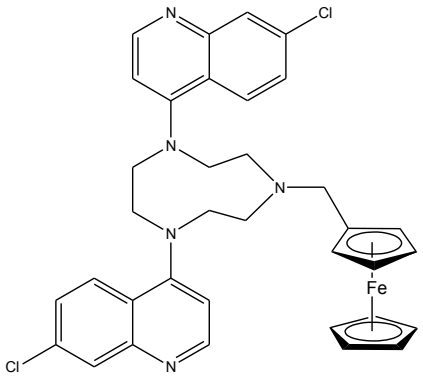
Another series of hybrid molecules that show promise are the trioxaferroquines. These compounds are antimalarial drugs containing 1,2,4-trioxane moieties covalently bound to an organometallic ferrocene derivative. The rationale behind the preparation of these compounds stems from two very potent antimalarials, namely artemisinin (1,2,4-trioxane) and ferroquine.[85] The preparation of these compounds was inspired and supported by the success of trioxaquine PA1103/SAR116242.[86] The antimalarial activity of several trioxaferroquines (**1.55**, Figure 1.26) and trioxaferrocenes (**1.56**, Figure 1.26) were evaluated against two CQ-resistant strains of *P. falciparum* (FcB1 and FcM29). The trioxaferroquines display good activity against both strains of the parasite. The corresponding trioxaferrocenes, however, did not exhibit any note-worthy activity. This further supports the importance of the 4-aminoquinoline moiety for antimalarial activity. *In vivo* studies also support the use of these compounds as antimalarial drugs due to the ability of one of the complexes to clear parasitemia in mice infected with *P. vinkei petteri*.[85]



1.55 **1.56**

Figure 1.26: An example of a trioxaferroquine and trioxaferrocene evaluated for antimalarial activity.

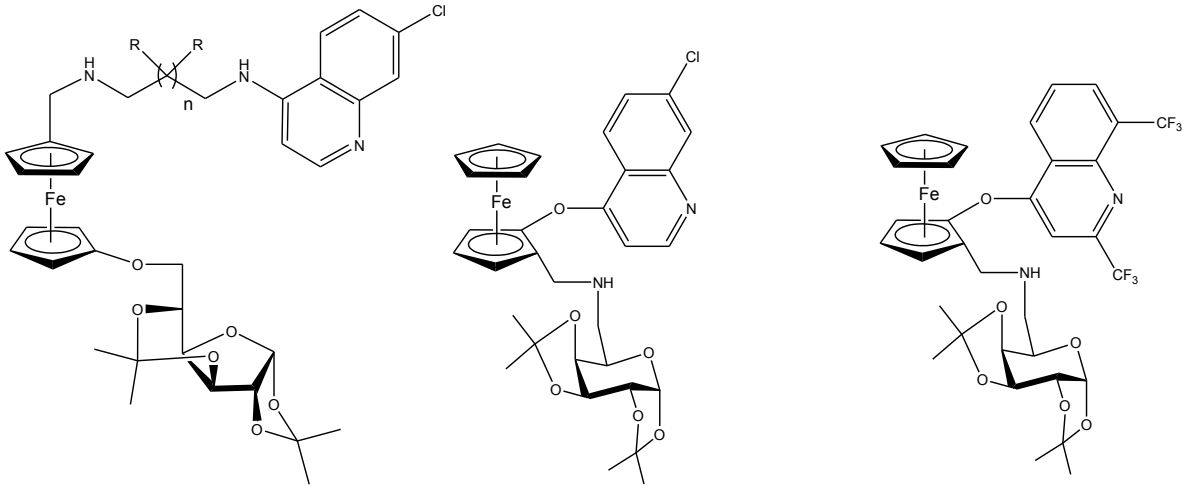
Ferrocene-containing triazacyclononane quinoline conjugates were also prepared and their activity tested against HB3 (CQ-sensitive) and Dd2 (CQ-resistant) *P. falciparum*. Compound **1.57** (Figure 1.27) showed potent activity against the Dd2 strain, but was less active against the sensitive strain.[87]



1.57

Figure 1.27: Triazacyclononane bisquinoline ferrocene conjugate active against CQ resistant *P. falciparum.*

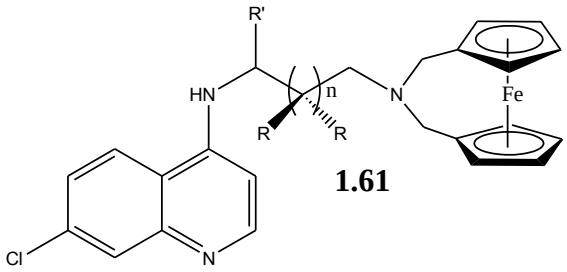
Ferrocene compounds incorporating a 7-chloroquinoline and a 1,2,3,5-(diisopropylidene-α-D-glucofuranose moiety (**1.58**, Figure 1.28) were prepared and tested against the D10, Dd2 and K1 strains of *P. falciparum*. Two of the compounds showed consistently high *in vitro* activity against the two resistant strains (Dd2 and K1), higher than chloroquine.[88] Ferrocene-containing carbohydrate derivatives of chloroquine (**1.59**, Figure 1.28) and mefloquine (**1.60**, Figure 1.28) were evaluated against the D10 and Dd2 *P. falciparum* strains. Incorporation of the carbohydrate moiety enhances the activity of the compounds, specifically in the resistant strains.[89]



1.58 **1.59** **1.60**

Figure 1.28: Diisopropylidene-α-D-glucofuranose and carbohydrate ferrocene quinolines

Ferrocenaphane analogues of ferroquine (**1.61**, Figure 1.29) have recently been prepared and tested against various strains of *P. falciparum.* The compounds were active against both choroquine-sensitive and chloroquine-resistant strains. These compounds were able to overcome resistance and this phenomenon was mainly attributed to the lipophilicity of the compounds, intramolecular hydrogen bonding and their compact size.[90]



1.61

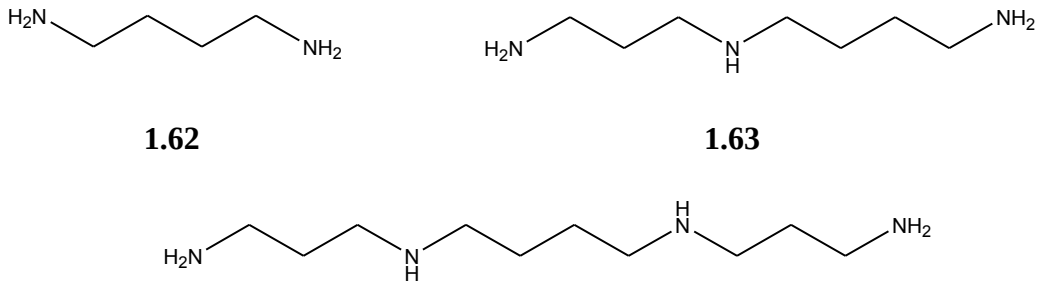
Figure 1.29: Ferrocenaphane 7-chloroquinolines that may overcome CQ resistance

The derivatisation of ferroquine, its analogues and other organometallic compounds has been a major contributor towards the quest for new organometallic compounds displaying antimalarial activity. The success of many of these compounds supports the development of various other organometallic molecules in order to evaluate their antimalarial activity in the

hope to obtain compounds possessing similar or more potent activity as well as those that possess distinct biological mechanisms to chloroquine.

1.5. Polyamines: potential drug delivery systems

Polyamines are compounds that play an important role in all living systems. They are generally defined as compounds containing two or more amine moieties. These compounds are naturally occurring and are found in mammalian cells. Putrescine (**1.62,** Figure 1.30), spermidine (**1.63**, Figure 1.30) and spermine (**1.64**, Figure 1.30) are found in most eukaryotes. These compounds are mainly responsible for maintaining cell viability.[91] Studies reveal that these systems are able to transport cytotoxic drugs into tumour cells.[92] Cells import polyamines by means of a polyamine transporter (PAT) in order to sustain cell growth. The transporter is able to tolerate modified polyamines, therefore drug incorporated polyamines are able to penetrate tumour cells.[93]



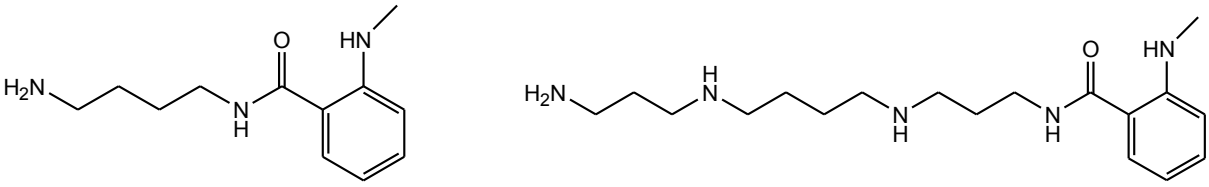
1.62 **1.63**

1.64

Figure 1.30: Naturally occurring polyamines involved in cell proliferation and differentiation.

Although polyamine research has been aimed mainly towards the discovery of selective drugs targeting human tumour cells, these compounds have also been found to exhibit selective uptake by *Plasmodium* infected erythrocytes. The erythrocytes were analysed and it appeared as though polyamine levels increased by more than 10 fold.[94,95] It has also been shown that a bis(benzyl) polyamine analogue, MDL27695, inhibits growth in CQ-sensitive and resistant strains of *P. falciparum*.[96] Polyamine amides (Figure 1.31) of *N*-methylanthranilic acid were synthesised in order to exploit the PAT system of parasites. These compounds are analogues

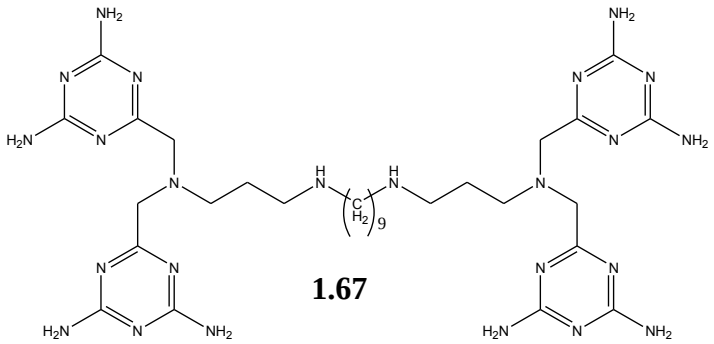
of spermidine, putrescine (**1.65**) and spermine (**1.66**). Intracellular uptake was monitored using fluorescent microscopy. The spermine derivative showed measurable accumulation.[97]



1.65 **1.66**

Figure 1.31: Polyamine amides of putrescine and spermine

A series of 1,3,5-triazine-substituted polyamines (**1.67**, Figure 1.32) have been screened against CQ-sensitive (NF54) and CQ-resistant (K1) strains of *P. falciparum*. The compounds were generally more active against the resistant strains. Incorporation of multiple triazine moieties resulted in enhanced activity.[98] In another study, tetrameric ferrocenylthiosemicarbazones conjugated to a polyamine backbone were evaluated against the W2 resistant strain of *P. falciparum*. Increased efficacy of the conjugated systems was observed compared to the non-conjugated precursor, suggesting that the polyamine is required for activity.[99]



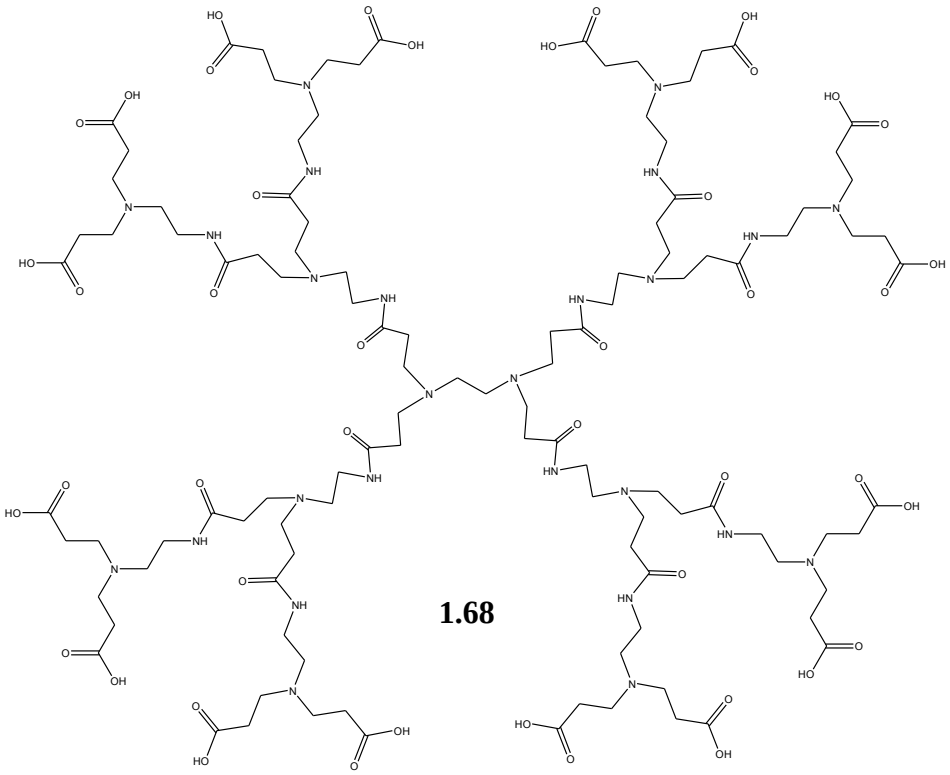
1.67

Figure 1.32: An example of a triazine polyamine that inhibits growth of *P. falciparum in vitro*.

Polyamines are desirable systems because they could be used as drug delivery agents in a similar manner to dendrimers. Dendrimers (**1.68**, Figure 1.33) are multivalent, tree-like, three dimensional molecules that contain several repeating units. These molecules have been used

extensively in polymer and material science; however, there is a growing interest in the use of these compounds as part of drug systems.[100]

Dendrimers have mainly been used in the delivery of cancer chemotherapeutic drugs.[101] Drug delivery can essentially be carried out by two distinct mechanisms: (i) by utilising host-guest chemistry, where the drug is introduced as the guest and released due to changes in pH, temperature or diffusion, or (ii) by degradation of a drug-dendrimer conjugate (where the drug is covalently linked to the dendrimer) *in vivo*. The first mechanism is less desirable because controlling the amount of drug loaded onto the dendrimer as part of a host-guest system can be problematic. The second mechanism would be favoured because dendrimers have a large number of surface groups that can be functionalised with drug molecules which leads to the formation of pro-drugs.[100] An example of a dendrimer-quinoline system can be seen with the PEGylated (PEG = polyethylene glycol) peptide dendrimeric carrier of chloroquine (Figure 1.34). The results revealed that loading of the drug onto the dendrimer reduced the haemolytic toxicity and cytotoxicity of CQ. Reduction of ring and trophozoite stages of *P. falciparum* was also observed.[102]



1.68

Figure 1.33: An example of a PAMAM (polyamido amine) dendrimer

Very few studies have been conducted where polyamines were used as carriers in order to afford potential antimalarial agents. This study looked at making use of various mono- and polyamines in order afford new imino- and amino-containing compounds with potential antiplasmodial activity. The rationale for this study was supported by the use of polymers (or multimeric structures) as drug delivery systems.[103] It must be noted that the compounds prepared in this study are not polymers or macromolecules, but the preparation of multimeric and multinuclear systems was influenced by the use of macromolecular systems as therapeutics. This project involved the design, synthetic preparation and biological evaluation of various mono- and multimeric organic and organometallic compounds. These compounds were prepared for their potential as antiplasmodial agents. In addition to this, their activity against other diseases including trichomoniasis and oesophageal cancer was also investigated. Preliminary mechanistic studies were performed in order to elucidate the possible mode of action of these compounds against *Plasmodium falciparum*.

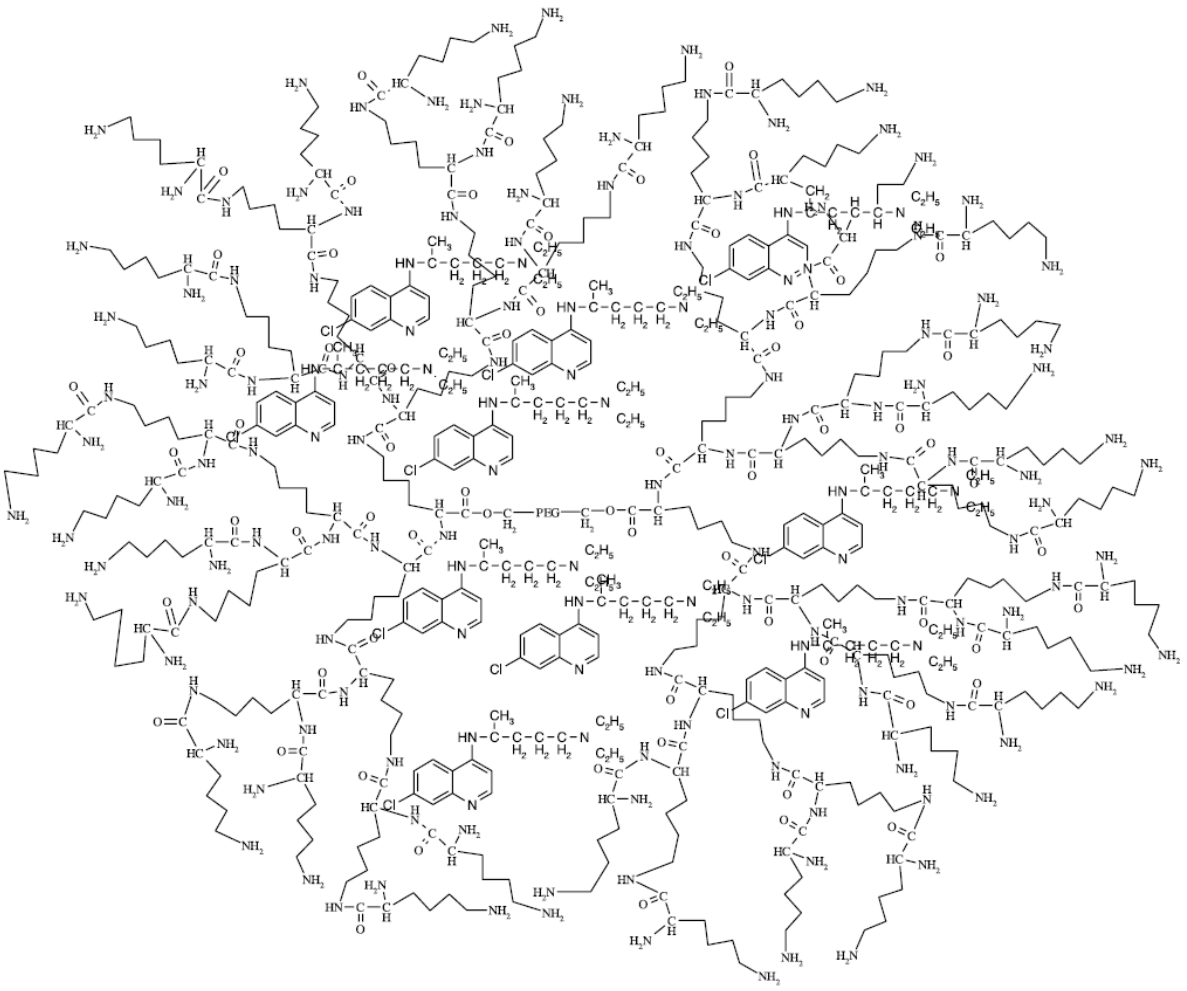


Figure 1.34: A pictorial representation of chloroquine loaded onto a PEG-lysine G5 dendrimer[101]

1.6. Aims and specific objectives

1.6.1. Aims

Many biological studies have been conducted on monomeric quinoline or mononuclear metal complexes of various quinolines. There is much scope for the development of multimeric systems towards the discovery of new compounds that may potentially possess antiplasmodial activity. The overall aims of the project were as follows:

1) To prepare various mono- and multimeric quinoline-containing compounds conjugated to various amine and polyamine scaffolds (Figure 1.35).
2) To prepare compounds containing one or more metal moiety by also making use of polyamine scaffolds.
3) To evaluate these compounds for biological activity in order to compare the activity of monomeric and multimeric systems.
4) To use physicochemical methods to investigate a possible mechanism of action for the compounds.

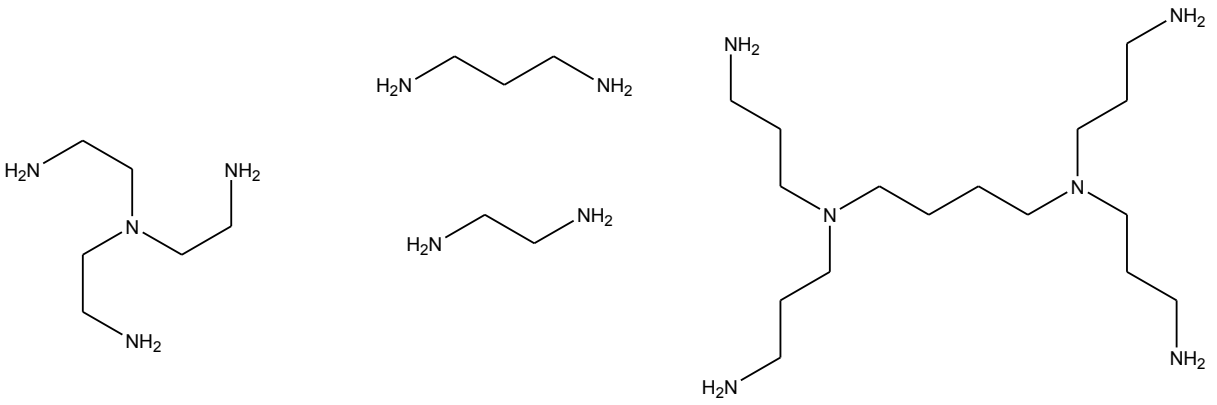


Figure 1.35: Polyamines used in this study**.**

1.6.2. Specific objectives

1) To prepare a series of mono- and multimeric quinoline and ferrocenyl compounds conjugated to amine and polyamine scaffolds (Figure 1.36). These compounds possess thiourea moieties which are also active antimalarial pharmacophores.

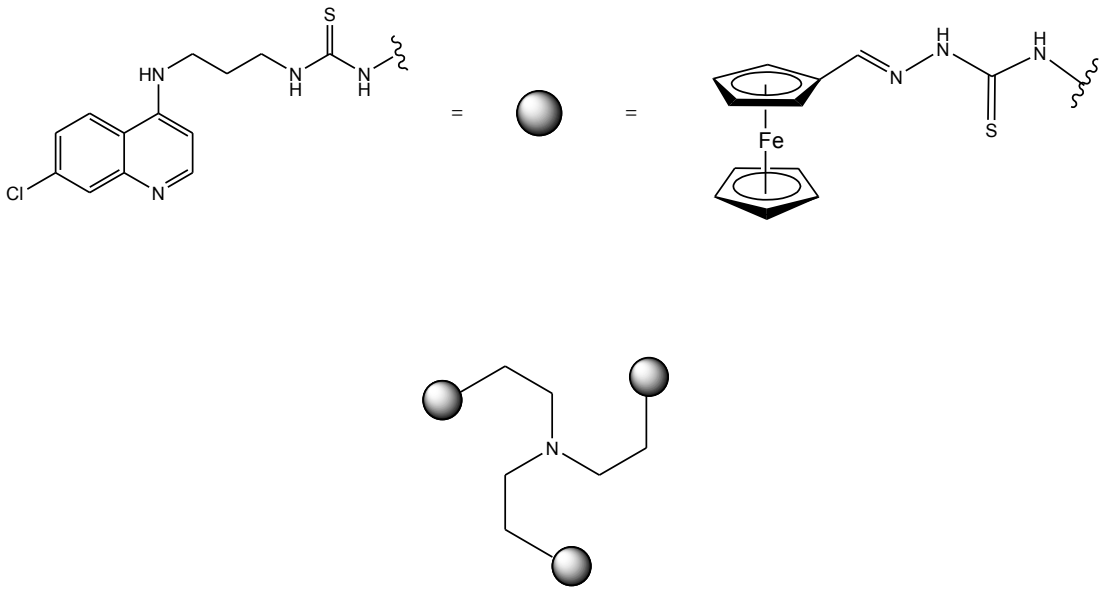
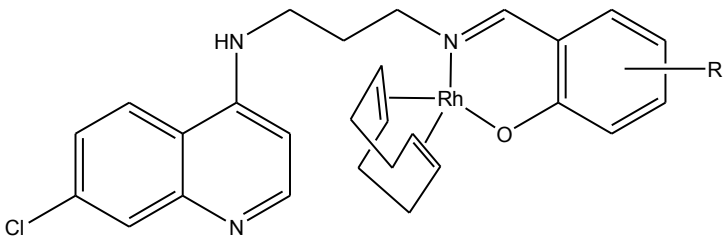


Figure 1.36: General structures of some thiourea compounds prepared in this study.

2) To prepare a series of mono- and binuclear Rh(I) quinoline salicylaldimine complexes and their corresponding ligands (Figure 1.37) in order to gain insight to any structure-activity relationships. Rh(I) was been chosen because very few studies have been conducted using this metal in malaria therapy. It also possesses square-planar geometry which may aid in the compounds ability to interact with haem.



R = electron withdrawing or donating groups

Figure 1.37: Mononuclear Rh(I) salicylaldimine complexes investigated in this study.

3) To prepare mono- and multinuclear ferrocene compounds (Figure 1.38) containing imino and amino moieties. Since ferroquine is one of the most promising

organometallic compounds for malaria therapy it was considered worthwhile to investigate the activity of compounds possessing ferrocenyl moieties.

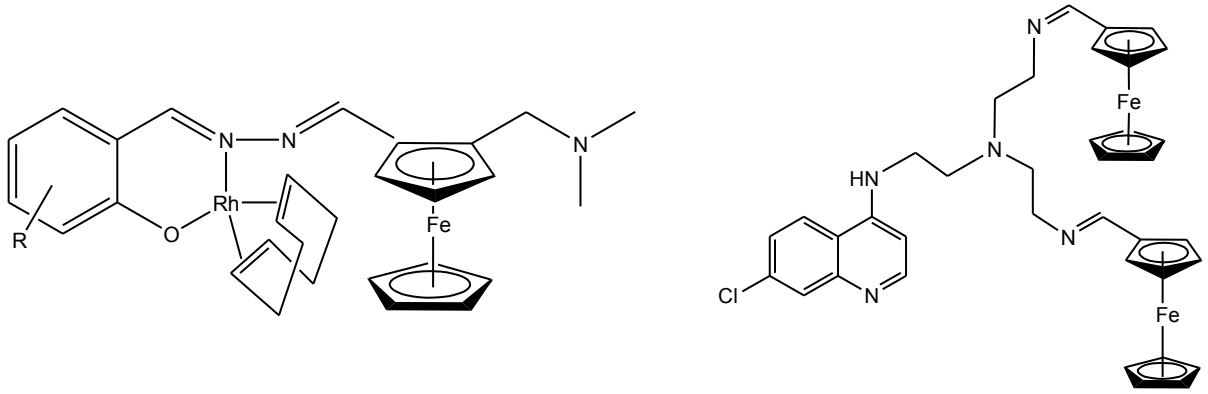


Figure 1.38: Some ferrocenyl derivatives investigated in this study.

4) To test these compounds against the CQ-sensitive (NF54) and CQ-resistant (Dd2 or K1) strains of *P. falciparum*. To evaluate the activity of these compounds against *Trichomonas vaginalis*, a parasite responsible for the sexually transmitted infection, trichomoniasis. To investigate the cytotoxicity of the compounds against WHCO1 oesophageal cancer cells. To use physicochemical methods to evaluate the extent to which these compounds are able to inhibit synthetic haemozoin (β-haematin) formation using an NP-40 detergent assay.

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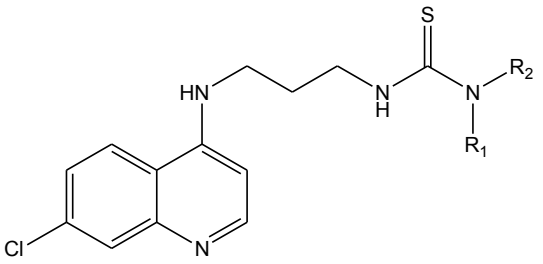
CHAPTER 2

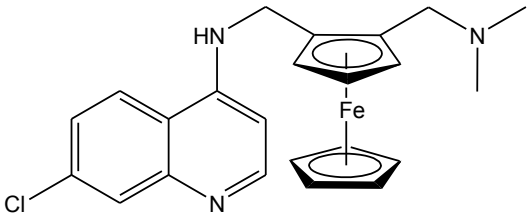
Synthesis, characterisation and biological evaluation of thiourea-based polyamines

2.1. Introduction

Thiourea-containing compounds are attractive for the design of new antiplasmodial agents. Thiosemicarbazones are a class of thioureas that possess a wide range of biological properties including antimalarial activity.[1-5] Many thiosemicarbazones display activity against protozoans by inhibiting various cysteine proteases as well as other targets.[5] Recent studies have shown that a series of quinoline-containing thioureas (Figure 2.1a) exhibited activity in both chloroquine-sensitive and resistant strains of *Plasmodium falciparum* (*P. falciparum*) *in vitro*. Ferrocene-containing compounds have also been found to possess biological activity including anticancer, antimalarial and antifungal.[6]



a



b

R_1 = alkyl, aryl;

R_2 = H, alkyl

Figure 2.1: (a) Monomeric quinoline thioureas showing activity against sensitive and resistant strains of *P. falciparum* and (**b**) ferroquine a ferrocene-containing analogue of chloroquine able to overcome CQ resistance.

In addition to this, thiosemicarbazones (TSCs) containing ferrocenyl moieties have proven to be active against parasites as well as certain tumours.[7,8] More recently ferrocenyl thiosemicarbazones conjugated to a poly(propyleneimine) (PPI) dendrimer were evaluated *in vitro* and displayed moderate antiplasmodial activity against the W2 chloroquine-resistant strain of *P. falciparum*.[9] It is suggested that the incorporation of the ferrocenyl moiety alters the lipophilic nature of molecules and therefore may enhance the activity of certain compounds due to increased uptake of the compounds into the digestive vacuole of the parasite. Ferroquine (Figure 2.1b) is an excellent example of a ferrocenyl-containing molecule that exhibits potent antiplasmodial activity against chloroquine-resistant strains of *P. falciparum*.[10]

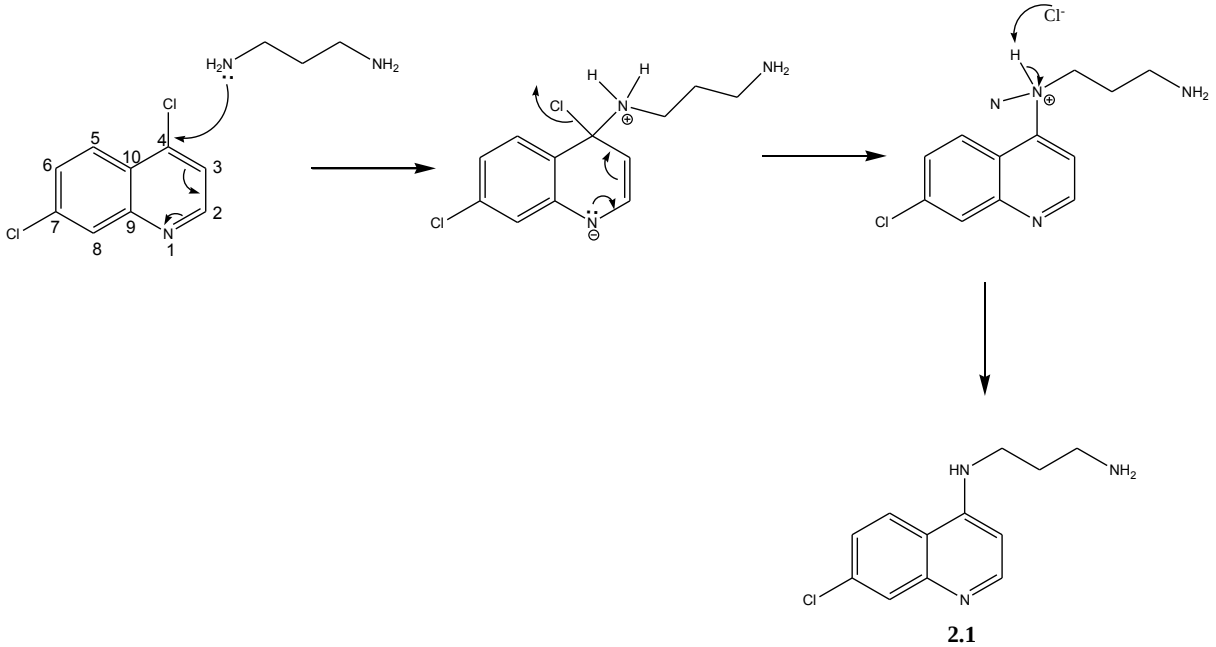
Since very little research has been geared towards investigating the biological activity (specifically antiplasmodial activity) of systems containing multiple biologically active motifs, this study investigated the synthesis, characterisation and biological evaluation of mono- and multimeric quinoline and ferrocene thiourea compounds. An increased number of biologically active moieties may result in enhanced activity. These moieties were incorporated onto polyamine systems in the hope of enhancing the transport of these motifs across membranes and in addition to this aid in the accumulation of these compounds inside of the digestive vacuole of the parasite due to pH trapping. The *in vitro* antiparasitic and antitumour activity as well as β-haematin inhibition activity of these compounds is described.

2.2. Synthesis and characterisation of mono- and multimeric quinoline thioureas 2.1 – 2.6

2.2.1. Synthesis

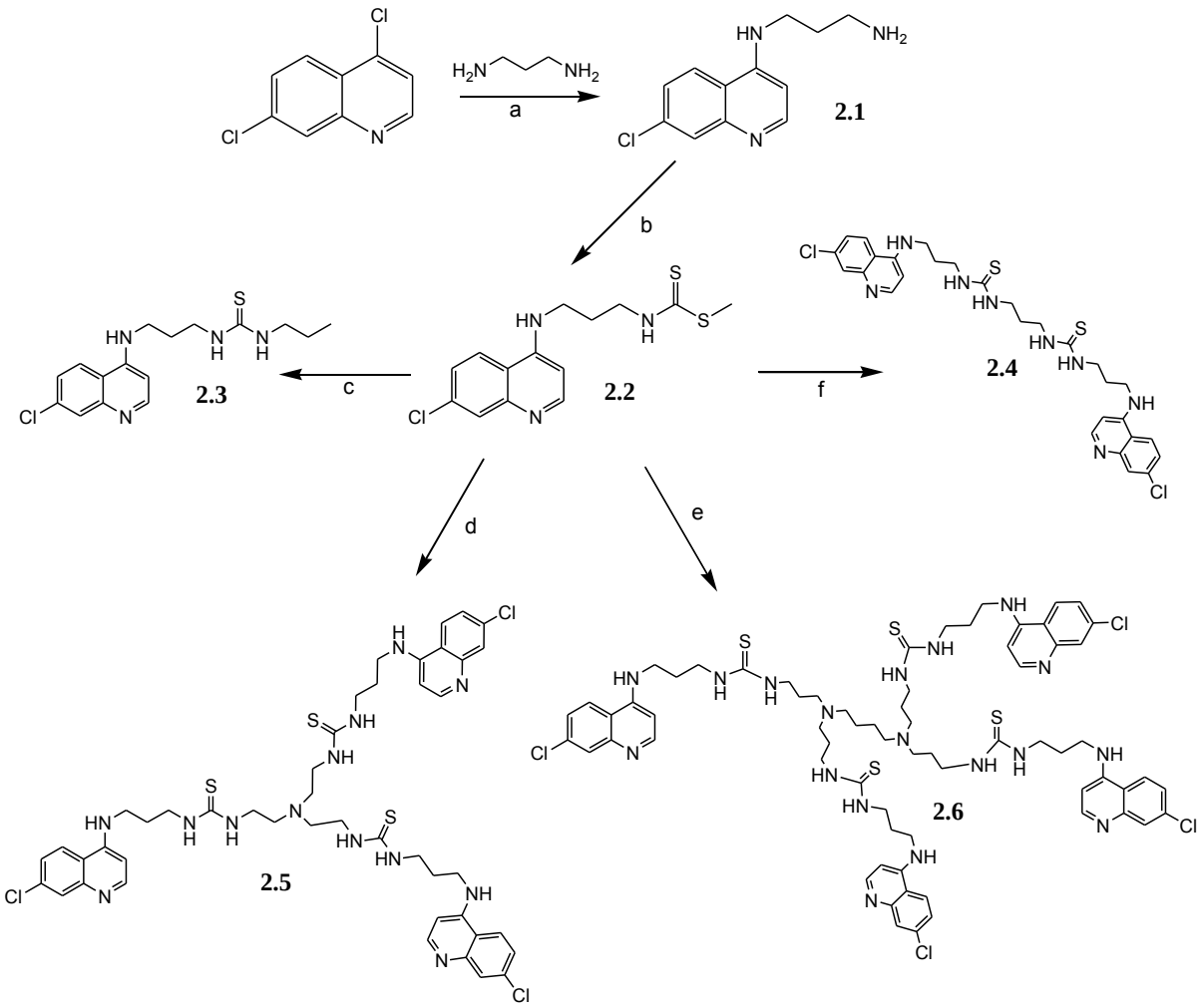
Four new 4-amino-7-chloroquinoline thioureas were synthesised using template procedures. 4,7-Dichloroquinoline was reacted with an excess of 1,3-diaminopropane via a nucleophillic aromatic substitution (S_NAr) reaction to afford *N'*-(7-chloroquinolin-4-yl)-propane-1,3-diamine (**2.1**, Figure 2.2). The chlorine atom at position 4 (numbering shown in Figure 2.2) of the quinoline ring is the favoured leaving group which is a consequence of the position of the quinoline nitrogen. The quinoline nitrogen is an electron sink which is able to accept a lone pair of electrons in order to allow attack at C-4 by the nucleophilic nitrogen (Figure 2.2).

N'-(7-Chloroquinolin-4-yl)-propane-1,3-diamine (**2.1**) was modified to form [3-(7-chloro-quinolin-4-ylamino)-propyl]-dithiocarbamic acid methyl ester (**2.2**) by means of a modified one-pot synthesis previously described by Katti *et al.*[11] *N*-(7-Chloroquinolin-4-yl)-propane-1,3-diamine (**2.1**) was reacted with carbon disulfide in the presence of KOH in isopropanol, followed by methylation with iodomethane at 0°C. Compounds **2.3** – **2.6** were obtained by subjecting **2.2** to nucleophillic substitution by various amines such as *n*-propylamine, 1,3-diaminopropane, tris(2-aminoethyl)amine and *N*,*N*,*N*′,*N*′-tetrakis(3-aminopropyl)-1,4-butanediamine (DAB-Am-4), respectively (Scheme 2.1). Compounds **2.3** – **2.6** were obtained as cream solids in various yields (12 – 85%).



2.1

Figure 2.2: Mechanism of nucleophillic aromatic substitution (S_NAr) to afford **2.1**.



Scheme 2.1: Synthesis of quinolines **2.1** – **2.6**. Reagents and conditions: (a) neat; (b) KOH (1 eq.), $NH_2NH_2{\cdot}H_2O$ (1 eq.), CS_2 (1 eq.), CH_3I (1 eq.) 0 – 5 °C; (c) *n*-propylamine, (d) tris(2-amino)ethyl amine; (e) DAB-Am-4; (f) 1,3-diaminopropane MeOH, reflux, 24 h.

2.2.2. Characterisation

The compounds were characterised using various techniques including ^{1}H, $^{13}C\{^{1}H\}$ (proton decoupled ^{13}C), heteronuclear single quantum coherence (HSQC) and correlation spectroscopy (COSY) nuclear magnetic resonance (NMR) spectroscopy, infrared (IR) spectroscopy and electrospray ionisation mass spectrometry (ESI-MS).

NMR Spectroscopy

Analysis of the ^{1}H NMR spectra of compounds **2.1** – **2.6** confirmed that the proposed compounds were obtained. The ^{1}H NMR spectrum of **2.1** was recorded using CD_3OD and was in agreement with the proposed structure. The integration of all of the proton resonances in the spectrum of **2.1** was consistent with the mono-substituted amine. The ^{1}H NMR spectra of **2.2** – **2.6** were recorded in DMSO-d_6. The spectrum of **2.2** supported formation of the thioester moiety due to the presence of a singlet at 2.46 ppm, which was assigned to the CH_3 group of the ester moiety. The absence of this particular signal in the spectra of **2.3 – 2.6** confirmed displacement of the -SCH_3 group of compound **2.2** in each case.

The spectrum of **2.3** displayed signals for the protons of the propyl chain at 0.86, 1.49 and 3.30 ppm for the -C***H***$_3$, -CH_2C***H***$_2CH_3$ and -NHC***H***$_2$- groups, respectively. Signals for the protons of the propyl chain of compound **2.4** were observed at 1.73 and 3.30 ppm for -CH_2C***H***$_2CH_2$- and -NHC***H***$_2$-, respectively. Signals for the ethyl moiety of the trimeric compound **2.5**, resonated at 2.66 and 3.44 ppm. Signals were significantly broader in the spectrum of compound **2.6**, in comparison to the spectra of the mono-, di- and trimeric counterparts. This may be attributed to the multimeric nature (containing four quinoline moieties) of this compound. Signals for the protons of the butyl core were observed at 1.32 and 2.35 ppm. Multiplets for the protons of the propyl groups resonated at 1.59, 2.35 and between 3.21 and 3.50 ppm (overlap of signals observed) for -CH_2C***H***$_2CH_2$-, -CH_2C***H***$_2$N- and -NHC***H***$_2$-, respectively.

The ^{1}H NMR spectrum of **2.6** is shown in Figure 2.3. For compounds **2.3** – **2.6**, signals for the newly formed N*H* group were assigned at approximately 7.19 ppm. The signal for H3 (numbering shown in Figure 2.2) of the quinoline moiety occurred as a doublet at approximately 6.4 ppm for these compounds. Proton H8 was assigned to a doublet at approximately 7.8 ppm in all cases. A doublet with a coupling constant of approximately 2 Hz was observed for the latter, due to coupling of H8 to H6. The signal for H6 appeared as a doublet of doublets in each spectrum, thus confirming long-range coupling within the aromatic system. The integration observed in each spectrum was consistent with the proposed structures.

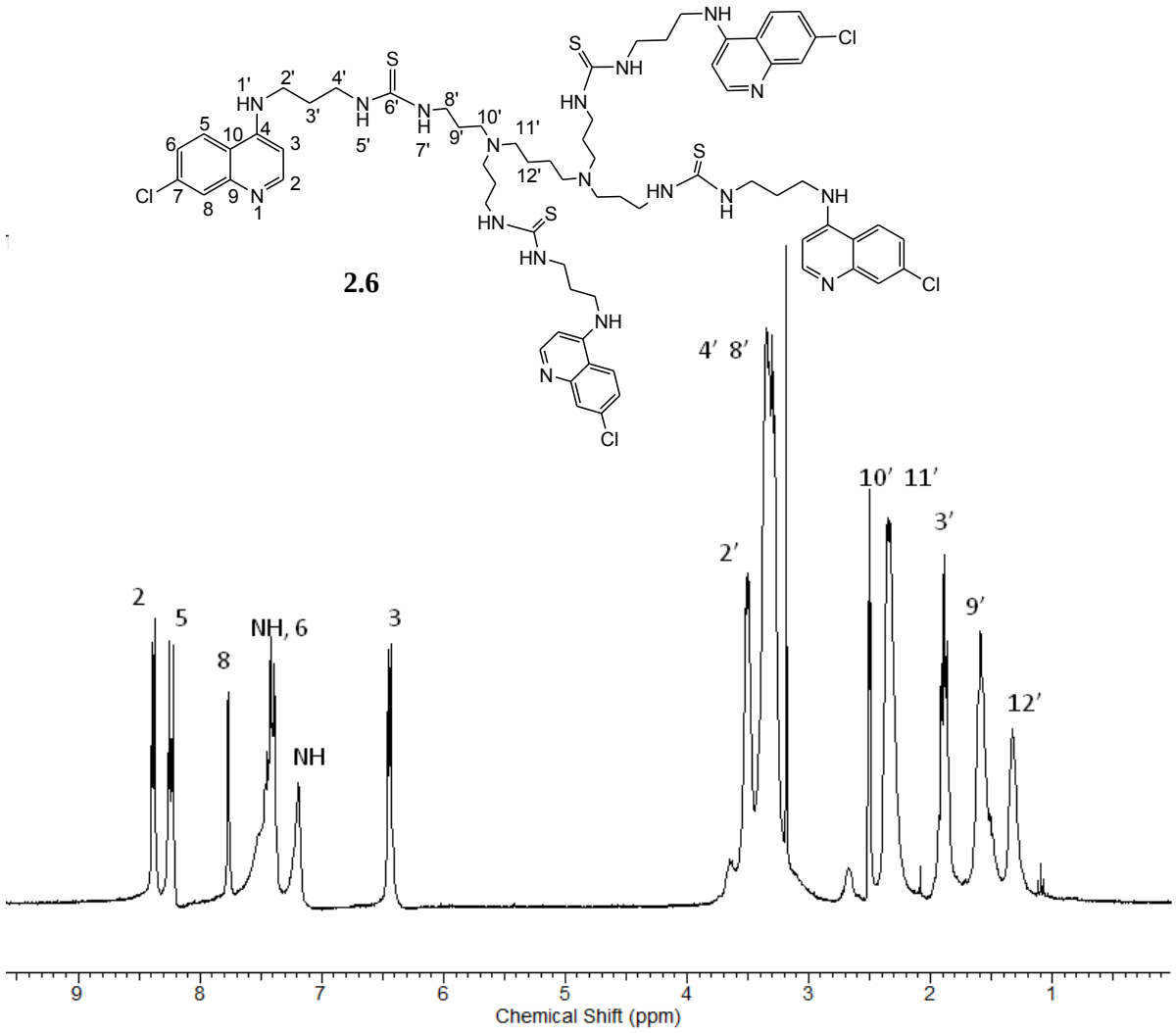


Figure 2.3: ^{1}H NMR spectrum of the tetrameric thiourea **2.6** in DMSO-d_6

Analysis of the $^{13}C\{^{1}H\}$ NMR spectra further confirmed the structures of these compounds. Resonances for the carbon atoms of the quinoline rings appeared in the region of 98 and 152 ppm. The thiocarbonyl signals for **2.3** - **2.6** were observed at approximately 182 ppm. In the case of **2.3**, signals affiliated with the aliphatic carbon atoms were found between 11.10 and 45.10 ppm. One signal was observed for carbon atoms C5 and C6 in the spectra of **2.3**, **2.5** and **2.6**. This was confirmed by a 2D HSQC experiment. Two cross-peaks were observed for this signal, confirming the same resonance for these two atoms. In the case of **2.4**, two distinct resonances were observed for C5 (123.67 ppm) and C6 (123.88 ppm). Signals attributed to the aliphatic carbon atoms were observed at similar resonances compared to **2.3** and occur between 40.79 and 40.93 ppm. The spectra of **2.5** and **2.6** were consistent with the proposed

structures and compared favourably with the monomeric and dimeric analogues. Signals for the aliphatic carbon atoms of **2.5** resonated between 27.30 and 55.98 ppm, while these resonances were observed between 24.73 and 53.75 ppm for **2.6**.

IR spectroscopy

Infrared spectroscopy is another useful tool used to identify functional groups that are present in various compounds. The infrared spectra of these compounds were recorded in the solid state and prepared as KBr pellets. The spectra of compounds **2.1** – **2.6** displayed absorption bands for the quinoline C=N between 1611 and 1613 cm^{-1}. Absorption bands for the thiocarbonyl (C=S) vibrations of **2.3** - **2.6** were observed between 1136 and 1138 cm^{-1}. This particular band in **2.2** appeared at a slightly higher frequency, 1141 cm^{-1}, compared to the rest of the compounds. This may be attributed to the presence of the adjacent electron donating $-SCH_3$ group, resulting in shortening of the C=S bond and therefore causing a slight increase in the wavenumber of this band.

ESI mass spectrometry

The new compounds **2.3** – **2.6** were submitted for ESI-MS analysis to further confirm the proposed structures. The samples were analysed in the positive mode. For compound **2.3**, a peak at *m/z* 337.1248 (100%) was observed and corresponds to the protonated form of the monomeric quinoline molecule. For **2.4**, a peak at *m/z* 315.0951 (100%) was assigned to $[M+2H]^{2+}$. Peaks at 513 (60%) corresponding to $[M+2Na]^{2+}$ and 1429 (70%) corresponding to $[M+H]^{+}$ were observed in the spectra of **2.5** and **2.6**, respectively. Sodium adducts are commonly observed when using this technique. In the mass spectra of **2.5** and **2.6** successive loss of the quinoline thiourea moieties were observed giving rise to fragmentation peaks. Figure 2.4 shows the mass spectrum of **2.6** as an example.

Based on the characterisation data outlined above, it was concluded that a series of mono- and multimeric quinoline thioureas were successfully synthesised using template procedures. NMR spectroscopy, IR spectroscopy and ESI-MS confirmed the integrity of these compounds.

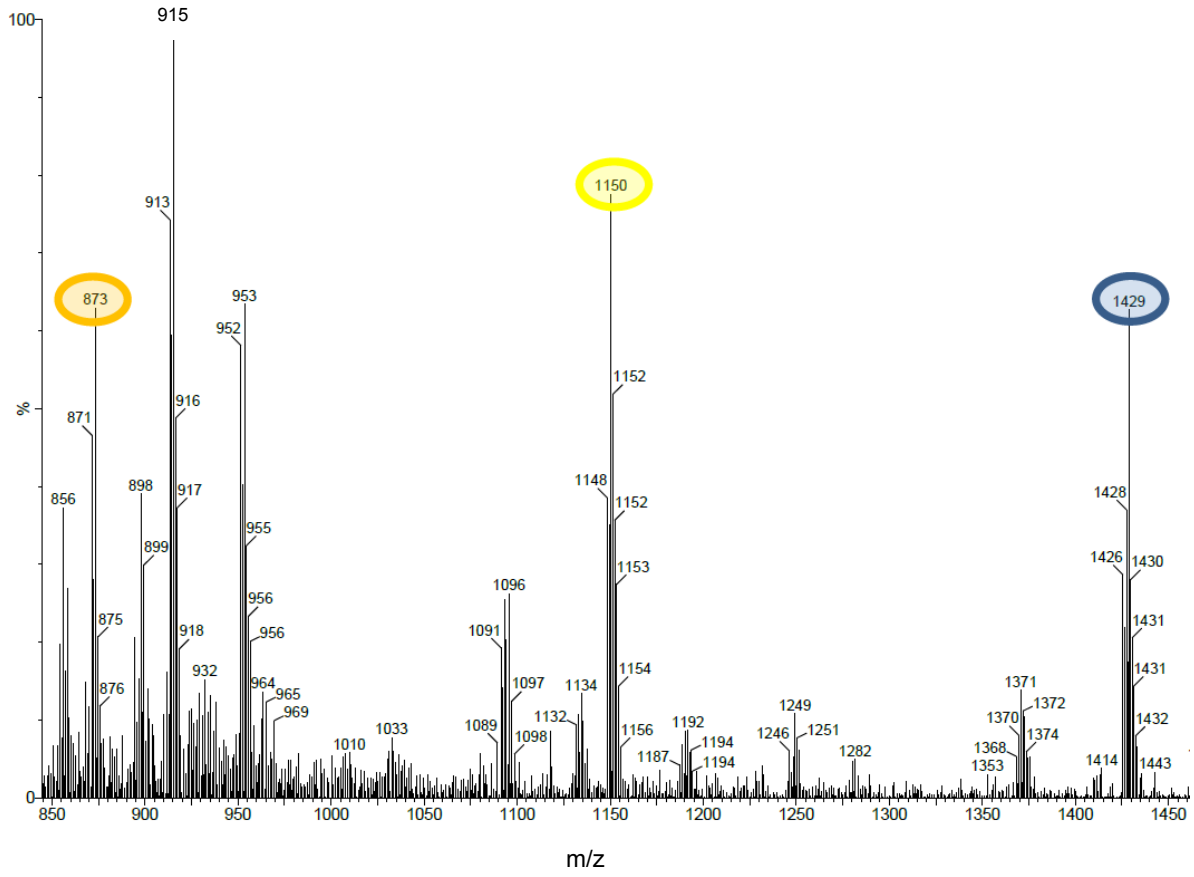


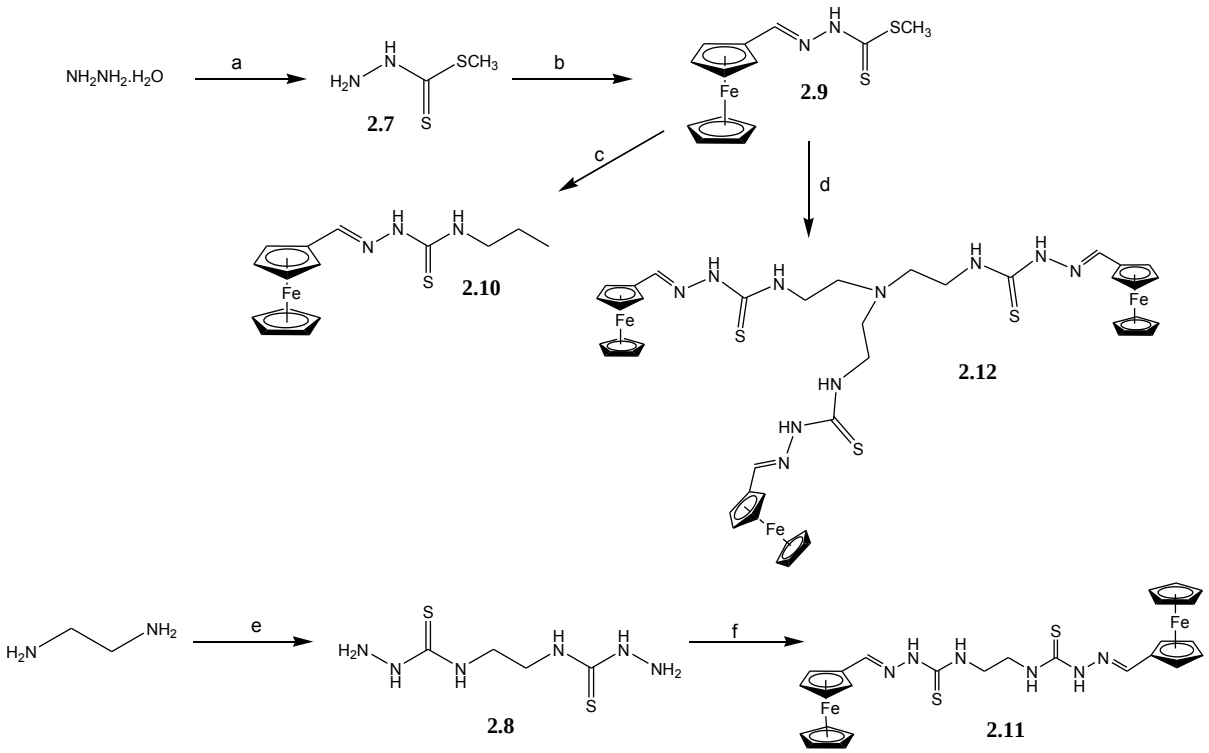
Figure 2.4: ESI mass spectrum of **2.6** obtained in the positive mode – highlighted peaks correspond to masses of the tetrameric (blue); trimeric (yellow) and dimeric (orange) fragments.

2.3. Synthesis and characterisation of mono- and multimeric ferrocene thioureas 2.7 – 2.12

2.3.1. Synthesis

Three ferrocenylimine thiourea compounds were prepared by the procedures outlined in Scheme 2.2. Methyl hydrazinecarbodithioate (**2.7**) was prepared in a similar manner to compound **2.1** using the method described by Klayman *et al.*[1] Hydrazine hydrate was treated with carbon disulphide in *i*-PrOH in the presence of KOH. Methylation with iodomethane afforded **2.7** in 32% yield. Ethane-1,2-dithiosemicarbazide (**2.8**) was prepared in order to afford a dimeric ferrocene derivative. This method also involved the preparation of the

dithiosemicarbazide from 1,2-ethanediamine via a diacid precursor. The diacid that formed *in situ* was then treated with excess hydrazine hydrate which displaced the carboxylic acid moiety by nucleophillic addition to the thiocarbonyl forming the desired dithiosemicarbazide. Subsequent reaction of **2.7** with ferrocenecarboxaldehyde in *i*-PrOH gave rise to thioester **2.9**.[12] The monomeric (**2.10)** and trimeric (**2.12**) ferrocene-thioureas were prepared by refluxing **2.9** with *n*-propylamine and tris(2-amino)ethyl amine in EtOH, respectively. The dimeric ferrocene-thiourea **2.11** was prepared by modification of the method outlined by Wiles *et al.*[13] whereby ethane-1,2-dithiosemicarbazide (**2.8**)[14] and ferrocenecarboxaldehyde were reacted at room temperature in water to afford the desired product. The compounds were isolated as red/orange powders (30 – 98%).



Scheme 2.2: Synthesis of ferrocenyl thioureas **2.9** – **2.12**. Reagents and conditions: (a) KOH (1 eq.), CS_2 (1 eq.), CH_3I (1 eq.) 0 – 5 °C; (b) ferrocenecarboxaldehyde, *i*-PrOH, reflux; (c) *n*-propylamine, EtOH, reflux; (d) tris(2-amino)ethyl amine, EtOH, reflux; (e) NaOH, CS_2, $NH_2NH_2{\cdot}H_2O$; (f) ferrocenecarboxaldehyde, H_2O, r.t.

2.3.2. Characterisation

NMR Spectroscopy

The ^{1}H NMR spectra obtained for compounds **2.9** – **2.12** were consistent with the proposed structures. Schiff-base condensation of **2.7** and ferrocenecarboxaldehyde was confirmed by the appearance of a singlet at 8.12 ppm attributed to the imine proton. A singlet for the CH_3 protons of the thioester moiety was observed at 2.50 ppm. Signals for the imine protons resonated between 7.89 and 7.90 ppm for **2.10 – 2.12**. These shifts were found upfield in comparison to the same signal observed for compound **2.9**. Signals for the -C***H***$_3$, CH_3C***H***$_2CH_2$- and -NHC***H***$_2CH_2$- protons of compound **2.10** were assigned at 0.86, 1.59 and 3.49 ppm, respectively. The C***H***$_2$ protons of the spacer for **2.11** (Figure 2.5) appeared as a singlet at 3.78 ppm, while two multiplets were assigned to the ethyl protons of **2.12** at 2.83 and 3.71 ppm. Singlets for the hydrazinic protons resonated between 11.11 and 11.29 ppm. Signals for the newly formed secondary amine were observed between 8.12 and 8.36 ppm. Signals for the protons of the unsubstituted cyclopentadienyl (Cp) ring of the ferrocenyl moieties were observed as singlets between 4.17 - 4.21 ppm for the mono-, di- and trimeric derivatives. These signals were shifted upfield compared to the same signal in the spectrum of compound **2.9** (4.24 ppm).

^{13}C{^{1}H} NMR spectroscopy was also used to further characterise these compounds. Signals for the thione carbon atoms (C=S) were assigned at approximately 177 ppm for compounds **2.10** – **2.12**. The C=N resonances were observed at 143.45, 143.96 and 143.70 ppm for the mono-, di- and trimeric ferrocenyl derivatives, respectively. Resonances for the carbon atoms of the unsubstituted Cp rings were assigned at 69.40, 69.43 and 69.44 ppm for **2.10** - **2.12**, respectively. Signals for the carbon atoms of the substituted Cp rings resonated at approximately 70.5 ppm for those closest to the imine moiety and at 68 ppm for those furthest from the imine moiety.

IR spectroscopy

Absorption bands attributed to the imine bond were observed at 1604, 1611 and 1606 cm^{-1} for **2.10**, **2.11** and **2.12**, respectively. These bands were found at a higher frequency compared to thioester **2.9** (1597 cm^{-1}). Absorption bands for the N-H vibrations were observed between

3437 and 3129 cm^{-1}. Absorption bands for the asymmetric ferrocene ring vibrations were also observed between 1103 and 1106 cm^{-1} for these compounds.

ESI mass spectrometry

Compounds **2.10** – **2.12** were analysed using ESI mass spectrometry. Base peaks at *m/z* 330.0724 and 601.0582 corresponding to the protonated molecular ion, $[M+H]^+$, were observed for **2.10** and **2.11**, respectively. A peak at *m/z* 957.1362 (70%) corresponding to $[M]^+$ was assigned in the spectrum of **2.12**.

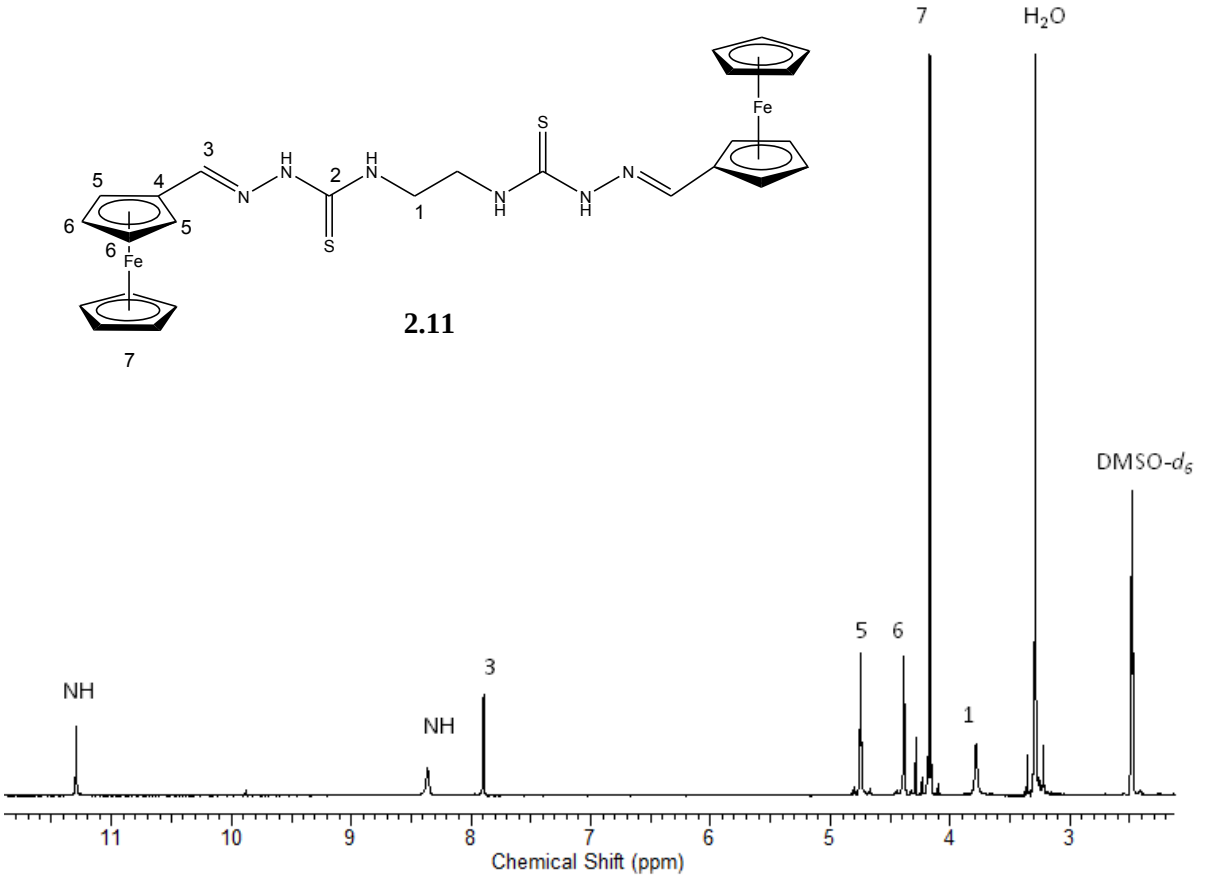


Figure 2.5: ^{1}H NMR spectrum of the dimeric ferrocene derivative **2.11** in DMSO-d_6

The NMR spectoscopic, IR spectroscopic and mass spectrometry data described above was in agreement with the proposed structures of the ferrocenyl compounds.

2.4. Biological and physicochemical evaluation

Once the synthesised compounds were characterised, their biological activity was investigated. Specifically, the compounds were screened *in vitro* against two strains of *P. falciparum,* namely the NF54 chloroquine-sensitive (CQS) strain and the Dd2 chloroquine-resistant (CQR) strain for antiplasmodial activity. The ability of the compounds to inhibit β-haematin (synthetic haemozoin) formation was also evaluated. In addition to this, the *in vitro* antitumour activity of the compounds was also investigated against WHCO1 oesophageal cancer. Lastly, the compounds were also screened against the G3 strain of the *T. vaginalis* parasite, which is responsible for the sexually transmitted disease trichomoniasis.

2.4.1. Prediction of the lipophilicity of compounds 2.3 – 2.6 and 2.10 – 2.12

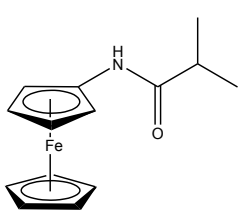
In order to gain some insight into the relationship between the lipophilicity of these compounds and their activity, the lipophilicity of the quinoline compounds at pH 7.4 and pH 4.8 were estimated using Marvinsketch V5.9.4. Due to the fact that this program cannot predict lipophilicity of metal complexes, the lipophilicity of the ferrocenyl compounds could not be evaluated in the same manner and therefore an alternative method was employed in order to estimate their logP.

To estimate the logP of the ferrocenyl-containing compounds **2.10** – **2.12**, modification of the fragmental approach proposed by Rekker *et al*[15] and Lanez *et al*[16-18] were used to predict the logP of the Fc compounds. The Rekker method is a calculation based on the decomposition of a molecule into substructures that possess theoretical hydrophobicity values. This approach was adapted to use theoretical values obtained from Marvinsketch as well as the experimental logP value of ferrocene (Fc) which is 2.66. In this case, the logP of the Fc-H fragment (ferrocene less one hydrogen atom) is required. The logP value of this fragment (f(Fc-H)) is 2.456 based on literature.[16] Two different logP methods found in the Marvinsketch V5.9.4 program (weighted – method 1 and Klop – method 2) were applied in order to predict the logP of benzyl versions (where Fc-H is replaced by C_6H_5) of ferrocenyl compounds **F1** - **F9** (Figure 2.6).

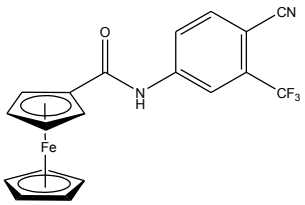
The logP of the Fc compounds were predicted using the following equation:

$$\mathbf{LogP_{(Fc\ derivative)} = LogP_{(benzene\ derivative)} - f_{(benzene\ fragment)} + f_{(fc\text{-}H)}}$$

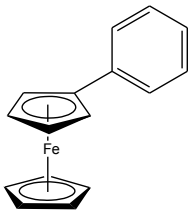
Where: LogP(benzyl derivative) was calculated using Marvinsketch
f(benzyl fragment) was obtained from Marvinsketch
f(fc-H) was obtained from literature[16]



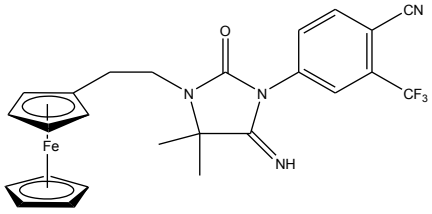
F1



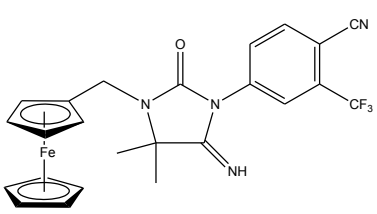
F2



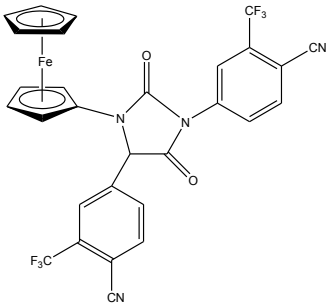
F3



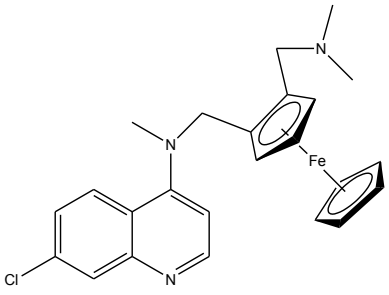
F4



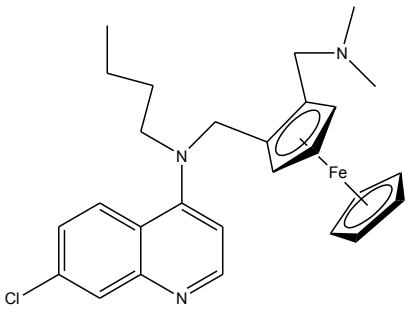
F5



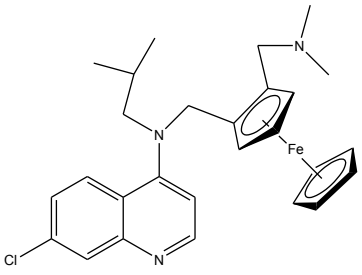
F6



F7



F8

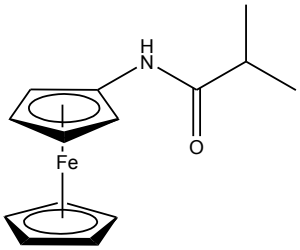


F9

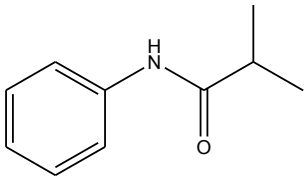
Figure 2.6: Reference ferrocene compounds **F1** – **F9** that were used to validate the adapted method

Calculations were carried out for all compounds and their predicted values compared to experimental values. Experimental values for **F1 - F9** were taken from literature.[16-18] XY scatter plots (Figure 2.7 and 2.8) were generated in order to assess the correlation between the experimental values and the estimated ones. An example of the calculation of the logP of **F1** using the weighted method is shown below:

Example using weighted method (method 1):



Fc derivative (F1)



benzene derivative

$$\mathbf{LogP_{(Fc\ derivative)}} = LogP_{(benzene\ derivative)} - f_{(benzene\ fragment)} + f_{(fc\text{-}H)}$$

$$= 2.45 - (0.33x5+0.02) + 2.456$$

$$= 3.24$$

Experimental value: 2.64.

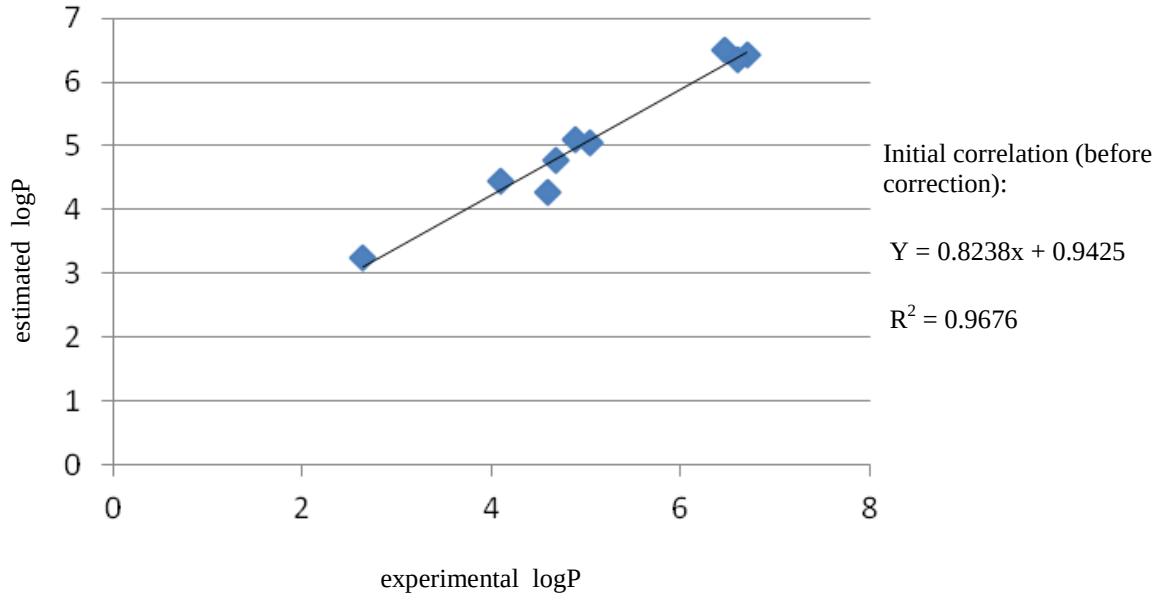


Figure 2.7: Correlation between estimated and experimental logP values using the weighted method

The XY scatter plot (Figure 2.7) resulted in good correlation ($R^2 > 0.9$), however, the slope was less than 1 and the intercept not equal to zero. A correction was then applied to the values. The same procedure was carried out on **F1** – **F9** using the Klop method (method 2). The results are shown in Table 2.1 and 2.2.

Table 2.1

LogP values obtained for compounds **F1** – **F9** using the weighted method

compound	experimental	estimated	corrected estimate
F1	2.64	3.24	2.79
F2	4.10	4.44	4.24
F3	4.59	4.26	4.03
F4	5.04	5.06	4.99
F5	4.68	4.77	4.65
F6	6.47	6.51	6.76
F7	4.89	5.10	5.05
F8	6.70	6.42	6.65
F9	6.60	6.34	6.55
	RMSD	**0.290**	**0.229**

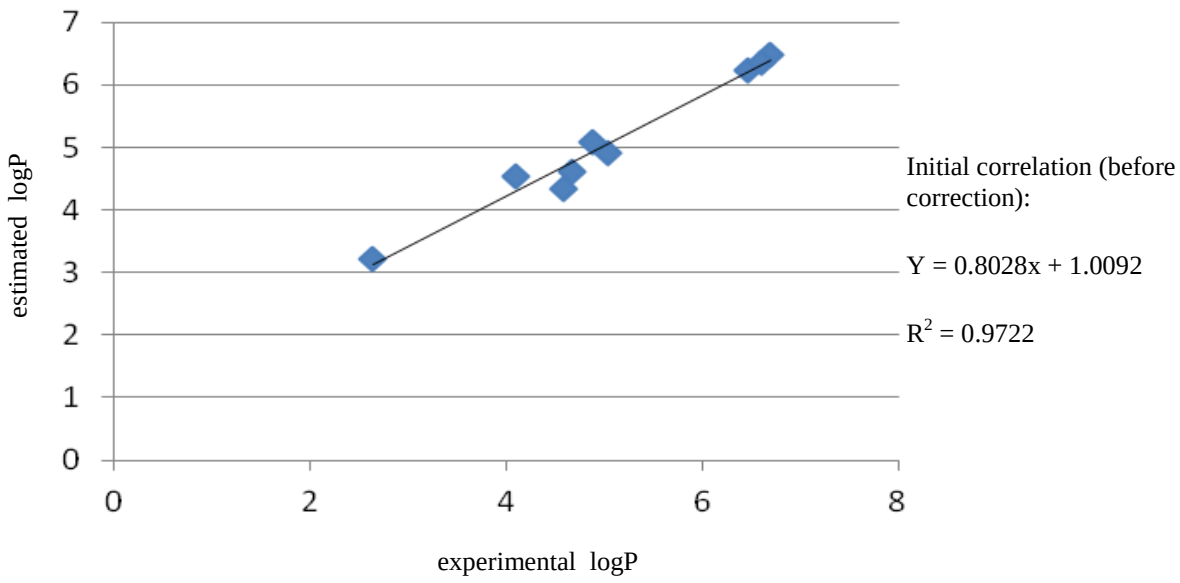


Figure 2.8: Correlation between estimated and experimental logP values using Klop method

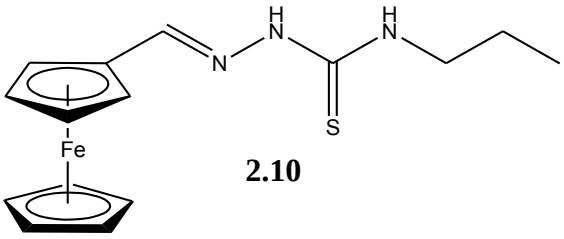
Table 2.2

LogP values obtained for compounds **F1** – **F9** using Klop method

compound	experimental	estimated	corrected estimate
F1	2.64	3.22	2.75
F2	4.1	4.54	4.40
F3	4.59	4.34	4.15
F4	5.04	4.92	4.87
F5	4.68	4.62	4.41
F6	6.47	6.24	6.52
F7	4.89	5.08	5.07
F8	6.7	6.47	6.80
F9	6.6	6.35	6.14
	RMSD	**0.301**	**0.269**

The corrected values were in close agreement with the experimental values using both methods. This therefore supported the use of this particular method to estimate the logP values of the Fc-containing compounds. Since the weighted method (Method 1) gave the smallest root mean square deviation (RMSD) after correction, this method was applied to estimate the logP of the ferrocenyl thioureas **2.10** – **2.12**. The estimated logP and selected logD values of compounds **2.3** – **2.6** and **2.10** – **2.12** are presented in Table 2.3.

Estimation of LogP of Fc thioureas (2.10 – 2.11)

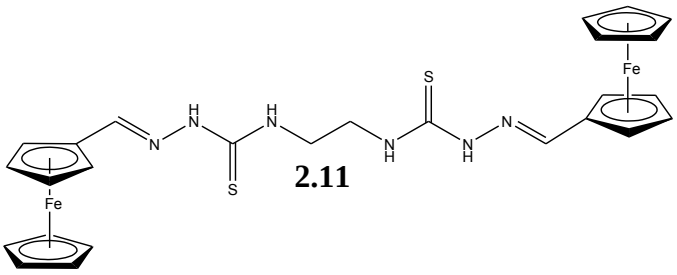


2.10

Initial prediction: 2.77 – (0.33x5+0.17) + 2.456 = 3.41

Corrected value: (3.41 – 0.9425) / 0.8238 = 2.99

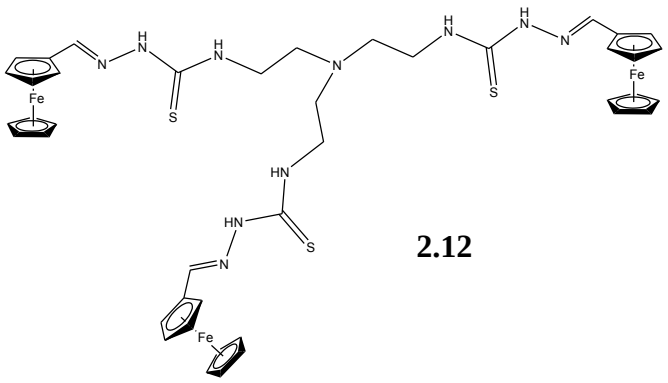
where 0.9425 = intercept and 0.8238 = slope



2.11

Initial prediction: 3.61 – 2(1.82) + 2(2.456) = 4.88

Corrected value: (4.88 – 0.9425) / 0.8238 = 4.78



2.12

Initial prediction: 5.34 – 3(1.82) + 3(2.456) = 7.25

Corrected value: (7.25 – 0.9425) / 0.8238 = 7.66

Table 2.3

Lipophilicity of thioureas at various pH

compound	logP	$logD_{7.4}$	$logD_{4.8}$
2.3	2.95	2.71	1.63
2.4	4.03	3.54	1.40
2.5	5.88	5.02	-0.22
2.6	8.62	2.78	-3.62
2.10	2.99	nd[g]	nd
2.11	4.78	nd	nd
2.12	7.66	nd	nd
CQ	4.63[h]	nd	nd

[g] not determined
[h] literature value[19]

2.4.2. Antiparasitic activity against *P. falciparum*.

Compounds **2.3 – 2.6** and **2.10** – **2.12** were evaluated for their antiplasmodial activity *in vitro* against the NF54 CQS and Dd2 CQR strains of *P. falciparum*. A dose-response experiment was performed on the compounds to determine the IC_{50} value, the concentration of the compound inhibiting 50% of parasite growth. The activity of these compounds were compared to the reference drug, chloroquine. The lower the IC_{50} value, the more active the compound is. The results are presented in Table 2.4.

Table 2.4

In vitro antiplasmodial activity of thioureas **2.3** – **2.6** and **2.10** – **2.12** against NF54 and Dd2 strains of *P. falciparum.*

compound	IC_{50} (µM) [a] ± SE NF54[b]	IC_{50} (µM) ± SE Dd2[c]	resistance index[d]
2.3	0.16 ± 0.03	0.26 ± 0.01	1.6
2.4	0.34 ± 0.03	0.23 ± 0.01	0.7
2.5	0.09 ± 0.01	0.52 ± 0.06	5.8
2.6	0.20 ± 0.01	0.51 ± 0.03	2.5
2.10	15.79 ± 1.28	16.22 ± 1.59	1.0
2.11	1.15 ± 0.07	31.88 ± 5.89	27.7
2.12	71.71 ± 9.93	5.63 ± 0.19	0.1
CQ	0.025 ± 0.01	0.14 ± 0.01	7
FQ	0.033 ± 0.01	0.019[e]	1.7

[a] IC_{50} represents the micromolar equivalents of test compounds required to inhibit parasite growth by 50%.

[b] NF54 chloroquine-sensitive strain of *P. falciparum.*

[c] Dd2 chloroquine-resistant strain of *P. falciparum*

[d] Resistance index (RI) = IC_{50}Dd2/IC_{50}NF54

[e] literature value[20]

Antiplasmodial activity against the NF54 CQ-sensitive strain of P. falciparum

Overall, the quinoline-containing compounds (**2.3** – **2.6)** showed better activity than the ferrocenyl compounds (**2.10** - **2.12)**. This suggests that the quinoline moiety has a greater influence on antiplasmodial activity compared to the ferrocenyl motif. This is consistent with

other potent 4-amino-7-chloroquinolines that have been used successfully to combat malaria. The trimeric quinoline, **2.5**, exhibited the best activity in the CQ sensitive strain, while the trimeric ferrocenyl compound (**2.12**), showed the lowest activity. Comparing the activity of quinolines **2.3**, **2.4** and **2.5**, the enhanced activity of the last may be due to the incorporation of a basic tertiary amino group, which is not present in **2.3** and **2.4**. This may aid in accumulation of **2.5** in the digestive vacuole of the parasite as a result of pH trapping. The slightly lower activity of **2.6**, which also contains basic tertiary amino groups, can probably be attributed to its highly lipophilic nature.

Compound **2.5** exhibited the best activity which suggests sufficient accumulation of this compound inside of the digestive vacuole. This appears to be a consequence of the $logD_{7.4}$ and logP values (Table 2.3). Compounds **2.3** and **2.6** exhibited similar activity in the sensitive strain, but these compounds were less active than **2.5**. This suggests that these compounds accumulate less than **2.5** based on their lower $logD_{7.4}$ values. Compound **2.4** has a higher $logD_{7.4}$ value compared to **2.3** and **2.6**, but appears to accumulate less based on the larger IC_{50} value. The mono- and trimeric ferrocenyl thioureas (**2.10** and **2.12**) exhibited the lowest activity of all the compounds at the tested concentration, while the dimeric ferrocenyl thiourea (**2.11**) displayed moderate activity in this strain. This compound has a logP value comparable to chloroquine. The low activity of **2.12** in this strain may be a combination of its highly lipophilic nature and the lack of the active quinoline moiety. The high lipophilicity may hinder its transport because it may be trapped in the lipid membrane and not accumulate sufficiently inside of the digestive vacuole.[21] The ferrocenyl precursor (**2.9**), was also screened, but was found to be inactive at the tested concentration. This particular compound is less lipophilic (logP = 3.72) than compound **2.11**. This suggests functionalisation with the various amines, in this case, leads to enhanced activity, but these compounds were less active than the quinoline thioureas.

Antiplasmodial activity against the Dd2 CQ-resistant strain of P. falciparum

Compounds **2.3**, **2.4** and **2.10** exhibited similar activities in both the sensitive and resistant strains of *P. falciparum*, while **2.5** and **2.6** showed slightly lower activity in the Dd2 strain compared to the NF54 strain. Compound **2.10** demonstrated consistent activity across both strains. The activity of compound **2.11** decreased significantly in the Dd2 strain compared to

the NF54 which was confirmed by the large resistance index (RI) of 27.7. The RI is valuable for the analysis of potential drugs. RI values are defined as the quotient of the IC_{50} value obtained in the resistant strain and the IC_{50} value obtained in the sensitive strain. Smaller RI values (1 or < 1) indicate that the parasite is equally or more active in the resistant strain compared to the sensitive strain. Figure 2.9 shows the RI values for compounds **2.3** – **2.6** and **2.10** – **2.12**, chloroquine and ferroquine. The activity of **2.12** increased significantly in the resistant strain, as this particular compound gave a RI of 0.1. In most cases, with the exception of **2.11**, the compounds showed a lower RI value compared to CQ. In this strain, lipophilicity appears to have a negligible effect on the activity of these compounds. Compounds **2.3** and **2.4** exhibited comparable activity, while compounds **2.5** and **2.6** showed similar activity in this strain of the parasite.

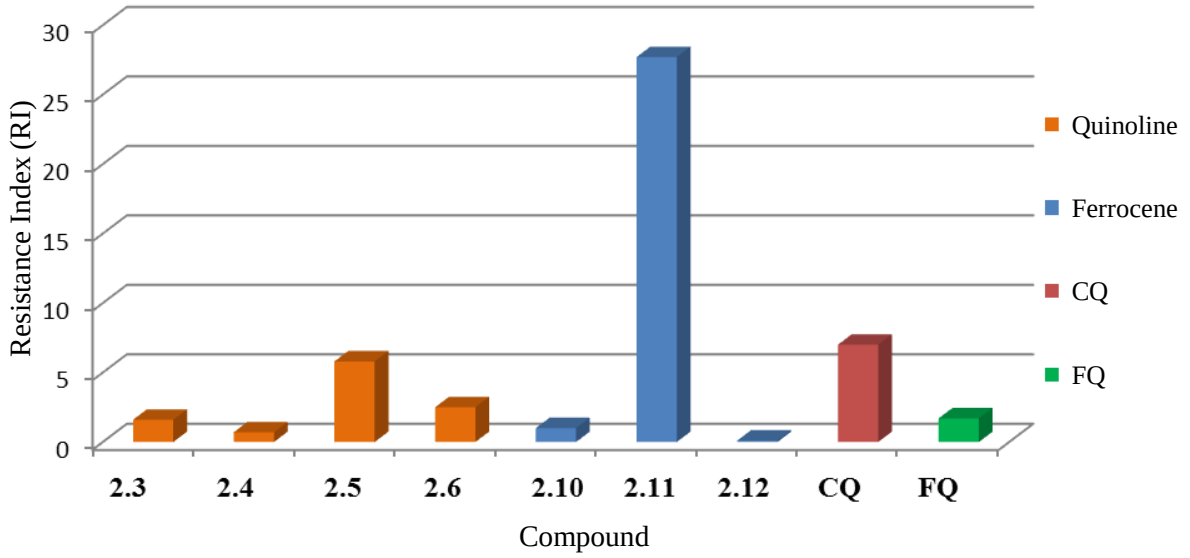


Figure 2.9: Resistance index values (RI) for compounds **2.3** – **2.6**, **2.10** – **2.12**, CQ and FQ

Since the activity of quinoline compounds **2.5** and **2.6** decreased in the resistant strain, this suggests that these compounds may encounter the same problems that CQ experiences. It is suggested that CQ accumulation is less in chloroquine-resistant strains compared to sensitive strains. This is associated with mutations in a gene encoding for the *P. falciparum* chloroquine-resistant transporter (PfCRT). This is a protein found in the membrane of the

digestive vacuole.[22,23] The mutation allows for some of the protonated form of CQ to be expelled from the digestive vacuole. Essentially, only the uncharged form of chloroquine is able to permeate membranes. Once it reaches the digestive vacuole, it is able to form mono- or di-protonated forms which results in accumulation in the digestive vacuole,[24,25] since these forms are not membrane permeable. This is not the case in CQ-resistant strains as CQ can be expelled. Perhaps due to the structural similarities between chloroquine and the quinoline thioureas discussed here, the thioureas may experience a similar fate in the resistant strain.

2.4.3. β-Haematin inhibition studies

The mode of action of chloroquine is believed to be inhibition of haemozoin crystal growth. Once chloroquine reaches the digestive vacuole, it binds to haematin (a toxic product of haemoglobin degradation) and prevents conversion into haemozoin. A build-up of free haematin results in damage to the parasite. Many 4-amino-7-chloroquinolines are believed to inhibit haemozoin crystal growth. In some cases, haemozoin itself is believed to be the target of antimalarials.[26,27] Since the quinoline derivatives prepared in this study showed promising antiplasmodial activity against the NF54 strain of *P. falciparum*, their ability to inhibit haem aggregation was evaluated in order to obtain some idea of their possible mode of action. The β-haematin (synthetic haemozoin) inhibition activity of the ferrocenyl compounds, including a tetrameric derivative (**2.13**, Figure 2.10) was also studied. Compound **2.13** showed activity against the W2 CQ-resistant strain of *P. falciparum* in a previous study.[9] Figure 2.11 depicts the data obtained from the β-haematin inhibition assay. The compounds were studied using a Nonidet P-40 (NP-40) detergent mediated assay.[28] Evidence suggests that haemozoin formation is not a spontaneous process. Research has shown that haem crystallisation occurs in the presence of neutral lipids found inside of the digestive vacuole of the parasite.[29,30] The neutral detergent, NP-40, was therefore used to mimic lipids and thus mediates β-haematin formation in the assay. The assay was performed in a 96-well plate and each compound tested in quadruplicate. Haematin was incubated with an increasing concentration of the drug in the presence of the detergent. The amount of synthetic haemozoin formed was then quantified using the colorimetric pyridine ferrochrome method published by Egan *et al*.[31] Pyridine is able to complex to free haematin, but not to β-haematin. This allowed for the amount of synthetic haemozoin formed, to be measured. Amodiaquine and chloroquine diphosphate

were used as controls in order to validate the data obtained. The IC_{50} values obtained for these two compounds (16 and 74 μM, respectively) compared favourably with literature.[28]

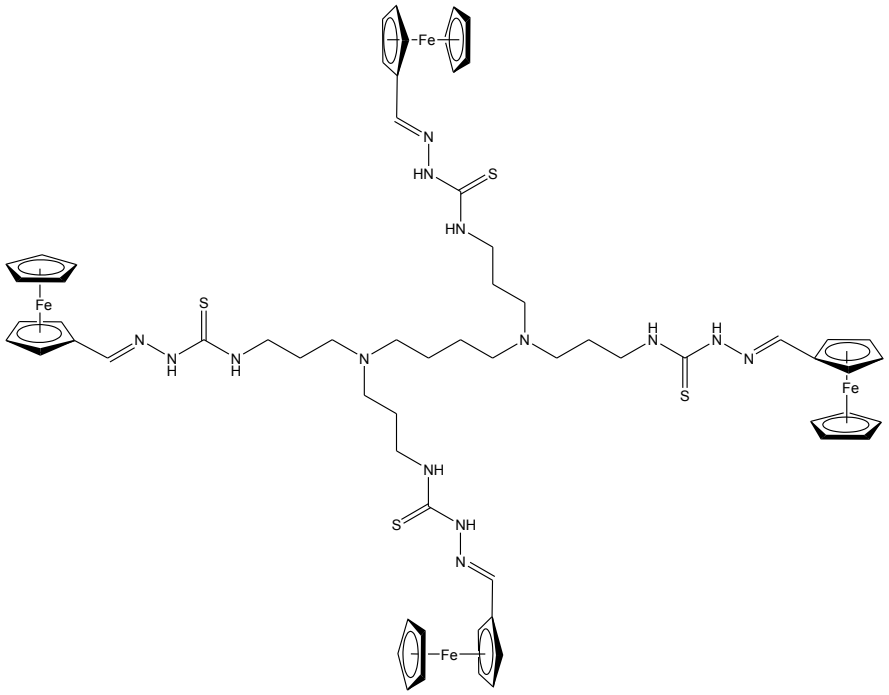


Figure 2.10: A tetrameric ferrocene thiourea (**2.13**) also evaluated for its β-haematin inhibition activity

The quinoline compounds inhibited β-haematin formation better than the ferrocenyl thioureas, specifically compound **2.6** (IC_{50} < 4.5 μM). This may be attributed to an increased number of quinoline moieties that are able to interact with haem thus inhibiting crystallisation.[32] Compounds **2.4** and **2.5** also appeared effective at inhibiting haem aggregation giving IC_{50} values of 4.5 and 6.5 μM, respectively. Compounds **2.12** and **2.13** inhibited β-haematin formation more modestly, giving IC_{50} values of 31 and 15 μM, respectively. With the exception of **2.10** and **2.11**, the compounds inhibited β-haematin formation to a greater extent compared to CQ. There appears to be a trend between the size of these molecules and their ability to inhibit haem aggregation. The larger, tetrameric molecules were able to inhibit β-haematin formation to a greater extent than their respective monomeric derivatives (Figure

2.11). No clear correlation is discernable between β-haematin inhibition activity and the *in vitro* antiplasmodial activity (Figure 2.12). This is probably due to differences in accumulation of these compounds inside of the parasite. Since the β-haematin inhibition study was conducted in a cell-free medium, other factors including lipophilicity and pH trapping could affect the uptake and accumulation of these compounds in parasites and therefore no correlation was observed.

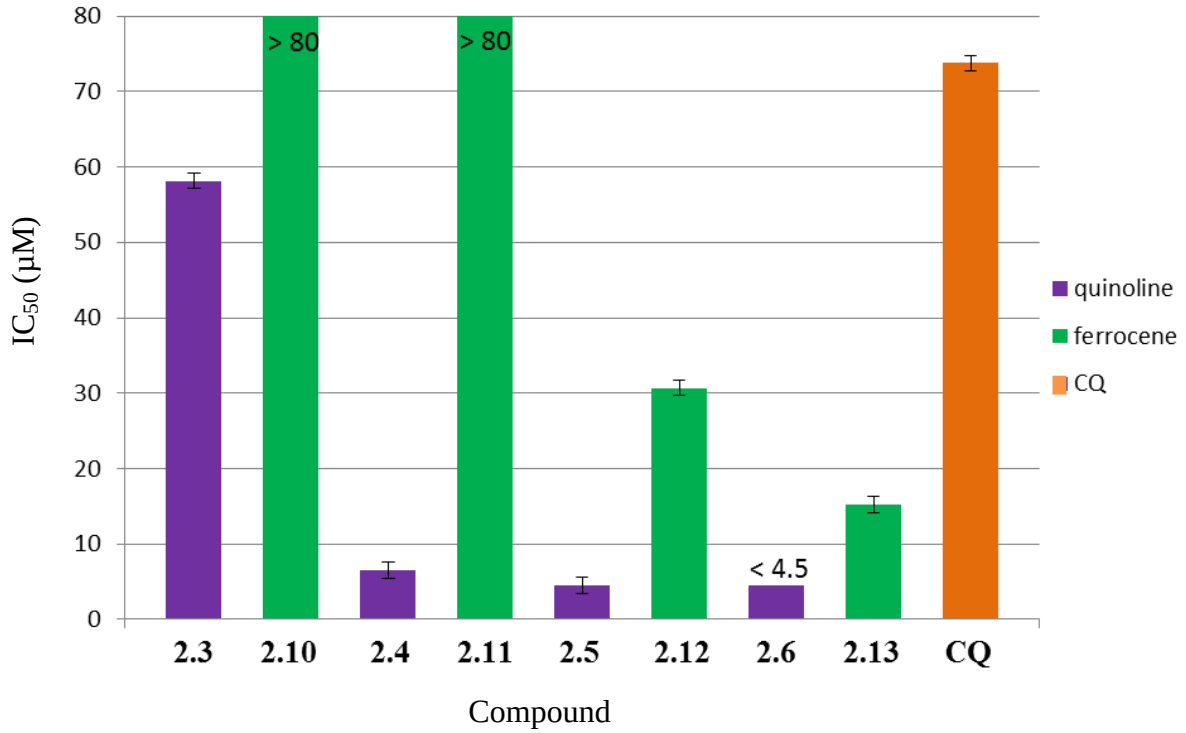


Figure 2.11: Graph depicting the IC_{50} values for compounds **2.3** - **2.6** and **2.10** – **2.13** towards β-haematin inhibition.

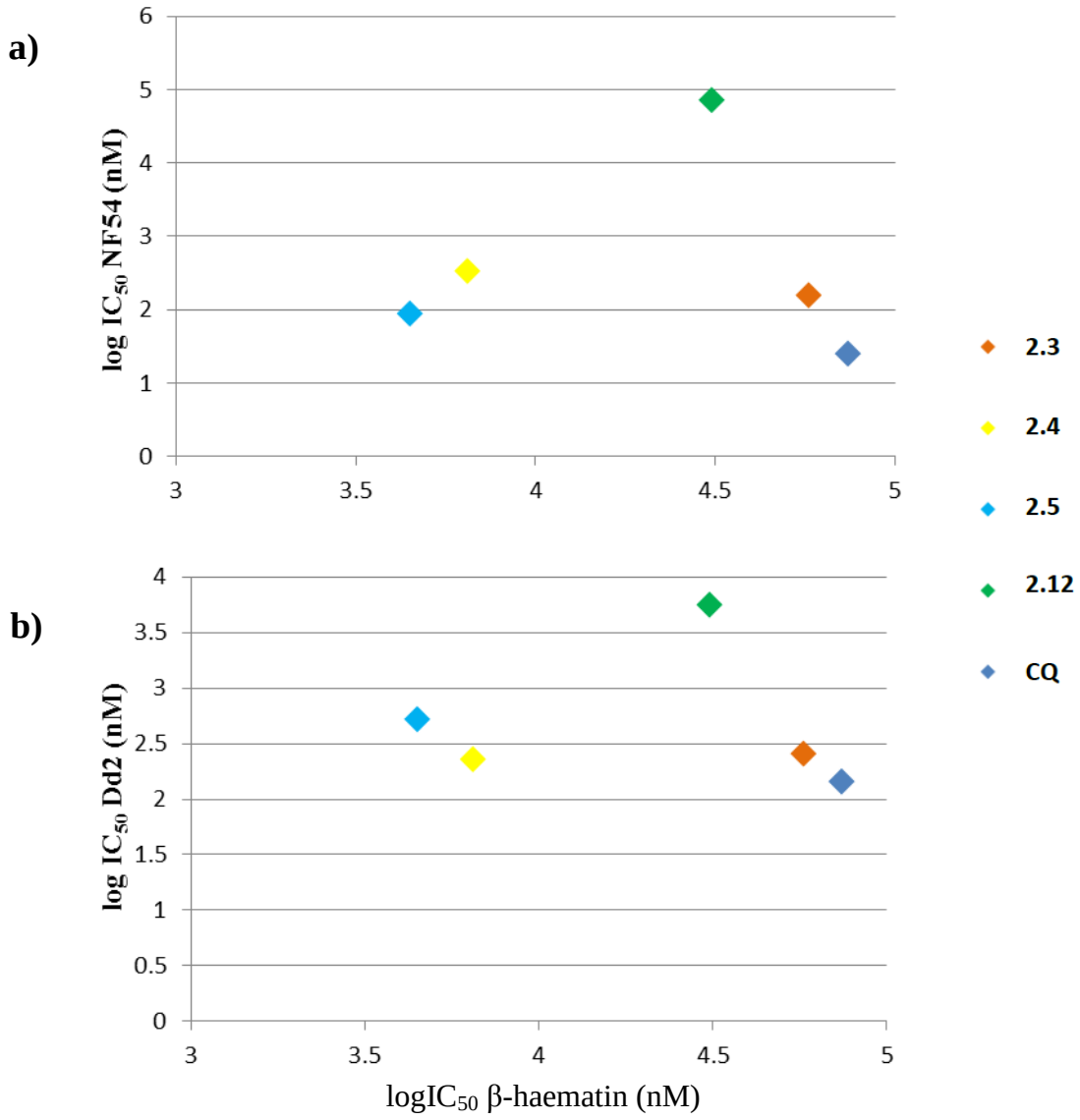


Figure 2.12: Plots of β-haematin inhibition activity vs antiplasmodial activity in the **a**) CQ sensitive strain (NF54) and **b**) CQ resistant strain (Dd2) for selected compounds.

2.4.4. *In vitro* cytotoxicity studies

The *in vitro* activity of compounds **2.3** – **2.6** and **2.10** – **2.11** were evaluated in the WHCO1 oesophageal cancer cell-line. Oesophageal cancer is the eighth most common cancer globally. The occurrence of oesophageal cancer is highest in Southern Africa. Southern Europe and Western Africa report the lowest incidences of oesophageal cancer.[33]

Many quinoline and ferrocene-containing compounds have been studied as potential anticancer agents.[34,35] Some ferrocene-containing compounds have been found to be highly effective against certain cancers.[35] The compounds were evaluated for their activity using a (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[36] The results obtained from this assay are shown in Table 2.5. The IC_{50} value of cisplatin against this cell-line is also given in the table.

Table 2.5

IC_{50} values obtained for **2.3** – **2.6** and **2.10** – **2.12** against WHCO1 cancer cells.[a]

compound	IC_{50} (μM)	95% confidence interval	selectivity index[b]	selectivity index[c]
2.3	43.8	36.2 - 52.9	273	168
2.4	38.4	30.5 - 48.2	113	167
2.5	6.2	5.2 - 7.5	69.0	11.9
2.6	0.99	0.9 - 1.1	4.95	1.94
2.10	55.7	31.8 - 97.8	3.53	3.44
2.11	inactive	-	-	-
2.12	inactive	-	-	-
Cisplatin	13.0[d]	-	-	-

[a] Values were determined from a dose response curve (assayed with MTT), IC_{50} represents the micromolar equivalents of test compounds required to inhibit 50% cell viability.

[b] IC_{50}WHCO1/IC_{50}NF54

[c] IC_{50}WHCO1/IC_{50}Dd2

[d] literature value[37]

With the exception of **2.11** and **2.12**, all the quinoline compounds (**2.3** - **2.6**) and **2.10** appeared to exhibit some form of activity against this particular cell-line. In particular, compounds **2.5** and **2.6** showed good activity, with IC_{50} values in the low micromolar range. In the case of the ferrocenyl thioureas, **2.10** displayed moderate activity (IC_{50} = 55.7 μM) against this cell-line. The quinoline compounds were more active than the ferrocenyl thioureas. Compound **2.6** exhibited very promising activity, giving an IC_{50} of about 1 μM. It appears in the case of the quinoline derivatives that the larger molecules (**2.5** and **2.6**) displayed enhanced activity compared to their mono- and dimeric counterparts. There also

appeared to be size-dependent activity for this class of compounds. This phenomenon is also observed for certain dendritic compounds whereby cytotoxicity is often generation-dependant, with higher generation dendrimers showing the best activity.[38-40] Studies carried out on natural polyamines show that these systems are able to transport cytotoxic drugs into tumour cells.[41] In this case, synthetic polyamines were under investigation, it has been shown that the polyamine transporter is able to tolerate modified polyamines thus the compound can be taken up by the cell.[42] This factor, in combination with the ability of these quinolines to interact with DNA, possibly by intercalation, may be a reason for the enhanced activity.[43] The activity of the compounds was also compared to cisplatin, a potent metal-containing anticancer agent, compounds **2.5** and **2.6** exhibited better activity than this drug in this particular cell-line.

The selectivity index (SI) is useful in order to gauge the selectivity towards a particular cell type. SI values concerning the WHCO1 cell-line and the two antiplasmodial strains (NF54 and Dd2) were calculated and are given in Table 2.5. The SI values were calculated by dividing the IC_{50} value obtained in the cancer cell-line by the IC_{50} value obtained in the respective *P. falciparum* strain. A large value suggests selectivity towards malaria parasites rather than cancer cells, i.e. the compounds are better antiplasmodial agents than they are antitumoural. Generally, the compounds showed similar selectivity, regardless of the antiplasmodial strain. Compounds **2.3** – **2.5** were more selective towards *P. falciparum* than WHCO1 cells. SI values in the range of 11.9 – 273 were observed for these compounds. Compounds **2.3** and **2.4** both gave SI values greater than 100. Compounds **2.6** and **2.10** exhibited lower SI values, which indicate similar antiplasmodial activity and cytotoxicity. This suggests that in these cases, antiplasmodial activity may be a consequence of their cytotoxicity.

The most cytotoxic compound, **2.6**, was further screened against normal KMST-6 human fibroblast, along with cisplatin, in order to ascertain the degree of toxicity of the compound (Table 2.6). This particular compound was more cytotoxic towards WHCO1 oesophageal cancer cells than the KMST-6 fibroblast. This is evidenced by the large SI value (93). In this case, the SI value was calculated by dividing the IC_{50} value obtained in normal tissue by the IC_{50} value obtained against cancer cells. A large value indicates that the compound decreases cell viability in WHCO1 cells to a larger degree compared to normal tissue. Cisplatin was also screened against the KMST-6 cell-line. Both compound **2.6** and cisplatin exhibited similar

toxicity against the KMST-6 cell-line, however, when the activity of cisplatin was compared across both the KMST-6 and WHCO1 cell-lines, cisplatin gave a lower SI value (7.30) compared to compound **2.6**. This suggests that whereas cisplatin's toxic nature may result in relatively poor selectivity against cancer cells, compound **2.6** is much more selective and worthy of further investigation.

Table 2.6

IC_{50} values against KMST-6 human fibroblast and WHCO1 cancer cells.

compound	IC_{50} (μM) ± SD KMST-6	IC_{50} (μM) WHCO1	selectivity index[a]
2.6	92 ± 4	0.99	93.0
cisplatin	96 ± 0.4	13.0	7.30

[a] IC_{50}KMST-6/IC_{50}WHCO1

2.4.5. Antiparasitic activity against *T. vaginalis*

Trichomonas vaginalis (*T. vaginalis*) is a protozoan responsible for the sexually transmitted disease trichomoniasis. The current FDA-approved treatment, metronidazole (M), Figure 2.13, experiences resistance in some patients.[44,45] Unfortunately, in many countries, there is no other licensed drug available for the treatment of trichomoniasis.

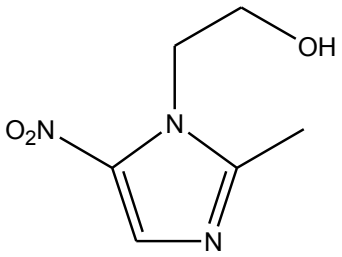


Figure 2.13: Metronidazole, the current FDA-approved treatment against *T. vaginalis*

This therefore supports research towards alternative therapies that are equipotent and are able to overcome resistance experienced by metronidazole. There has recently been an interest in obtaining suitable alternative therapies for this infection.[45] More recently metal-based compounds have shown promise as potential candidates against trichomoniasis.[46-48] The application of metal complexes as potential antiparasitics against *T. vaginalis* has been sparsely explored.

A preliminary study to evaluate the activity of the afore-mentioned compounds (**2.3** – **2.6** and **2.10 – 2.13**) against this particular parasite was conducted. The compounds were screened against the G3 strain of *T. vaginalis* at a drug concentration of 10 μM. Figure 2.14 shows the data obtained from this preliminary screen. The graph depicts the percentage parasite growth inhibition for the screened compounds along with the data obtained for thioester **2.9** and metronidazole.

Studying the quinoline compounds, the monomeric thiourea **2.3** showed the lowest percentage growth inhibition, while the tetrameric compound **2.6**, showed the highest inhibition in this series, followed by **2.4** and then compound **2.5**. A similar trend was observed for the ferrocenyl thioureas. The tetrameric compound **2.13** showed the highest percentage inhibition, followed by the dimeric and then the trimeric derivative. The monomeric ferrocene derivatives **2.9** (thioester) and **2.10** displayed the lowest inhibition in this series. The thioester showed slightly lower percent growth inhibition compared to the amine-functionalised derivative **2.10**.

Trends in percent parasite inhibition for compound **2.3** – **2.6** and **2.9** – **2.13**:

mono < ***trimeric*** < ***dimeric*** < ***tetrameric***

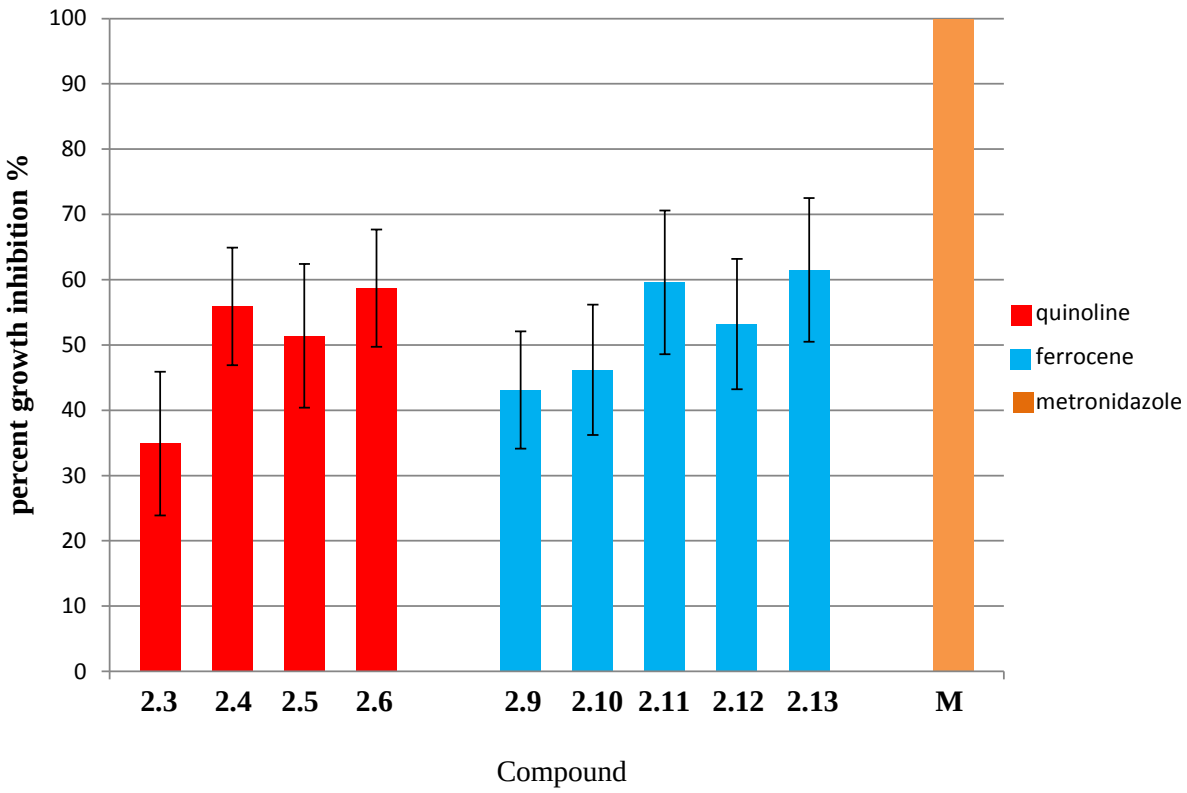


Figure 2.14: Percent growth inhibition for compounds **2.3** – **2.6** and **2.9** – **2.13** against *T. vaginalis.*

Overall, the tetrameric compounds (**2.6** and **2.13**) showed the highest percent inhibition in each series. On average the ferrocenyl compounds inhibited parasite growth slightly better compared to the quinoline compounds. The lipophilic nature of the ferrocene moiety may be a factor contributing to the slightly enhanced activity of the organometallic derivatives. Although many of these compounds were able to inhibit more than 50% of parasite growth, their activity was not comparable to metronidazole.

2.5. Summary

A series of mono- and multimeric quinoline (**2.1** – **2.6**) and ferrocene-containing (**2.9** – **2.12**) thioureas were prepared. The quinoline compounds were synthesised by displacement of the $-SCH_3$ group of the quinoline-containing precursor by various amines. Two of the ferrocenyl compounds (**2.10** and **2.12**) were prepared using similar methodology, while the dimeric ferrocene thiourea was obtained via a Schiff-base condensation reaction between

ferrocenecarboxaldehyde and ethane-1,2-dithiosemicarbazide. The compounds were characterised using ^{1}H, $^{13}C\{^{1}H\}$, HSQC and COSY NMR spectroscopy, infrared spectroscopy and ESI mass spectrometry.

The synthesised compounds were evaluated for their biological activity *in vitro*. The compounds were screened against two strains of *P. falciparum*, the NF54 CQ-sensitive and Dd2 CQ-resistant strains of *P. falciparum*. The quinoline thioureas displayed good activity against the sensitive strain, while the ferrocene-containing compounds were less active. The quinoline compounds displayed lower resistance index values compared to chloroquine but only the dimeric derivative exhibited a RI value less than 1. All of the quinoline compounds were able to inhibit β-haematin formation, with the tetrameric derivative (**2.6**) displaying the best activity. No correlation between the β-haematin inhibition activity and the antiplasmodial activity was observed. The quinoline compounds also showed moderate to good activity against WHCO1 oesophageal cancer cells. The tetrameric quinoline thiourea (**2.6**) was found to be the most active compound in this cell-line. This compound was also found to be more active in cancer cells compared to normal cells. In the case of the *T. vaginalis* parasite growth inhibition assay, the ferrocenyl compounds showed slightly enhanced activity compared to the quinoline thioureas, but not comparable to metronidazole.

2.6. References

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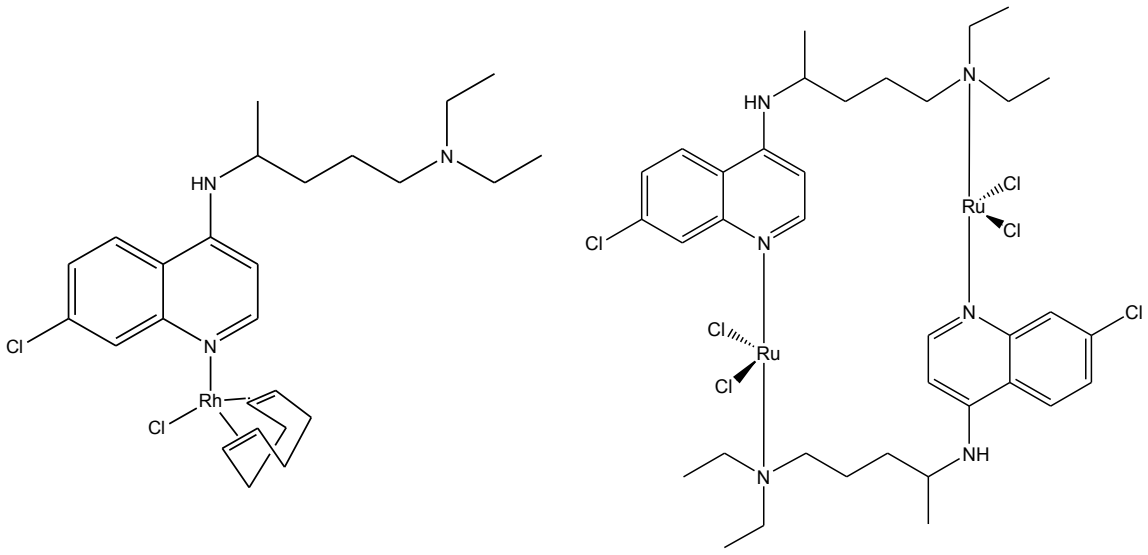
CHAPTER 3

Synthesis, characterisation and biological evaluation of mono- and bis-salicylaldimine quinolines and their Rh(I) complexes

3.1. Introduction

One of the first organometallic complexes that was screened against malarial parasites was [RhCl(COD)CQ] (Figure 3.1a). The *in vitro* activity of this compound against *Plasmodium berghei* (*P. berghei*) was promising.[1] A ratio of 0.96 for the IC_{50} value of chloroquine diphosphate (CQDP)/IC_{50} of the complex indicates very little difference between the activity of CQ and the rhodium complex against *P. berghei*. *In vivo*, this complex reduced parasitemia to a greater extent to that of CQ. Based on this, other metal-based antimalarials were prepared and tested for activity.[2,3] A binuclear ruthenium chloroquine complex $[RuCl_2(CQ)]_2$ (Figure 3.1b) was evaluated and found to be potent against *P. berghei*, showing enhanced activity compared to CQDP.[1]



a **b**

Figure 3.1: (**a**) [RhCl(COD)CQ] and (**b**) $[RuCl_2(CQ)]_2$ exhibiting activity against *P. berghei*.

More recently, ruthenium arene half sandwich complexes have received attention for their biological activity.[4-6] In one instance, ruthenium *p*-cymene complexes have been prepared and tested for their activity against various plasmodial strains.[7] In these cases, the ruthenium moiety is not bonded to the quinoline nitrogen (which plays a role in antimalarial activity), but is coordinated to the ligand in a bidentate manner in the lateral side chain. One of the complexes, a salicylaldimine quinoline complex, shown in Figure 3.2, exhibited promising activity against the CQ-sensitive strain of *P. falciparum*.[7]

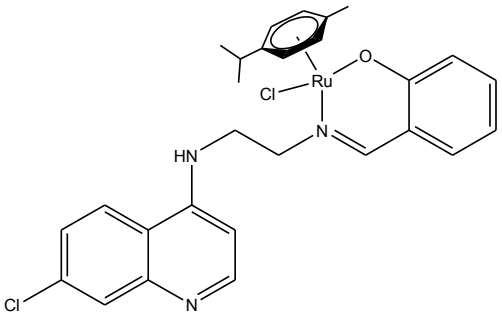


Figure 3.2: Ruthenium *p*-cymene quinoline complex that showed promising activity against malaria parasites.

Based on the promising activity of [RhCl(COD)CQ] and since the antimalarial activity of only a few Rh(I) complexes have been reported,[1,8,9] there is scope to investigate the activity of new Rh(I) compounds as potential antiplasmodial agents. Rh(I) complexes have also been shown to possess biological activity including anticancer[10] and anti-trypanosomal activity.[11] For example, binuclear rhodium compounds of the form $[Rh_2(COD)_2L]^{2+}$ or $[Rh_2(COT)_2L]^{2+}$, where COT = 1,3-1,5-cyclooctatetraene and L = pentamidine (Figure 3.3), were screened against *Trypanosoma brucei brucei*. This particular protozoan is the cause of sleeping sickness. The corresponding Ir(I) complexes were also prepared and evaluated, but the Rh(I) compounds showed enhanced activity compared to the Ir(I) compounds. Generally, the rhodium complexes showed activity 4-times greater than the iridium compounds.[11]

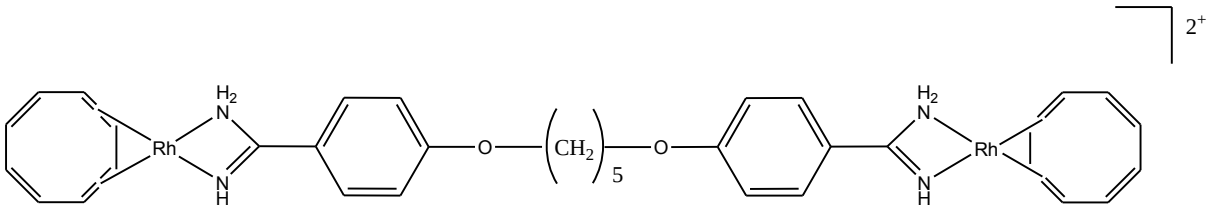


Figure 3.3: Binuclear Rh(I) complex possessing activity against the parasite *Trypanosoma brucei brucei*.

Very little work has been done to investigate the effect of multinuclearity on antimalarial activity. In the case of $[RuCl_2(CQ)]_2$ (Figure 3.1b), the increased number of metal centres and quinoline moieties appeared to have a favourable effect on antiplasmodial activity. In this chapter, the preparation, characterisation and biological properties of mononuclear and binuclear Rh(I) salicylaldiminato quinoline complexes and their corresponding ligands were investigated.

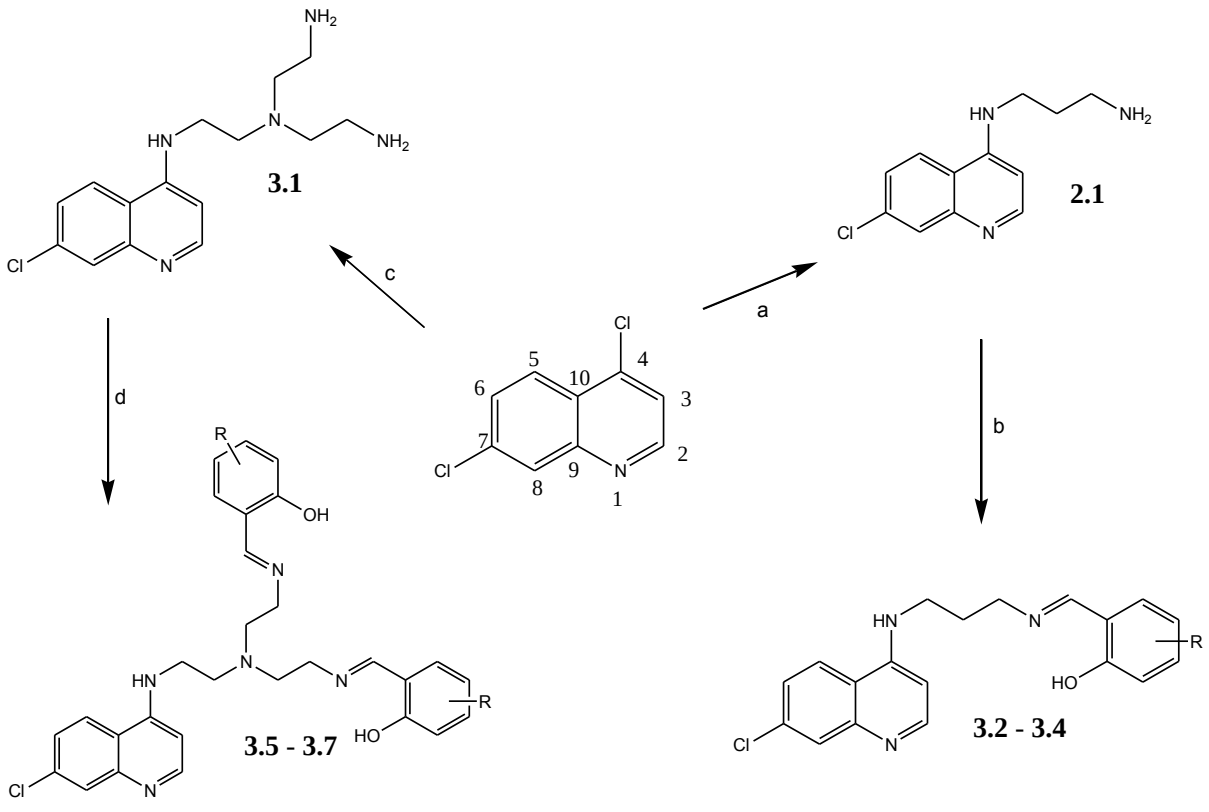
3.2. Synthesis and characterisation of mono- and dimeric salicylaldimine quinoline ligands 3.1 – 3.7

3.2.1. Synthesis

A series of monomeric and dimeric salicylaldimine quinoline ligands were prepared using standard Schiff-base chemistry. Salicylaldehyde and two substituted salicylaldehydes were condensed with *N'*-(7-chloroquinolin-4-yl)-propane-1,3-diamine (**2.1**) and *N*-(7-chloroquinolin-4-yl)-tris(2-aminoethyl)amine (**3.1**) to afford mono- and dimeric salicylaldimines, respectively (Scheme 3.1). *N*-(7-Chloroquinolin-4-yl)-tris(2-aminoethyl)amine was prepared by a nucleophilic aromatic substitution (S_NAr) reaction of 4,7-dichloroquinoline and an excess of tris(2-amino)ethylamine (11 mol equivalents) under an argon atmosphere to yield a monofunctionalised product after the work up process. The amines were reacted with the appropriate aldehydes giving rise to the desired ligands. In the case of the monomeric ligands, *N'*-(7-chloroquinolin-4-yl)-propane-1,3-diamine (**2.1**) was reacted with 5-chlorosalicylaldehyde, *o*-vanillin and salicylaldehyde in a 1:1 stoichiometric ratio in diethyl ether to afford the desired monosalicylaldimine ligands (**3.2 – 3.4**). *N*-(7-Chloroquinolin-4-yl)-tris(2-aminoethyl)amine (**3.1**) was reacted with the same aldehydes in

ethanol in a 1:2 stoichiometric ratio to afford the desired dimeric salicylaldimines (**3.5 – 3.7**). The preparation of these ligands is outlined in Scheme 3.1.

In the case of the monomeric salicylaldimine ligands (**3.2 – 3.4**), the compounds precipitated from solution. The dimeric salicylaldimine ligands (**3.5 – 3.7**) were soluble in ethanol and were therefore precipitated with diethyl ether. All compounds were isolated by filtration. The ligands were obtained as yellow powders in moderate to good yields (40 - 83%). The monomeric salicylaldimine ligands exhibited slightly enhanced solubility in common organic solvents such as dichloromethane and acetone compared to the dimeric systems. All the ligands were readily soluble in alcohols and dimethyl sulfoxide. The compounds were insoluble in diethyl ether, hexane and pentane.



R = 5-Cl (**3.2** and **3.5**); 3-OMe (**3.3** and **3.6**); H (**3.4** and **3.7**)

Scheme 3.1: Synthesis of quinoline ligands **2.1** and **3.1** - **3.7**. Reagents and conditions: (a) 1,3-propanediamine (5 eq.), neat 5 h; (b) aldehyde (1 eq.), diethyl ether, 16 h, r.t; (c) tris(2-aminoethyl)amine (11 eq.), neat, 24 h, 90°C; (d) aldehyde (2 eq.), EtOH, 16 h, r.t.

3.2.2. Characterisation

The ligands were characterised by ^{1}H, $^{13}C\{^{1}H\}$, HSQC and COSY NMR spectroscopy, IR spectroscopy, electrospray ionisation (ESI) and electron impact (EI) mass spectrometry.

NMR Spectroscopy

The ^{1}H NMR spectra of **3.2** – **3.7** were recorded in $CDCl_3$ and were consistent with the structures of the proposed ligands. The ^{1}H NMR spectrum of **3.2** is shown in Figure 3.4. Selected ^{1}H and $^{13}C\{^{1}H\}$ NMR resonances are given in Table 3.1 (numbering of the quinoline ring is shown in Scheme 3.1). Schiff-base condensation was confirmed by the appearance of a signal for the imine resonances between 8.30 and 8.38 ppm for **3.2** – **3.4** and between 7.99 and 8.29 ppm for **3.5** – **3.7**. The imine signal was observed upfield in the 5-Cl derivatives compared to the 3-OMe and unsubstituted salicylaldimine compounds. The same signal in the unsubstituted compounds was more deshielded compared to the 3-OMe and 5-Cl compounds.

Table 3.1

Selected ^{1}H and $^{13}C\{^{1}H\}$ NMR resonances for ligands **3.2** – **3.7.**

compound	*H*C=N	N*H*	*H*2	*H*8	HC=N	*C*2	*C*8
3.2	8.30	5.07	8.52	7.94	164.65	151.98	128.88
3.3	8.35	5.12	8.50	7.92	165.86	152.04	128.82
3.4	8.38	5.10	8.52	7.94	165.83	152.03	129.01
3.5	7.99	5.96	8.37	7.84	165.58	151.39	127.15
3.6	8.16	-	8.30	8.06	166.71	154.26	125.79
3.7	8.29	7.33	8.09	8.04	166.78	154.73	129.58

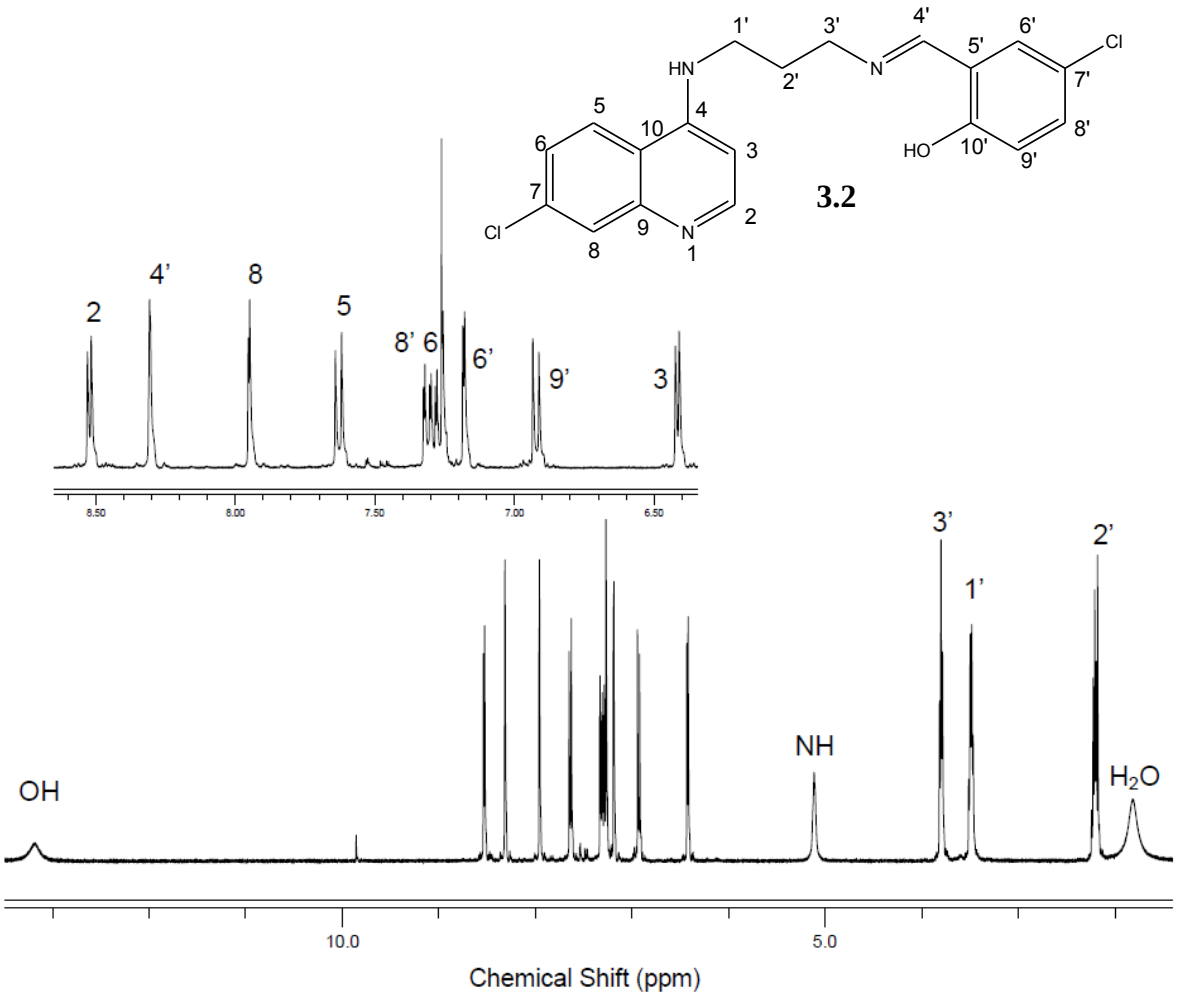


Figure 3.4: ^{1}H NMR spectrum of **3.2** in $CDCl_3$.

The NH signals in the spectra of the monomeric derivatives (**3.2** – **3.4**) were more shielded compared to those observed in the dimeric salicylaldimine compounds. In the case of **3.6**, the NH signal was not observed. The deshielding could possibly be explained by the occurrence of intramolecular hydrogen bonding in the dimeric derivatives. The NH proton is able to hydrogen bond with the nitrogen atom of the tertiary amine (Figure 3.5), forming a 5-membered ring. This could possibly be a reason for the difference in this signal position between the monomeric and dimeric derivatives. Resonances for H2 were observed between 8.50 and 8.52 for **3.2** – **3.4** and 8.09 and 8.37 for **3.5** – **3.7**. Signals for H8 appeared as doublets at approximately 8 ppm for these ligands. This splitting pattern is attributed to long range coupling of H8 to H6.

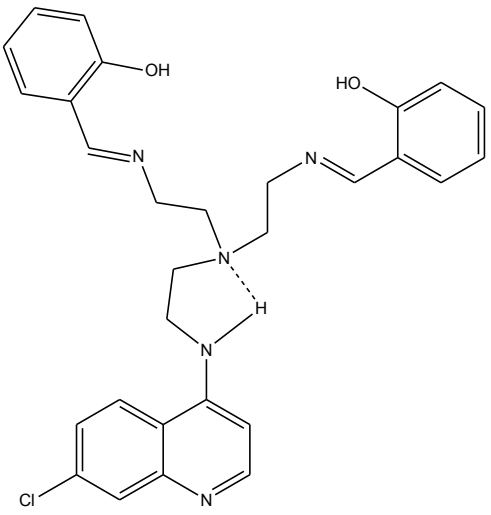


Figure 3.5: Possible intramolecular H-bonding that may occur in the dimeric derivatives.

$^{13}C\{^{1}H\}$ NMR spectroscopy was used to further confirm that the desired compounds were obtained. The spectra of **3.2** – **3.4** were recorded in $CDCl_3$, while the spectra of **3.5** – **3.7** were recorded in DMSO-d_6 (due to lower solubility in $CDCl_3$). Resonances for the CH_2 carbon atoms of the aliphatic chains were assigned between 29.96 and 57.40 ppm for **3.2** – **3.4** and between 41.28 and 56.62 ppm for **3.5** – **3.7**. Signals for the imine carbon atoms were observed between 164.65 and 166.78 ppm for these ligands. Signals for C8 appeared between 125.79 and 129.01 ppm. Signals for C2 appeared between 151.39 and 154.73 ppm. Analysis of the NMR spectroscopic data confirmed that the desired ligands were successfully synthesised.

IR Spectroscopy

The infrared spectra of these ligands were recorded in the solid state as KBr pellets. Upon condensation of the respective precursors, two absorption bands were observed for the two C=N stretching frequencies. Bands for the newly formed C=N bond was observed at a higher frequency compared to the C=N of the quinoline ring. An absorption band for the Schiff-base C=N stretching frequency appeared between 1631 and 1648 cm^{-1}, while the absorption band for the quinoline C=N stretching frequency appeared between 1609 and 1615 cm^{-1}. Absorption bands for the NH stretching frequencies were observed in the range of 3238 – 3424 cm^{-1}.

Mass spectrometry

The molecular weights of these compounds were confirmed using ESI or EI mass spectrometry (Table 3.2). Compounds **3.2** – **3.5** were analysed using ESI-MS in the positive mode, while **3.6** and **3.7** were analysed using EI-MS. The mass spectra of **3.2**, **3.3** and **3.5** showed peaks corresponding to the protonated molecular ion, $[M+H]^+$. The spectra of **3.4**, **3.6** and **3.7** exhibited peaks corresponding to $[M]^+$. This data further confirmed synthesis of the desired ligands.

Table 3.2

Mass spectrometry data obtained for ligands **3.2** – **3.7**.

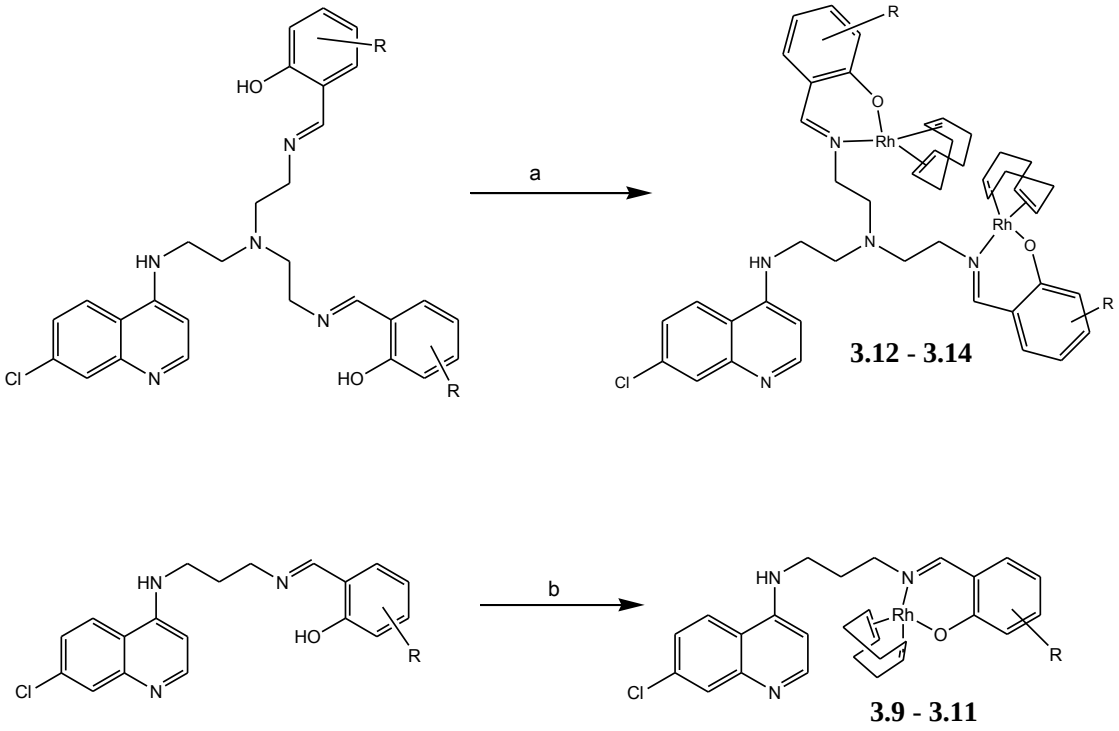
compound	peak observed	assignment
3.2	374.0824	$[M+H]^+$
3.3	370.1319	$[M+H]^+$
3.4	339.0903	$[M]^+$
3.5	584.1404	$[M+H]^+$
3.6	574.9313	$[M]^+$
3.7	514.9832	$[M]^+$

Upon synthesis and characterisation of the ligands, the next step was to prepare their corresponding Rh(I) complexes.

3.3. Synthesis and characterisation of mono- and binuclear Rh(I) salicylaldimine quinoline complexes 3.9 – 3.14

3.3.1. Synthesis

The afore-mentioned ligands **3.2** – **3.7** were subsequently reacted with $[RhCl(COD)]_2$ (**3.8**)[12] to afford the desired mono- and binuclear Rh(I) salicylaldimine quinoline complexes **3.9** – **3.14** (Scheme 3.2).



R = 5-Cl (**3.9**, **3.12**); 3-OMe (**3.10**, **3.13**); H (**3.11**, **3.14**)

Scheme 3.2: Synthesis of Rh(I) salicylaldimine complexes **3.9** – **3.14**. Reagents and conditions: (a) Ligand (1 eq.), excess NaH, $[RhCl(COD)]_2$ (1 eq.), DCM, 3 – 6 h, r.t. (b) Ligand (2 eq.), excess NaH, $[RhCl(COD)]_2$ (1 eq.), DCM, 3 – 6 h, r.t.

A dilute solution of the ligand in DCM was prepared. To this, excess NaH was added. During initial attempts, Et_3N was used as the base instead, but the base proved difficult to remove during the work-up. The base was then changed to NaH, resulting in efficient removal of

excess base using water. The role of the base is to deprotonate the OH group of the salicylaldimine moiety to facilitate coordination at this particular site upon addition of the metal precursor. Mononuclear complexes **3.9 – 3.11** were obtained by reaction of the ligand and the metal precursor in a 2:1 stoichiometric ratio, while binuclear complexes **3.12 – 3.14** were obtained using a 1:1 ligand to metal precursor ratio. The complexes were obtained as yellow powders in moderate to good yields (52 – 79%).

3.3.2. Characterisation

The complexes were characterised by the same techniques as the ligands.

NMR Spectroscopy

The ^{1}H NMR spectra for complexes **3.9** – **3.14** were recorded in $CDCl_3$. Analysis of the spectra suggested that the desired compounds were obtained. The ^{1}H NMR spectrum of complex **3.11** and its corresponding ligand (**3.4**) is shown in Figure 3.6.

Coordination of the metal to the ligand was confirmed by an upfield shift of the signal for the imine proton compared to the free ligand (Table 3.3). The upfield shift is due to shielding as a result of the formation of the Rh-N bond. The resonance for the phenolic proton was absent in the spectra of the complexes. Both the shifting of the imine proton signal and the loss of the signal for the phenolic proton suggests coordination in a bidentate manner by the imine nitrogen and the phenolic oxygen. In complexes **3.9** – **3.14** four distinct resonances for the protons of the COD moiety were observed. The CH_2 protons of the COD moiety exhibited chemical non-equivalence. The *exo* and *endo* methylene resonances of the COD moiety (Figure 3.7) were assigned at approximately 1.8 and 2.3 ppm, respectively.

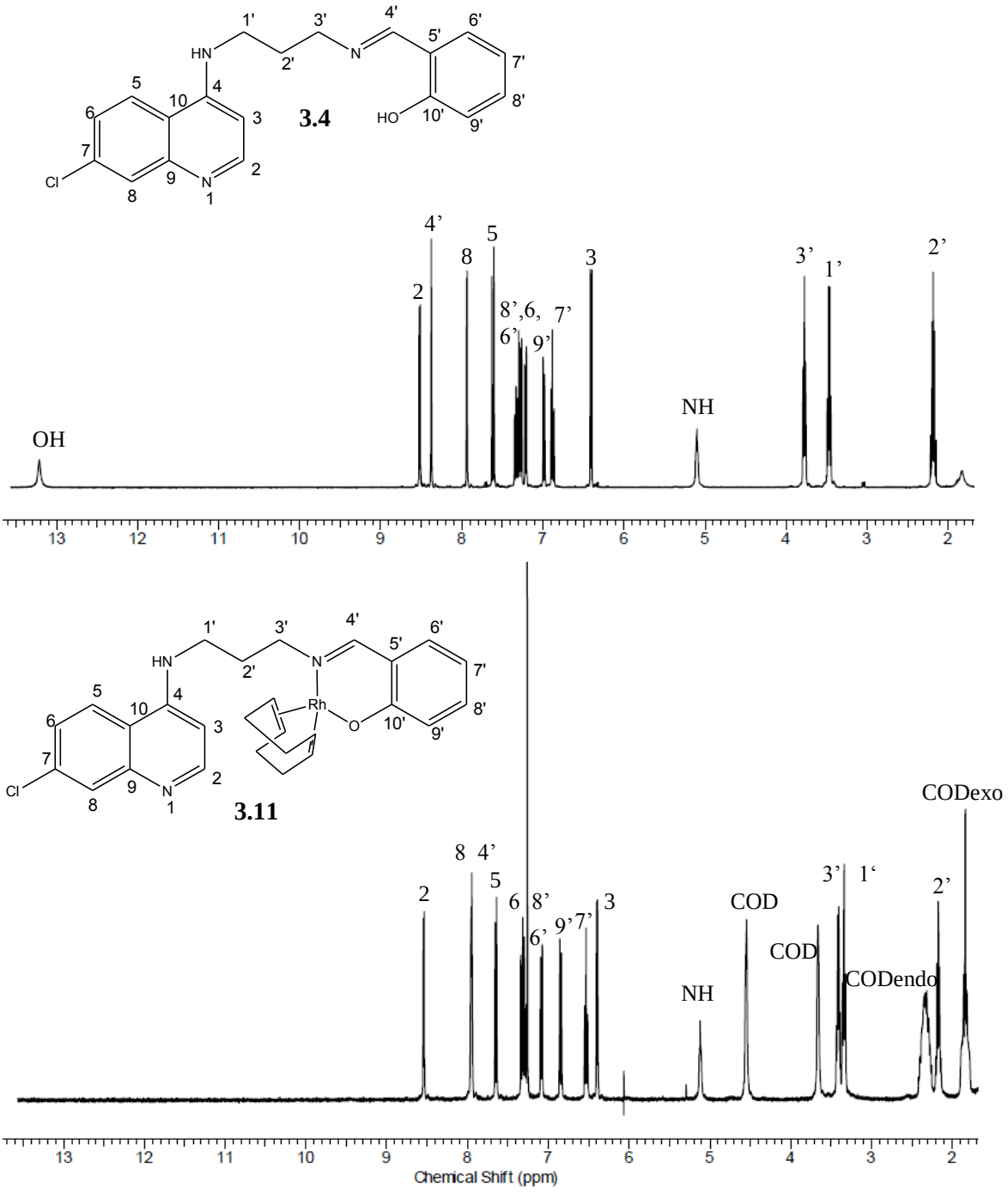


Figure 3.6: ^{1}H NMR spectra of **3.4** and **3.11** in $CDCl_3$

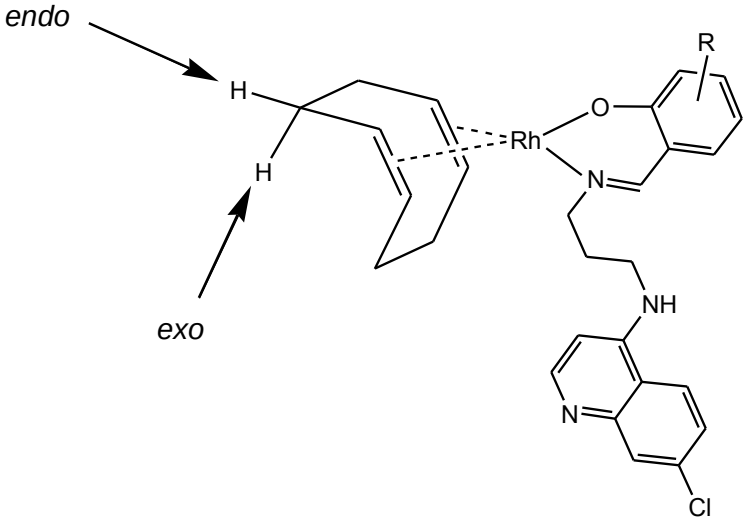


Figure 3.7: Structure indicating the *exo* and *endo* methylene protons

A non-uniform magnetic field is commonly observed in π systems.[13,14] When electrons in π systems encounter an applied field, a magnetic field that causes non-uniformity is induced. This in turn influences the chemical shifts of surrounding nuclei by either causing a shielding or deshielding effect, depending on the position of the nuclei in this field. Figure 3.8 shows the various shielded and deshielded regions associated with alkenes. The shielded area (blue) is observed above and below the plane of the double bond, whereas the deshielded region (pink) occurs in the plane of this bond. This may explain why the signal for the *exo* methylene protons (situated in the shielded region) appeared upfield compared to the *endo* methylene signal. The olefinic hydrogen atoms were more deshielded compared to the methylene protons due to their occurrence in the deshielded region (blue).

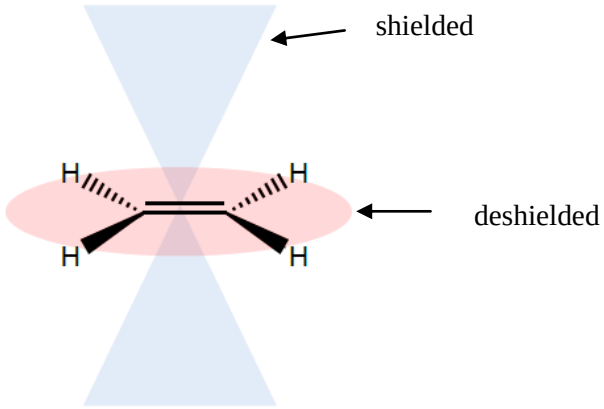


Figure 3.8: Shielded and deshielded regions of alkenes.

Signals for the olefinic protons of the COD moiety appeared at approximately 3.6 and 4.5 ppm for the complexes. Two multiplets were observed for these protons in the complexes, while these protons appeared as one multiplet in the spectrum of the $[RhCl(COD)]_2$ precursor. This is attributed to the *trans* effect induced by the *N,O* ligand. The signal further downfield was assigned to the proton *trans* to the imine nitrogen, while the signal found more upfield was attributed to the proton *trans* to the phenolic oxygen. This phenomenon is consistent with other *N,O*-Rh(I) complexes.[15-19] This further confirmed coordination to the imine nitrogen and phenolic oxygen rather than to the quinoline nitrogen.

$^{13}C\{^1H\}$ NMR spectroscopy was also used to characterise these complexes. Coordination was confirmed by the appearance of signals for the aliphatic carbon atoms of the COD moiety at approximately 28.8 ppm for the mononuclear complexes **3.9** – **3.11** and 28.9 ppm for the binuclear complexes **3.12** – **3.14**. The second COD resonances were observed at approximately 31.7 ppm for these complexes. The olefinic carbon atoms appeared as two signals. These signals are doublets (see Table 3.2) due to carbon-rhodium coupling as a result of the 100% natural abundance of ^{103}Rh and its nuclear spin of ½. The signals for these carbon atoms were assigned at approximately 71 and 86 ppm, respectively. A resonance at 71 ppm was assigned to the carbon atoms found *trans* to *O*, while the signal at 86 ppm accounts for the carbon atoms *trans* to *N*. The $^{103}Rh–^{13}C$ spin-spin coupling constants for *C trans* to *O* were approximately 14 Hz, while the coupling constant for *C trans* to *N* was approximately 12 Hz. The splitting patterns and coupling constants are consistent with data observed for similar Rh-*N,O* complexes.[15-19]

Table 3.3

Selected ^{1}H and $^{13}C\{^{1}H\}$ NMR data obtained for complexes **3.9** – **3.14**.

compound	$\underline{H}C=N$ ($\underline{H}C=N_{ligand}$)	$\underline{H}$-COD_{exo}; $\underline{H}$-COD_{endo}	$\underline{H}C=CH_{COD}$; $HC=C\underline{H}_{COD}$	$H\underline{C}=CH_{COD}$ (m, J_{C-Rh} Hz); $HC=\underline{C}H_{COD}$ (m, J_{C-Rh} Hz)
3.9	7.87 (8.30)	1.84; 2.34	3.66; 4.54	71.66 (d, 14.09); 86.04(d, 11.86)
3.10	7.95 (8.35)	1.85; 2.35	3.68; 4.66	71.21 (d, 14.12); 85.49 (d, 11.78)
3.11	7.94 (8.38)	1.84; 2.34	3.66; 4.54	71.29 (d, 14.08); 85.70 (d, 11.96)
3.12	7.71 (7.99)	1.84; 2.37	3.51; 4.52	71.48 (d, 14.03); 86.08 (d, 11.53)
3.13	7.75 (8.16)	1.83; 2.36	3.51; 4.64	71.04 (d,14.18); 85.47 (d, 11.68)
3.14	7.76 (8.29)	1.82; 2.36	3.51; 4.53	71.13 (d, 13.69); 85.67 (d, 11.67)

IR Spectroscopy

Infrared spectroscopy was used to further confirm formation of the Rh(I) complexes **3.9** – **3.14**. The most obvious difference between the spectra of the ligands and the complexes was that in the spectra of the ligands, two absorption bands were observed for the two different C=N environments. In the case of the complexes, one absorption band was observed. Upon coordination, the absorption band that was initially at a higher frequency (~ 1630 cm^{-1}) in the ligands, shifted towards lower frequency (similar to that of the $C=N_{quinoline}$). This was expected upon coordination to the imine nitrogen. The nitrogen atom donates electrons to the rhodium centre, resulting in the elongation of the C=N bond which causes a shift of this band to lower wavenumbers. The spectra suggest that both C=N absorption bands appeared at a similar frequency and therefore only one distinguishable absorption band was observed in the spectra of the complexes. The spectra shown in Figure 3.9 highlight the region where

absorption bands for the C=N frequencies appear for compounds **3.7** and **3.14**. Absorption bands for the C=N stretching frequencies of the complexes appeared between 1604 and 1613 cm^{-1}. Absorption bands for the N-H frequencies were observed between 3362 and 3439 cm^{-1}. The NH absorption bands appeared to remain constant upon coordination.

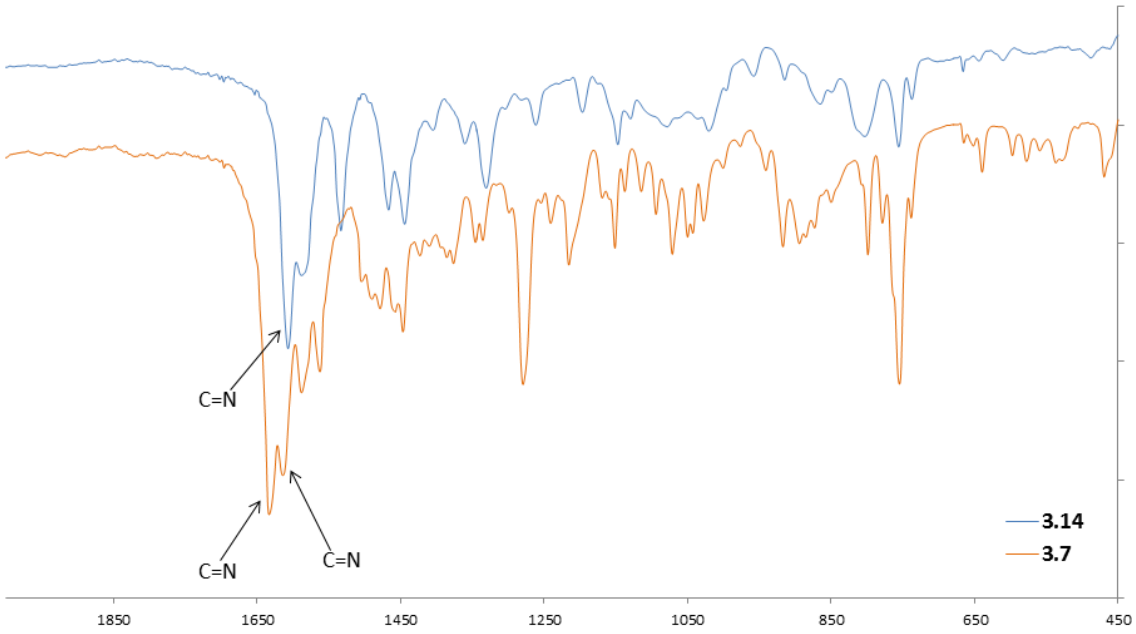


Figure 3.9: Infrared spectra of ligand **3.7** (orange) and complex **3.14** (blue) obtained in the solid state.

Mass Spectrometry

Mass spectrometry was also used to characterise these complexes (See Table 3.4). Both ESI (recorded in the positive mode) and EI mass spectrometry (for complexes **3.9** - **3.11**) was used to analyse these compounds. In all cases the molecular ion peak or a peak for the molecule's protonated form was observed in the spectra of the complexes. Complexes **3.9**, **3.10** and **3.11** were analysed using EI mass spectrometry. The spectra of the mononuclear complexes showed peaks corresponding to $[M]^+$ at *m/z* 582.7133, 578.7728 and 548.6803, respectively. Complexes **3.12**, **3.13** and **3.14** were analysed using ESI mass spectrometry. The spectra of **3.13** and **3.14** exhibited peaks at 996.2199 and 936.2004 corresponding to $[M+H]^+$. A peak in the spectrum of **3.12** at 1004.1231 was assigned to the protonated molecular ion, $[M+H]^+$.

Table 3.4

Mass spectrometry data obtained for complexes **3.9** – **3.14**.

compound	peak observed	assignment
3.9	582.7133	$[M]^+$
3.10	578.7728	$[M]^+$
3.11	548.6803	$[M]^+$
3.12	1004.1231	$[M+H]^+$
3.13	996.2199	$[M+H]^+$
3.14	936.2004	$[M+H]^+$

The above-mentioned characterisation data clearly supports preparation of the desired rhodium(I) quinoline complexes. Based on satisfactory correlation of the characterisation data and the proposed structures, the biological activity of these compounds were studied and the data are described in the next section.

3.4. Biological and physicochemical evaluation

3.4.1. Antiparasitic activity against *P. falciparum*

The *in vitro* antiplasmodial activity of ligands **3.2** – **3.7** and complexes **3.9** – **3.14** were evaluated against chloroquine-sensitive (NF54) and chloroquine-resistant (K1) strains of *Plasmodium falciparum*. Chloroquine (CQ) and ferroquine (FQ) were used as reference drugs. The data obtained from this evaluation are shown in Table 3.5.

Table 3.5

In vitro antiplasmodial activity and resistance indices of compounds **3.2** – **3.7**, **3.9** – **3.14,** CQ and FQ against the NF54 CQ-sensitive and the K1 CQ-resistant strains of *P. falciparum*.

compound	IC_{50}[a] ± SE NF54[b] (µM)	IC_{50} ± SE K1[c] (µM)	resistance index[d]
3.2	0.052 ± 0.001	0.72 ± 0.089	13.7
3.3	0.055 ± 0.001	0.65 ± 0.083	11.8
3.4	0.072 ± 0.006	0.68 ± 0.017	9.4
3.5	4.9 ± 0.28	2.3 ± 0.26	0.4
3.6	4.9 ± 0.37	1.8 ± 0.18	0.4
3.7	7.0 ± 0.48	4.5 ± 0.78	0.6
3.9	0.098 ± 0.009	0.87 ± 0.055	8.8
3.10	0.16 ± 0.020	1.3 ± 0.19	7.7
3.11	0.091 ± 0.009	1.8 ± 0.061	19.6
3.12	7.9 ± 0.37	2.9 ± 0.35	0.4
3.13	4.8 ± 0.075	2.2 ± 0.12	0.4
3.14	4.0 ± 0.12	1.5 ± 0.14	0.4
CQ	0.025 ± 0.005	0.30 ± 0.038	11.8
FQ	0.033 ± 0.010	0.014[e]	0.5

[a] IC_{50} represents the micromolar equivalents required to inhibit parasite growth by 50%

[b] NF54 chloroquine-sensitive strain of *P. falciparum;* n = number of data sets averaged, n = 3

[c] K1 chloroquine-resistant strain of *P. falciparum*

[d] Resistance index (RI) = IC_{50}K1/IC_{50}NF54

[e] Literature IC_{50} value against K1[20]

Antiplasmodial activity against the NF54 CQ-sensitive strain of P. falciparum

The monomeric salicylaldimine ligands (**3.2** – **3.4**) showed good activity against the NF54 strain of *P. falciparum*. Compounds that exhibit IC_{50} values lower than 1 µM would be considered as potential antimalarial candidates. The monomeric ligands exhibited IC_{50} values less than 1 µM. The 5-Cl (**3.2**) and 3-OMe (**3.3**) derivatives exhibited similar IC_{50} values,

while the IC_{50} value obtained for the unsubstituted derivative (**3.4**) was slightly higher. The activity observed for these compounds are comparable to the activity of chloroquine (IC_{50} = 0.025 µM) and ferroquine (IC_{50} = 0.033 µM) against the same strain. The dimeric salicylaldimine ligands (**3.5** – **3.7**) were far less active than their monomeric counterparts. The monomeric ligands were at least 90-times more active than the dimeric compounds. A similar trend in activity was observed for the dimeric compounds as the monomeric systems. The 5-Cl (**3.5**) and 3-OMe (**3.6**) derivatives exhibited similar activity and showed slightly enhanced activity compared to the unsubstituted analogue (**3.7**). This suggests that the substituents play a minor role in activity in this case.

Corresponding rhodium COD complexes were prepared in order to establish whether incorporation of the metal moiety is beneficial towards antiplasmodial activity since this would alter the lipophilic properties of the compounds. Table 3.6 shows the ratio between the IC_{50} value obtained for the complex and the IC_{50} value obtained for the ligand.

Table 3.6

Ratio of the activity between ligands (**3.2** – **3.7**) and complexes (**3.9** – **3.14**) against NF54 CQ-sensitive *P. falciparum.*

complex (ligand)	IC_{50} ratio[a]
3.9 (3.2)	1.9
3.10 (3.3)	3
3.11 (3.4)	1.3
3.12 (3.5)	1.6
3.13 (3.6)	1.0
3.14 (3.7)	0.6

[a] IC_{50}(complex)/IC_{50}(ligand)

A value close to 1 suggests that the ligand and the complex exhibit similar activity. A value greater than 1 suggests that complexation lowers activity. A value less than 1 indicates that complexation enhances the activity of the compound. Comparing the activity of the monomeric ligands (**3.2** – **3.4**) and mononuclear complexes (**3.9** – **3.11**), it was found that the compounds exhibited ratios between 1.3 and 3. Complex **3.10** (R = H) and complex **3.9** (R =

5-Cl) appeared to be less active than their corresponding ligands (ratios of 3.0 and 1.9, respectively. The unsubstituted complex **3.11** showed similar activity to its ligand as a ratio of approximately 1.3 was obtained. Although most of the complexes were less active than their ligands, the compounds exhibited high activity giving IC_{50} values much lower than 1 µM. With respect to the binuclear complexes, complex **3.13** (R = 3-OMe) and its ligand (**3.6**) exhibited similar activity giving an IC_{50} ratio of approximately 1, suggesting that complexation has little to no effect on activity in this case. The 5-Cl derivative, complex **3.12**, was observed to be slightly less active compared to its free ligand (ratio = 1.6), while complex **3.14**, the unsubstituted derivative, was approximately 1.8 times more active than its corresponding ligand (IC_{50} ratio = 0.6). The latter appeared to be the only case where incorporation of the metal had a favourable effect on activity against this *P. falciparum* strain.

The mononuclear complex possessing the 3-OMe moiety (**3.9**) exhibited lower activity (IC_{50} = 0.16 µM) compared to the 5-Cl (IC_{50} = 0.098 µM) and unsubstituted (IC_{50} = 0.091 µM) derivatives. In the binuclear complexes, it was found that the compound with the 5-Cl group (**3.12**) was least active, followed by the 3-OMe derivative (**3.13**). The unsubstituted compound, **3.14**, was the most active binuclear complex.

The CQ-Rh(I) COD complex mentioned in Figure 3.1a was tested previously by Sànchez-Delgado *et al*. against *Plasmodium Berghei* and exhibited similar activity (IC_{50} = 73 nM) to CQ.[1] Although the values obtained for these complexes cannot directly be compared to that of the CQ derivative mentioned (since different strains of *Plasmodium* were used), the mononuclear complexes (**3.9** – **3.11**) showed similar activity to this complex (IC_{50} values between 91 and 160 nM). The quinoline moiety appears to be the driving force behind the activity of these compounds since the free ligands also exhibited good activity.

Antiplasmodial activity against the K1 CQ-resistant strain of P. falciparum

Similar trends in activity were observed for this strain. The monomeric ligands (**3.2** – **3.4**) were the most active. The dimeric ligands, mononuclear complexes and binuclear complexes exhibited activity in the same range (1.3 – 2.9 µM). All the tested compounds were found to be less active than chloroquine and ferroquine in this strain.

The monomeric ligands (**3.2** – **3.4**) were the most active compounds exhibiting IC_{50} values less than 1 µM. These values are comparable to one another (IC_{50} values between 0.65 and 0.72 µM). The substituent has a negligible effect on activity. The mononuclear complexes (**3.9** – **3.11**) were slightly less active than their ligands (IC_{50} ratios shown in Table 3.7). In this strain, the 5-Cl complex (**3.9**) exhibited similar activity to its free ligand (IC_{50} ratio = 1.2), while the rest of the mononuclear complexes were less active than their ligands. The dimeric ligands (**3.5** – **3.7**) showed moderate to weak activity against this strain. The 3-OMe derivative (**3.6**) exhibited better activity than the 5-Cl and the unsubstituted salicylaldimine derivative. Two of the binuclear complexes (**3.12** and **3.13**) exhibited comparable activity to their free ligands based on their IC_{50} ratio (1.3 and 1.2, respectively). Once again the unsubstituted derivative (**3.14**) showed improved activity upon complexation (IC_{50} ratio = 0.3).

Table 3.7

Ratio of the activity between ligands (**3.2** – **3.7**) and complexes (**3.9** – **3.14**) against K1 CQ-resistant *P. falciparum.*

complex (ligand)	IC_{50} ratio[a]
3.9 (3.2)	1.2
3.10 (3.3)	1.9
3.11 (3.4)	2.6
3.12 (3.5)	1.3
3.13 (3.6)	1.2
3.14 (3.7)	0.3

[a] IC_{50}(complex)/IC_{50}(ligand)

Overall, the monomeric ligands and mononuclear complexes showed decreased activity in the resistant strain compared to the sensitive strain. Figure 3.10 gives the resistance indices for the compounds. Ligands **3.2** – **3.4** and complexes **3.9** – **3.11**, which contain one salicylaldimine moiety, exhibited larger RI values compared to the dimeric salicylaldimine compounds **3.5** – **3.7** and **3.12** – **3.14**. Ligands **3.2** – **3.4** and their corresponding complexes (**3.9** – **3.11**)

displayed similar antiplasmodial behaviour to CQ. In the sensitive strain, the compounds showed excellent activity but in the resistant strain, the activity decreased more than 10-fold.

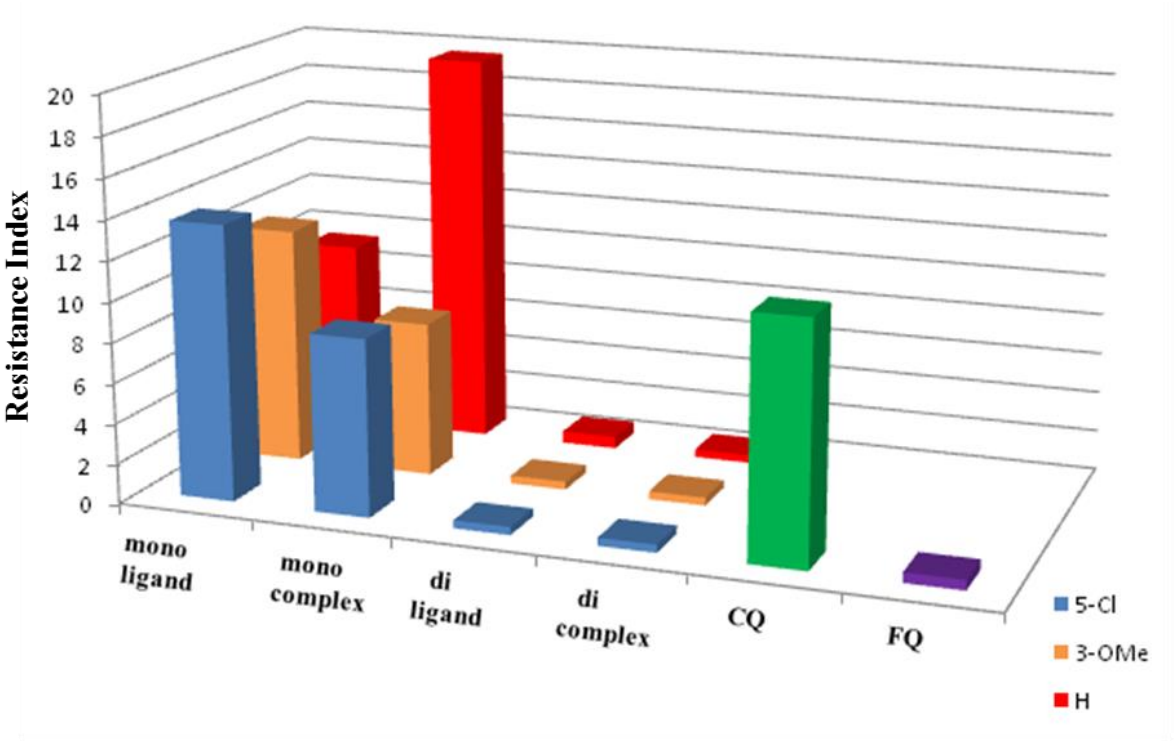


Figure 3.10: Resistance indices for compounds **3.2** – **3.7** and **3.9** – **3.14**

The monomeric ligands and complexes demonstrated similar activity profiles to that of chloroquine because these compounds showed very good activity against the sensitive strain, but were less active in the resistant strain. This was not observed for the dimeric derivatives. The dimeric compounds showed similar or enhanced activity in the resistant strain compared to the sensitive strain. Ferroquine appeared to follow a similar activity profile to the dimeric compounds. In the case of ferroquine it is believed that this compound's activity in the resistant strain is due to its lipophilic character and in addition to this, an intramolecular hydrogen bond (Figure 3.11) between the tertiary amino group and the secondary amino group aids in the formation of a folded conformation, which results in an enhancement of its transport through membranes.[21,22]

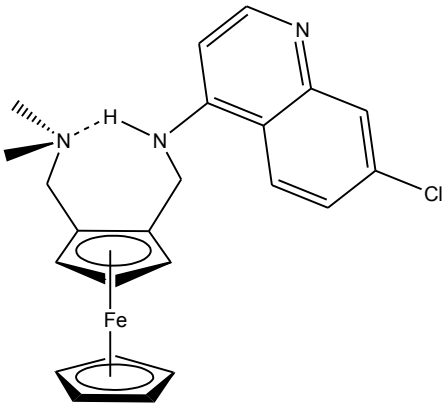


Figure 3.11: Intra-molecular bonding observed in ferroquine.

As suggested in section 3.2.2 of this chapter, the dimeric systems may also possess intramolecular hydrogen bonding based on the shift of the NH signal. If the intramolecular H-bonding aids in the transport of these molecules across membranes, perhaps this is a reason for the similar activities observed for these compounds in the resistant and the sensitive strains. This was not observed for the monomeric derivatives. The lower activity of the dimeric compounds, however, compared to the monomeric compounds may be a consequence of their highly lipophilic nature.

To more closely compare the activity of these compounds to the susceptibility and resistance of chloroquine, a plot of the IC_{50} values obtained from this study is shown in Figure 3.12. CQ susceptibility is defined as values less than 0.1 μg/ml (194.1 nM), while values greater than 1 μg/ml (1941 nM) define resistance.[23,24] The plot includes these margins for a better comparison. All the monomeric salicylaldimine compounds **3.2** – **3.4** lie within the margin defining CQ resistance. Their activity and *in vitro* behaviour is therefore comparable to chloroquine. Ferroquine, the most active compound, exhibited similar behaviour *in vitro* across both strains. The dimeric compounds **3.5** – **3.7** were far less active compared to the monomeric compounds. This may be a consequence of their size or lipophilicity. Lipophilicity plays a role in the transport of the drug across membranes into the digestive vacuole of the parasite. If the compound is too lipophilic, this may hinder its transport. The predicted logP values of selected compounds are shown in Table 3.8.

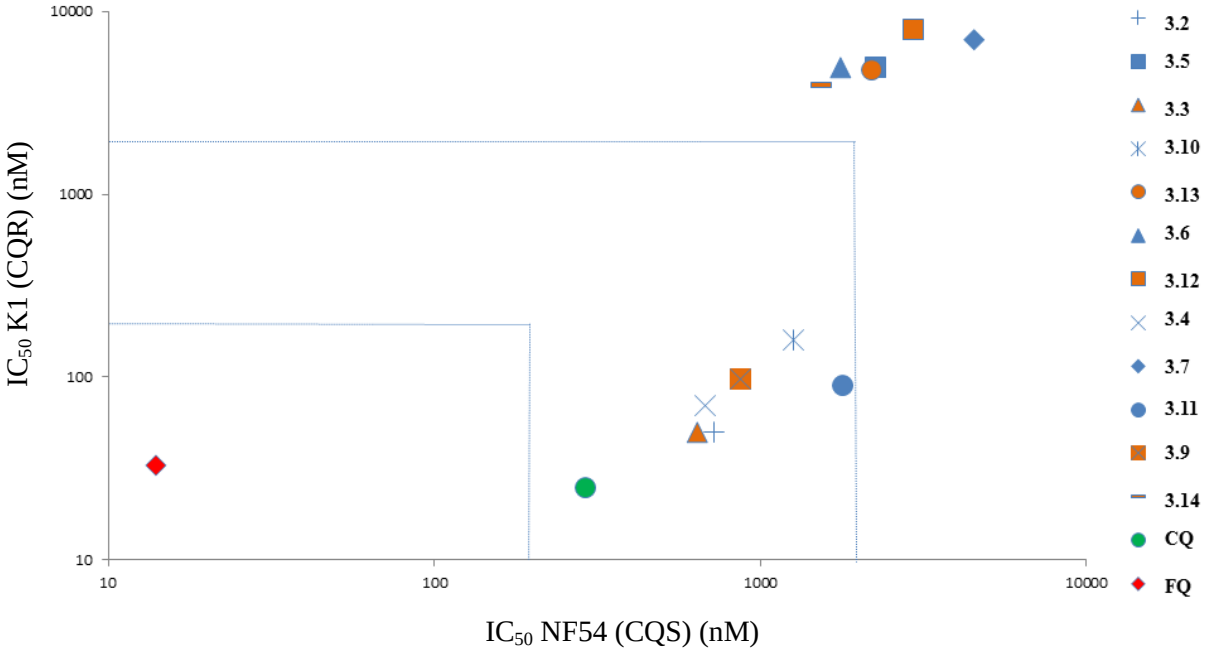


Figure 3.12: Scatter plot depicting the correlation between the NF54 CQ-sensitive strain and the K1 CQ-resistant strain. The margins (shown in blue) represent CQ susceptibility (194.1 nM) and CQ resistance (1941 nM), respectively.

Table 3.8

Predicted logP values of compounds **3.2** – **3.7**

compound	logP[a]	$logD_{4.7}$	$logD_{7.4}$
3.2	4.32	2.11	4.02
3.3	3.56	1.07	3.30
3.4	3.71	1.05	3.43
3.5	6.33	1.92	5.47
3.6	4.81	0.23	4.01
3.7	5.12	0.34	4.31
1,5-cyclooctadiene	2.83	2.83	2.83

[a]logP and logD values predicted using MarvinSketch V5.9.4

Generally, the monomeric ligands (**3.2** – **3.4**) exhibited lower logP values compared to their dimeric counterparts (**3.5** – **3.7**). Comparing the lipophilicity to the activity (given as $logIC_{50}$)

against the sensitive strain (Figure 3.13), the monomeric compounds were more active compared to the dimeric salicylaldimines. As can be seen in Figure 3.13, compounds that have logP values between 3.5 and 4.3 appear to exhibit good antiplasmodial activity.

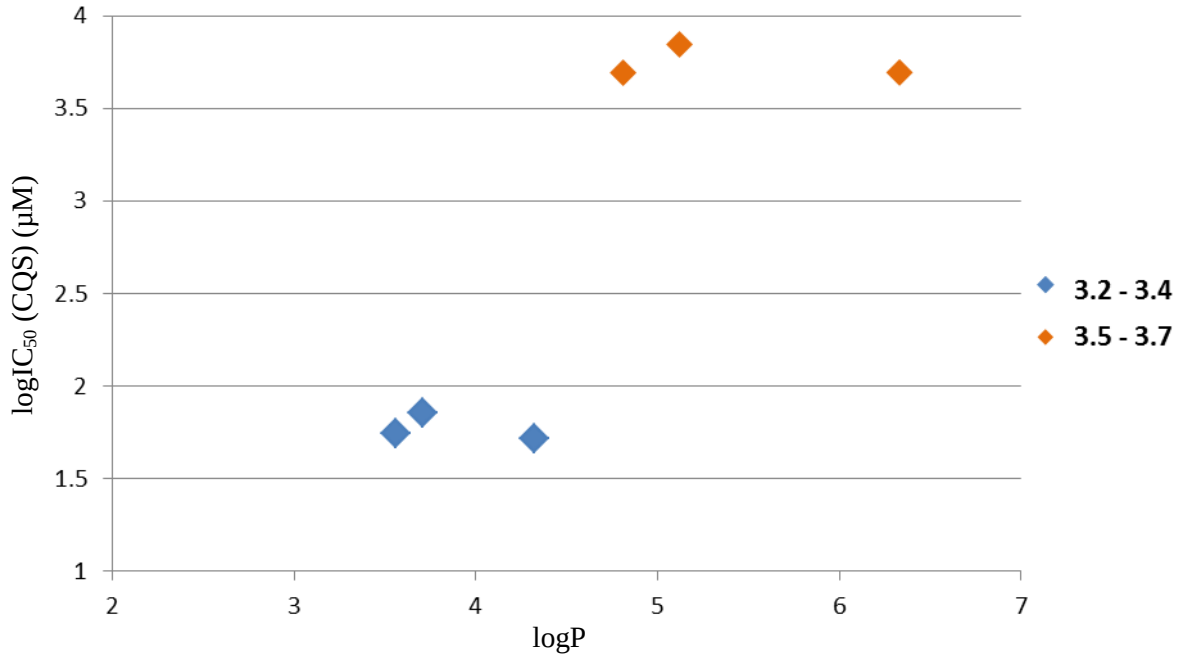


Figure 3.13: Plot of logP vs the $logIC_{50}$ obtained for the NF54 CQS strain of *P. falciparum*.

The logP value of the 1,5-cyclooctadiene moiety was predicted to be 2.83 (Table 3.8). The addition of the cyclooctadiene moiety to the ligands would therefore result in an overall increase in the lipophilicity of the complexes compared to the free ligands. This may be a reason for the slight decrease in the activity of the complexes compared to the ligands in some cases. Of course many other factors, including vacuolar accumulation play a role in the activity of these compounds.

3.4.2. β-Haematin inhibition studies

Inhibition of haemozoin formation remains an important target for the development of potential antimalarials. Since other successful quinoline compounds, including chloroquine, are believed to inhibit haemozoin formation, it was expected that the prepared compounds would act by means of a similar mechanism.[25] The NP-40 detergent mediated assay was used to establish if these compounds inhibit β-haematin (synthetic haemozoin) formation.[26]

Both the ligands (**3.2** – **3.7**) and the complexes (**3.9** – **3.14**) were screened and Figure 3.14 shows dose-response curves obtained for compounds **3.3** and **3.10**. This figure depicts the relationship between the absorbance of a haem-pyridine complex with an increasing drug concentration. At higher drug concentrations, the absorbance was higher, due to the presence of the haem-pyridine complex,[27] hence, inhibition of β-haematin formation occurred at higher drug concentration. The curves were generated using GraphPad Prism software.[28]

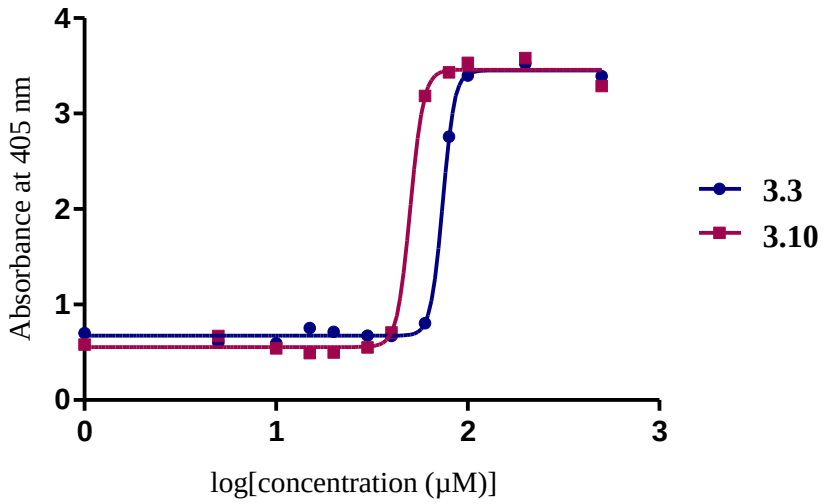


Figure 3.14: Dose-response curves obtained for ligand **3.3** and its complex **3.10** using the NP-40 detergent mediated assay

The monomeric ligands **3.2** – **3.4** were found to exhibit the lowest β-haematin inhibition activity of all the tested compounds in this series. The data are presented in Figure 3.15. The monomeric salicylaldimine ligands showed comparable activity with slight differences. The 5-Cl derivative (**3.2**) displayed slightly higher activity (IC_{50} = 64 µM) compared to the methoxy (IC_{50} = 74 µM) derivative (**3.3**), while the unsubstituted salicylaldimine (IC_{50} = 84 µM) was the least active ligand (**3.4**). The monomeric ligands exhibited similar β-haematin inhibition activity as chloroquine. The dimeric ligands (**3.5** – **3.7**) were found to be approximately twice as active compared to their monomeric counterparts. The R group appeared to have little to no significant effect on β-haematin inhibition activity unlike with the monomeric ligands. The IC_{50} values for these compounds were observed in the range of 33 and 35 µM. The higher activity suggests that these compounds inhibit β-haematin crystallisation to a greater extent compared to the monomeric ligands.

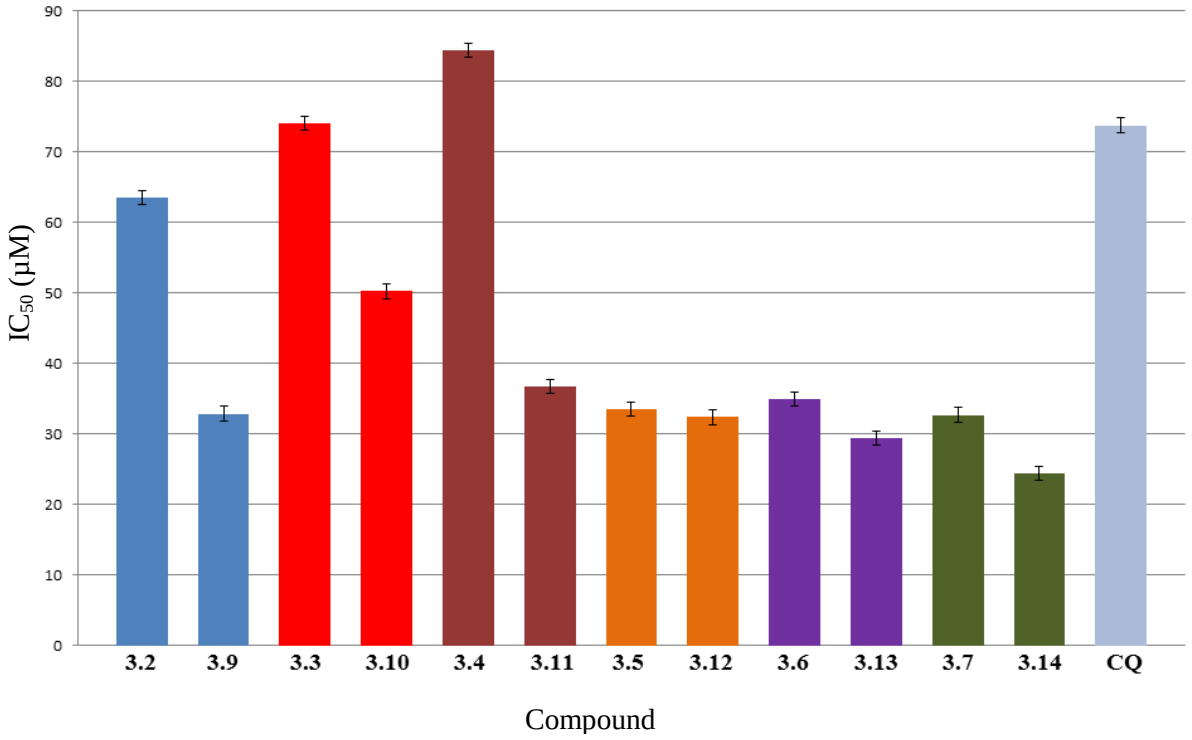


Figure 3.15: IC_{50} values (µM) obtained from β-haematin inhibition studies for the salicylaldimine quinoline compounds (**3.2** – **3.7** and **3.9** - **3.14**). Same coloured pairs represent ligand and its corresponding metal complex.

The mononuclear complexes **3.9** – **3.11** exhibited enhanced activity compared to their corresponding ligands. The methoxy derivative (**3.10**) showed the lowest activity of the series (IC_{50} = 50 µM), while the 5-Cl (**3.9**) and unsubstituted (**3.11**) derivatives exhibited comparable activity (IC_{50} = 33 and 37 µM, respectively). The binuclear complexes **3.12** – **3.14**, exhibited similar activity to their ligand counterparts. IC_{50} values were observed between 24 and 32 µM. In this case, the unsubstituted salicylaldimine complex **3.14** exhibited the highest activity. The mononuclear complexes (with the exception of **3.10**) possessed similar activity to the binuclear complexes.

Overall, it appears that incorporation of the metal fragment onto these systems results in enhancement of the β-haematin inhibition activity of the free ligands. This may be a consequence of the geometry of the metal fragment and increased lipophilicity. The metal

complexes possess a square planar geometry about the metal centre, which introduces a planar system about the salicylaldimine moiety, which may be a reason for the increased activity. This may favour interactions between haem and the compounds.

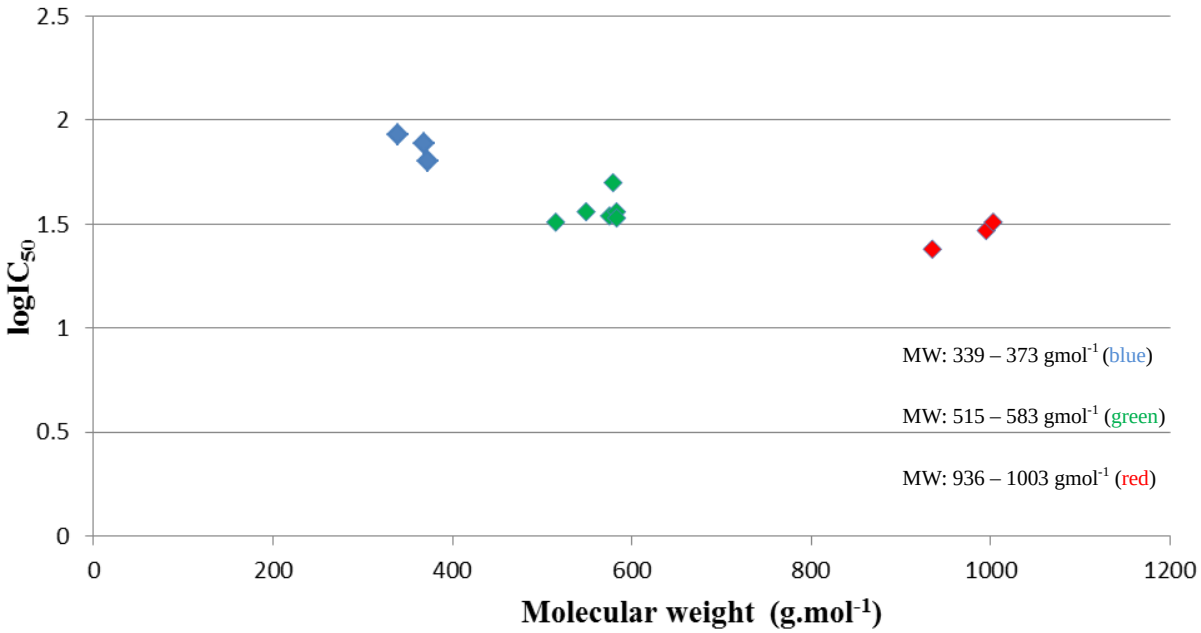


Figure 3.16: Relationship between the molecular weight of the tested compounds and $logIC_{50}$.

On closer inspection of the activity of these compounds, it was observed that compounds with similar molecular weights behave similarly. A plot comparing $logIC_{50}$ and molecular weight is shown in Figure 3.16. The monomeric ligands (**3.2** – **3.4**) have molecular weights in the range of 339 – 373 $g.mol^{-1}$. These compounds were the least active and gave $logIC_{50}$ values of approximately 1.9. Compounds **3.5** – **3.11**, which are the dimeric salicylaldimine ligands and mononuclear complexes, have molecular weights in the range of 515 and 583 $g.mol^{-1}$. These compounds gave $logIC_{50}$ values of approximately 1.5. Compounds **3.13** and **3.14** were the most active and have large molecular weights. Based on this, there appears to be some relationship between the size of the compound and its ability to inhibit β-haematin formation. This is consistent with data obtained for the compounds described in chapter 2. An explanation for this may be that lipophilicity influences β-haematin inhibition activity. The monomeric ligands are less lipophilic than the dimeric ligands and were therefore less effective at inhibiting β-haematin formation. The mononuclear complexes are more lipophilic

than their corresponding ligands due to incorporation of the lipophilic COD moiety. As a result, the mononuclear complexes showed improved β-haematin inhibition compared to its ligands. The dimeric ligands, mononuclear complexes and binuclear complexes exhibited comparable β-haematin inhibition activity. It may be that the increased lipophilicity is a reason for this enhanced activity. No simple correlation between β-haematin inhibition and antiplasmodial activity was observed. For instance, the monomeric ligands (**3.2** – **3.4**) were the most active compounds in both the NF54 CQ-sensitive and K1 CQ-resistant strains, but these compounds were the least effective β-haematin inhibitors. In addition to this, the least active compounds (dimeric salicylaldimines) *in vitro* displayed the best β-haematin inhibition.

It would be expected that antiplasmodial activity be related to the compound's ability to inhibit haemozoin formation, however, the ability of the drug to be transported into the digestive vacuole and accumulate therein is an important factor that governs the extent of antiplasmodial activity.

3.4.3. *In vitro* cytotoxicity studies

The compounds described in this chapter were also screened against the WHCO1 oesophageal cancer cell-line. Ideally, the compounds should exhibit selectivity towards malaria parasites. If no selectivity is observed, the antiplasmodial activity may be a consequence of the cytotoxicity of these compounds. Many traditional quinoline-containing antimalarials, including chloroquine, have also been investigated as antitumour agents against human breast cancer cells.[29] The results pertinent to this study are depicted in Table 3.9.

The tested compounds showed moderate cytotoxicity against this cell-line. The compounds did however exhibit greater activity compared to cisplatin against this cell-line (IC_{50} = 13.0 μM).[30] The dimeric salicylaldimine compounds **3.5**, **3.7** and **3.13** exhibited the highest cytotoxicity. With the exception of the monomeric 3-OMe salicylaldimine ligand (**3.3**) and its corresponding dimeric derivative (**3.6**), the monomeric salicylaldimine compounds exhibited slightly lower cytotoxicity compared to the dimeric derivatives (Figure 3.17).

Table 3.9

IC_{50} values obtained for compounds **3.2 – 3.7** and **3.9 – 3.14** against WHCO1 cancer cells.

compound	IC_{50} (μM)	95% confidence interval	selectivity index[a]	selectivity index[b]
3.2	8.5	7.5 - 9.2	163	11.8
3.3	8.8	7.8 - 9.9	160	13.5
3.4	8.4	7.2 - 9.6	116	12.3
3.5	5.9	5.2 - 6.8	1.20	2.63
3.6	11.1	9.7 - 12.8	2.25	6.31
3.7	4.7	4.3 - 5.2	0.674	1.04
3.9	8.7	7.9 - 9.7	89.2	10.1
3.10	11.3	9.5 - 13.6	68.7	8.97
3.11	9.8	8.8 - 10.9	107	5.45
3.12	7.8	6.3 - 9.5	0.975	2.63
3.13	3.9	3.4 - 4.4	0.802	1.76
3.14	7.6	7.2 – 8.0	1.91	4.96
Cisplatin	13.0[c]	-	-	-

[a] (IC_{50}WHCO1/IC_{50}NF54)

[b] (IC_{50}WHCO1/IC_{50}K1)

[c] IC_{50} value obtained for cisplatin against WHCO1 cancer cells.[30]

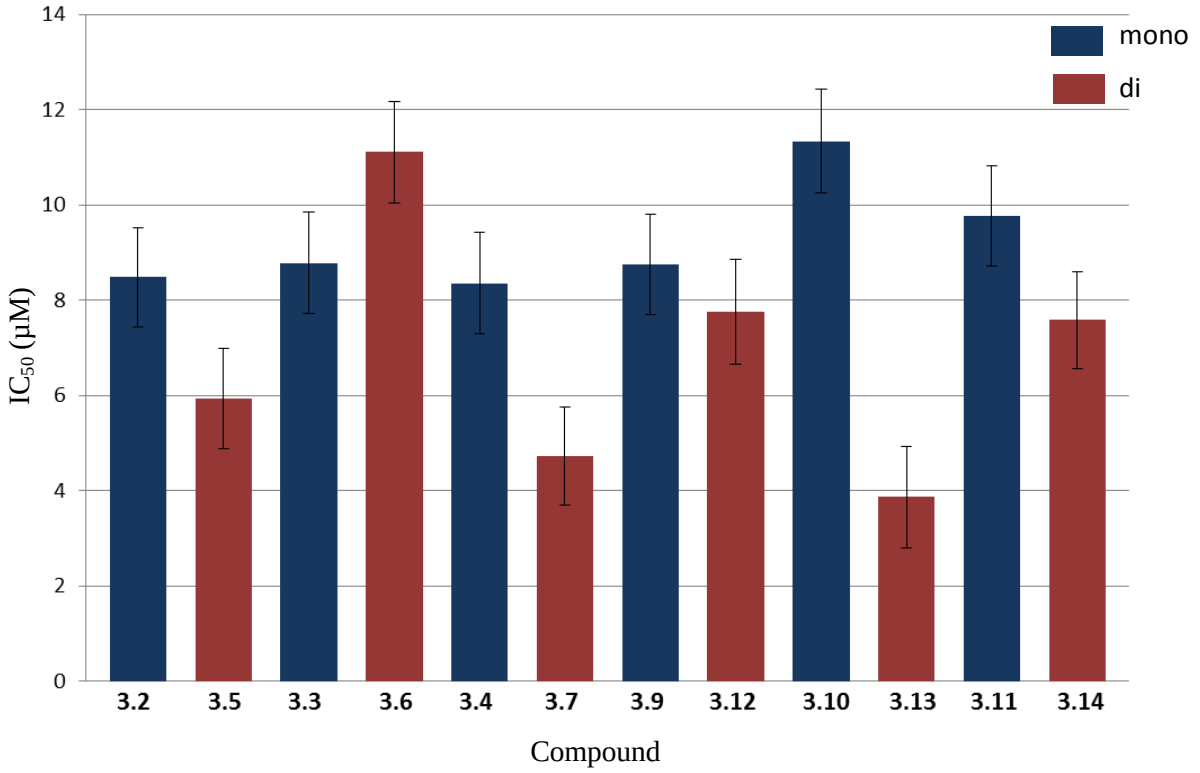


Figure 3.17: Graph comparing the cytotoxicity between monomeric and dimeric salicylaldimines.

The dimeric derivatives are considered to be polyamines because they contain more than one amino group. Studies carried out on polyamine systems reveal that these compounds are capable of transporting cytotoxic drugs into tumour cells.[31] Tumour cells are able to import polyamines by means of a polyamine transporter (PAT) in order to sustain their growth. The transporter is able to tolerate modified polyamines, therefore drug-incorporated polyamines may be able to penetrate the tumour cells via the PAT.[32] This may be a reason for the enhanced activity of some of the dimeric salicylaldimine compounds.

A similar trend in anticancer activity was observed for the quinoline derivatives discussed in chapter 2. Similarly, there are examples of other systems where activity appears to be a size-dependent phenomenon. Dendritic ruthenium arene compounds have been shown to display a similar effect. The multimeric systems show enhanced activity compared to monomeric systems.[33,34]

The monomeric ligands **3.2** – **3.4**, displayed comparable cytotoxicity. The substituent did not appear to affect activity significantly in this case. The dimeric ligands **3.5** (where R = 5-Cl) and **3.7** (where R = H) exhibited enhanced activity compared to the dimeric ligand where R = 3–OMe. The unsubstituted dimeric salicylaldimine ligand **3.7** showed the highest activity in the series. The mononuclear rhodium complexes exhibited a similar trend in activity compared to the dimeric ligands. Complex **3.9** (R = 5-Cl) and complex **3.11** (R = H) showed slightly increased activity compared to the methoxy-containing derivative (**3.10**). The binuclear rhodium complexes showed an opposing trend in anticancer activity compared to the mononuclear analogues. The 5-Cl substituted (**3.12**) and unsubstituted salicylaldimine (**3.14**) exhibited lower activity compared to the methoxy-containing derivative (**3.13**). Complex **3.13** was the most active compound of the series.

Comparing activity of the ligands and the metal complexes, the complexes generally exhibited slightly lower cytotoxicity compared to the free ligands. The only case where this was not observed is with ligand **3.6** and its corresponding rhodium complex **3.13**.

Selectivity indices (SI) were calculated for these compounds for both the chloroquine-sensitive strain (NF54) and the chloroquine-resistant strain (K1) of *P. falciparum*. Figure 3.18 compares the selectivity indices for the tested compounds (numerical values are shown in Table 3.9).

Firstly, against the NF54 strain of *P. falciparum*, the monomeric ligands (**3.2** – **3.4**) showed high selectivity towards malaria parasites giving rise to SI values greater than 100 (shown in blue). The mononuclear complexes (**3.9** – **3.11**) also exhibited high selectivity towards this strain, although the SI values were somewhat lower compared to the free ligands (SI: 69 – 107). This is a consequence of the slightly lower activity of the complexes against the NF54 strain of *P. falciparum* compared to the ligands. The dimeric ligands and complexes displayed similar SI values giving values in the range of 0.7 and 2.2. Compared to the SI values of the monomeric compounds, these compounds exhibited much lower SI values. These values are also close to 1 which suggests that the antimalarial activity (NF54 strain) is comparable to the activity against the WHCO1 cell-line suggesting little to no selectivity. This insinuates that cytotoxicity may be an explanation for the antimalarial activity of the dimeric compounds.

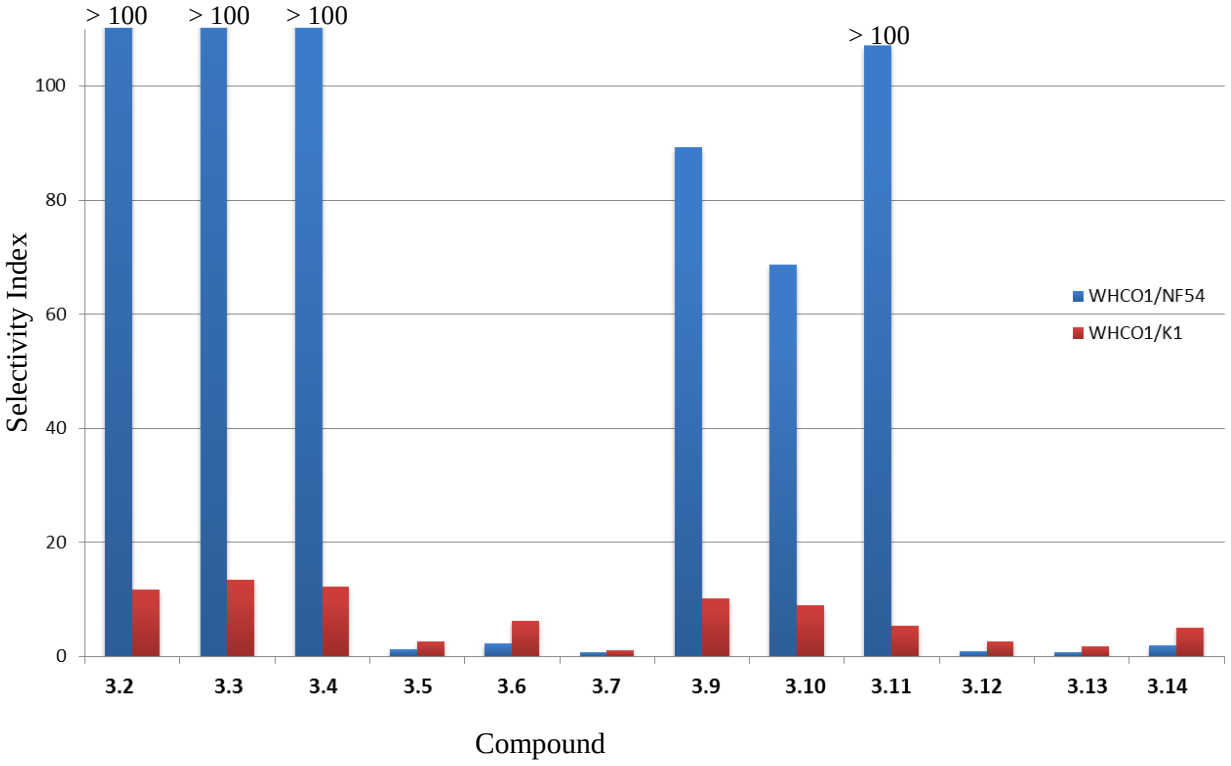


Figure 3.18: Selectivity indices obtained for the monomeric and dimeric salicylaldimines

When looking at the SI values obtained for the K1 strain, a large difference is observed for the monomeric salicylaldimine ligands and mononuclear complexes compared to the NF54 strain. In some cases a 10-fold decrease in selectivity was observed for these compounds when comparing the two strains. This is due to the lower activity of these compounds in the resistant strain compared to the sensitive strain. The SI values obtained for the dimeric salicylaldimine ligands and complexes are consistent with those obtained for the sensitive strain. This suggests that these dimeric compounds may act by means of a cytotoxic mechanism.

Further testing of the most cytotoxic compound **3.13**, was conducted against normal healthy human fibroblast KMST-6. This was done in order to provide further insight into whether these compounds act against all cell types or perhaps these compounds may be potential anticancer agents. The data obtained from this study is given in Table 3.10. The selectivity index was also determined.

Table 3.10

IC_{50} values against KMST-6 human fibroblast and WHCO1 cancer cells.

compound	IC_{50} (µM) ± SD KMST-6	IC_{50} (µM) WHCO1	selectivity index[a]
3.13	92 ± 4	3.9	23.6
cisplatin	96 ± 0.4	13.0	7.30

[a] IC_{50}KMST-6/IC_{50}WHCO1

As can be seen by the IC_{50} values obtained for **3.13** against the two cell-lines, the complex exhibited higher activity in the WHCO1 cancer cell-line compared to the normal fibroblast. A selectivity index of 23.6 was determined for this compound, indicating selectivity towards cancer cells. Comparing the SI value to that of cisplatin, the complex exhibited higher selectivity than cisplatin towards WHCO1 cancer cells. This suggests that complex **3.13** may not be toxic towards all cell types and its activity should be investigated against various other cell-lines.

3.4.4. Antiparasitic activity against *T. vaginalis*

The salicylaldimine ligands (**3.2** – **3.7**) and their complexes (**3.9** – **3.14**) described in this chapter were screened for potential activity against the G3 strain of *T. vaginalis*. The data are presented in Figure 3.19. All the compounds were screened at a concentration of 50 µM.

At the tested concentration, more than half of the tested compounds exhibited less than 50% parasite inhibition. The dimeric 3-OMe ligand (**3.6**) displayed the lowest activity giving a percentage inhibition of about 10%. With respect to the monomeric ligands (**3.2** – **3.4**), the 3-OMe substituted (**3.3**) and unsubstituted (**3.4**) ligands exhibited comparable activity (49% inhibition observed). The 5-Cl derivative (**3.2**) showed slightly lower activity in this series. Contrary to this, the 5-Cl derivative of the dimeric ligands (**3.5** – **3.7**), mononuclear complexes (**3.9** – **3.11**) and binuclear complexes (**3.12** – **3.14**) exhibited the best activity in each series of compounds. The 5-Cl binuclear complex (**3.12**) exhibited the best activity overall, comparable to metronidazole at the tested concentration (100% inhibition). Slightly less active was the 3-OMe binuclear complex (**3.13**).

It is noteworthy that the compounds containing the 5-Cl derivative (with the exception of **3.2**) showed enhanced activity compared to the compounds where R = 3-OMe and R = H. Metronidazole is a 5-nitroimidazole compound, its activity appears to be dependent on the presence of the electron-withdrawing NO_2 group.[35] Perhaps in this case the electron withdrawing 5-Cl moiety imparts a similar biological effect as the 5-NO_2 group for metronidazole. It has been suggested that free radical production may be a possible mode of action for this drug.[35] The chlorido substituent has previously been shown to enhance the antiparasitic activity of some metal–containing compounds towards trichomoniasis. For example, palladium(II) thiosemicarbazones (Figure 3.20a) containing this group showed considerable parasite inhibition against the T1 strain of *T. vaginalis*.[36] In a separate study, ruthenium–arene thiosemicarbazone complexes possessing a chlorido moiety on the aryl ring (Figure 3.20b) exhibited promising activity against the G3 strain of *T. vaginalis*. It was also observed that the binuclear complexes **3.12 – 3.14**) displayed enhanced activity compared to their corresponding free ligands (**3.5 – 3.7**).

One of the possible reasons why the compounds containing the 5-Cl group displayed good activity may be a consequence of their lipophilicity. As shown in Table 3.8, the 5-Cl ligands are more lipophilic compared to the rest of the ligands in each case. In addition to this, incorporation of cyclooctadiene moieties further increases the lipophilic character of the complexes. As a result, the binuclear complexes (**3.12** – **3.14**) exhibited moderate to high parasite inhibition. This would also account for the enhanced activity of the binuclear complexes compared to their ligands. This phenomenon was also evident for some compounds described in chapter 2. The more lipophilic ferrocene-containing compounds showed slightly enhanced inhibition compared to organic derivatives.[37]

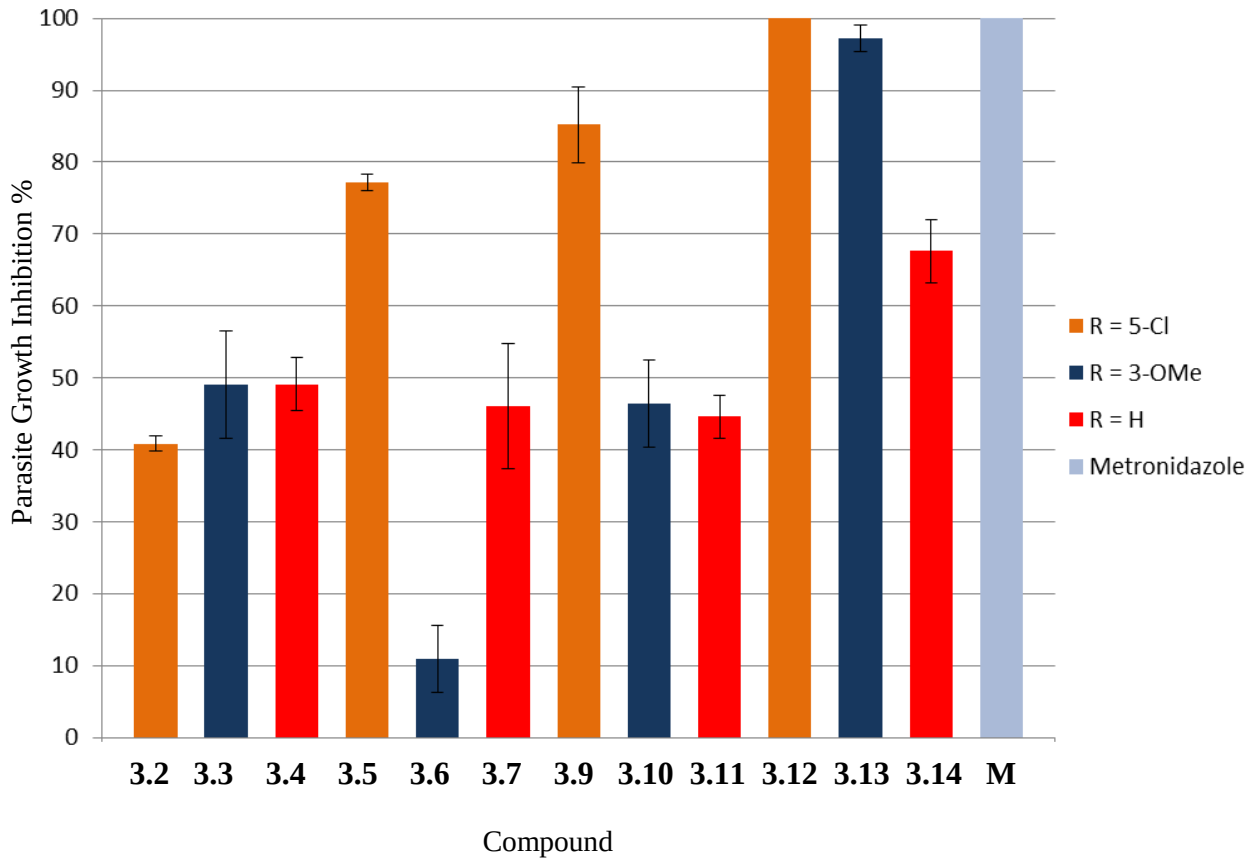
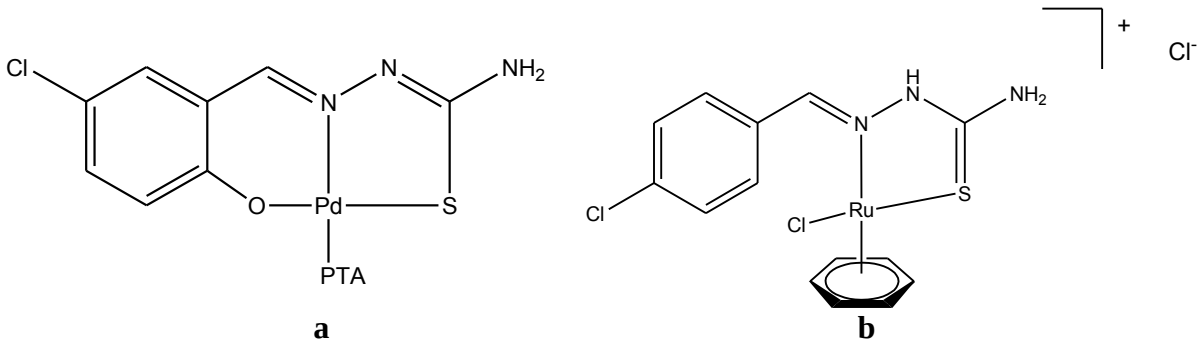


Figure 3.19: Percentage parasite inhibition of salicylaldimine ligands and their corresponding Rh(I) complexes.

Lipophilicity has been shown to be an important factor that influences antiparasitic activity. For example, 2-pyridyl pyrimidines tested for their antiplasmodial and antileishmanial activity have shown a correlation between increased lipophilicity and good activity.[38] In another study, some lipophilic tetracyclines showed enhanced parasite growth inhibition over non-lipophilic derivatives against various strains of *T. vaginalis*.[39] In many cases, it is believed that the uptake of these compounds is dependent on the lipophilicity. Increased lipophilicity aids in the transport of the compounds across various cell membranes and in turn, results in sufficient accumulation of compounds inside of cells.



PTA = phosphatriazaadamantane

Figure 3.20: (**a**) Palladium(II) thiosemicarbazone complex and (**b**) ruthenium-arene complex showing promising activity against *T. vaginalis*.

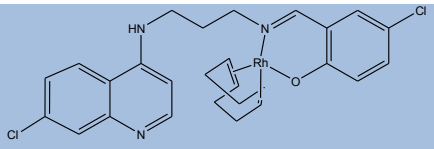
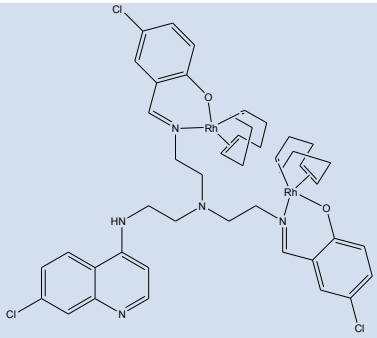
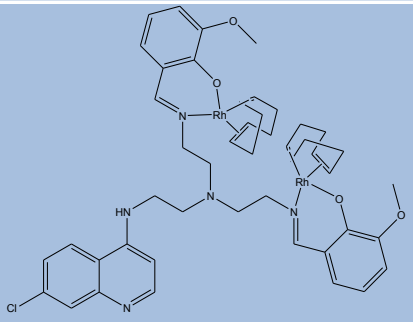
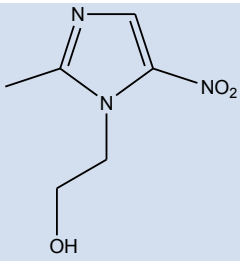
Compounds **3.9**, **3.12** and **3.13** exhibited parasite inhibition greater than 80%. These compounds were further evaluated in order to obtain IC_{50} values for a better comparison of their activity to metronidazole. The IC_{50} values determined from this study are shown in Table 3.11. Complex **3.12** exhibited the highest activity giving an IC_{50} value of 4.8 µM. Comparatively, this compound was found to be less active than metronidazole (IC_{50} = 0.72 µM) despite both of these compounds showing 100% parasite growth inhibition at 50 µM. Metronidazole is therefore more effective than **3.12** because a lower concentration of the drug can be used to obtain high parasite growth inhibition. Complex **3.13** displayed similar activity to **3.12** as an IC_{50} value of 6.1 µM was observed for this compound. The percent growth inhibition observed for this compound was approximately 97%. The 5-Cl mononuclear complex (**3.9**) exhibited activity 2-fold lower than **3.13** (IC_{50} = 12 µM).

Despite the fact that the tested compounds were found not to possess activity comparable to metronidazole, some valuable insight has been obtained from this study. Firstly, it is further emphasised that the chlorido substituent plays an important role in antiparasitic activity, including activity against *T.vaginalis*. This further supports the preparation of compounds possessing this particular moiety. Furthermore it was also observed that the lipophilicity of the compound is also important. In this case, increased lipophilicity, due to the multimeric nature of the compounds, appeared to affect parasite growth inhibition favourably. In addition to this, metal complexes remain an attractive class of compounds as alternative antiparasitic therapies

due to their enhanced parasite growth inhibition properties compared to their corresponding ligands.

Table 3.11

IC_{50} values obtained for selected compounds against the G3 strain of *T. vaginalis in vitro*.

compound	structure	IC_{50}(μM)[a] ± SE
3.9		12 ± 0.06
3.12		4.8 ± 0.54
3.13		6.1 ± 0.88
Metronidazole		0.72[b]

[a] IC_{50} represents the micromolar equivalents of test compounds required to inhibit parasite growth by 50%.

[b] IC_{50} value taken from reference 40.

3.5. Summary

A small series of mono- (**3.2** – **3.4**) and bis-imine (**3.5** – **3.7**) quinoline ligands based upon different salicylaldehydes were prepared using template procedures. The ligands contained either electron withdrawing groups (5-Cl derivatives), electron donating substituents (3-OMe derivatives) or these ligands were unsubstituted salicylaldimine derivatives. The salicylaldimine ligands were afforded in various yields (40 – 83%). Once the ligands were successfully obtained, their corresponding neutral Rh(I) 1,5-cyclooctadiene complexes (**3.9** – **3.14**) were prepared (yields: 52 – 79%). The ligands and the complexes were characterised using standard spectroscopic and analytical techniques including NMR and IR spectroscopy, ESI or EI mass spectrometry. The characterisation data suggested coordination of the metal to the ligand in a bidentate manner to the imine nitrogen and the phenolic oxygen and not at the quinoline nitrogen.

The ligands and their complexes were screened for their antiplasmodial activity *in vitro* against two strains of *P. falciparum*. Against the chloroquine-sensitive strain (NF54), the monomeric ligands (**3.2** – **3.4**) and the mononuclear complexes (**3.9** – **3.11**) exhibited enhanced activity compared to their dimeric counterparts (**3.5** – **3.7** and **3.12** – **3.14**). In the resistant strain (K1), the monomeric ligands and complexes experienced a loss of activity as was evident by the large RI values, suggesting that these complexes possess a similar activity profile to that of chloroquine. The dimeric derivatives maintain the same activity in the resistant strain compared to the sensitive strain. In some cases, there was a slight enhancement in activity.

The compounds were evaluated for their ability to inhibit β-haematin formation in order to elucidate a possible mechanism of action for these compounds. There appeared to be a relationship between the size of the molecules and their ability to inhibit β-haematin formation. The monomeric ligands (**3.2** – **3.4**) were less efficient at inhibiting β-haematin formation but exhibited similar activity to CQ. Their corresponding rhodium complexes (**3.9** – **3.11**) appeared to inhibit β-haematin formation to a larger extent. The dimeric salicylaldimine ligands (**3.5** – **3.7**) and complexes (**3.12** – **3.14**) also showed enhanced activity compared to the monomeric ligands. This may be a consequence of increased lipophilicity of the larger molecules.

The compounds were also screened for their cytotoxicity against WHCO1 oesophageal cancer cells in order to evaluate their activity. In most cases, the dimeric derivatives (**3.5** – **3.7** and **3.12** – **3.14**) showed increased cytotoxicity compared to their monomeric counterparts. The most cytotoxic complex was the 3-OMe binuclear Rh(I) complex (**3.13**). Generally, with the exception of the latter complex, the metal complexes were slightly less cytotoxic than their corresponding ligands. Comparing the antiplasmodial activity to the cytotoxicity, the monomeric compounds showed selectivity towards malaria parasites. The dimeric compounds appeared to show similar activity towards malaria parasites and cancer cells. This suggests that cytotoxicity may be a reason for the antiplasmodial activity as well.

The antiparasitic activity of the compounds against the G3 strain of *T. vaginalis* was also evaluated. It was observed that the binuclear Rh(I) complexes (**3.12** – **3.14**) exhibited enhanced activity compared to the monomeric counterparts as well as their corresponding ligands. The 5-Cl bis-salicylaldimine Rh(I) complex (**3.12**) showed the highest activity against the parasite. Increased lipophilicity appears to promote enhanced activity. In addition to this, it was also observed that compounds possessing the 5-Cl moiety exhibited promising antiparasitic activity.

Overall, the prepared compounds, specifically the monomeric derivatives, showed some potential as antimalarial agents. One major downfall is that the monomeric compounds showed decreased activity in the CQ-resistant strain of *P. falciparum* compared to the sensitive strain. The next step would be to attempt to enhance the activity of these compounds in the resistant strain. This could possibly be done by altering the lipophilic properties of the compounds by incorporating ferrocene as part of these systems. This may help overcome cross-resistance experienced by these compounds in a similar way that ferroquine is able to overcome the resistance experienced by chloroquine.

3.6. References

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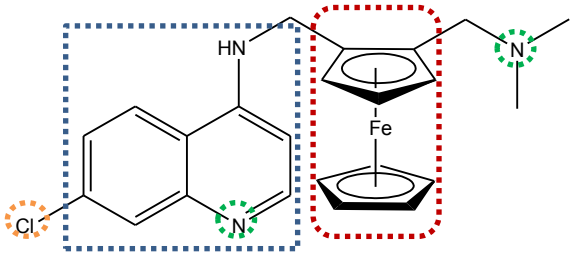
CHAPTER 4

Synthesis, characterisation and biological evaluation of ferrocenyl-containing quinolines and salicylaldimines

4.1. Introduction

As mentioned previously, malarial parasites rapidly develop resistance towards many antimalarials, treatment of this disease remains problematic. Ferroquine (Figure 4.1) has the ability to overcome resistance experienced by chloroquine and is therefore an important model for the development of other compounds that are equipotent and that are able to overcome resistance. Ferroquine has recently completed phase IIb clinical trials.[1] Its mode of action is not fully understood, but it is believed that this compound is also able to hinder haemozoin formation like chloroquine due to the presence of the 4-aminoquinoline moeity.[2] Ferroquine is also able to accumulate more efficiently inside of the digestive vacuole of the parasite compared to chloroquine.[3] Once inside of the digestive vacuole, ferroquine inhibits haemozoin formation strongly and is also able to generate reactive oxygen species (ROS). The generation of ROS permanently damages the parasite.[3-5]



Fc moiety results in the compound overcoming PfCRT resistance possibly by means of ROS generation

Weak bases aid in vacuolar accumulation

4-aminoquinoline necessary for haemozoin crystal growth inhibition

Electron withdrawing group for correct charge distribution and haemozoin crystal growth inhibition

Figure 4.1: Ferroquine, a potent antimalarial that has completed Phase IIb clinical trials

Non-quinoline based ferrocenes have also been investigated as antiplasmodial agents. For example, ferrocenyl chalcones were evaluated against the K1 strain of *P. falciparum.* The compounds exhibited activity in the low micromolar range. The derivative containing a 4-nitro-phenyl group (Figure 4.2) showed the highest activity. The ferrocenyl motif appeared to contribute favourably towards antiplasmodial activity. Its redox behaviour may be a reason for this.[6]

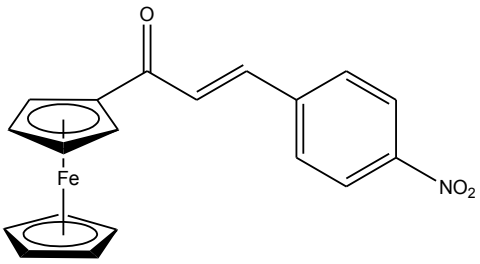
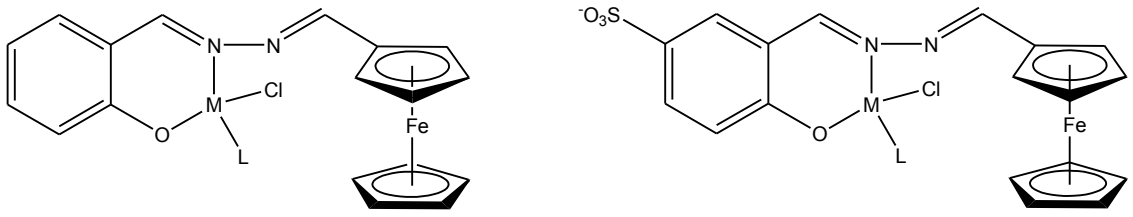


Figure 4.2: Ferrocenyl chalcone exhibiting antiplasmodial activity against the K1 strain of *P. falciparum*

More recently, ferrocenyl azines and various metal complexes thereof (Figure 4.3) were synthesised and evaluated for their antiplasmodial activity. Neutral as well as anionic salicylaldimine ruthenium(II), rhodium(III) and iridium(III) complexes were tested in order to establish potential activity.[7,8] The neutral compounds (Figure 4.3a) exhibited moderate activity against the NF54 chloroquine-sensitive strain of *P. falciparum*, giving values in the low micromolar range. The metal complexes displayed enhanced activity compared to the salicylaldimine ligand.[7] In a separate study, water-soluble anionic versions (Figure 4.3b) were evaluated, but these compounds were less active than their neutral analogues.[8] It appears that the enhanced water-solubility diminishes activity, possibly due to insufficient uptake of the compounds by the parasite. A way to remedy this would be to enhance their lipophilicity, which may in turn result in increased activity. In addition to this, incorporation of a metal with square-planar geometry may enhance interactions with haematin/haemozoin, which may result in enhanced activity. This is discussed in the current chapter.



a **b**

M = Ru(II); Rh(III); Ir(III)

L = *p*-cymene; Cp*

Figure 4.3: Ferrocenyl azines that showed moderate to weak antiplasmodial activity

In addition to antiparasitic activity; various metallocenes, including ferrocene-containing compounds, have also exhibited a wide range of biological properties.[9] This includes antitumour properties.[9] Many ferrocene-based compounds have attracted interest due to their chemical stability and non-toxic nature, making them suitable for biological use. Ferrocene itself is also easily functionalised in order to afford diverse variations. All of these factors need to be considered when developing new therapies. In this chapter, ferrocenyl moieties have been incorporated as part of various systems in the hope to afford biologically active compounds.

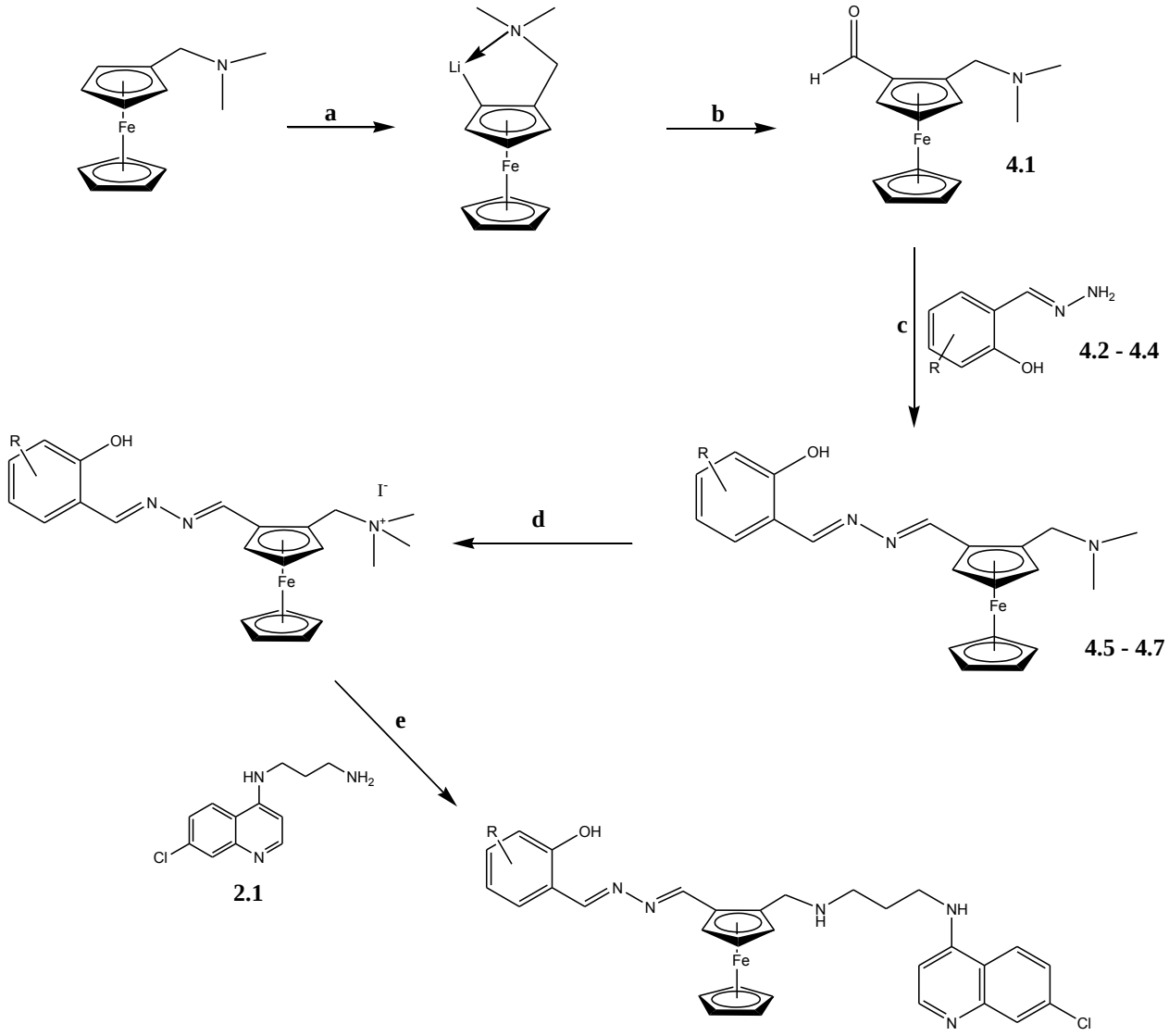
4.2. Synthesis and characterisation of ferrocenyl salicylaldimines 4.5 – 4.7, quinolines 4.9 – 4.11 and some Rh(I) complexes 4.12 – 4.14

4.2.1. Synthesis

Since the monomeric salicylaldimine systems mentioned in chapter 3 showed promising antiplasmodial activity in the NF54 CQ-sensitive strain of *P. falciparum*, it was decided to attempt to improve on the activity of these compounds in the resistant strain (since these compounds showed loss of activity in the resistant strain compared to the sensitive strain). This was proposed by incorporating ferrocenyl moieties as part of these systems. This would hopefully result in improved activity in the resistant strain, as can be seen with the activity of ferroquine compared to chloroquine. The initial idea to prepare salicylaldimine ferrocenyl quinolines involved the method outlined in Scheme 4.1. This procedure involved the preparation of ferrocenyl-containing salicylaldimines **4.5** (R = 5-Cl), **4.6** (R = 3-OMe) and **4.7**

(R = H), followed by quaternisation of the tertiary amine using iodomethane to form the desired quaternary ammonium salts. The salts could then be reacted with *N'*-(7-chloroquinolin-4-yl)-propane-1,3-diamine (**2.1**) in the hope to afford the desired quinoline-containing ferrocenyl salicylaldimines. Compounds **4.5** – **4.7** were prepared by means of Schiff-base condensation reactions between the (dimethylamino)methyl ferrocenecarboxaldehyde (**4.1**) and various salicylaldehyde hydrazones (**4.2** (R = 5-Cl), **4.3** (R = 3-OMe), **4.4** (R = H)).

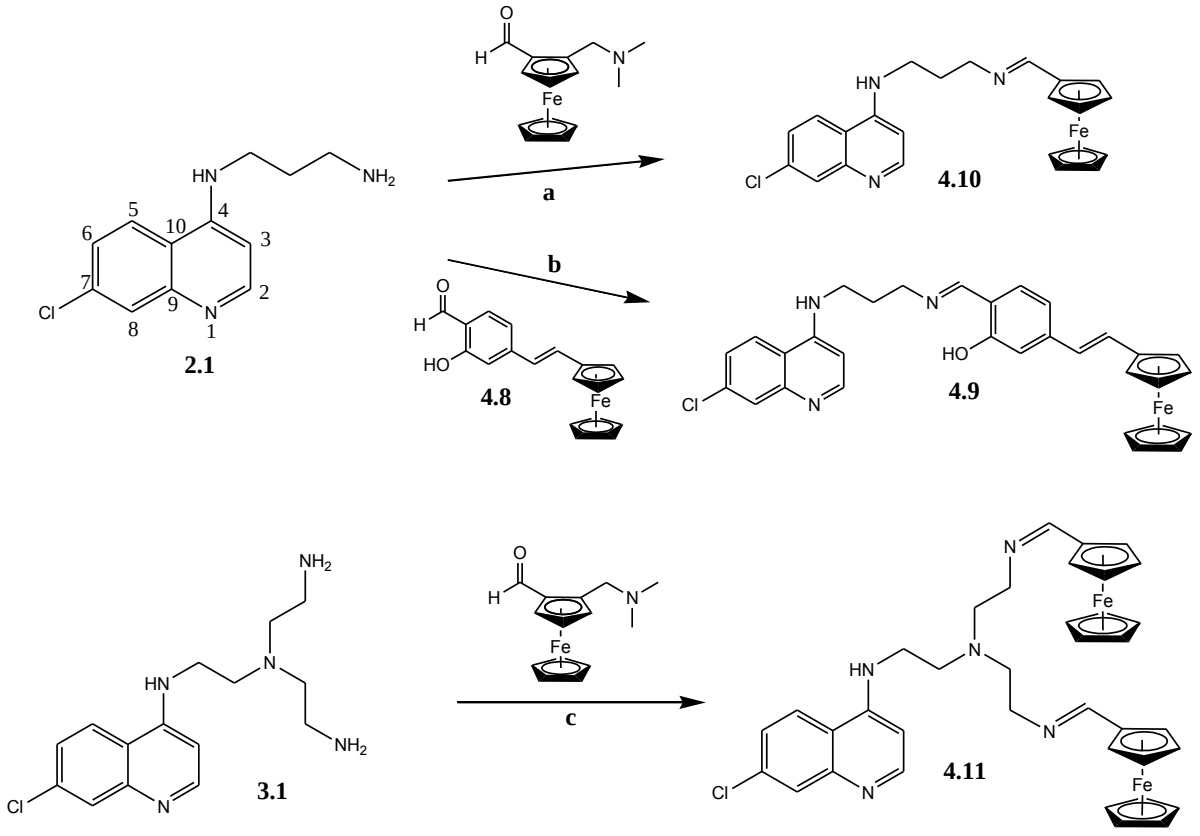
Compound **4.1** was prepared by means of *ortho*-lithiation of (dimethylamino)methyl ferrocene using *n*-butyllithium in hexanes to afford the lithiated species *in situ* (Scheme 4.1). The dimethylamino group is *ortho*-directing and thus the *ortho*-metalated product was obtained.[10] The lithium derivative was then reacted with *N*,*N*-dimethylformamide giving, after the work-up procedure, the desired 1,2-disubstituted product **4.1**. Formation of the 1,2-disubstituted derivative is mainly attributed to the added stability of the lithiated intermediate. The dimethylamino group is able to stabilise the alkali metal. Aldehyde **4.1** was then reacted with either 5-chlorosalicylaldehyde hydrazone (**4.2**), 3-methoxysalicylaldehyde hydrazone (**4.3**) or salicylaldehyde hydrazone (**4.4**) in diethyl ether to afford salicylaldimine ferrocenes **4.5** – **4.7**. The hydrazones were prepared by modification of literature procedures.[11,12] The hydrazones were condensed with **4.1** in a 1:1 stoichiometric ratio. After purification by means of silica gel chromatography, ferrocenyl salicylaldimines **4.5** – **4.7** were obtained as red oils in moderate to good yields (66 – 88%). These ligands were soluble in common solvents including diethyl ether, dichloromethane, chloroform and dimethyl sulfoxide.



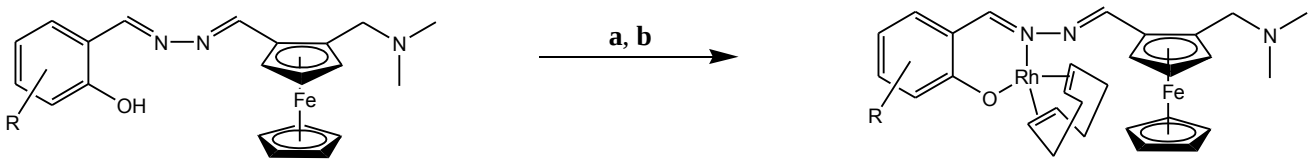
Scheme 4.1: Synthesis of ferrocenyl derivatives **4.1** and **4.5** – **4.7**. Reagents and conditions: (a) (dimethylamino)methyl ferrocene (1 eq.), *n*-BuLi (1.45 eq.), Et_2O, 16 h, r.t. (b) DMF (1.25 eq), 4 h, r.t. (c) appropriate salicylaldehyde hydrazone (1 eq.), Et_2O, 16 h, r.t. (d) MeI (1.1 eq.), $(CH_3)_2CO$, 4 h, r.t. (e) *N'*-(7-chloroquinolin-4-yl)-propane-1,3-diamine, CH_3CN, r.t. or reflux.

Quaternisation of the tertiary amine of **4.5** was carried out using iodomethane and the resulting ammonium salt was reacted with *N'*-(7-chloroquinolin-4-yl)-propane-1,3-diamine (**2.1**) in acetonitrile under reflux conditions in the presence of K_2CO_3. The ferrocenyl precursor appeared unstable upon heating at elevated temperatures. The reaction was also attempted at ambient temperature for a prolonged time period, but starting material was recovered. It was then decided that since the substituent on the aryl ring of the salicylaldimine quinoline compounds discussed in chapter 3 did not have a significant influence on antiplasmodial activity, the ferrocenyl and salicylaldimine moieties could then be incorporated differently onto the quinoline component (Scheme 4.2). *N'*-(7-Chloroquinolin-4-yl)-propane-1,3-diamine (**2.1**) was reacted with the ferrocenyl salicylaldehyde **4.8**[13] via a Schiff-base condensation reaction (Scheme 4.2) to afford the salicylaldimine ferrocenyl quinoline **4.9** in good yield. In addition to this, a non-salicylaldimine containing mono-ferrocenyl quinoline (**4.10**) and a bis-ferrocenyl quinoline (**4.11**) were also prepared (Scheme 4.2) in order to probe the importance of the salicylaldimine motif for biological activity (see later sections). Compound **4.9** was specifically prepared in order to compare the effect of having a salicylaldimine, quinoline and ferrocene moiety present in the same compound to compare its activity to those compounds mentioned in chapter 3 (**3.2** – **3.4**) and compound **4.10**. Compounds **4.10** and **4.11** were prepared by means of Schiff-base condensation reactions between ferrocenecarboxaldehyde and *N'*-(7-chloroquinolin-4-yl)-propane-1,3-diamine (**2.1**) in the case of **4.10** or *N*-(7-chloroquinolin-4-yl)-tris(2-aminoethyl)amine (**3.1**) in the case of **4.11**.

The corresponding rhodium(I) cyclooctadiene complexes of ferrocenyl salicylaldimine ligands **4.5** – **4.7** were also prepared (Scheme 4.3) in order to evaluate the effect of the metal on biological activity compared to the ligands. Rh(I) was chosen due to its square-planar geometry. This may aid in interactions with haem. Complexes **4.12** – **4.14** were prepared by firstly deprotonation of the phenolic proton using excess NaH in dichloromethane, followed by addition of 0.5 eq of $[RhCl(COD)]_2$ (**3.8**).[14] The complexes were obtained as orange-red powders in excellent yields (94 – 98%). The complexes were soluble in organic solvents such as dichloromethane, chloroform and dimethyl sulfoxide.



Scheme 4.2: Synthesis of ferrocenyl quinolines **4.9** – **4.11**. Reagents and conditions: (a) Compound **2.1** (1 eq.), ferrocenecarboxaldehyde (1.1 eq.), Et_2O, 16 h, r.t. (b) Compound **2.1** (1 eq.), ferrocenyl aldehyde **4.8** (1 eq.), DCM/MeOH, 16 h, r.t. (c) Compound **3.1** (1 eq.), ferrocenecarboxaldehyde (2 eq.), EtOH, 16 h, r.t.



4.5 - 4.7 **4.12 - 4.14**

R = 5-Cl (**4.5**, **4.12**); 3-OMe (**4.6**, **4.13**); H (**4.7**, **4.14**)

Scheme 4.3: Synthesis of ferrocenyl salicylaldimine Rh(I) complexes. Reagents and conditions: (a) Salicylaldimine ligand (1 eq.), NaH (excess), DCM, 1.5 h, r.t. (b) $[RhCl(COD)]_2$ (0.5 eq.), 3 h, r.t.

4.2.2. Characterisation

All the synthesised compounds were characterised using standard techniques such as ^{1}H and $^{13}C\{^{1}H\}$ NMR spectroscopy, infrared spectroscopy and high resolution (HR) ESI mass spectrometry. 2D NMR spectroscopy was also used in the assignment of some signals.

NMR spectroscopy

The ^{1}H NMR spectra of these compounds were recorded in $CDCl_3$ and the spectra were consistent with the proposed structures. Selected ^{1}H NMR resonances for compounds **4.5** – **4.7** and **4.9** – **4.11** are given in Table 4.1. The details are discussed in the subsequent text.

Table 4.1

Selected ^{1}H NMR resonances for **4.5** – **4.7** and **4.9** – **4.14**

compound	HC=N (Fe)	HC=N	OH	H2a (m, $^{2}J_{H\text{-}H}$ Hz)	H2b (m, $^{2}J_{H\text{-}H}$ Hz)	Cp_{unsub}
4.5	8.66	8.59	11.88	3.29 (d, 13.01)	3.73 (12.92)	4.18
4.6	8.66	8.67	12.18	3.32 (d, 12.61)	3.74 (13.01)	4.19
4.7	8.66	8.67	11.89	3.30 (d, 12.97)	3.72 (12.96)	4.18
compound	**H2**	**H3**	**H8**	**HC=N**	**NH**	**Cp_{unsub}**
4.9	8.54	6.43	7.93	8.35	5.10	4.16
4.10	8.50	6.36	7.93	8.18	6.76	4.18
4.11	8.48	6.33	7.89	8.10	6.66	4.31
compound	**HC=N (Fe)**	**HC=N**	**H-COD_{exo}; H-COD_{endo}**		**HC=CH_{COD}; HC=CH_{COD}**	**Cp_{unsub}**
4.12	7.98	7.58	1.95; 2.46		3.98; 4.60	4.20
4.13	8.00	7.68	1.95; 2.47		3.98; 4.70	4.20
4.14	8.01	7.67	1.95; 2.48		3.98; 4.62	4.21

The spectra of precursors **4.1 – 4.4** compared favourably with literature.[10-12] In the case of precursor **4.1**, the structure of the product was confirmed by the appearance of a signal at 10.03 ppm, which is indicative of the proton of the aldehyde moiety. This particular signal was absent in the spectra of (dimethylamino)methyl ferrocene. Successful synthesis of hydrazones **4.2** – **4.4** was confirmed by the appearance of signals for the imine protons in the range of 7.77 and 7.86 ppm. Further to this, broad singlets at about 5.5 ppm confirmed that the mono-condensed products were obtained. These signals were assigned to the protons of the NH_2 group. The method outlined for the synthesis of compounds **4.5** – **4.7** results in the formation of racemic mixtures. The chirality of ligands **4.5** – **4.7** was evident in their ^{1}H NMR spectra. The CH_2 protons adjacent to the dimethylamino group (assigned as H2) are diastereotopic, thus two signals were observed for these protons. In an achiral environment, these protons would be observed as one singlet, but since the metallocene exhibits planar chirality due to the fact that the ferrocene is 1,2-disubstituted, the protons are non-equivalent. The environments above and below the substituted cyclopentadienyl ring are different. This resulted in one of the methylene protons (e.g. H2a) to be closer to the Fe centre compared to the other (e.g. H2b). This chemical non-equivalence causes the protons to couple to each other. This therefore resulted in the appearance of two doublets with coupling constants of about 13 Hz in each case.

Schiff-base condensation for **4.5** – **4.7** was confirmed by the appearance of two imine proton signals in each spectrum. A singlet for the proton of the newly formed imine bond was observed at 8.66 ppm for ligands. The imine signal adjacent to the salicylaldimine moiety appeared in the region between 8.59 and 8.67 ppm for **4.5** – **4.7**, while signals for the hydroxyl protons of these ligands were observed between 11.88 and 12.18 ppm. Three signals were observed for the protons of the substituted Cp ring in the range of 4.5 and 4.9 ppm. Singlets for the unsubstitued Cp ring were assigned at *ca*. 4.18 ppm. For compounds **4.9** – **4.11**, Schiff-base condensation was confirmed by the appearance of a singlet at 8.35, 8.18 and 8.10 in the spectra of **4.9**, **4.10** and **4.11**, respectively, for the imine proton. The integration was in agreement with the proposed compounds. The ^{1}H NMR spectrum of **4.9** is depicted in Figure 4.4.

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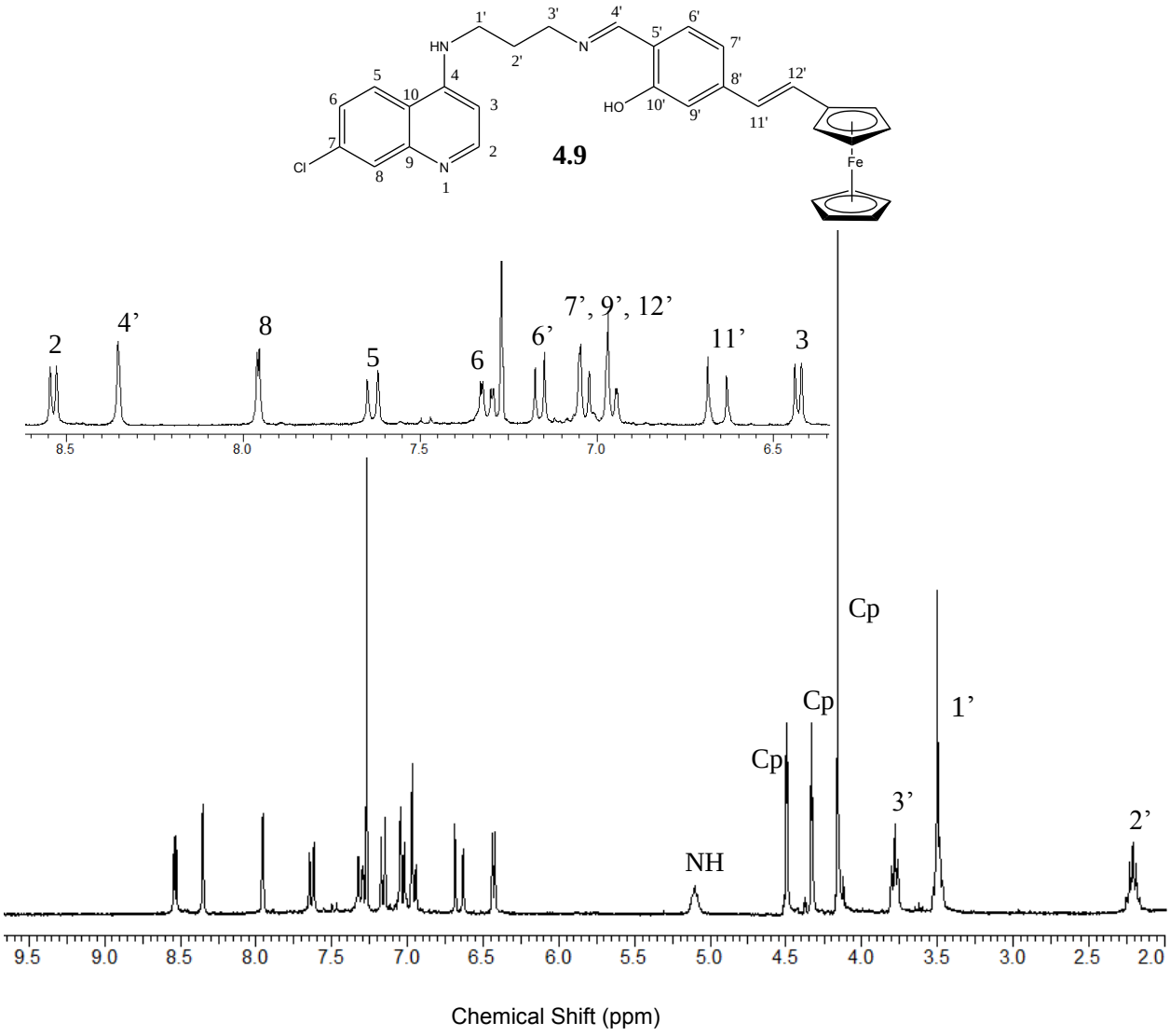


Figure 4.4: ^{1}H NMR spectrum of compound **4.9** in $CDCl_3$

Signals for the aromatic protons of the quinoline motif appeared in the expected region for **4.9** – **4.11**. A doublet was observed for each H2 proton (numbering of the quinoline ring shown in Scheme 4.2) of the quinoline ring and occurred in the region of 8.48 and 8.54 ppm, while the signal for H3 was observed between 6.33 and 6.43 ppm. A broad signal was assigned to the NH proton in each case. For compound **4.9**, this particular signal was more shielded than the same signal in the spectra of **4.10** and **4.11**. In the case of **4.9**, this signal resonated at 5.10 ppm, while in the case of **4.10** and **4.11** it was seen at 6.66 and 6.76 ppm, respectively. Signals for the protons of the unsubstituted Cp ring were observed between 4.16 and 4.31 ppm. A typical AA’BB’ spin system was seen in the spectra for the substituted Cp ring. In the case of

4.9 and **4.10**, two triplets were observed in each spectrum for the $H_{\alpha,\alpha'}$ and $H_{\beta,\beta'}$ protons of the Cp ring. These signals resonated as two multiplets in the spectrum of **4.11**

The ^{1}H NMR spectra of compounds **4.12** – **4.14** were in agreement with the proposed complexes. The ^{1}H NMR spectrum of complex **4.13** can be seen in Figure 4.5. Firstly, the broad singlet attributed to the phenolic proton in the spectra of the ligands were absent from the spectra of the complexes. This suggested coordination to the metal via the phenolic oxygen. An upfield shift was observed for the signals of both imine protons in the spectra of the complexes. A shift of about 1 ppm was observed for the imine proton adjacent to the salicylaldimine moiety. This confirmed bidentate coordination to the phenolic oxygen and salicylaldimine nitrogen. As a result, the imine proton resonance adjacent to the ferrocenyl motif shifted upfield by about 0.6 ppm. In addition to this, complexation was further confirmed by the presence of signals for the cyclooctadiene (COD) moiety in the expected region. Four signals were observed due to the *trans* influence of the nitrogen and oxygen donor atoms of the ligand. The COD_{exo} protons were seen as a multiplet at 1.95 ppm, while the COD_{endo} signals were observed at approximately 2.47 ppm. Two multiplets for the olefinic protons were assigned at 3.98 and *ca*. 4.6 ppm. This compared favourably with the spectra of the complexes discussed in chapter 3 and similar complexes found in literature.[15-19] The signal for the unsubstituted Cp ring remained constant upon coordination, it was observed at approximately 4.20 ppm.

The $^{13}C\{^{1}H\}$ NMR spectra of ligands **4.5 – 4.7** and **4.9 – 4.14** further supported formation of the desired compounds. The expected number of signals was observed in each spectrum. Table 4.2 gives selected $^{13}C\{^{1}H\}$ NMR data. The imine carbon signals of the salicylaldimine moiety of ligands **4.5** – **4.7** were observed in the region between 162.99 and 163.91 ppm. This signal was particularly deshielded in the case of the 5-chloro derivative (163.91 ppm) (**4.5**) compared to the unsubstituted derivative (**4.7**), which was attributed to the electron withdrawing ability of the substituent. The carbon signals of the imine adjacent to the ferrocenyl moiety resonated between 161.23 and 162.60 ppm. The CH_2 carbon signals were seen at approximately 57.1 ppm, while the carbon atoms of the unsubstituted Cp ring resonated around 70 ppm. For ligands **4.9** – **4.11**, the imine carbon atom signals were observed in the range of 162.00 and 165.37 ppm. The signals of the quinoline ring were consistent in all three spectra. Signals for the carbon atoms C2, C3 and C8, resonated at

approximately 152, 99 and 128 ppm, respectively. These data are also consistent with data obtained for the quinoline compounds described in chapter 3.

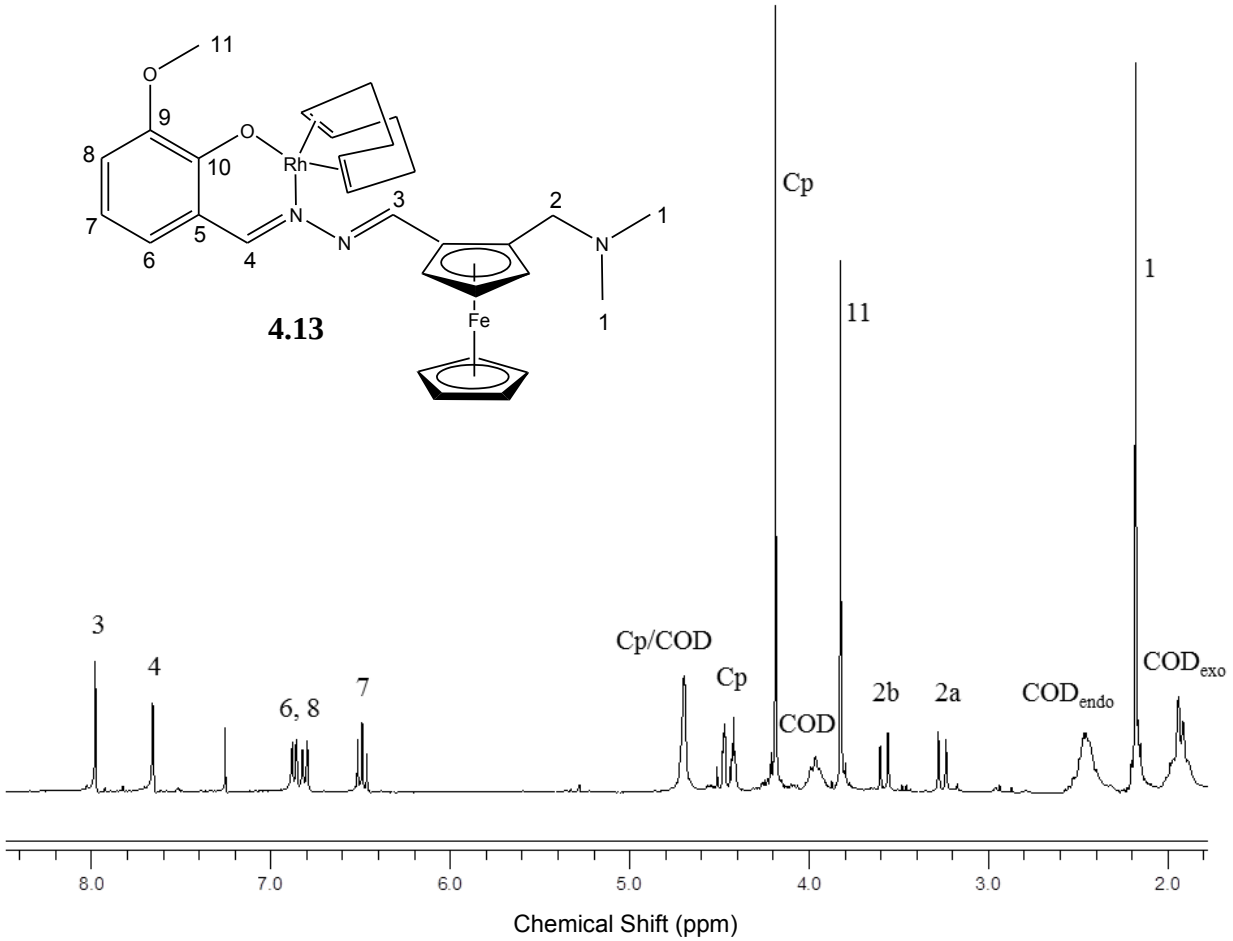


Figure 4.5: ^{1}H NMR spectrum of complex **4.13** in $CDCl_3$

Table 4.2

Selected $^{13}C\{^{1}H\}$ NMR resonances for **4.5** – **4.7** and **4.9** – **4.14**

compound	H*C*=N (Fe)	H*C*=N	*C*H$_2$	NCH$_3$	Cp$_{unsub}$
4.5	161.23	163.91	57.18	44.96	70.12
4.6	162.54	163.22	57.11	44.87	70.09
4.7	162.60	162.99	57.18	45.01	70.06
compound	**H*C*=N**	***C*2**	***C*3**	***C*8**	**Cp$_{unsub}$**
4.9	165.37	151.97	99.11	128.81	69.32
4.10	162.00	152.17	98.57	128.63	69.16
4.11	162.04	151.95	99.01	128.31	69.05
compound	**H*C*=N (Fe)**	**H*C*=N**	**H*C*=CHCOD (m, *JC-Rh* Hz); HC=*C*HCOD (m, *JC-Rh* Hz)**		**Cp$_{unsub}$**
4.12	157.65	152.06	73.38 (d, 13.36); 85.43 (d, 12.12)		70.03
4.13	157.55	152.99	72.77 – 73.35 (m); 84.86 (10.71)		70.00
4.14	157.57	153.12	72.86 (d, 13.46); 85.02 (d, 12.12)		69.99

Complexation of ligands **4.5** – **4.7** was confirmed by the appearance of signals for the COD moiety in the expected regions. In the case of compounds **4.12** and **4.14**, two doublets were observed for the olefinic carbon atoms at about 73 ppm (for olefinic carbon atom *trans* to oxygen) and 85 ppm (for olefinic carbon atoms *trans* to nitrogen). In the case of compound **4.13**, a doublet at 85 ppm was assigned to the olefinic carbon atoms *trans* to nitrogen, while a multiplet was observed in the region of 72.77 – 73.35 ppm for the olefinic carbon atoms *trans* to oxygen. The aliphatic carbon atoms of the COD moiety appeared at 29 and 31 ppm for compounds **4.12** – **4.14**.

IR spectroscopy

Infrared spectroscopy further confirmed that the desired ligands and complexes were obtained. Characteristic bands for the azine (-C=N-N=C-) motif of **4.5** – **4.7** were observed in the range of 1616 and 1618 cm^{-1}. In each case, one strong absorption band appeared for both C=N bonds, this suggests an overlap of the two frequencies. Absorption bands for the C=C aromatic moieties were observed between 1587 and 1582 cm^{-1}. In the case of complexes **4.12** – **4.14**, these bands shifted to lower wavenumber, due to the metal withdrawing electron density away from the nitrogen of the azine moiety. This in turn causes elongation of the C=N bond, and therefore a shift was observed. Figure 4.6 shows the IR spectra of ligand **4.7** and its corresponding complex **4.14**. In the case of the complexes, bands for the azine moiety were observed around 1600 cm^{-1}. Absorption bands for the C=C stretching frequencies also shifted to lower wavenumbers compared to the ligands. These absorption bands appeared between 1574 and 1576 cm^{-1}. Schiff-base condensation to afford **4.9** – **4.11** was confirmed by the appearance of absorption bands at 1611, 1638 and 1639 cm^{-1} for the C=N stretching frequencies, respectively. Absorption bands for the C=N stretching frequencies of the quinoline ring of **4.10** and **4.11** appeared at 1613 and 1611 cm^{-1}, respectively. This particular absorption band for **4.9** overlaps with the Schiff-base C=N stretching frequency and therefore only one absorption band was observed.

Mass spectrometry

High resolution ESI mass spectrometry was used to confirm the molecular weights of the synthesised compounds. Analysis of the mass spectra of ligands **4.5** – **4.7** and **4.9** – **4.11**, confirmed that the desired compounds were obtained. With the exception of **4.9**, the spectra of the ligands showed base peaks for the protonated molecular ion ($[M+H]^+$). In the case of **4.9**, a base peak corresponding to $[M+2H]^{2+}$ was observed. Peaks corresponding to $[M+H]^+$ were observed in the spectra of complexes **4.12** – **4.14**. In all cases, the assignments were in close agreement with the calculated values.

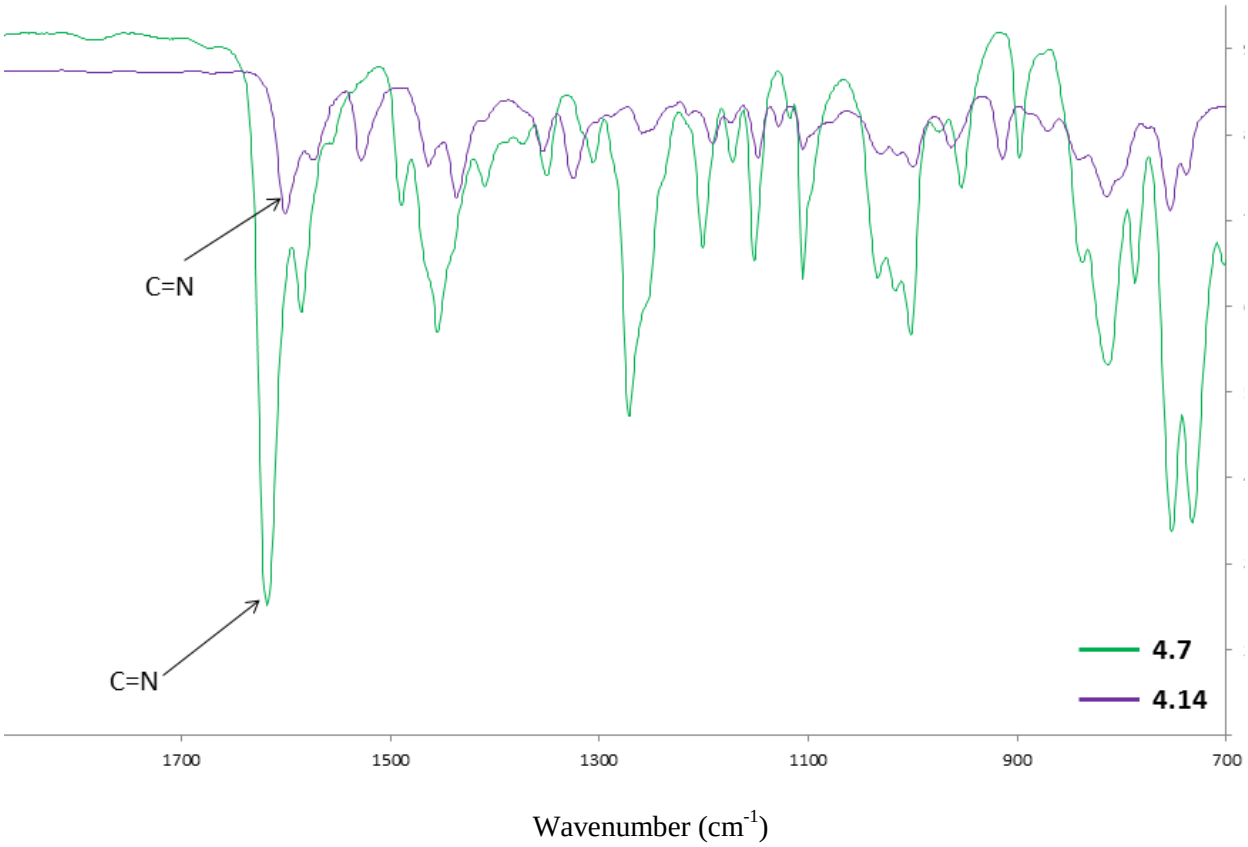


Figure 4.6: IR spectra of ligand **4.7** (green) and corresponding complex **4.14** (purple) obtained in the solid state.

The afore-mentioned characterisation data clearly supports preparation of the proposed ferrocenyl quinolines, salicylaldimines and rhodium(I) complexes. Based on satisfactory correlation of this data and the proposed structures, the biological activities of these compounds were investigated and the data are described in the next section.

4.3. Biological and physicochemical evaluation

4.3.1. Antiparasitic activity against *P. falciparum*

Many quinoline-based compounds are effective antimalarials, but resistance remains a major issue. As discussed previously, ferrocenyl compounds such as ferroquine are able to counteract this resistance. Chapter 1 highlighted various examples of ferrocene-containing derivatives that exhibited promising antiplasmodial activity. The compounds discussed in this chapter (quinoline and non-quinoline) were therefore screened for activity against two strains

i.e. the NF54 CQS and K1 CQR strains of *P. falciparum*. It was expected that the non-quinoline compounds would not experience the same fate as many quinoline-containing compounds regarding resistance. The results obtained from a preliminary screen are presented in Table 4.3. The values of CQ and FQ are also given.

Table 4.3

In vitro antiplasmodial activity and resistance indices of compounds **4.5** – **4.7**, **4.9** – **4.14,** CQ and FQ against the NF54 CQ-sensitive and the K1 CQ-resistant strains of *P. falciparum*.

compound	IC_{50}[a] ± SE NF54[b] (µM)	IC_{50} ± SE K1[c] (µM)	resistance index[d]	logP[e]
4.5	32 ± 2	14 ± 2	0.44	4.04
4.6	101 ± 36	13 ± 2	0.13	3.12
4.7	11 ± 5	50 ± 36	4.50	3.31
4.9	nd[f]	0.82 ± 0.28	nd	6.97
4.10	0.083 ± 0.01 (n = 2)	0.59 ± 0.03 (n = 2)	7.11	4.51
4.11	0.72 ± 0.04	0.65 ± 0.04 (n = 2)	0.90	7.36
4.12	18 ± 7	3.9 ± 0.04	0.22	nd
4.13	9.6 ± 2.5	8.1 ± 1.9	0.85	nd
4.14	7.9 ± 4.2	11 ± 3	1.41	nd
CQ	0.025	0.30	11.84	4.63[g]
FQ	0.033	0.014[g]	0.42	5.1[g]

[a]IC_{50} represents the micromolar equivalents required to inhibit parasite growth by 50%

[b]NF54 chloroquine-sensitive strain of *P. falciparum;* n = number of data sets averaged, n = 3,

[c]K1 chloroquine-resistant strain of *P. falciparum;* n = number of data sets averaged, n = 3,

[d]Resistance index (RI) = IC_{50}K1/IC_{50}NF54

[e]logP predicted using the method described in chapter 2

[f]compound not active at the tested concentration (1000 ng/ml)

[g]Literature value[20]

Antiplasmodial activity against the NF54 CQ-sensitive strain of P. falciparum

The data obtained from this screen are preliminary findings. Compound **4.10**, a quinoline–containing derivative, was found to be the most active, exhibiting an IC_{50} value of 0.083 µM. Compound **4.11** displayed slightly lower activity but still exhibited an IC_{50} value less than

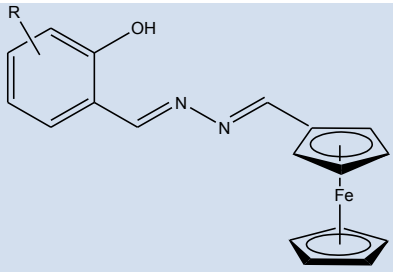
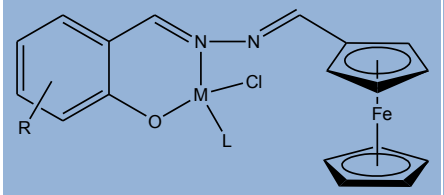
1 µM. One of the reasons for its lower activity may be a consequence of its high lipophilicity. A predicted logP of 7.36 was observed for this compound. Compounds that are too lipophilic may experience difficulty accumulating inside of the digestive vacuole of the parasite because they might interact more with various cell membranes and therefore may not be transported effectively. Surprisingly, the remaining quinoline derivative, **4.9** was not active at the tested concentration (1000 ng/ml). This was an unexpected result and further tests are required in order to quantify its activity.

The activity of the afore-mentioned quinolines was also compared to the activity of ligands **3.2** – **3.7** discussed in chapter 3. Monomeric salicylaldimine ligands **3.2** – **3.4** exhibited potent activity (0.052 – 0.072 µM) in this strain, in the same range as CQ and FQ. Compound **4.10** also displayed similar activity to these compounds which suggests that the salicylaldimine motif does not contribute significantly towards the activity of **3.2** – **3.4**. Compound **4.11** also showed a significant increase in activity compared to dimeric salicylaldimines **3.5** – **3.7**. This suggests that substitution of the salicylaldimine moiety by ferrocene, results in enhanced activity in this case.

The ferrocenyl azine ligands **4.5** – **4.7** exhibited variable activity ranging from 11 to 101 µM. The complexes (**4.12** – **4.14**) exhibited activity in the low micromolar range and were more active than their respective ligands. In each case, the unsubstituted salicylaldimine derivative was the most active. These compounds, however, did not exhibit comparable activity to **4.10**. The lower activity was mainly attributed to these compounds not possessing a bioactive quinoline moiety. The activity of **4.5** – **4.7** and **4.12** – **4.14** were also compared to the activity of similar ferrocenyl azines depicted in Table 4.4. The IC_{50} values were taken from literature.[7,8] One series of compounds were neutral (**4.15** and **4.17** – **4.19**), while the other was a series of water-soluble, anionic compounds (**4.16** and **4.20** – **4.21**). When the activities of compounds **4.5** – **4.7** were compared to the activity of **4.15** and **4.16** in this particular strain, compounds **4.5** and **4.15** were found to exhibit similar antiplasmodial activity. Compound **4.6** was least active, while **4.7** exhibited the highest activity. Complexes **4.12** – **4.14** were moderately active but complexes **4.17** – **4.19** displayed better activity.

Table 4.4

Antiplasmodial activity of similar ferrocenyl azine derivatives (**4.15** – **4.22**) against the NF54 CQS strain.[7,8]

compound	structure	IC_{50} (μM)
4.15: R = H **4.16**: R = 5-SO_3^-		**4.15**: 33 **4.16**: 55
4.17: R = H, M = Ru(II), L = *p*-cymene **4.18**: R = H, M = Rh(III), L = Cp* **4.19**: R = H, M = Ir(III), L = Cp* **4.20**: R = 5-SO_3^-, M = Ru(II), L = *p*-cymene **4.21**: R = 5-SO_3^-, M = Rh(III), L = Cp*		**4.17**: 3.9 **4.18**: 5.8 **4.19**: 6.0 **4.20**: 47 **4.21**: 57

Complexes **4.12** – **4.14** and **4.17** – **4.19** all displayed enhanced activity compared to the water-soluble derivatives **4.20** and **4.21**. From this data it was evident that lipophilicity plays an important role on the activity of these compounds. The water-soluble derivatives **4.20** and **4.21**, are probably not able to accumulate sufficiently inside of the digestive vacuole of the parasite due to their hydrophilicity and were therefore less active. A correct balance between hydrophilicity and lipophilicity is required in order for compounds to be transported effectively into the parasite. Incorporation of the (dimethylamino)methyl group as part of these systems did not appear to have a significant effect on activity. The substituents on the salicylaldimine ring also did not appear to affect activity favourably. The unsubstituted derivative showed the best activity in each family. All of the non-quinoline based compounds that were tested did not exhibit activity comparable to CQ or FQ in this strain and none exhibited IC_{50} values lower than 1 μM.

Antiplasmodial activity against the K1 CQ-resistant strain of P. falciparum

In order to obtain information about the behaviour of these compounds in CQR strains, all the compounds were screened against the K1 strain of *P. falciparum*. The data are given in Table 4.4. Overall, compounds **4.9** – **4.11** exhibited good activity against this strain of *P.*

falciparum, giving values less than 1 µM (0.82, 0.59 and 0.65 µM, respectively). This is similar to data obtained for ligands **3.2** – **3.4** in the same strain. Figure 4.7 depicts the resistance index values for the Fe-based compounds and also for **3.2** – **3.7**.

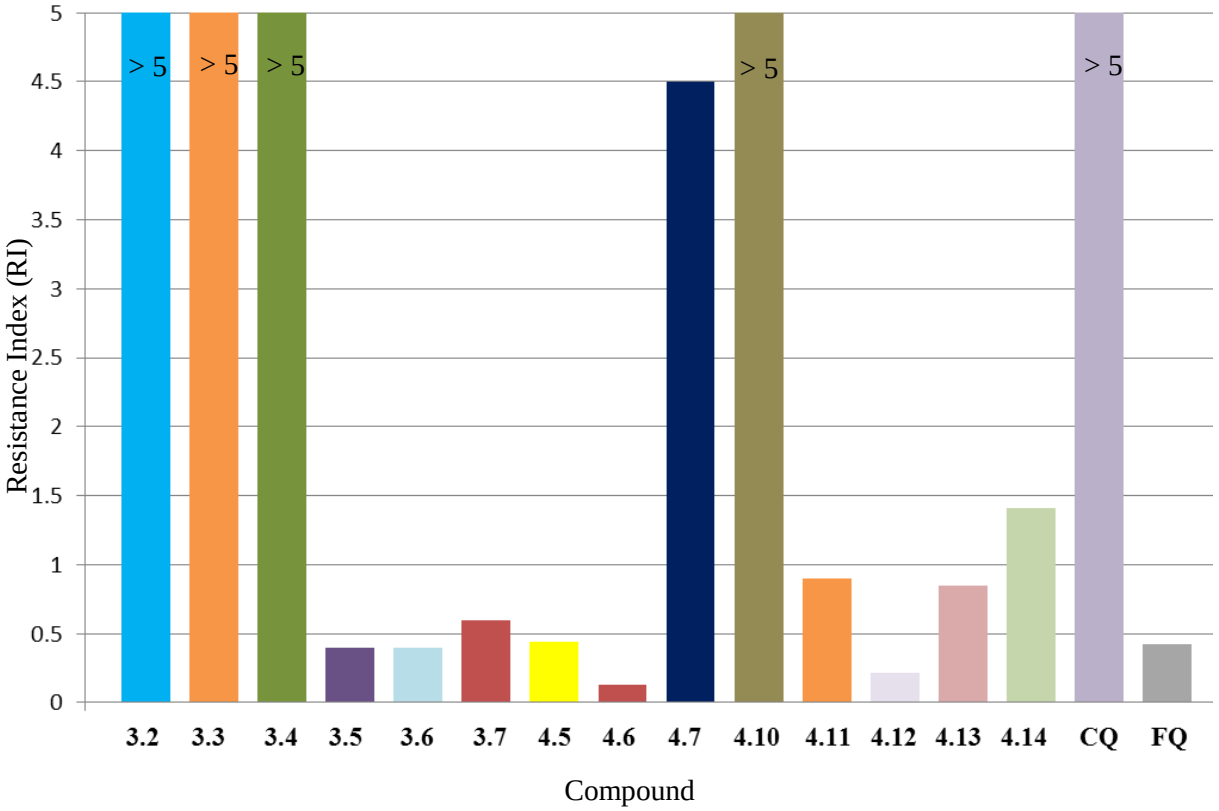


Figure 4.7: Resistance indices for compounds **3.2** – **3.7**, **4.5** – **4.7**, **4.9** – **4.14**, CQ and FQ

Compound **4.9** was more active in the resistant strain compared to the sensitive strain and therefore an IC_{50} value could be obtained at the tested concentration. Contrary to this, the activity of **4.10** decreased in the resistant strain compared to the sensitive strain, this was also observed for the salicylaldimine derivatives **3.2** – **3.4** mentioned in chapter 3. As can be observed in Figure 4.7, compounds possessing the tris(2-aminoethyl)amine scaffold, **3.5** – **3.7** and **4.11** exhibited resistance indices lower than values obtained for **3.2** – **3.4** and CQ. As mentioned in chapter 3, intramolecular hydrogen bonding may occur in these systems, similar to FQ. This may aid in these compounds overcoming the cross-resistance experienced by the monomeric analogues. Compound **4.11** was more active than its corresponding salicylaldimine derivatives **3.5** – **3.7** (IC_{50} = 2.3, 1.8 and 4.5 µM, respectively) in the resistant strain. This was also observed in the sensitive strain. All ferrocenyl azines, with the exception of **4.7** and its corresponding complex (**4.14**), exhibited increased activity in the resistant strain.

RI values were less than 1 in these cases. In this strain the unsubstituted compounds were less active compared to the 5-Cl and 3-OMe derivatives. The activity of **4.7** decreased 4.5 times in the K1 strain compared to the NF54 strain. The activity of **4.14** also declined slightly. The 5-Cl complex (**4.12**) exhibited the best activity in this series. Despite the observation that most of these compounds exhibited enhanced activity in this strain, none of the compounds displayed activity close to FQ, CQ or even compounds **3.2** – **3.4**, further emphasising the importance of the quinoline moiety for antiplasmodial activity.

4.3.2. β-Haematin inhibition studies

The compounds described in this chapter were also screened for potential β-haematin inhibition activity using the NP-40 detergent mediated assay in order to rationalise their antiplasmodial activity. Figure 4.8 depicts the β-haematin inhibition activity for compounds **4.5** – **4.7** and **4.9** – **4.14**.

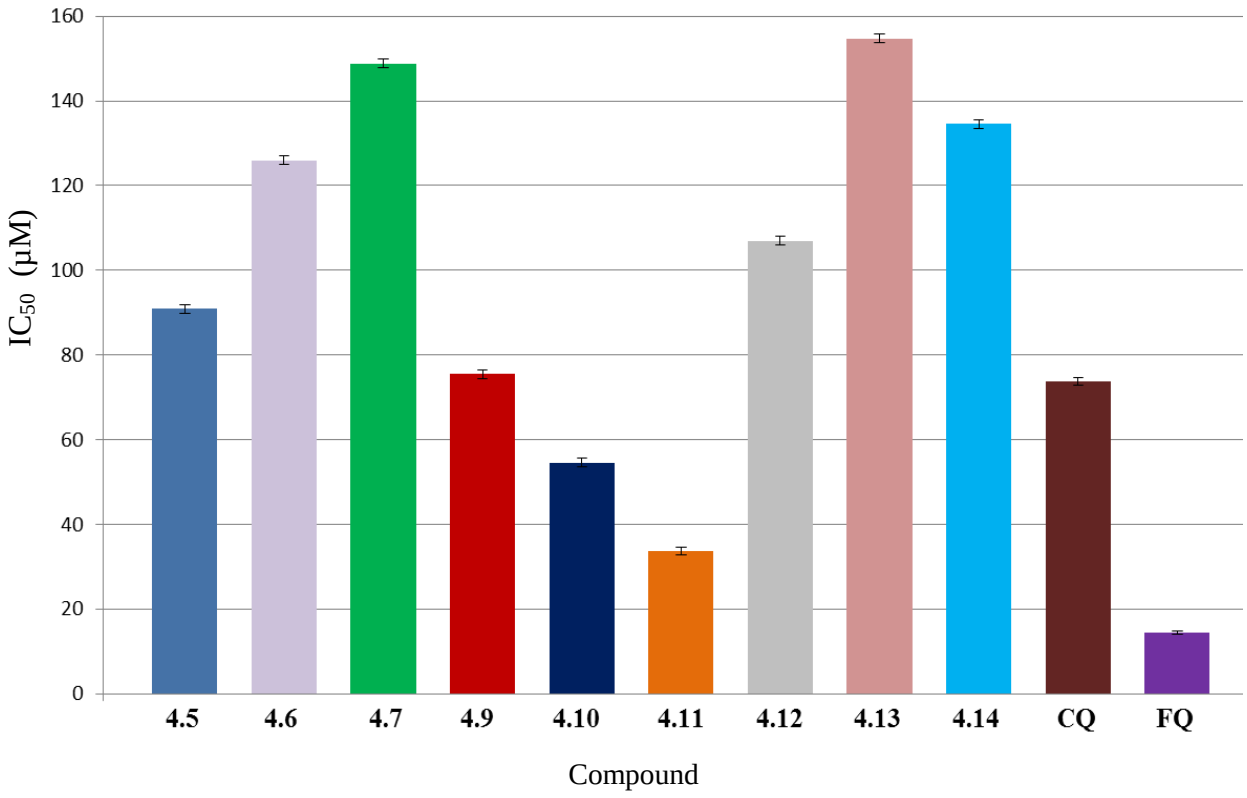


Figure 4.8: IC_{50} values obtained for the salicylaldimine ferrocenyl ligands (**4.5** – **4.7**) and complexes (**4.12** – **4.14**), quinoline ferrocenes (**4.9** – **4.11**), CQ and FQ towards β-haematin inhibition activity.

Compounds **4.5** – **4.7** weakly inhibited β-haematin formation, displaying IC_{50} values greater than 90 μM. This was expected because these compounds did not possess quinoline moieties, which is usually required for strong β-haematin inhibition activity. The activity of the ligands decreased upon complexation for **4.12** and **4.13**. The activity of **4.7** however**,** improved slightly when complexed. The ligand and complex possessing the 5-Cl group showed the best activity in each series. This is consistent with data obtained for the β-haematin inhibition activity of the monomeric salicylaldimine ligands (**3.2** – **3.4**) and complexes (**3.9 – 3.11**) discussed in chapter 3. Compound **4.9** exhibited similar activity to chloroquine in this study, while ferroquine displayed the highest β-haematin inhibition activity. Compound **4.10** showed slightly improved activity compared to **4.9**, while compound **4.11** exhibited high activity.

The β-haematin inhibition activity of the ferrocenyl azines **4.5** – **4.7** and **4.12 – 4.14** were compared to that of similar ferrocenyl derivatives depicted in Table 4.4. The β-haematin inhibition activities of these compounds were taken from literature.[7,8] Compounds **4.15** and **4.17** – **4.19** exhibited enhanced β-haematin inhibition activity compared to **4.5** – **4.7** and **4.12** – **4.14**. Compounds **4.5** – **4.7** and **4.12** – **4.14** did however exhibit enhanced activity compared to similar water-soluble derivatives **4.16**, **4.20** and **4.21**. It is apparent from this information that lipophilicity plays a role in inhibition of haem aggregation. From information acquired in earlier chapters, it was expected that compounds that were more water-soluble would exhibit lower β-haematin inhibition activity. Since compounds **4.5** – **4.7** and **4.12** – **4.14** exhibited low β-haematin inhibition activity, their antiplasmodial activity may be a consequence of their ability to generate free radicals. This has been shown to be the case in some ferrocene-based compounds that exhibited antiplasmodial activity.[6] Ferroquine is also believed to be able to generate hydroxyl radicals from H_2O_2 in the parasitic digestive vacuole by means of a Fenton-like reaction. This causes severe damage to the membranes of the parasite.[21] The quinoline compounds (**4.9** – **4.11**) were able to inhibit β-haematin formation to a larger extent compared to the non-quinoline derivatives. No simple correlation can be made between the β-haematin inhibition and antiplasmodial activity, intracellular factors such as compound uptake and accumulation probably also greatly affects the antiplasmodial activity of these compounds confounding simple correlation.

4.3.3. *In vitro* cytotoxicity studies

Metallocenes have been known to possess various biological activities.[22,23] Many ferrocenyl compounds specifically, have exhibited antitumour and cytotoxic activities.[24-27] One of a few promising ferrocenyl-based drug candidates discovered was hydroxyferrocifen (Figure 4.9).[25] This was discovered when the organic drug hydroxytamoxifen was altered by substituting its phenyl ring with a ferrocene unit and screened for activity.[25]

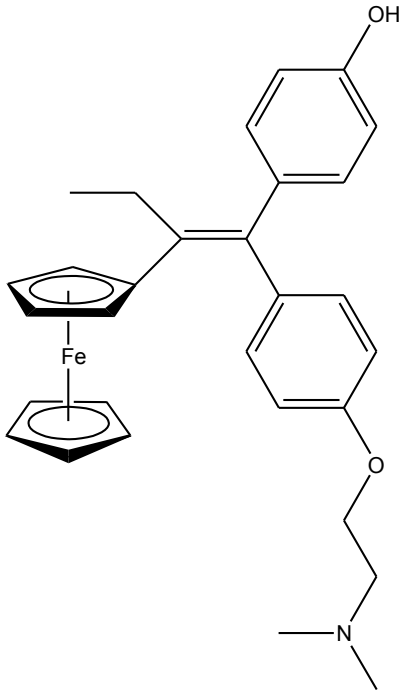


Figure 4.9. Hydroxyferrocifen, a potential ferrocene-based anticancer agent

Based on this, all the ferrocenyl compounds described in this chapter were screened for cytotoxicity against WHCO1 oesophageal cancer cells. This was also carried out in order to gain insight about the selectivity of these compounds towards malarial parasites. All of the ferrocenyl quinoline derivatives exhibited activity against this particular cell-line. Compound **4.12** was the only non-quinoline compound that exhibited cytotoxic activity. The rest of the non-quinoline derivatives (**4.5** – **4.7**, **4.13** and **4.14**) were not active at the tested concentration. CQ and FQ were also tested for their activity in this cell-line. The data are presented in Table 4.5.

Table 4.5

IC_{50} values obtained for **4.9 - 4.12**, CQ and FQ against WHCO1 cancer cells[a]

compound	IC_{50} (µM)	95% confidence interval	selectivity index[b]	selectivity index[c]
4.9	10.0	8.4 – 11.8	nd	12
4.10	5.8	5.1 – 6.6	70	10
4.11	8.0	7.2 – 9.0	11	12
4.12	9.7	9.0 – 10.4	0.54	2
CQ	6.3	5.7 – 6.9	252	21
FQ	17.3	13.1 – 22.8	523	1234
cisplatin	13.0[d]	-	-	-

[a] Values were determined from a dose response curve (assayed with MTT), IC_{50} represents the micromolar equivalents of test compounds required to inhibit 50% cell viability.

[b] IC_{50}WHCO1/IC_{50}NF54

[c] IC_{50}WHCO1/IC_{50}Dd2

[d] literature value[28]

Compound **4.10** was the most cytotoxic, exhibiting an IC_{50} value of 5.8 µM. Compound **4.9** and **4.12** displayed similar cytotoxicity to each other, with **4.11** showing slightly higher activity to **4.9**. CQ and **4.10** exhibited comparable activity, while FQ was the least cytotoxic against this cell-line (IC_{50} = 17.3 µM). All the compounds, with the exception of FQ, exhibited higher cytotoxicity than cisplatin. The activity of **4.10** was compared to that of monomeric salicylaldimines **3.2 – 3.4** and **3.9** – **3.11**. Compound **4.10** showed increased cytotoxicity compared to these salicylaldimine derivatives. The activity of **4.9** was compared to that of **3.2** – **3.4** and showed slightly lower cytotoxicity compared to the salicylaldimine derivatives. Compound **4.11** displayed similar activity to the monomeric salicylaldimines **3.2** – **3.4**. The enhanced activity of the quinolines against this cell-line compared to the non-quinoline compounds may be due to their ability to target DNA. Many quinoline-based compounds have been shown to interact with DNA by means of intercalation.[29]

Selectivity indices (SI) were calculated for these compounds for both the chloroquine-sensitive strain (NF54) and the chloroquine-resistant strain (K1) of *P. falciparum*. Figure 4.10 compares the selectivity indices for the tested compounds (numerical values are shown in Table 4.5).

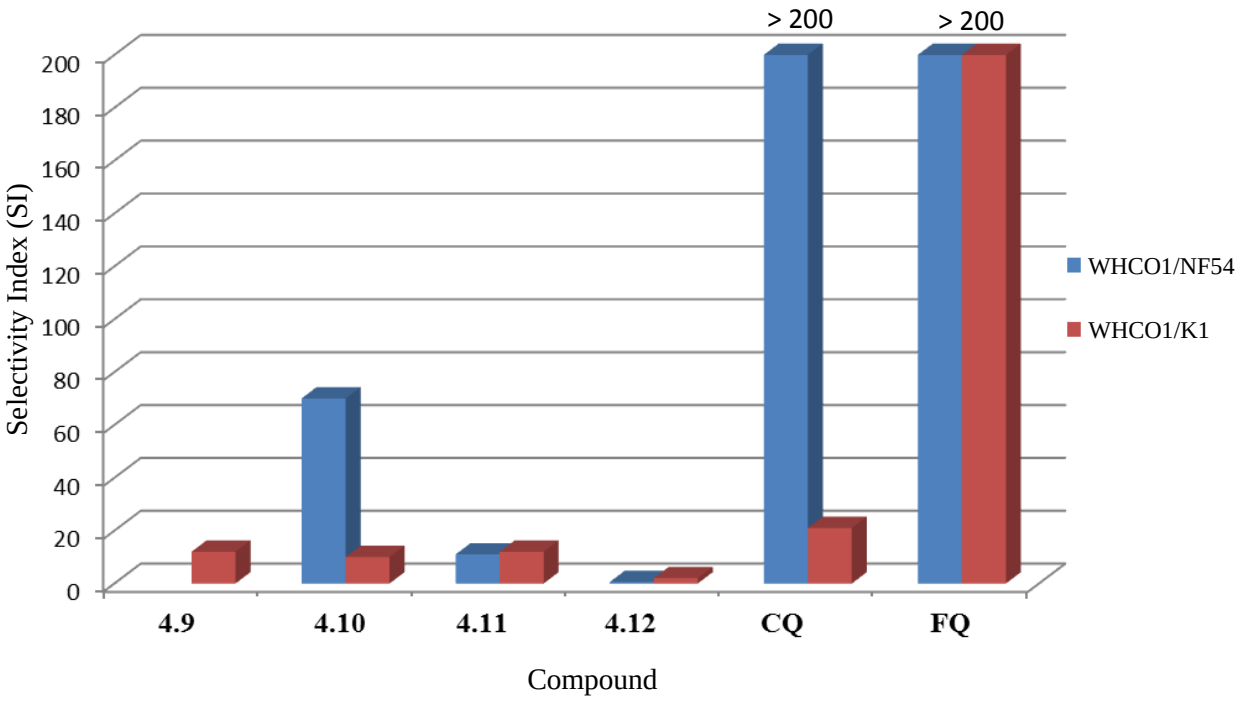


Figure 4.10: Selectivity indices obtained for **4.9** – **4.12**, CQ and FQ.

CQ and FQ exhibited large SI values (> 200) when comparing this cell-line and the CQS strain of *P. falciparum*. These compounds displayed potent activity against the NF54 strain and therefore exhibited large SI values. These SI values are greater than those observed for ligands **3.2** – **3.4** (116 – 163) for the same strain. Compound **4.10** exhibited lower selectivity than CQ and FQ towards the CQS strain, but this is due to the compound being less active than CQ and FQ against the NF54 strain and in addition to this, it exhibited the highest cytotoxicity. Compounds **4.11** exhibited slightly lower selectivity than **4.10** towards the NF54 strain, but this was a consequence of its lower antiplasmodial activity compared to **4.10**. Compound **4.12** did not exhibit any selectivity towards the CQS strain. In all cases, with the exception of FQ, low selectivity was observed towards the CQR strain of *P. falciparum*. This is mainly a consequence of low antiplasmodial activity of these compounds in the CQ-resistant strain. This was also observed for the monomeric salicylaldimines **3.2** – **3.4** and **3.9** – **3.11**. In the cases where low selectivity was observed, the cytotoxicity of the compounds may explain the antiplasmodial data obtained. In addition to intracellular factors, the cytotoxicity of these compounds may explain why no simple correlation could be discerned between β-haematin inhibition activity and antiplasmodial activity.

4.3.4. Antiparasitic activity against *T. vaginalis*

Since most of the non-quinoline based compounds prepared in this study did not exhibit any appreciable antiplasmodial activity, these compounds, in addition to the quinoline-containing compounds, were screened against the G3 strain of *T.vaginalis* at a concentration of 50 µM in order to evaluate activity against this parasite. The data obtained from this study is presented in Figure 4.11. Chloroquine and ferroquine were also evaluated for potential activity. The percentage parasite growth inhibition activity of metronidazole (M) is also given.

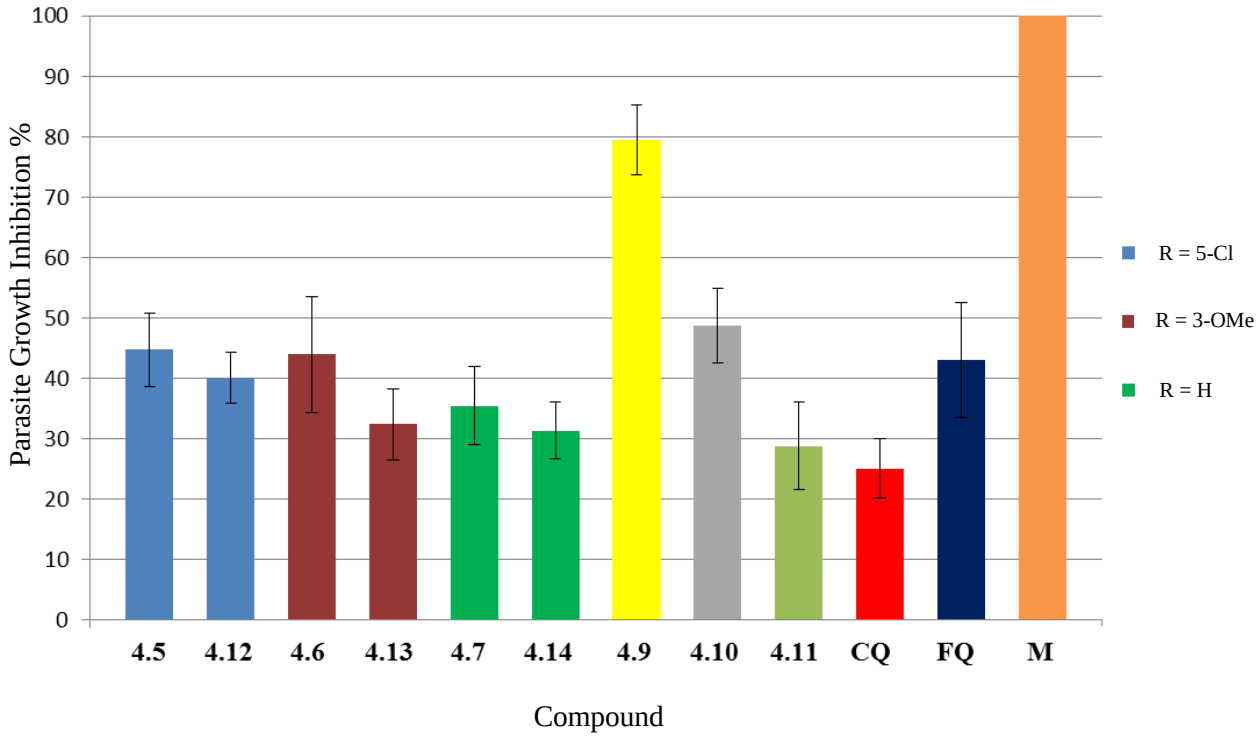


Figure 4.11: Percentage parasite inhibition of salicylaldimine ferrocenyl ligands (**4.5** – **4.7**) and complexes (**4.12** – **4.14**), quinoline ferrocenes (**4.9** – **4.11**), CQ, FQ and M. (same coloured pairs represent ligand and corresponding Rh(I) complex)

With the exception of metronidazole and compound **4.9**, the tested compounds caused less than 50% parasite growth inhibition. Chloroquine exhibited the lowest activity, causing approximately 25% parasite growth inhibition. On the other hand, compound **4.9** displayed the highest activity by inducing about 79% parasite growth inhibition. The second most active compound was **4.10**. This compound inhibited parasite growth by about 49%. The ferrocenyl salicylaldimine ligands **4.5** – **4.7**, inhibited parasite growth modestly. The corresponding metal complexes **4.12** – **4.14**, exhibited lower activity compared to their respective ligands.

Compound **4.5** and **4.12**, the 5-Cl substituted derivatives, exhibited slightly higher parasite growth inhibition compared to the unsubtituted and 3-OMe derivatives. Ferroquine displayed similar activity to ferrocenyl ligands **4.5** and **4.6**. None of the tested compounds exhibited activity comparable to metronidazole at the tested concentration.

The low activity of some of these compounds may be a result of their lipophilicity. Ligands **4.5** – **4.7** exhibited lipophilicities in the range of 3.12 – 4.04, which is low compared to the logP of compound **4.9**, the most active compound. The latter has a logP equal to 6.97. Based on this, it was expected that compound **4.11** would exhibit enhanced activity due to its high lipophilicity (logP = 7.36), but this was not the case. It seemed that the activity of compound **4.9** may be attributed to the various moieties present in the molecule. This compound contains a quinoline, ferrocene as well as a salicylaldimine moiety. Amalgamation of these moieties appeared to significantly increase the activity of the compound against this parasite. Evidence for this was seen for **4.10** and FQ. These compounds do not contain salicylaldimine moieties but do possess quinoline and ferrocene functionalities. These compounds exhibited lower activities than compound **4.9**. Quinoline salicylaldimines **3.2** – **3.4** (discussed in chapter 3) not possessing the ferrocenyl moiety were also less active than compound **4.9**, but exhibited comparable activity to compound **4.10**. The ferrocenyl azines **4.5** – **4.7** and **4.12** – **4.14** also exhibited similar activities to compounds **3.2** – **3.4**.

4.4. Summary

New ferrocenyl azines (**4.5** – **4.7**) containing a (dimethylamino)methyl moiety and their respective Rh(I) complexes (**4.12** – **4.14**) as well as ferrocenyl quinolines (**4.9** – **4.11**) were synthesised and characterised using various spectroscopic and spectrometric techniques. These techniques included ^{1}H and ^{13}C NMR spectroscopy, IR spectroscopy and ESI-mass spectrometry. The data suggested formation of the desired compounds. The ferrocenyl azine ligands were afforded in moderate to good yields (67 – 88 %), while the complexes were afforded in excellent yields (94 – 98%). These ferrocenyl derivatives were prepared in order to compare their activity to the non-ferrocenyl salicylaldimines mentioned in chapter 3. Compound **4.9** was prepared in order to investigate the effect on activity by combining the salicylaldimine, quinoline and ferrocenyl moieties as part of the same system.

A preliminary antiplasmodial screen was conducted against the NF54 CQS and K1 CQR strains of *P. falciparum*. The ferrocenyl azine ligands **4.5** – **4.7** exhibited weak to moderate activity across both parasite strains. Upon complexation the activity increased in all cases but not sufficiently to compare to CQ and FQ. Quinoline **4.10** displayed good activity in the NF54 strain but decreased when examined in the resistant strain. Compound **4.9** did not exhibit activity at the tested concentration in the sensitive strain, but further tests should be conducted in order to quantify its activity. This compound did exhibit increased activity in the resistant strain. Compound **4.11** exhibited good activity in the sensitive strain but lower than **4.10**, possibly a consequence of its lipophilicity. Compounds **4.9** – **4.11** exhibited good activity in the resistant strain, giving IC_{50} values less than 1 μM. Compound **4.10** exhibited similar activity to the monomeric salicylaldimines **3.2** – **3.4** in the sensitive strain. Compounds **4.9** – **4.11** exhibited similar activities to **3.2** – **3.4** in the resistant strain.

The compounds were also screened for their ability to inhibit β-haematin formation using the NP-40 detergent mediated assay. The quinoline-based derivatives exhibited the highest β-haematin inhibition activity. Specifically, compound **4.11** displayed the highest activity. Compound **4.9** exhibited similar activity to CQ, while **4.10** was slightly more active. The ferrocenyl azines **4.5** – **4.7** and **4.12** – **4.14** showed weak β-haematin inhibition activity (IC_{50} values > 90 μM) but compounds possessing the 5-Cl substituent exhibited slightly higher activity to those that did not have this group. There did not appear to be a simple correlation between β-haematin inhibition activity and antiplasmodial activity, but the most potent antiplasmodials (quinoline-based) exhibited higher β-haematin inhibition activity than the non-quinolines, which were less active in the parasite.

The compounds were tested against the WHCO1 cancer cell-line. All of the quinoline-based compounds exhibited activity against this cell-line, including CQ and FQ. Only one of the ferrocenyl azines (**4.12**) displayed activity at the tested concentration. Compound **4.10** exhibited the highest cytotoxicity. CQ and FQ showed high selectivity towards the NF54 CQS strain compared to the WHCO1 cell-line. Compounds **4.10** and **4.11** exhibited moderate selectivity towards the NF54 strain, while **4.12** did not exhibit selectivity towards malarial parasites. Based on this data, it appears that the antiplasmodial activity may be a result of the cytotoxicity of some of these compounds.

Lastly, the compounds were screened against the G3 strain of *T. vaginalis*. All of the compounds, with the exception of **4.9**, exhibited less than 50% parasite growth inhibition. Comparing the activity of the ferrocenyl azine ligands (**4.5** – **4.7**) and complexes, the complexes (**4.12** – **4.14**) exhibited slightly lower activity than their respective ligands. The ferrocenyl azines exhibited lower lipophilicity, which may have been the reason for their low activity. The 5-Cl substituent appeared to slightly enhance the activity of the ferrocenyl azines. Compound **4.9** displayed the highest activity and also a calculated logP of 6.97. The combination of the quinoline, ferrocene and salicylaldimine moieties, in addition to its higher lipophilicity, appeared to have a favourable effect on parasite growth inhibition compared to the rest of the compounds. The activity of this compound was also greater than the activity exhibited by the monomeric salicylaldimines **3.2** – **3.4** mentioned in chapter 3 at the same concentration.

4.5. References

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CHAPTER 5

Conclusions and future outlook

5.1. Conclusions

The aim of this project was to prepare various quinoline and non-quinoline based monomeric and multimeric compounds as potential antiplasmodial agents. Specifically, various amines and polyamines were incorporated as part of these systems. Mono- and multimeric quinoline and ferrocenyl-based thioureas were synthesised by template procedures. Mono- and dimeric salicylaldimine ligands containing a quinoline moiety and corresponding Rh(I) complexes were also prepared by straightforward synthetic procedures. In addition to this, ferrocenyl quinolines as well as ferrocenyl-containing salicylaldimines were synthesised. Selected Rh(I) complexes of the latter were also prepared. All these compounds were characterised using ^{1}H and $^{13}C\{^{1}H\}$ NMR spectroscopy, infrared spectroscopy and mass spectrometry.

All compounds were screened for antiplasmodial activity against the NF54 CQS strain of *P. falciparum*. In addition to this, the compounds were either screened against the Dd2 or K1 CQR strains of *P. falciparum*. *P. falciparum* is the most deadly causative parasite of malaria. In most cases, compounds possessing the quinoline moiety showed moderate to good activity against the CQS strain of *P. falciparum*. In many cases, however, the compounds exhibited lower activity in the CQR strain. This was observed for the monomeric salicylaldimine ligands and complexes. This phenomenon was less severe for the quinoline-based thioureas. In the case where the compound possessed the quinoline, ferrocene and salicylaldimine motifs, enhanced activity was observed in the CQR strain compared to the sensitive strain. With regards to the dimeric salicylaldimine ligands and complexes, it was found that incorporating the second salicylaldimine moiety did not favourably affect the antiplasmodial activity. Incorporation of the metal also did not appear to have a large influence on activity. The lower activity of the dimeric compounds may have been a consequence of their increased lipophilicity. Molecules that are too lipophilic may not accumulate sufficiently inside of the digestive vacuole due to strong interactions with cell membranes. These compounds did however show similar activity in both the CQS and CQR strains of *P. falciparum*, indicating that these compounds may be able to circumvent CQ resistance. Generally, compounds that

did not possess the quinoline motif, specifically the ferrocenyl azines, exhibited weak antiplasmodial activity compared to those that did.

Some of the prepared compounds were potent β-haematin inhibitors. The most potent compounds were those containing more than one quinoline moiety. The most potent being the tetrameric quinoline thiourea. Increasing size and lipophilicity appeared to enhance β-haematin inhibition activity. Surprisingly, two non-quinoline based ferrocenyl thioureas showed moderate β-haematin inhibition activity, this may be attributed to the increased lipophilicity of these compounds. In the case of the salicylaldimine compounds, the dimeric salicylaldimines exhibited higher activity than the monomeric derivatives. Also the mononuclear Rh(I) complexes exhibited greater activity than their corresponding monomeric salicylaldimine ligands. This suggests that incorporation of the lipophilic COD moiety enhances activity. This could also be attributed to the planar nature of the Rh(I) metal ion. The non-quinoline salicylaldimine ferrocenes weakly inhibited β-haematin formation, but the quinoline-containing ferrocenyl compounds exhibited higher activity. Generally, compounds possessing the 5-Cl substituent exhibited slightly better activity than the unsubstituted derivatives and the derivatives containing the 3-OMe group. No correlation between antiplasmodial activity and β-haematin inhibition activity could be established but the most active compounds in the parasite displayed higher β-haematin inhibition activity. This was mainly attributed to the different conditions used for the two assays (since the β-haematin inhibition assay is conducted in a cell-free medium).

The compounds were also screened against the WHCO1 oesophageal cancer cell-line. This was conducted in order to elucidate information about the toxicity of the compounds. Two of the quinoline-based thioureas were cytotoxic, showing IC_{50} values < 10 μM, the tetrameric derivative was the most cytotoxic. The ferrocenyl thioureas did not exhibit high cytotoxicity. The salicylaldimine quinolines also displayed cytotoxicity in the low micromolar range. The monomeric salicylaldimine quinolines appeared to be more selective towards the NF54 strain of the *P. falciparum* compared to cancer cells. The antimalarial activity of the dimeric compounds may be a consequence of their cytotoxicity since these compounds exhibited similar antiplasmodial and anticancer activity. The most cytotoxic thiourea and salicylaldimine compound were tested against KMST-6 human fibroblast. This was to gauge the toxicity of these compounds in normal cells. Both compounds were weakly cytotoxic in

this particular cell-line. This suggested selectivity of the afore-mentioned towards cancer cells. The ferrocenyl quinoline-based compounds all exhibited cytotoxicity against the WHCO1 cell-line, while the ferrocenyl azines were inactive against this cell-line. The activity of the quinoline compounds may be a consequence of their ability to interact with DNA by intercalation or their ability to generate free radicals. It appears that the antiplasmodial activity of some of these compounds may also be a consequence of their cytotoxicity.

The compounds were also evaluated for antiparasitic activity against *T. vaginalis*. In the case of the thioureas, the ferrocenyl compounds exhibited slightly enhanced activity compared to the quinoline-based thioureas. The most effective compound that inhibited parasite growth was the tetrameric ferrocenyl thiourea. The dimeric derivative was slightly less effective. Only one of the quinoline-based ferrocenyl compounds exhibited good activity against this parasite. The combination of the salicylaldimine, ferrocene and quinoline motifs resulted in enhanced activity compared to compounds not possessing all three of these moieties. Compounds possessing the 5-Cl moiety in the quinoline-based and non-quinoline salicylaldimines exhibited enhanced parasite growth inhibition. This further confirmed the importance of this electron withdrawing group on antiparasitic activity, specifically against this parasite. In addition to this, increased lipophilicity appeared to be another factor that contributes favourably towards activity against this parasite. Cytotoxicity may also play a role in the activity of these compounds against this strain. For example, the most cytotoxic binuclear Rh(I) salicylaldimine complex exhibited high parasite growth inhibition activity as well. This has also been observed for other compounds.

5.2. Future outlook

Based on the data provided, some suggestions regarding future work can be made.

Firstly, it is clear that the quinoline motif remains essential for potent antiplasmodial activity and should still be considered when designing alternative antimalarial therapies due to its ability to strongly inhibit β-haematin formation.

Secondly, incorporation of polyamines as part of quinoline-containing systems may be advantageous in diminishing cross-resistance. Careful attention must be paid to the number of

quinoline moieties incorporated. Too many, may result in an unwanted increase in the toxicity of the compounds, making them less suitable antiplasmodial agents.

The lipophilicity of the compounds is also important to consider. Incorporating ferrocene alters the lipohilicity of compounds and in the case of ferroquine (and some of the compounds prepared in this study), results in consistent activity across sensitive and resistant strains. Incorporation of polyamines may result in increased lipophilicity of the compounds and this may lower accumulation of the compound inside of the digestive vacuole but to remedy this, water-soluble groups can be incorporated as part of these systems, which may counteract this effect and result in enhanced activity of the compound. The compound depicted in Figure 5.1 incorporates these suggestions.

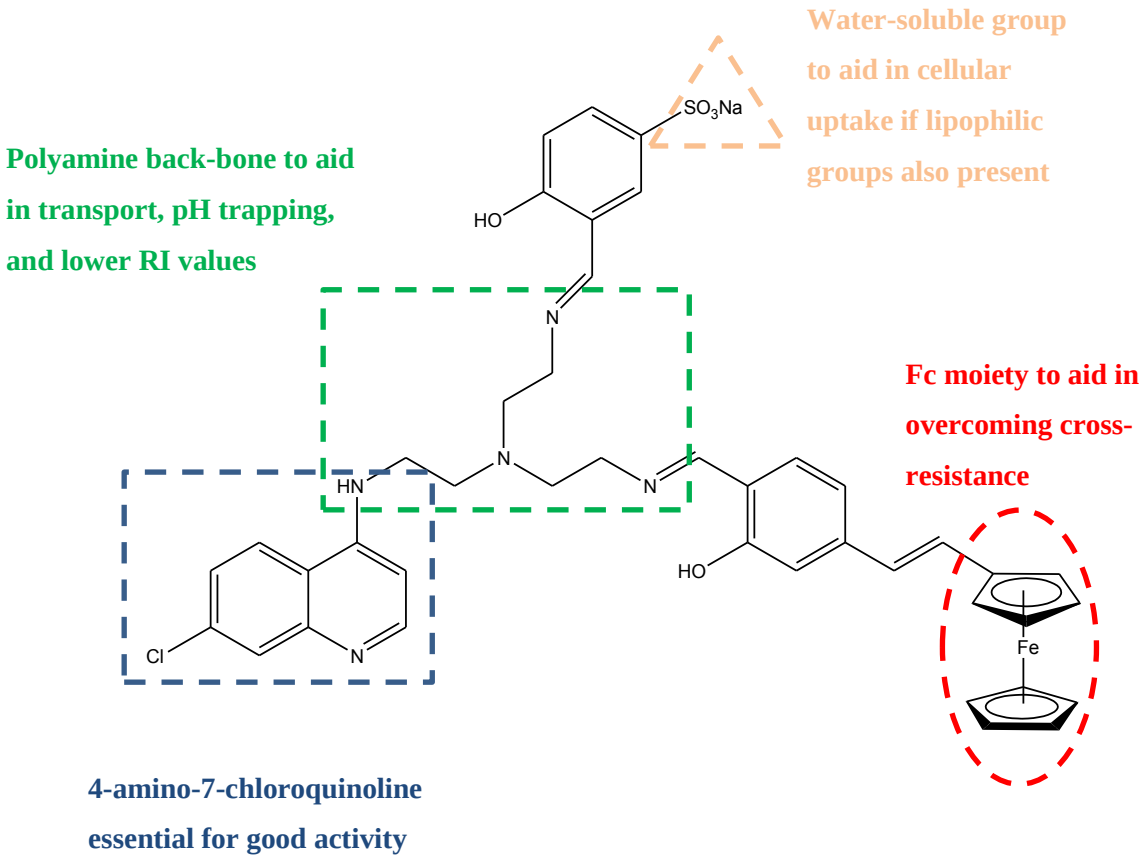


Figure 5.1: Rationale that can be used in the design of potential antimalarials

Some of the compounds prepared in this study exhibited high cytotoxicity especially in the case of the multimeric thioureas. In the case of the tetrameric thiourea, activity decreased

when screened against normal cells. This suggests selectivity toward the oesophageal cancer cell-line. The next step would be to screen this compound against various other normal and cancer cell-lines to establish a cytotoxicity profile. The mode of action of this complex can be ascertained by performing DNA-binding studies (i.e. viscosity experiments, DNA denaturing studies, UV absorption studies), since quinoline compounds have been known to intercalate with this biomolecule.

Further work regarding the antiparasitic activity of the 5-Cl salicylaldimine Rh(I) complex against *T. vaginalis* should be carried out in order establish its possible target in the parasite. A more detailed structure-activity study should also be performed using various derivatives of the ferrocenyl compound containing the salicylaldimine and quinoline groups in order to gain more insight about these types of compounds for this application.

CHAPTER 6

Experimental

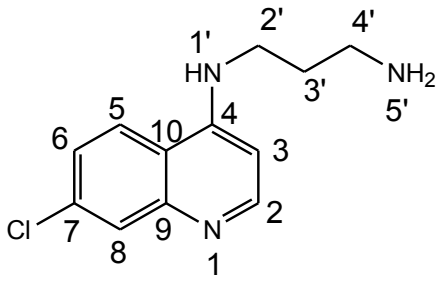
6.1. General

All reagents and solvents were obtained from commercial sources (Sigma-Aldrich, Merck, and Kimix) and, unless otherwise stated, were used as received. $[RhCl(COD)]_2$ (**3.8**)[1] and ferrocenyl salicylaldehyde (**4.8**)[2] were prepared according to literature procedures. Nuclear magnetic resonance (NMR) spectra were recorded using a Varian Mercury 300 spectrometer (^1H at 300.077 MHz, $^{13}C\{^1H\}$ at 75.454 MHz), a Varian Unity 400 spectrometer (^1H at 399.953 MHz, $^{13}C\{^1H\}$ at 100.577 MHz) or a Bruker 400 FT spectrometer (^1H at 400.200 MHz, $^{13}C\{^1H\}$ at 100.600 MHz) at 30.0°C. Coupling constants are reported in Hz. Infrared (IR) spectra were determined using a Perkin Elmer Spectrum 100 FT-IR spectrometer and was carried out in the solid state using KBr pellets or using Attenuated Total Reflectance (ATR). In some cases, samples were analysed as neat oils using NaCl cells. Low resolution and high resolution (HR) ESI-mass spectrometry was used to further characterise new compounds and determinations were carried out using a Waters API Quattro instrument in the positive mode. EI mass spectrometry was used to confirm the molecular weights of new compounds and samples were analysed using a JEOL GCmateII apparatus. Melting points were recorded using a Reichert – Jung Thermovar or a Buchi B-540 melting point apparatus and are uncorrected.

6.2. Experimental details for Chapter 2

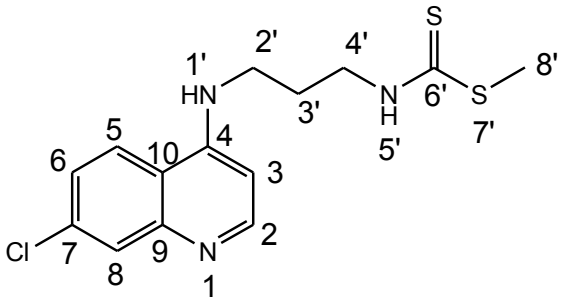
6.2.1. Synthetic methods and characterisation of quinoline thioureas 2.1 – 2.6

*N'- (7-Chloroquinolin-4-yl)-propane-1,3-diamine (**2.1**)*[3]



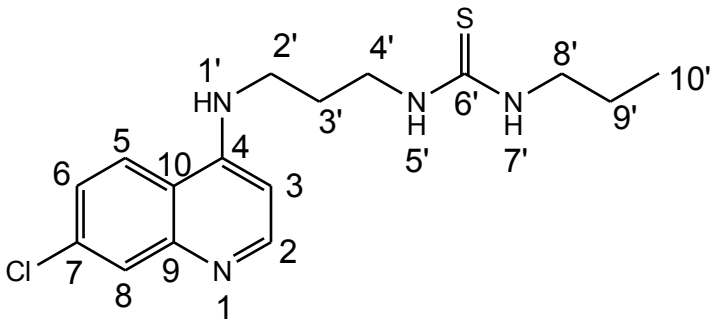
4,7-Dichloroquinoline (0.978 g, 4.94 mmol) and 1,3-propanediamine (2.1 ml) were heated to 80 °C in a flask with gentle stirring. This was continued for 1 hour and the temperature was then increased to 139 °C and stirring continued for 4 hours. The product was cooled to room temperature and 1 N NaOH (10 ml) was added. The mixture was then extracted with DCM (5 x 50 ml). The organic layer was then washed with H_2O (2 x 50 ml) and dried over $MgSO_4$. Evaporation of the solvent results in a beige powder as the desired product **2.1** (1.00 g, 86%). **^{1}H NMR (300.066 MHz, CD_3OD-d_4):** (δ, ppm) 1.88 (2H, m, H-3'); 2.78 (2H, t, $^3J_{H\text{-}H}$ = 6.96, H-4'); 3.40 (2H, t, $^3J_{H\text{-}H}$ = 6.70, H-2'); 6.49 (1H, d, $^3J_{H\text{-}H}$ = 5.68, H-3); 7.35 (1H, dd, $^3J_{H\text{-}H}$ = 9.02, $^4J_{H\text{-}H}$ = 2.19, H-6); 7.75 (1H, d, $^4J_{H\text{-}H}$ = 2.15, H-8); 8.04 (1H, d, $^3J_{H\text{-}H}$ = 9.03, H-5); 8.32 (1H, d, $^3J_{H\text{-}H}$ = 5.65, H-2). **IR (KBr):** (ν_{max}/cm^{-1}) 3238 (N-H); 1611 (C=N).

*3-[7-Chloroquinolin-4-ylamino)-propyl]-dithiocarbamic acid methyl ester (**2.2**)*[4]



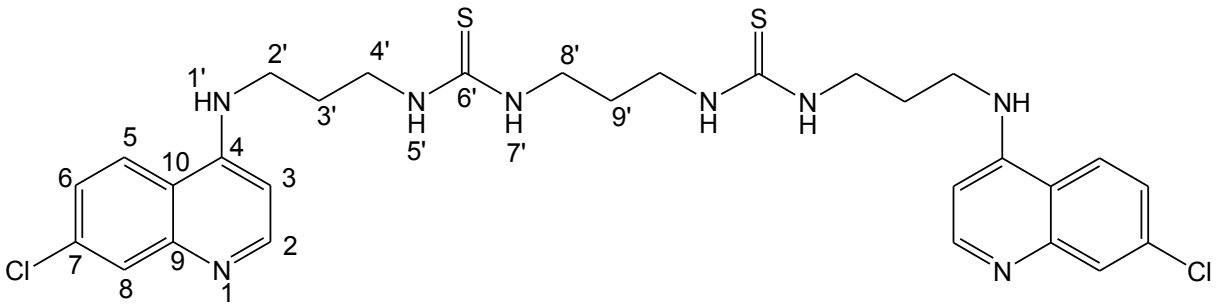
To a cooled solution of KOH (0.317 g, 5.64 mmol) in H_2O (15 ml), isopropanol (10 ml) and DMSO (5 ml), compound **2.1** (1.11 g, 4.70 mmol) was added. Ice-cooled CS_2 (0.34 ml) was added dropwise over a 1 hour period (< 10 °C) whilst stirring. The mixture was stirred for an additional 2 hour period at room temperature. Ice-cooled iodomethane (0.35 ml) was added dropwise over 1.5 hour period and stirred for a further 1.5 hours at room temperature. The cream precipitate was then collected by filtration, washed with water and dried *in vacuo*, giving rise to a pale yellow powder as the desired compound (**2.2**) (0.891 g, 58%). **^{1}H NMR (400.220 MHz, DMSO-d_6):** (δ, ppm) 2.01 (2H, m, H-3'); 2.46 (3H, s, H-8'); 3.36 (2H, m, H-4'); 3.75 (2H, m, H-2'); 6.44 (1H, d, $^3J_{H\text{-}H}$ = 5.60, H-3); 7.31 (1H, br s, NH); 7.39 (1H, dd, $^3J_{H\text{-}H}$ = 8.98, $^4J_{H\text{-}H}$ = 2.20, H-6); 7.74 (1H, d, $^4J_{H\text{-}H}$ = 2.19, H-8); 8.17 (1H, d, $^3J_{H\text{-}H}$ = 9.04, H-5); 8.33 (1H, d, $^3J_{H\text{-}H}$ = 5.52, H-2), 9.95 (1H, s, NH). **IR (KBr):** (ν_{max}/cm^{-1}) 1611 (C=N); 1141 (C=S).

Mono-quinoline thiourea ***(2.3)***



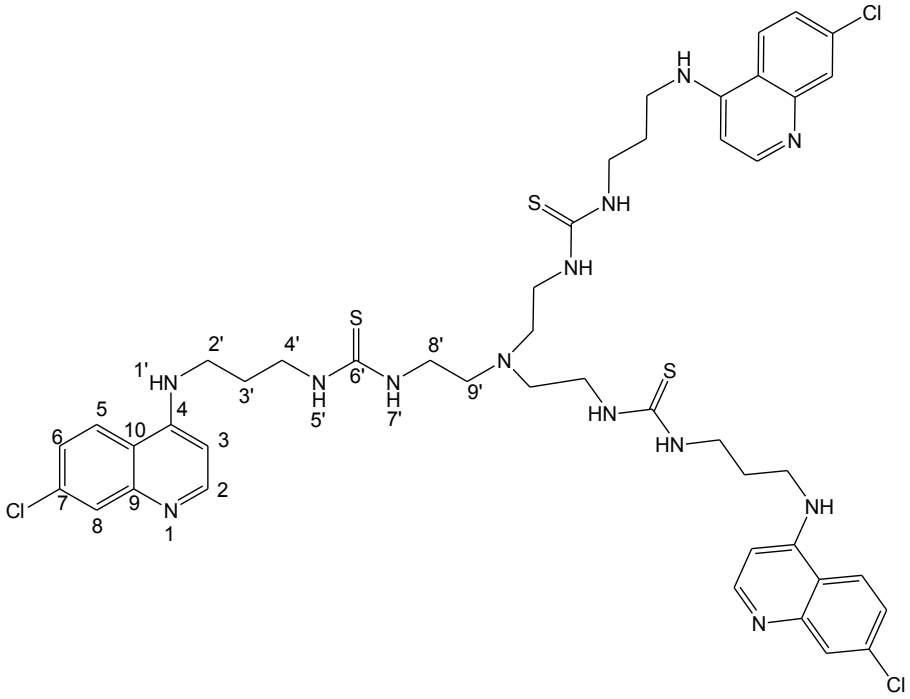
Compound **2.2** (0.354 g, 1.09 mmol) was dissolved in MeOH (20 ml), to this *n*-propylamine (0.15 ml) was added dropwise with stirring. The solution was refluxed for 24 hours and cooled to room temperature. The solvent was removed *in vacuo* and diethyl ether added to the residue. The residue was washed with diethyl ether and the supernatant decanted. This was repeated until the supernatant remained clear. The oily residue was dried under vacuum giving rise to the desired product (**2.3**) as a cream solid (0.220 g, 60%). **^{1}H NMR (300.066 MHz, DMSO-*d*$_6$):** (δ, ppm) 0.86 (3H, t, $^{3}J_{H\text{-}H}$ = 7.36, H-10'); 1.49 (2H, m, H-9'); 1.90 (2H, m, H-3'); 3.30 (4H, m, H-4' and H-8'); 3.52 (2H, m, H-2'); 6.47 (1H, d, $^{3}J_{H\text{-}H}$ = 5.45, H-3); 7.19 (1H, br s, NH); 7.35 (2H, m, NH); 7.42 (1H, dd, $^{3}J_{H\text{-}H}$ = 8.99, $^{4}J_{H\text{-}H}$ = 2.24, H-6); 7.80 (1H, d, $^{4}J_{H\text{-}H}$ = 2.20, H-8); 8.24 (1H, d, $^{3}J_{H\text{-}H}$ = 9.03, H-5); 8.40 (1H, d, $^{3}J_{H\text{-}H}$ = 5.40, H-2). **^{13}C{^{1}H} NMR (75.454 MHz, DMSO- *d*$_6$):** (δ, ppm) 11.10 (C-10'); 21.85 (C-9'); 27.59 (C-3'); 40.34 (C-4'); 41.13 (C-2'); 45.10 (C-8'); 98.51 (C-3); 117.37; 123.82 (C-5, C-6); 127.36 (C-8); 133.19; 148.98; 149.87; 151.72 (C-2); 181.99 (C-6'). **IR (KBr):** (ν_{max}/cm^{-1}) 3435 (N-H); 1613 (C=N); 1136 (C=S). **ESI-MS:** *m/z* 337.1248 (100%, [M+H]$^{+}$). **Elemental Analysis** $C_{16}H_{21}ClN_4S{\cdot}1.5H_2O$ calculated: C 52.81, H 6.65, N 15.40%; found: C 53.06; H 6.96; N 16.21%.

*Bis-quinoline thiourea (**2.4**)*



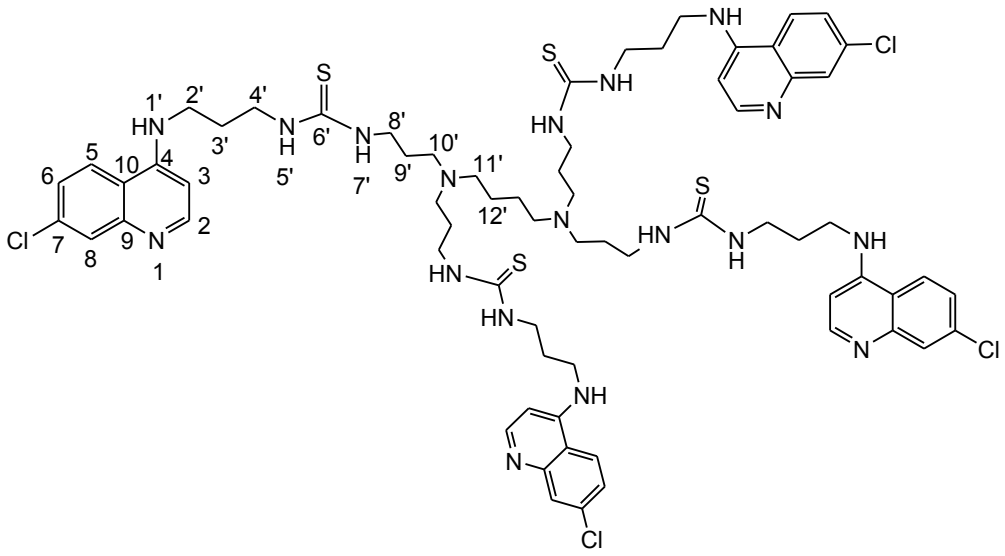
Compound **2.2** (1.49 g, 4.56 mmol) was dissolved in MeOH (20 ml), to this 1,3-propanediamine (0.13 ml) was added dropwise with stirring. The solution was refluxed for 24 hours and cooled to room temperature. The solvent was removed *in vacuo* and acetone added to the residue. The mixture was washed with acetone and the supernatant decanted. This was repeated until the supernatant remained clear. The gummy matter was then washed with diethyl ether to remove the acetone. The diethyl ether was then decanted and the residue dried under vacuum giving rise to the product (**2.4**) as a beige powder (0.818 g, 85%). **M.p.** 109 - 111°C. **^{1}H NMR (300.066 MHz, DMSO-d_6):** (δ, ppm) 1.73 (2H, m, H-9'); 1.90 (4H, m, H-3'); 3.30 (4H, m, H-8'); 3.40 (4H, m, H-4'); 3.51 (4H, m, H-2'); 6.46 (2H, d, $^{3}J_{H\text{-}H}$ = 5.44, H-3); 7.19 (2H, m, NH); 7.37-7.47 (6H, m, NH, 6'); 7.77 (2H, d, $^{4}J_{H\text{-}H}$ = 2.14, H-8); 8.24 (2H, d, $^{3}J_{H\text{-}H}$ = 9.03, H-5); 8.40 (2H, d, $^{3}J_{H\text{-}H}$ = 5.40, H-2). **^{13}C{^{1}H} NMR (75.454 MHz, DMSO-d_6):** (δ, ppm) 27.41 (C-3'); 28.51 (C-9'); 40.79-40.93 (C-2', C-4', C-8'); 98.45 (C-3); 117.18; 123.67 (C-5); 123.88 (C-6); 127.03 (C-8); 133.31; 148.66; 149.87; 151.60 (C-2); 181.68 (C-6'). **IR (KBr):** (ν_{max}/cm^{-1}) 3258 (N-H); 1613 (C=N); 1138 (C=S). **ESI-MS:** *m/z* 629.1812 (20%, $[M+H]^{+}$); 315.0951 (100%, $[M+2H]^{2+}$). **Elemental Analysis** $C_{29}H_{34}Cl_2N_8S_2{\cdot}2H_2O$ calculated: C 52.32, H 5.75, N 16.83%; found: C 52.51; H 5.95; N 15.13%.

*Tris-quinoline thiourea (**2.5**)*



Compound **2.2** (0.415 g, 1.27 mmol) was dissolved in MeOH (20 ml), to this tris(2-aminoethyl)amine (0.06 ml) was added dropwise with stirring. The solution was refluxed for 24 hours and cooled to room temperature. The solvent was removed *in vacuo* giving rise to a pale orange solid. The solid was washed with acetone and the supernatant decanted. This was repeated until the supernatant remained clear. The residue was washed with diethyl ether, the ether was then decanted and the product dried under vacuum. The product (**2.5**) was isolated as a cream-yellow solid (0.242 g, 58%). **M.p.** 134 - 136°C. **^{1}H NMR (300.066 MHz, CD_3OD-d_4):** (δ, ppm) 1.96 (6H, m, H-3'); 2.66 (12H, m, H-4', H-9'); 3.44-3.74 (12H, m, H-2', H-8'); 6.44 (3H, m, H-6); 7.31 (3H, m, H-6); 7.71 (3H, m, H-8); 7.99 (3H, m, H-5); 8.28 (3H, m, H-2). **^{13}C{^{1}H} NMR (75.454 MHz, DMSO-d_6):** (δ, ppm) 27.49 (C-3'); 41.13 (C-4'); 41.58 (C-2'); 52.92 (C-8'); 54.41 (C-9'); 98.46 (C-3); 117.33; 123.75 (C-5, C-6); 127.29 (C-8); 133.12; 148.92; 149.81; 151.64 (C-2); 182.23 (C-6'). **IR (KBr):** (ν_{max}/cm^{-1}) 3280 (N-H); 1611 (C=N); 1138 (C=S). **ESI-MS:** *m/z* 980 (5%, $[M+H]^+$); 513 (60%, $[M+2Na]^{2+}$). **Elemental Analysis** $C_{45}H_{54}Cl_3N_{13}S_3 \cdot 3H_2O$ calculated: C 52.29, H 5.85, N 17.62%; found: C 52.00; H 6.38; N 16.60%.

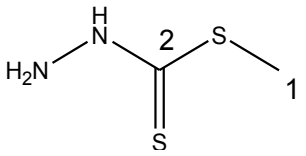
*Tetra-quinoline thiourea (**2.6**)*



Compound **2.2** (0.888 g, 2.72 mmol) and G1 DAB dendrimer (0.164 g, 0.518 mmol) were combined in MeOH (20 ml). The solution was refluxed for 24 hours and cooled to room temperature. The supernatant was decanted giving a yellow oily residue which was dried *in vacuo*. The residue was washed with acetone, followed by diethyl ether. The supernatant was decanted and the oil dried under vacuum giving rise to the product (**2.6**) as a cream powder (92.0 mg, 12%). **M.p.** 115 - 117°C. **^{1}H NMR (300.066 MHz, DMSO-d_6):** (δ, ppm) 1.32 (4H, m, H-12'); 1.59 (8H, m, H-9'); 1.89 (8H, m, H-3'); 2.35 (12H, m, H-10', H-11'); 3.21-3.45 (16H, m, H-4' and H-8'); 3.50 (8H, m, H-2'); 6.44 (4H, br d, $^{3}J_{H\text{-}H}$ = 5.45, H-3); 7.19 (4H, br s, NH); 7.41 (12H, m, NH, H-6); 7.77 (4H, d, $^{4}J_{H\text{-}H}$ = 2.15, H-8); 8.25 (4H, m, H-5); 8.38 (4H, d, $^{3}J_{H\text{-}H}$ = 5.34, H-2). **^{13}C{^{1}H} NMR (100.600 MHz, d_6-DMSO):** (δ, ppm) 24.73 (C-12'); 26.78 (C-9'); 28.07 (C-3'); 41.42-42.87 (C-2', C-4', C-8'); 51.57 (C-10'); 53.75 (C-11'); 99.10 (C-3); 117.96; 124.47 (C-5, C-6); 127.95 (C-8); 133.82; 149.55; 150.45; 152.33 (C-2); 182.28 (C-6'). **IR (KBr):** (ν_{max}/cm^{-1}) 3272 (N-H); 1611 (C=N); 1138 (C=S). **ESI-MS:** *m/z* 1429 (70%, [M+H]$^{+}$). **Elemental Analysis** $C_{68}H_{88}Cl_4N_{18}S_4{\cdot}5H_2O$ calculated: C 53.81, H 6.51, N 16.61%; found: C 53.90; H 6.42; N 15.02%.

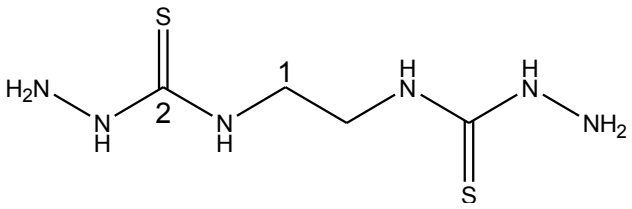
6.2.2. Synthetic methods and characterisation of ferrocene thioureas 2.7 – 2.12

*Methyl hydrazinecarbodithioate (**2.7**)*[5]



To a cooled solution of KOH (5.98 g, 0.107 mmol) in H_2O (25 ml), isopropanol (25 ml), $NH_2NH_2{\cdot}H_2O$ (3.32 ml) was added. Ice-cooled CS_2 (6.44 ml) was added dropwise whilst stirring giving rise to a yellow solution. The mixture was stirred for an additional 2.5 hour period at room temperature. Ice-cooled iodomethane (6.64 ml) was added dropwise over a 1 hour period and stirred for a further 1.5 hours at room temperature. The precipitate was then collected by filtration, washed with water and dried *in vacuo*, giving rise to a cream powder as the product **2.7** (4.17 g, 32%). **^{1}H NMR (300.066 MHz, DMSO-*d*$_6$):** (δ, ppm) 2.36 (3H, s, H-1); 5.02 (2H, s, NH_2); 10.73 (1H, br s, NH). **IR (KBr):** (ν_{max}/cm^{-1}) 3154 (N-H); 1598 (N-H); 704 (C-S).

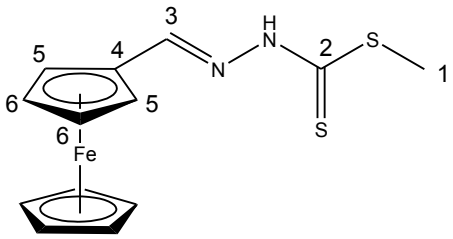
*Ethane-1,2-dithiosemicarbazide (**2.8**)*[6]



1,2-Diaminopropane (1.68 mL, 25.0 mmol) was added to NaOH (2.16 g, 49.0 mmol) in water (40 ml). To this solution, CS_2 (4.50 ml, 75.0 mmol) was added and the mixture stirred for 4 hours, after which the organic layer disappeared. The solution was treated with sodium chloroacetate (5.77 g, 49.5 mmol) and the mixture was stirred for a further 16 hours at room temperature. The yellow solution was then acidified with 2 M HCl (5 ml) and an excess of

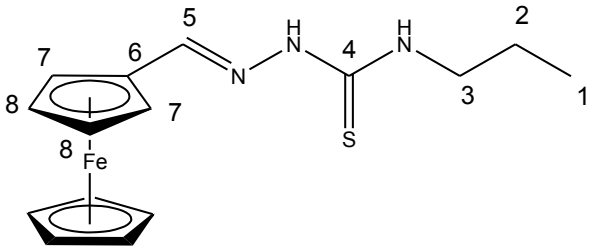
$NH_2NH_2 \cdot H_2O$ (11 ml) was added. The mixture was heated at 90 ℃ for 2 hours. The precipitate that was formed was collected by filtration, washed with water and dried *in vacuo*. The product (**2.8**) was isolated as a white powder (2.07 g, 40%). **^{1}H NMR (399.953 MHz, DMSO-*d*$_6$):** (δ, ppm) 3.63 (4H, s, H-1); 4.41 (4H, s, NH_2); 7.97 (2H, s, NH); 8.50 (2H, s, NH).

*Mono-ferrocene methyl ester (**2.9**)*[7]



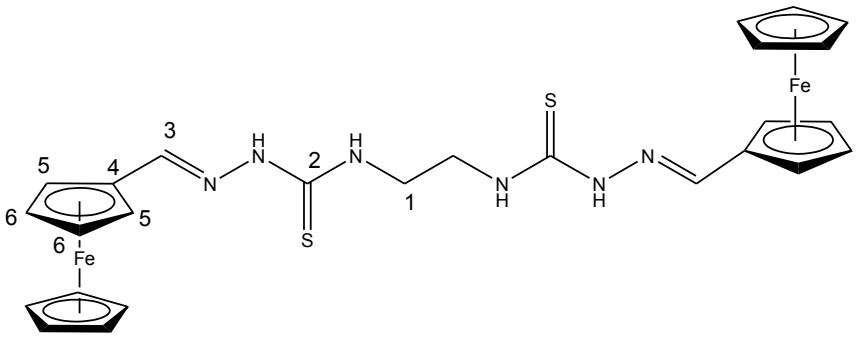
Methyl hydrazinecarbodithioate **2.7** (0.156 g, 1.28 mmol) was suspended in *i*PrOH (15 ml) and treated with ferrocenecarboxaldehyde (0.274 g, 1.29 mmol) at room temperature. The resulting solution was stirred for 24 hours at room temperature. The resulting precipitate was filtered and washed with cold *i*PrOH. The product (**2.9**) was isolated as a red powder (0.356 g, 87%). **^{1}H NMR (399.935 MHz, DMSO-*d*$_6$):** (δ, ppm) 2.50 (3H, m, H-1); 4.24 (5H, s, Cp_{unsub}); 4.51 (2H, m, Cp_{sub} H-6); 4.67 (2H, m, Cp_{sub} H-5); 8.12 (1H, s, H-3); 12.98 (1H, br s, NH). **IR (KBr):** (ν_{max}/cm^{-1}) 3121 (N-H); 1597 (C=N); 812 (C=S).

*Mono-ferrocene thiourea (**2.10**)*



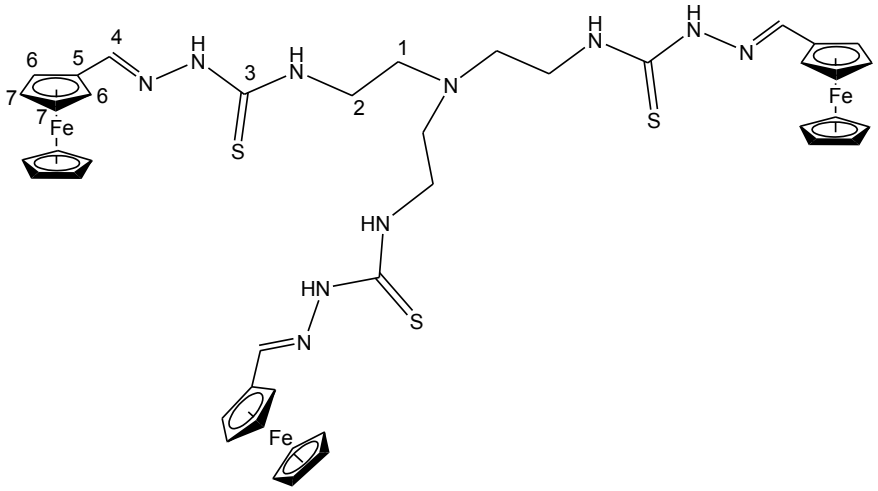
Compound **2.9** (0.197 g, 0.618 mmol) was suspended in EtOH (20 ml). *n*-Propylamine (0.06 ml) was added to the suspension and the resulting solution was refluxed under Argon for 12 hours. The solvent was removed *in vacuo* and the residue dissolved in DCM (15 ml). The organic layer was washed with water until the water layer remained clear. The solvent was removed *in vacuo* and the residue was dissolved in a minimum amount of diethyl ether. To this, petroleum ether was added and the product was allowed to precipitate at -20°C. The product (**2.10**) was isolated as a red-brown powder (62.0 mg, 30%). **M.p.** 98 - 101°C. **^{1}H NMR (399.953, DMSO-*d*$_6$):** (δ, ppm) 0.86 (3H, t, $^{3}J_{H\text{-}H}$ = 7.50, H-1); 1.59 (2H, m, H-2); 3.49 (2H, m, H-3); 4.21 (5H, s, Cp_{unsub}); 4.42 (2H, m Cp_{sub} H-8); 4.72 (2H, m, Cp_{sub} H-7); 7.89 (1H, s, H-5); 8.12 (1H, br s, NH); 11.11 (1H, s, NH). **^{13}C{^{1}H} NMR (100.635 MHz, DMSO-*d*$_6$):** (δ, ppm) 11.69 (C-1); 22.68 (C-2); 45.54 (C-3); 68.02 (C-8); 69.40 (Cp_{unsub}); 70.43 (C-7); 79.59 (C-6); 143.45 (C-5); 176.75 (C-4). **IR (KBr):** (ν_{max}/cm^{-1}) 3371 (N-H); 3129 (N-H); 1604 (C=N); 816 (C=S); 1090 (ferrocene). **ESI-MS:** *m/z* 330.0724 (100%, $[M+H]^+$). **Elemental Analysis** $C_{15}H_{19}FeN_3S$ calculated: C 54.72, H 5.82, N 12.76%; found: C 53.99; H 5.73; N 10.84%.

*Bis-ferrocene thiourea (**2.11**)*[8]



Ethane-1,2-dithiosemicarbazide **2.8** (0.160 g, 0.767 mmol) was suspended in H_2O (25 ml). To this, a few drops of 10.1 M HCl was added. Ferrocenecarboxaldehyde (0.331 g, 1.55 mmol) was added to the suspension and the reaction mixture stirred for 24 hours at room temperature. The precipitate was filtered, washed with water and dried *in vacuo*. The product (**2.11**) was isolated as an orange powder (0.453 g, 98%). **M.p.** 217°C decomposes without melting. **^{1}H NMR (300.066 MHz, DMSO-*d*$_6$):** (δ, ppm) 3.78 (4H, s, H-1); 4.17 (10H, s, Cp_{unsub}); 4.39 (4H, t, $^3J_{H\text{-}H}$ = 1.83, Cp_{sub} H-6); 4.74 (4H, t, $^3J_{H\text{-}H}$ = 1.84, Cp_{sub} H-5); 7.90 (2H, s, H-3); 8.36 (2H, br s, NH); 11.29 (2H, s, NH). **^{13}C{^{1}H} NMR (100.635 MHz, DMSO-*d*$_6$):** (δ, ppm) 44.01 (C-1); 68.19 (C-6); 69.43 (Cp_{unsub}); 70.49 (C-5); 79.43 (C-4); 143.96 (C-3); 177.23 (C-2). **IR (KBr):** (ν_{max}/cm^{-1}) 3437 (N-H); 3267 (N-H); 1611 (C=N); 816 (C=S). **ESI-MS:** *m/z* 601.0582 (100%, $[M+H]^+$). **Elemental Analysis** $C_{26}H_{28}Fe_2N_6S_2 \cdot H_2O$ calculated: C 50.50, H 4.56, N 13.59%; found: C 50.67, H 4.71, N 13.85%.

*Tris-ferrocene thiourea (**2.12**)*



Compound **2.9** (0.506 g, 1.59 mmol) was suspended in absolute EtOH (20 ml) under argon. To this mixture, tris(2-aminoethyl)amine (0.08 ml) was added dropwise and the resulting solution refluxed for 24 hours. The solution was cooled to room temperature, and the solvent removed under reduced pressure. The residue was dissolved in DCM (15 ml) and extracted with water until the aqueous layer was clear. The organic extract was evaporated to dryness giving rise to the product (**2.12**) as a red powder (0.226 g, 46%). **M.p.** 124 - 127 °C. **^{1}H NMR (300.066 MHz, DMSO-*d*$_6$):** (δ, ppm) 2.83 (6H, m, H-1); 3.71 (6H, m, H-2); 4.18 (15H, s, Cp_{unsub}); 4.41 (6H, m, Cp_{sub} H-7); 4.69 (6H, m, Cp_{sub} H-6); 7.90 (3H, s, H-4); 8.15 (3H, br s, NH); 11.22 (3H, s, NH). **^{13}C{^{1}H} NMR (100.635 MHz, DMSO-*d*$_6$):** (δ, ppm) 43.37 (C-2); 53.27 (C-1); 68.03 (C-7); 69.44 (Cp_{unsub}); 70.51 (C-6); 79.42 (C-5); 143.70 (C-4); 176.73 (C-3). **IR (KBr):** (ν_{max}/cm^{-1}) 3431 (N-H); 3366 (N-H); 1606 (C=N); 818 (C=S); 1103 (ferrocene). **ESI-MS:** *m/z* 957.1362 (70%, $[M+H]^+$). **Elemental Analysis** $C_{42}H_{48}Fe_3N_{10}S_3$ calculated: C 52.73, H 5.06, N 14.64%; found: C 52.63, H 5.54, N 13.98%.

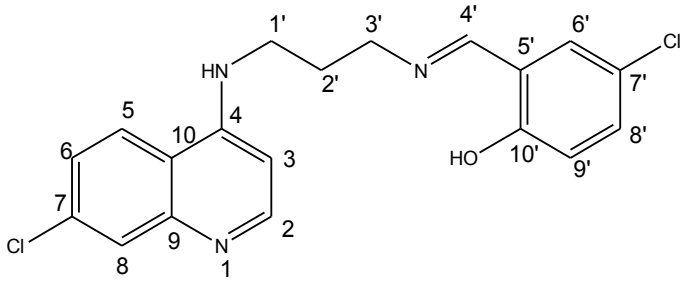
6.3. Experimental details for Chapter 3

6.3.1. Synthetic methods and characterisation of 4-amino-7-chloroquinoline mono-imines 3.2 – 3.4

6.3.1.1. General method to prepare ligands 3.2 – 3.4

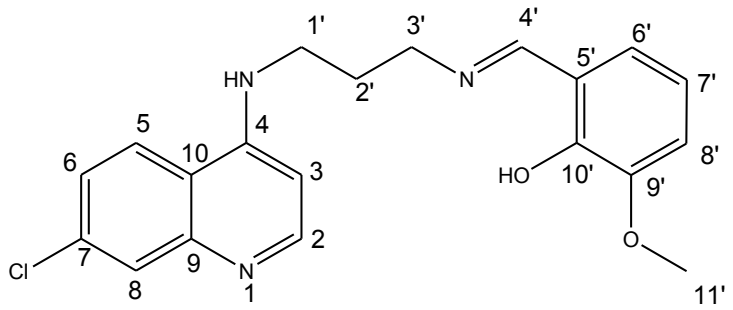
(N'-(7-Chloroquinolin-4-yl)-propane-1,3-diamine) (**2.1**) was suspended in diethyl ether (10 ml). To this, a slight excess of the desired salicylaldehyde in diethyl ether (10 ml) was added dropwise and the resulting mixture stirred at room temperature for 16 hours. The resulting precipitate was filtered using a Büchner funnel and washed with diethyl ether to remove excess aldehyde. The product was then dried *in vacuo*.

*Mono-5-chlorosalicylaldimine quinoline (**3.2**)*



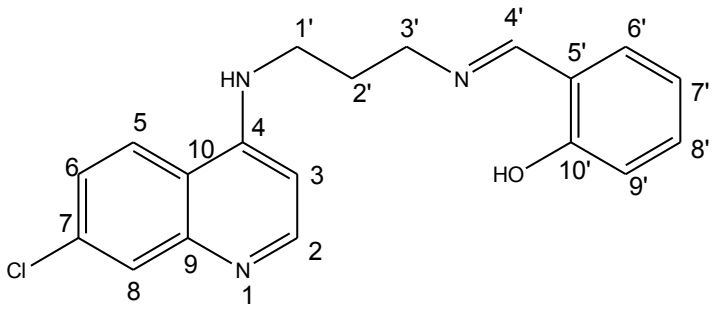
N'-(7-Chloroquinolin-4-yl)-propane-1,3-diamine (**2.1**) (0.329 g, 1.40 mmol) was reacted with 5-chlorosalicylaldehyde (0.263 g, 1.68 mmol). The product (**3.2**) was obtained as a pale yellow powder (0.423 g, 81%). **M.p.** 173 - 176°C. **^{1}H NMR (399.951 MHz, CDCl$_3$):** (δ, ppm) 2.18 (2H, m, H-2'); 3.46 (2H, m, H-1'); 3.78 (2H, t, $^{3}J_{H\text{-}H}$ = 6.44, H-3'); 5.07 (1H, br s, NH); 6.40 (1H, d, $^{3}J_{H\text{-}H}$ = 5.38, H-3); 6.91 (1H, d, $^{3}J_{H\text{-}H}$ = 8.80, H-9') ; 7.17 (1H, d, $^{4}J_{H\text{-}H}$ = 2.58, H-6'); 7.24-7.33 (2H, m, H-6, H-8'); 7.61 (1H, d, $^{3}J_{H\text{-}H}$ = 8.94, H-5); 7.94 (1H, d, $^{4}J_{H\text{-}H}$ = 2.13, H-8); 8.30 (1H, s, H-4'); 8.52 (1H, d, $^{3}J_{H\text{-}H}$ = 5.36, H-2), 13.18 (1H, br s, OH). **^{13}C{^{1}H} NMR (100.635 MHz, CDCl$_3$):** (δ, ppm) 29.92 (C-2'); 41.28 (C-1'); 57.35 (C-3'); 99.13 (C-3); 117.12; 118.59 (C-9'); 119.33; 120.71 (C-5); 123.48; 125.45 (C-6); 128.88 (C-8); 130.45 (C-6'); 132.38 (C-8'); 134.97; 149.13; 149.46; 151.98 (C-2); 159.53; 164.65 (C-4'). **IR (KBr):** (ν_{max}/cm^{-1}) 3238 (N-H); 1637 (C=N); 1615 (C=N) quinoline. **ESI-MS:** *m/z* 374.0824 (100%, [M+H]$^{+}$). **Elemental Analysis** $C_{19}H_{17}Cl_2N_3O$ calculated: C 60.97; H 4.58; N 11.23%, found: C 61.11; H 4.88; N 11.33%.

*Mono-3-methoxysalicylaldimine quinoline (**3.3**)*



N'-(7-Chloroquinolin-4-yl)-propane-1,3-diamine (**2.1**) (0.231 g, 0.979 mmol) was reacted with *o-vanillin* (0.165 g, 1.08 mmol). The product (**3.3**) was obtained as a yellow powder (0.291 g, 80%). **M.p.** 145 - 149°C. **^{1}H NMR (399.951 MHz, $CDCl_3$):** (δ, ppm) 2.16 (2H, m, H-2'); 3.47 (2H, m, H-1'); 3.76 (2H, t, $^{3}J_{H\text{-}H}$ = 6.76, H-3'); 3.91 (3H, s, H-11'); 5.12 (1H, br s, NH); 6.40 (1H, d, $^{3}J_{H\text{-}H}$ = 5.39, H-3); 6.81 (2H, m, H-8', H-7'); 6.94 (1H, m, H-6'); 7.28 (1H, dd, $^{4}J_{H\text{-}H}$ = 2.17, $^{3}J_{H\text{-}H}$ = 8.94, H-6); 7.63 (1H, d, $^{3}J_{H\text{-}H}$ = 8.99, H-5); 7.92 (1H, d, $^{4}J_{H\text{-}H}$ = 2.14, H-8); 8.35 (1H, s, H-4'); 8.50 (1H, d, $^{3}J_{H\text{-}H}$ = 5.32, H-2); 13.69 (1H, br s, OH). **^{13}C{^{1}H} NMR (100.635 MHz, $CDCl_3$):** (δ, ppm) 29.96 (C-2'); 41.25 (C-1'); 56.16 (C-11'); 56.97 (C-3'); 99.12 (C-3); 114.33 (C-8'); 117.18; 118.29 (C-7'); 118.50; 120.89 (C-5); 122.93 (C-6'); 125.33 (C-6); 128.82 (C-8); 134.86; 148.48; 149.18; 149.52; 151.50; 152.04 (C-2); 165.86 (C-4'). **IR (KBr):** (ν_{max}/cm^{-1}) 3424 (N-H); 1631 (C=N); 1611 (C=N) quinoline. **ESI-MS:** *m/z* 370.1319 (100%, $[M+H]^+$). **Elemental Analysis** $C_{20}H_{20}ClN_3O_2 \cdot 2H_2O$ calculated: C 59.19; H 5.96; N 10.35%, found: C 59.13; H 5.59; N 10.60%.

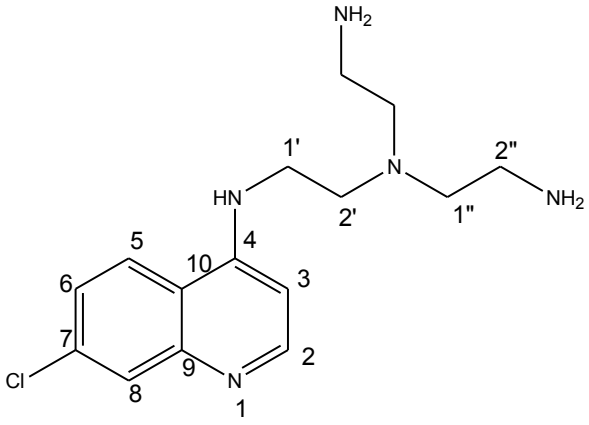
*Mono-salicylaldimine quinoline (**3.4**)*



N'-(7-Chloroquinolin-4-yl)-propane-1,3-diamine (**2.1**) (0.644 g, 2.73 mmol) was reacted with salicyladehyde (0.337 g, 2.76 mmol). The product (**3.4**) was obtained as a pale yellow powder (0.773 g, 83%). **M.p.** 174 - 176°C. **^{1}H NMR (399.951 MHz, $CDCl_3$):** (δ, ppm) 2.18 (2H, m, H-2'); 3.47 (2H, m, H-1'); 3.77 (2H, t, $^{3}J_{H\text{-}H}$ = 6.71, H-3'); 5.10 (1H, br s, NH); 6.40 (1H, d, $^{3}J_{H\text{-}H}$ = 5.37, H-3); 6.88 (1H, td, $^{4}J_{H\text{-}H}$ = 1.09, $^{3}J_{H\text{-}H}$ = 7.53, H-7'); 6.98 (1H, d, $^{3}J_{H\text{-}H}$ = 8.30, H-9'); 7.21 (1H, dd, $^{4}J_{H\text{-}H}$ = 1.69, $^{3}J_{H\text{-}H}$ = 7.65, H-6'); 7.28 (1H, dd, $^{4}J_{H\text{-}H}$ = 2.19, $^{3}J_{H\text{-}H}$ = 8.93, H-6); 7.33 (1H, m, H-8'); 7.61 (1H, d, $^{3}J_{H\text{-}H}$ = 8.96, H-5); 7.94 (1H, d, $^{4}J_{H\text{-}H}$ = 2.16, H-8); 8.38 (1H, s, H-4'); 8.52 (1H, d, $^{3}J_{H\text{-}H}$ = 5.34, H-2); 13.21 (1H, br s, OH). **^{13}C{^{1}H} NMR (100.635 MHz, $CDCl_3$):** (δ, ppm) 30.11 (C-2'); 41.48 (C-1'); 57.40 (C-3'); 99.15 (C-3); 117.06 (C-9'); 117.24; 118.71; 118.81 (C-7'); 120.67 (C-5); 125.36 (C-6); 129.01 (C-8); 131.31 (C-6'); 132.52 (C-8'); 134.90; 149.35; 149.49; 152.03 (C-2); 161.03; 165.83 (C-4'). **IR (KBr):** (ν_{max}/cm^{-1}) 3260 (N-H); 1634 (C=N); 1613 (C=N) quinoline. **EI-MS:** *m/z* 339.0903 (87%, $[M]^{+}$). **Elemental Analysis** $C_{19}H_{18}ClN_3O$ calculated: C 67.15; H 5.33; N 12.37%, found: C 66.90; H 5.07; N 12.70%.

6.3.2. Synthetic methods and characterisation of 4-amino-7-chloroquinoline bis-imines 3.1 and 3.5 – 3.7

*N-(7-Chloroquinolin-4-yl)-tris(2-aminoethyl)amine (**3.1**)*[9]

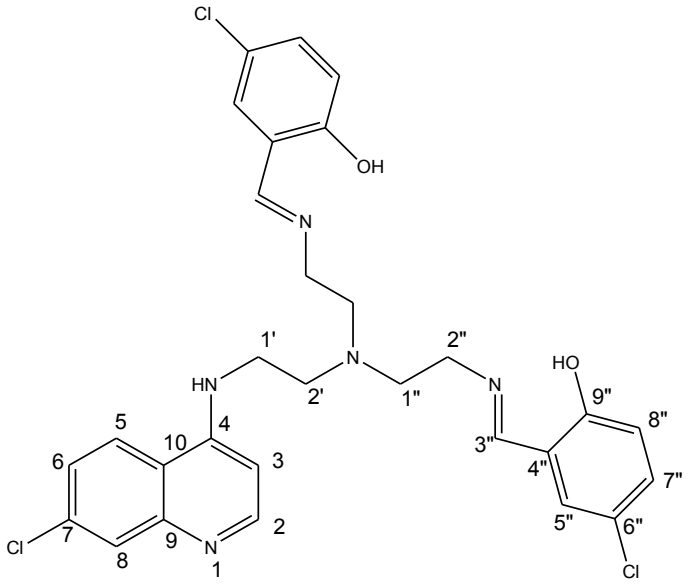


A mixture of 4,7-dichloroquinoline (0.588 g, 2.97 mmol) and tris(2-aminoethyl)amine (5 ml) was heated to 90 °C for 7 hours with stirring. The reaction was quenched with 1N NaOH (20 ml) and extracted with DCM (3 x 50 ml), followed by brine. The combined organic layers were dried using anhydrous $MgSO_4$. The drying agent was filtered off and the solvent removed *in vacuo* to give an orange semi-solid (**3.1**) (0.576 g, 63%). **^{1}H NMR (399.951, $CDCl_3$):** (δ, ppm) 1.48 (4H, br s, NH_2); 2.47-2.60 (4H, m, H-1"); 2.65-2.83 (6H, m, H-2", H-2'); 3.26 (2H, m, H-1'); 6.30 (1H, d, $^3J_{H\text{-}H}$ = 5.40, H-3); 6.64 (1H, br s, NH); 7.25 (1H, dd, $^4J_{H\text{-}H}$ = 2.73, $^3J_{H\text{-}H}$ = 8.91, H-6); 7.82-7.94 (2H, m, H-5, H-8); 8.45 (1H, d, $^3J_{H\text{-}H}$ = 5.37, H-2).

6.3.2.1 General method to prepare ligands 3.5 – 3.7

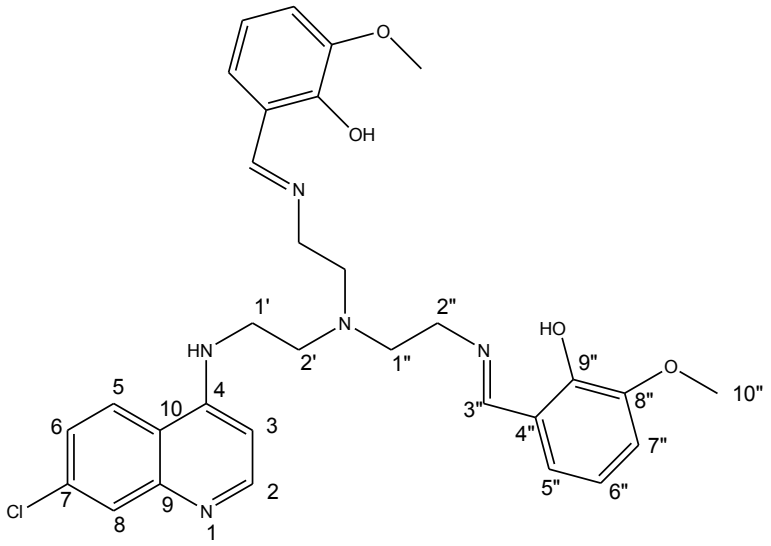
N-(7-Chloroquinolin-4-yl)-tris(2-aminoethyl)amine (**3.1**) was dissolved in EtOH (5 ml). To this, the appropriate aldehyde in EtOH (10 ml) was added and the resulting solution stirred at room temperature for 16 hours. The solvent was removed under reduced pressure and the residue washed continuously with diethyl ether. The resulting solid was then dried *in vacuo*.

*Bis-5-chlorosalicylaldimine quinoline (**3.5**)*



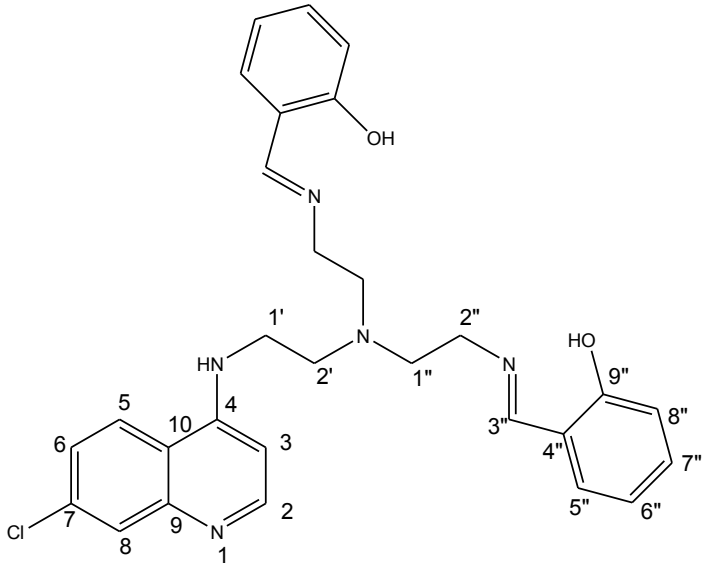
N-(7-Chloroquinolin-4-yl)-tris(2-aminoethyl)amine (**3.1**) (0.105 g, 0.339 mmol) was reacted with 5-chlorosalicylaldehyde (0.117 g, 0.747 mmol). The product (**3.5**) was isolated as a yellow powder (0.122 g, 61%). **M.p.** 154 - 158°C. **^{1}H NMR (399.951, CDCl$_3$):** (δ, ppm) 2.83 (2H, m, H-2'); 2.88 (4H, m H-1"); 3.31 (2H, m, H-1'); 3.57 (4H, m, H-2"); 5.96 (1H, br s, NH); 6.26 (1H, d, $^{3}J_{H\text{-}H}$ = 5.53, H-3); 6.61 (2H, d, $^{3}J_{H\text{-}H}$ = 8.81, H-8"); 6.67 (1H, dd, $^{4}J_{H\text{-}H}$ = 2.04, $^{3}J_{H\text{-}H}$ = 8.86, H-6); 6.72 (2H, d, $^{4}J_{H\text{-}H}$ = 2.61, H-5"); 7.09-7.12 (3H, m, H-5, H-7"); 7.84 (1H, d, $^{4}J_{H\text{-}H}$ = 2.11, H-8); 7.99 (2H, s, H-3"); 8.37 (1H, d, $^{3}J_{H\text{-}H}$ = 5.79, H-2). **^{13}C{^{1}H} NMR (100.635 MHz, DMSO-*d*$_6$):** (δ, ppm) 41.28 (C-1'); 52.62 (C-2'); 54.88 (C-1"); 56.36 (C-2"); 99.22 (C-3); 117.50; 119.37 (C-8"); 119.51; 121.68; 124.05 (C-5); 124.74 (C-6); 127.15 (C-8); 130.78 (C-5"); 132.53 (C-7"); 134.26; 148.37; 150.76; 151.39 (C-2); 161.27; 165.58 (C-3"). **IR (KBr):** (ν_{max}/cm^{-1}) 3312 (N-H); 1648 (C=N); 1609 (C=N) quinoline. **ESI-MS**: *m/z* 584.1404 (30%, [M+H]$^{+}$); 292.5737 (100%, [M+2H]$^{2+}$). **Elemental Analysis** $C_{29}H_{28}Cl_3N_5O_2{\cdot}2H_2O$ calculated: C 56.09; H 5.19; N 11.28%, found: C 55.66; H 4.96; N 11.21%.

*Bis-3-methoxysalicylaldimine quinoline (**3.6**)*



N-(7-Chloroquinolin-4-yl)-tris(2-aminoethyl)amine (**3.1**) (0.199 g, 0.647 mmol) was reacted with *o*-vanillin (0.197 g, 1.293 mmol). The product (**3.6**) was filtered and isolated as a yellow powder (0.149 g, 40%). **M.p.** 149 - 153°C. **^{1}H NMR (399.951, CDCl$_3$):** (δ, ppm) 2.89-2.99 (6H, m, H-2', H-1"); 3.42 (2H, m, H-1'); 3.64 (4H, m, H-2"); 3.84 (6H, s, H-10"); 6.34 (1H, d, $^{3}J_{H\text{-}H}$ = 6.25, H-3); 6.49-6.58 (4H, m, H-6", H-7"); 6.72 (1H, dd, $^{4}J_{H\text{-}H}$ = 1.79, $^{3}J_{H\text{-}H}$ = 8.89, H-6); 6.76 (2H, dd, $^{4}J_{H\text{-}H}$ = 1.79, $^{3}J_{H\text{-}H}$ = 7.62, H-5"); 7.67 (1H, d, $^{3}J_{H\text{-}H}$ = 8.95, H-5); 8.06 (1H, m, H-8); 8.16 (2H, s, H-3"); 8.30 (1H, d, $^{3}J_{H\text{-}H}$ = 6.13, H-2). **^{13}C{^{1}H} NMR (100.635 MHz, DMSO-*d*$_6$):** (δ, ppm) 41.91 (C-1'); 52.90 (C-2'); 55.21 (C-1''); 55.90 (C-2"); 56.42 (C-10"); 99.16 (C-3); 115.58 (C-7"); 116.84; 117.24 (C-6"); 118.49; 123.56 (C-5); 123.83 (C-5"); 125.15 (C-6); 125.79 (C-8); 136.09; 147.44; 147.84; 149.01; 153.16; 154.26 (C-2); 166.71 (C-3"). **IR (KBr):** (ν_{max}/cm^{-1}) 3414 (N-H); 1635 (C=N); 1613 (C=N) quinoline. **EI-MS:** *m/z* 574.9313 (5%, [M]$^{+}$). **Elemental Analysis** $C_{31}H_{34}ClN_5O_4{\cdot}3H_2O$ calculated: C 59.09; H 5.44; N 11.11%, found: C 59.52; H 6.05; N 11.53%.

*Bis-salicylaldimine quinoline (**3.7**)*



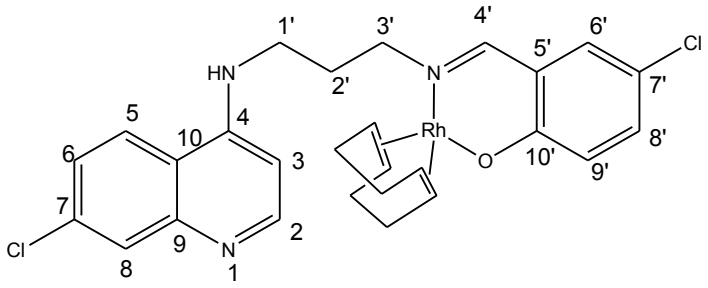
N-(7-Chloroquinolin-4-yl)-tris(2-aminoethyl)amine (**3.1**) (0.151 g, 0.491 mmol) was reacted with salicylaldehyde (0.126 g, 1.04 mmol). The product (**3.7**) was filtered and isolated as a yellow powder (0.113 g, 44%). **M.p.** 147 - 150°C. **^{1}H NMR (399.951, CDCl$_3$):** (δ, ppm) 2.94-3.01 (6H, m, H-2', H-1"); 3.43 (2H, m, H-1'); 3.66-3.72 (4H, m, H-2"); 6.29 (1H, d, $^{3}J_{H\text{-}H}$ = 6.63, H-3); 6.49 (1H, dd, $^{4}J_{H\text{-}H}$ = 2.08, $^{3}J_{H\text{-}H}$ = 8.96, H-6); 6.67-6.71 (4H, m, H-8", H6"); 6.92 (2H, dd, $^{4}J_{H\text{-}H}$ = 1.53, $^{3}J_{H\text{-}H}$ = 7.63, H-5"); 7.19 (2H, m, H-7"); 7.33 (1H, br s, NH); 7.36 (1H, d, $^{3}J_{H\text{-}H}$ = 9.04, H-5); 8.04 (1H, d, $^{4}J_{H\text{-}H}$ = 2.07, H-8); 8.09 (1H, d, $^{3}J_{H\text{-}H}$ = 6.53, H-2); 8.29 (2H, s, H-3"). **^{13}C{^{1}H} NMR (100.635 MHz, DMSO-*d*$_6$):** (δ, ppm) 41.87 (C-1'); 52.45 (C-2'); 54.96 (C-1''); 56.62 (C-2"); 99.00 (C-3); 117.04 (C-8"); 118.53 (6"); 118.82; 121.05; 125.67 (C-5); 126.52 (C-6); 129.58 (C-8); 131.88 (C-5"); 132.68 (C-7"); 136.79; 137.37; 146.64; 154.73 (C-2); 161.61; 166.78 (C-3"). **IR (KBr):** (ν_{max}/cm^{-1}) 3414 (N-H); 1633 (C=N); 1615 (C=N) quinoline. **EI-MS:** *m/z* 514.9832 (6%, [M]$^{+}$). **Elemental Analysis** $C_{29}H_{30}ClN_5O_2 \cdot 2.5H_2O$ calculated: C 62.08; H 6.29; N 12.48%, found: C 62.00; H 5.89; N 13.68%.

6.3.3. Synthetic methods and characterisation of 4-amino-7-chloroquinoline mono-imine Rh(I) complexes 3.9 – 3.11

6.3.3.1. General method to prepare complexes 3.9 – 3.11

The appropriate ligand was dissolved in dry DCM (30 ml) and to this NaH was added in excess. The mixture was stirred at room temperature for 1.5 hours under argon. A solution of $[RhCl(COD)]_2$ in DCM (5 ml) was then added dropwise to the mixture and stirred for 3 hours at room temperature. After this time, water (20 ml) was added to the reaction mixture and the organic extract washed with water (3 x 20 ml). The organic layer was dried over anhydrous $MgSO_4$. The drying agent was removed by filtration and the solvent of the resulting filtrate reduced under vacuum. The product was precipitated with *n*-hexane and filtered using a Büchner funnel.

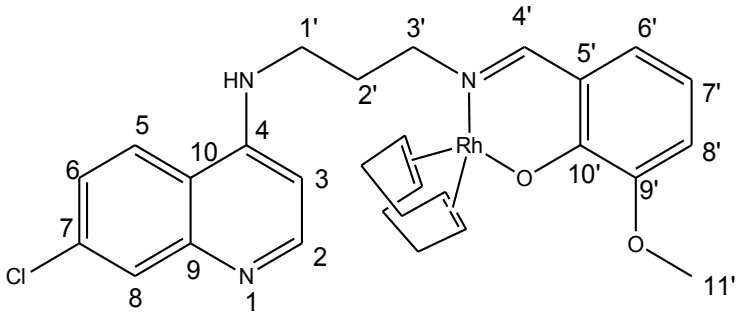
*Mono-5-chlorosalicylaldimine quinoline Rh(I)complex (**3.9**)*



Compound **3.2** (81.4 mg, 0.217 mmol), NaH (6.24 mg, 0.258 mmol) and $[RhCl(COD)]_2$ (53.4 mg, 0.108 mmol) were reacted. The product (**3.9**) was isolated as a bright yellow powder (85.5 mg, 68%). **M.p.** 130 - 134°C. **^{1}H NMR (399.951 MHz, $CDCl_3$):** (δ, ppm) 1.84 (4H, m, CH_2 $(COD)_{exo}$); 2.17 (2H, m, H-2'); 2.34 (4H, m, CH_2 $(COD)_{endo}$); 3.33 (2H, t, $^3J_{H\text{-}H}$ = 7.28, H-1'); 3.41 (2H, m, H-3'); 3.66 (2H, m, C*H*=CH (COD)); 4.54 (2H, m, CH=C*H* (COD)); 5.08 (1H, br s, NH); 6.41 (1H, d, $^3J_{H\text{-}H}$ = 5.25, H-3); 6.78 (1H, d, $^3J_{H\text{-}H}$ = 9.04, H-9'); 7.05 (1H, d, $^4J_{H\text{-}H}$ = 2.72, H-6'); 7.21 (1H, dd, $^4J_{H\text{-}H}$ = 2.83, $^3J_{H\text{-}H}$ = 9.36, H-8'); 7.34 (1H, dd, $^4J_{H\text{-}H}$ = 2.13, $^3J_{H\text{-}H}$ = 8.98, H-6); 7.64 (1H, d, $^3J_{H\text{-}H}$ = 8.90, H-5); 7.87 (1H, s, H-4'); 7.97 (1H, d, $^4J_{H\text{-}H}$ = 2.03, H-8); 8.56 (1H, d, $^3J_{H\text{-}H}$ = 5.18, H-2). **^{13}C{^{1}H} NMR (100.635 MHz, $CDCl_3$):** (δ, ppm) 28.81 (COD); 31.63 (COD); 32.66 (C-2'); 40.87 (C-1'); 57.19 (C-3'); 71.66 (d, $^1J_{Rh\text{-}C}$ = 14.09,

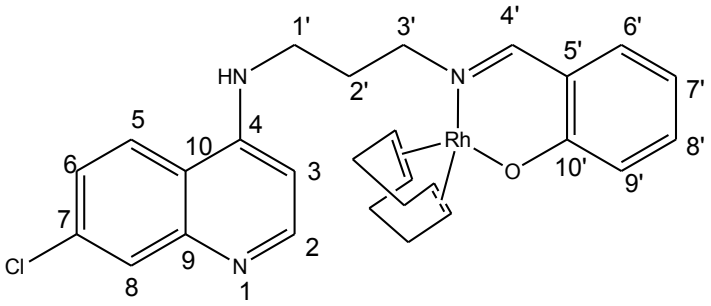
COD); 86.04 (d, $^{1}J_{Rh\text{-}C}$ = 11.86, COD); 99.17 (C-3); 117.25; 118.45; 119.53; 121.01 (C-5); 123.25 (C-9'); 125.50 (C-6); 128.92 (C-8); 132.87 (C-6'); 134.85 (C-8'); 135.04; 149.23; 149.28; 152.05 (C-2); 158.15; 164.96 (C-4'). **IR (KBr):** (ν_{max}/cm^{-1}) 3435 (N-H); 1609 (C=N). **EI-MS:** *m/z* 582.7133 (30%, $[M]^{+}$). **Elemental Analysis** $C_{27}H_{28}Cl_2N_3ORh$ calculated: C 55.50; H 4.83; N 7.19%, found: C 55.15; H 5.34; N 6.64%.

*Mono-3-methoxysalicylaldimine quinoline Rh(I)complex (**3.10**)*



Compound **3.3** (91.3 mg, 0.247 mmol), NaH (7.08 mg, 0.295 mmol) and $[RhCl(COD)]_2$ (61.3 mg, 0.124 mmol) were reacted. The product (**3.10**) was isolated as a bright yellow powder (93.2 mg, 65%). **^{1}H NMR (399.951 MHz, CDCl$_3$):** (δ, ppm) 1.85 (4H, m, CH_2 $(COD)_{exo}$); 2.16 (2H, m, H-2'); 2.35 (4H, m, CH_2 $(COD)_{endo}$); 3.33 (2H, t, $^{3}J_{H\text{-}H}$ = 6.89, H-1'); 3.39 (4H, m, H-3'); 3.68 (2H, m, C*H*=CH (COD)); 3.81 (3H, s, H-11') 4.66 (2H, m, CH=C*H* (COD)); 5.14 (1H, br s, NH); 6.38 (1H, d, $^{3}J_{H\text{-}H}$ = 5.33, H-3); 6.46 (1H, t, $^{3}J_{H\text{-}H}$ = 7.80, H-7'); 6.73 (1H, dd, $^{4}J_{H\text{-}H}$ = 1.55, $^{3}J_{H\text{-}H}$ = 8.14, H-8') ; 6.85 (1H, dd, $^{2}J_{H\text{-}H}$ = 1.58, $^{3}J_{H\text{-}H}$ = 7.52, H-6'); 7.34 (1H, dd, $^{4}J_{H\text{-}H}$ = 2.05, $^{3}J_{H\text{-}H}$ = 8.87, H-6); 7.66 (1H, d, $^{3}J_{H\text{-}H}$ = 8.95, H-5); 7.95 (2H, m, H-4', H-8); 8.53 (1H, d, $^{3}J_{H\text{-}H}$ = 5.30, H-2). **^{13}C{^{1}H} NMR (100.635 MHz, CDCl$_3$):** (δ, ppm) 28.81 (COD); 31.68 (COD); 32.66 (C-2'); 40.89 (C-3'); 56.74 (C-1'); 57.01 (C-11'); 71.21 (d, $^{1}J_{Rh\text{-}C}$ = 14.12, COD); 85.49 (d, $^{1}J_{Rh\text{-}C}$ = 11.78, COD); 99.09 (C-3); 113.65 (C-7'); 115.82 (C-6'); 117.26; 119.11; 121.15 (C-5); 125.46; (C-6); 126.84 (C-8'); 128.82 (C-8); 132.22; 134.97; 149.21; 149.34; 151.24; 152.04 (C-2); 165.78 (C-4'). **IR (KBr):** (ν_{max}/cm^{-1}) 3439 (N-H); 1613 (C=N). **EI-MS:** *m/z* 578.7728 (2%, $[M]^{+}$). **Elemental Analysis** $C_{28}H_{31}ClN_3O_2Rh$ calculated: C 57.99; H 5.39; N 7.25%, found: C 57.19; H 6.11; N 5.36%.

*Mono-salicylaldimine quinoline Rh(I)complex (**3.11**)*



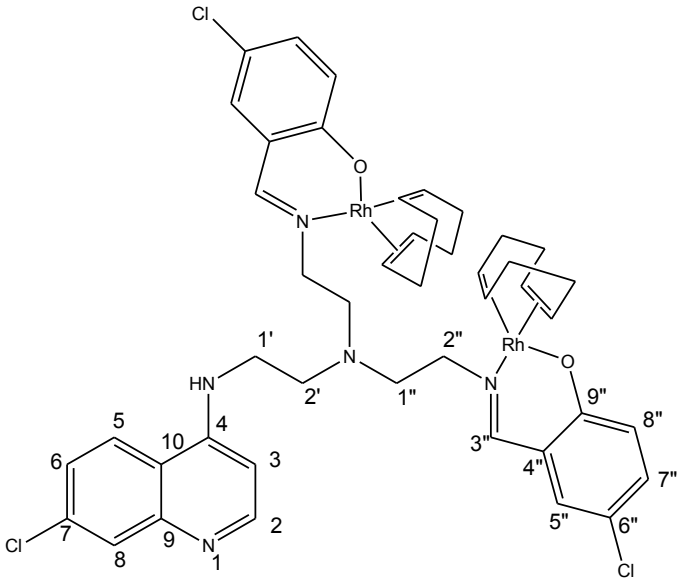
Compound **3.4** (0.108 g, 0.318 mmol), NaH (15.0 mg, 0.620 mmol) and $[RhCl(COD)]_2$ (0.078 mg, 0.159 mmol) were reacted. The product (**3.11**) was isolated as a bright yellow powder (90.0 mg, 52%). **M.p.** 127 - 129°C. **^{1}H NMR (399.951 MHz, $CDCl_3$):** (δ, ppm) 1.84 (4H, m, CH_2 $(COD)_{exo}$); 2.17 (2H, m, H-2'); 2.34 (4H, m, CH_2 $(COD)_{endo}$); 3.35 (2H, t, $^3J_{H\text{-}H}$ = 6.83, H-1'); 3.41 (4H, m, H-3'); 3.66 (2H, m, C*H*=CH (COD)); 4.54 (2H, m, CH=C*H* (COD)); 5.12 (1H, br s, NH); 6.39 (1H, d, $^3J_{H\text{-}H}$ = 5.36, H-3); 6.53 (1H, t, $^3J_{H\text{-}H}$ = 7.88, H-7'); 6.85 (1H, d, $^3J_{H\text{-}H}$ = 8.51, H-9') ; 7.09 (1H, dd, $^4J_{H\text{-}H}$ = 1.76, $^3J_{H\text{-}H}$ = 7.93, H-6'); 7.27-7.34 (2H, m, H-6, H-8'); 7.65 (1H, d, $^3J_{H\text{-}H}$ = 8.98, H-5); 7.94 (2H, m, H-4', H-8); 8.54 (1H, d, $^3J_{H\text{-}H}$ = 5.29, H-2). **^{13}C{^{1}H} NMR (100.635 MHz, $CDCl_3$):** (δ, ppm) 28.83 (COD); 31.67 (COD); 32.66 (C-2'); 40.98 (C-3'); 57.02 (C-1'); 71.29 (d, $^1J_{Rh\text{-}C}$ = 14.08, COD); 85.70 (d, $^1J_{Rh\text{-}C}$ = 11.96, COD); 99.13 (C-3); 114.62 (C-7'); 117.26; 119.02; 121.10 (C-5); 121.63 (C-9'); 125.47; (C-6); 128.89 (C-8); 134.90 (C-6'); 135.01 (C-8'); 149.24; 149.34; 152.06 (C-2); 166.02 (C-4); 166.37. **IR (KBr):** (ν_{max}/cm^{-1}) 3431 (N-H); 1607 (C=N). **EI-MS:** *m/z* 548.6803 (15%, $[M]^+$). **Elemental Analysis** $C_{27}H_{29}ClN_3ORh$ calculated: C 58.97; H 5.32; N 7.64%, found: C 58.61; H 5.64; N 6.30%.

6.3.4. Synthetic methods and characterisation of 4-amino-7-chloroquinoline bis-imine Rh(I) complexes 3.12 – 3.14

6.3.4.1. General method to prepare complexes 3.12 – 3.14

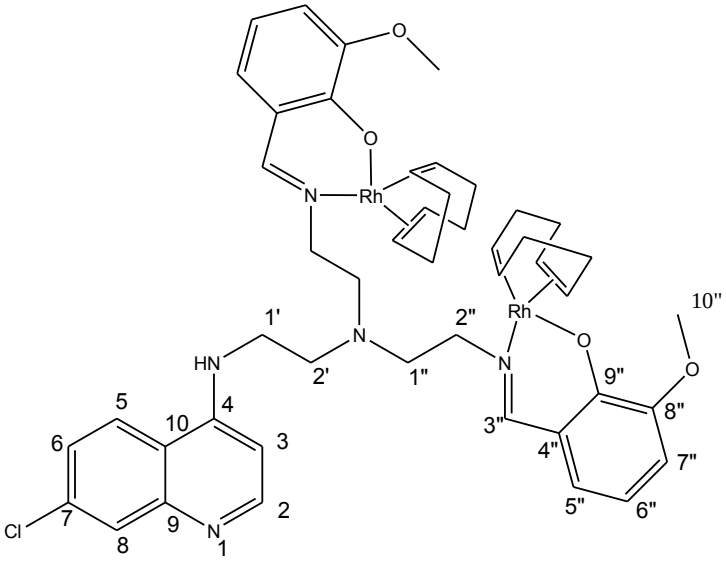
The appropriate ligand was dissolved in dry DCM (30 ml) and to this NaH was added in excess. The mixture was stirred at room temperature for 1.5 hours under argon. A solution of $[RhCl(COD)]_2$ in DCM (5 ml) was then added dropwise to the mixture and stirred for 5-6 hours at room temperature. After this time, water (20 ml) was added to the reaction mixture and the organic extract washed with water (3 x 20 ml). The organic layer was dried over anhydrous $MgSO_4$. The drying agent was removed by filtration and the solvent of the resulting filtrate reduced under vacuum. The product was precipitated with *n*-hexane and filtered using a Büchner funnel.

*Bis-5-chlorosalicylaldimine quinoline Rh(I)complex (**3.12**)*



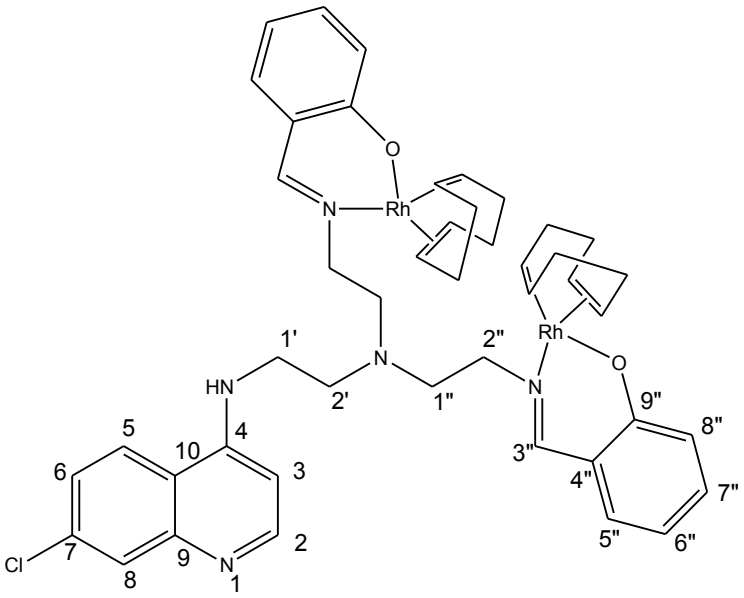
Compound **3.5** (107 mg, 0.184 mmol), NaH (16.6 mg, 0.692 mmol) and $[RhCl(COD)]_2$ (90.2 mg, 0.183 mmol) were reacted. The product (**3.12**) was isolated as a bright yellow powder (148 mg, 79%). **M.p.** 162 - 165°C. **^{1}H NMR (399.935, $CDCl_3$):** (δ, ppm) 1.84 (8H, m, CH_2 $(COD)_{exo}$); 2.37 (8H, m, CH_2 $(COD)_{endo}$); 2.88 (6H, m, H-1", 2'); 3.17 (4H, m, H-2"); 3.46 (2H, m, H-1'); 3.51 (4H, m, C*H*=CH (COD)); 4.52 (4H, m, CH=C*H* (COD)); 5.23 (1H, br s, NH); 6.37 (1H, d, $^3J_{H\text{-}H}$ = 5.52, H-3); 6.72 (2H, d, $^3J_{H\text{-}H}$ = 9.02, H-8"); 7.03 (2H, d, $^4J_{H\text{-}H}$ = 2.61, H-5"); 7.18 (2H, dd, $^4J_{H\text{-}H}$ = 2.64, $^3J_{H\text{-}H}$ = 9.05, H-7") 7.26-7.31 (2H, m, H-5, H-6); 7.71 (2H, s, H-3"); 7.93 (1H, m, H-8); 8.53 (1H, d, $^3J_{H\text{-}H}$ = 5.01, H-2). **^{13}C{^{1}H} NMR (100.635 MHz, $CDCl_3$):** (δ, ppm) 28.93 (COD); 31.67 (COD); 40.88 (C-1'); 53.96 (C-2'); 57.89 (C-2"); 58.21 (C-1"); 71.48 (d, $^1J_{Rh\text{-}C}$ = 14.03, COD); 86.08 (d, $^1J_{Rh\text{-}C}$ = 11.53, COD); 99.31 (C-3); 118.61; 119.54; 120.66 (C-5); 120.90; 123.30 (C-8"); 125.73 (C-6); 128.94 (C-8); 132.75 (C-5"); 134.88 (C-7"); 135.04; 149.25; 149.32; 151.99 (C-2); 159.20; 164.81 (C-4'). **IR (KBr):** (ν_{max}/cm^{-1}) 3427 (N-H); 1607 (C=N). **ESI-MS (HR)** *m/z* 1004.1231 (55%, $[M+H]^+$ requires 1004.1218). **Elemental Analysis** $C_{45}H_{50}Cl_3N_5O_2Rh_2$ calculated: C 53.88; H 5.02; N 6.98%, found: C 54.08; H 5.50; N 6.09%.

*Bis-3-methoxysalicylaldimine quinoline Rh(I)complex (**3.13**)*



Compound **3.6** (90.2 mg, 0.156 mmol), NaH (13.1 mg, 0.546 mmol) and $[RhCl(COD)]_2$ (77.5 mg, 0.157 mmol) were reacted. The product (**3.13**) was isolated as a bright yellow powder (121 mg, 78%). **M.p.** 149 - 153°C. **^{1}H NMR (399.935, $CDCl_3$):** (δ, ppm) 1.83 (8H, m, CH_2 $(COD)_{exo}$); 2.36 (8H, m, CH_2 $(COD)_{endo}$); 2.87 (6H, m, H-1", H-2'); 3.18 (4H, m, H-2"); 3.43 (2H, m, H-1'); 3.51 (4H, m, C*H*=CH (COD)); 3.77 (6H, s, H-10"); 4.64 (4H, m, CH=C*H* (COD)); 5.19 (1H, br s, NH); 6.32 (1H, d, $^3J_{H\text{-}H}$ = 5.46, H-3); 6.46 (2H, t, $^3J_{H\text{-}H}$ = 7.75, H-6"); 6.64 (2H, dd, $^4J_{H\text{-}H}$ = 1.42, $^3J_{H\text{-}H}$ = 8.07, H-7"); 6.84 (2H, dd, $^4J_{H\text{-}H}$ = 1.54, $^3J_{H\text{-}H}$ = 7.58, H-5"); 7.06-7.20 (2H, m, H-5, H-6); 7.75 (2H, s, H-3"); 7.90 (1H, m, H-8); 8.48 (1H, d, $^3J_{H\text{-}H}$ = 5.15, H-2). **^{13}C{^{1}H} NMR (100.635 MHz, $CDCl_3$):** (δ, ppm) 28.90 (COD); 31.68 (COD); 41.12 (C-1'); 53.64 (C-2'); 56.65 (C-2"); 58.08 (C-1", C-10'); 71.04 (d, $^1J_{Rh\text{-}C}$ = 14.18, COD); 85.47 (d, $^1J_{Rh\text{-}C}$ = 11.68, COD); 99.11 (C-3); 113.74 (C-6"); 115.65 (C-5"); 117.28; 119.08; 121.23 (C-5); 125.34 (C-6); 126.77 (C-7"); 128.70 (C-8); 134.78; 149.21; 149.35; 151.30 151.99 (C-2); 158.01; 165.58 (C-3"). **IR (KBr):** (ν_{max}/cm^{-1}) 3362 (N-H); 1604 (C=N). **ESI-MS (HR)** *m/z* 996.2199 (20%, $[M+H]^+$, requires 996.2209). **Elemental Analysis** $C_{47}H_{56}ClN_5O_4Rh_2 \cdot H_2O$ calculated: C 55.66; H 5.76; N 6.90%, found: C 55.20; H 5.93; N 6.02%.

*Bis-salicylaldimine quinoline Rh(I)complex (**3.14**)*

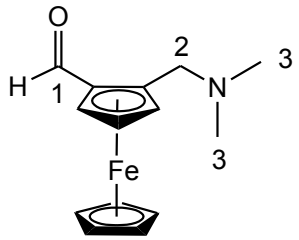


Compound **3.7** (0.065 g, 0.126 mmol), NaH (9.00 mg, 0.412 mmol) and $[RhCl(COD)]_2$ (61.0 mg, 0.125 mmol) were reacted. The product (**3.14**) was isolated as a bright yellow powder (76.0 mg, 65%). **^{1}H NMR (399.935, $CDCl_3$):** (δ, ppm) 1.82 (8H, m, CH_2 $(COD)_{exo}$); 2.36 (8H, m, CH_2 $(COD)_{endo}$); 2.88 (6H, m, H-1", H-2'); 3.19 (4H, m, H-2"); 3.47 (2H, m, H-1'); 3.51 (4H, m, C*H*=CH (COD)); 4.53 (4H, m, CH=C*H* (COD)); 5.28 (1H, br s, NH); 6.34 (1H, d, $^3J_{H\text{-}H}$ = 5.05, H-3); 6.53 (2H, t, $^3J_{H\text{-}H}$ = 7.47, H-6"); 6.80 (2H, d, $^3J_{H\text{-}H}$ = 8.53, H-8"); 7.01 (2H, d, $^3J_{H\text{-}H}$ = 7.64, H-5"); 7.19-7.34 (4H, m, H-6", H-5, H-6); 7.76 (2H, s, H-3"); 7.92 (1H, m, H-8); 8.51 (1H, d, $^3J_{H\text{-}H}$ = 5.64, H-2). **^{13}C{^{1}H} NMR (100.635 MHz, $CDCl_3$):** (δ, ppm) 28.95 (COD); 31.69 (COD); 40.94 (C-1'); 53.63 (C-2'); 57.95 (C-2"); 58.06 (C-1"); 71.13 (d, $^1J_{Rh\text{-}C}$ =13.69, COD); 85.67 (d, $^1J_{Rh\text{-}C}$ = 11.67, COD); 99.22 (C-3); 114.70 (C-6"); 117.29; 119.04; 121.09 (C-5); 121.66 (C-8"); 125.45 (C-6); 128.78 (C-8); 134.88 (C-7"; C-5"); 149.20; 149.35; 152.01 (C-2); 165.78 (C-3"); 166.35. **IR (KBr):** (ν_{max}/cm^{-1}) 3418 (N-H); 1606 (C=N). **ESI-MS (HR)** *m/z* 936.2004 (28%, $[M+H]^+$, requires 936.1998). **Elemental Analysis** $C_{45}H_{52}ClN_5O_2Rh_2{\cdot}2H_2O$ calculated: C 55.59; H 5.81; N 7.20%, found: C 55.81; H 5.80; N 6.49%.

6.4. Experimental details for Chapter 4

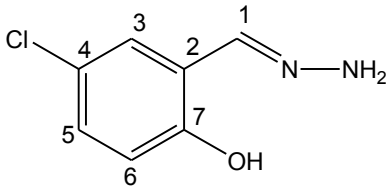
6.4.1. Synthetic methods and characterisation of ferrocenyl ligands and precursors 4.1 – 4.7

*(Dimethylamino)methyl ferrocenecarboxaldehyde (**4.1**)*[10]



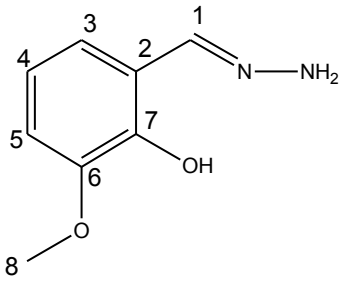
To a solution of (dimethylamino)methyl ferrocene (2.00 g, 8.23 mmol) in anhydrous diethyl ether (20 ml), *n*-butyllithium (6 ml, 12.0 mmol) was added slowly at -78°C under an argon atmosphere. The solution was allowed to warm to room temperature and stirred for a further 18 hours. After this time, an orange precipitate was observed. Anhydrous DMF (0.8 ml, 10.3 mmol) was added dropwise to the mixture. The resulting mixture was stirred at room temperature for an additional 4 hours. The reaction was quenched with water (20 ml). The aqueous layer was washed with diethyl ether (3 x 15 ml) and the combined organic extracts dried using sodium sulphate. The solution was filtered by gravity and the solvent removed under vacuum. The product was purified by means of column chromatography. Diethyl ether: petroleum ether: triethylamine (70:20:10) was used to elute the product. Compound **4.1** was obtained as a dark red oil (1.50 g, 67%). **^{1}H NMR (399.951 MHz, $CDCl_3$):** (δ, ppm) 2.15 (6H, s, H-3); 3.28 (1H, d, $^{2}J_{H\text{-}H}$ = 13.18, H-2a); 3.76 (1H, d, $^{2}J_{H\text{-}H}$ = 13.18, H-2b); 4.16 (5H, s, Cp_{unsub}); 4.48 (1H, m, Cp_{sub}); 4.54 (1H, m, Cp_{sub}); 4.71 (1H, m, Cp_{sub}); 10.03 (1H, s, H-1). **^{13}C{^{1}H} NMR (100.635 MHz, $CDCl_3$):** (δ, ppm) 44.67 (C-3); 56.46 (C-2); 70.08 (Cp); 70.28 (Cp); 71.72 (Cp); 75.74 (Cp); 77.73; 86.48; 193.10 (C-1). **IR (NaCl cells):** (ν_{max}/cm^{-1}) 1675 (C=O); 1443 (N-CH_3).

*5-Chlorosalicylaldehyde hydrazone (**4.2**)*[11]



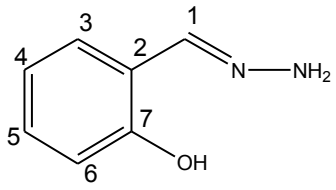
To a stirred solution of hydrazine hydrate (2 ml) in absolute EtOH (70 ml), a solution of 5-chlorosalicylaldehyde (1.24 g, 7.95 mmol) in absolute EtOH (30 ml) was added dropwise over a 2 hour period. After this time, the solvent was reduced and the solution kept at -20°C for 16 hours. After this time, a precipitate formed and was filtered. The product was dried *in vacuo*. Compound **4.2** was obtained as a white powder (0.434 g, 32%). **^{1}H NMR (399.951 MHz, $CDCl_3$):** (δ, ppm) 5.51 (2H, br s, NH_2); 6.87 (1H, d, $^3J_{H\text{-}H}$ = 8.79, H-6); 7.05 (1H, d, $^4J_{H\text{-}H}$ = 2.56, H-3); 7.14 (1H, dd, $^4J_{H\text{-}H}$ = 2.56, $^3J_{H\text{-}H}$ = 8.79, H-5); 7.77 (1H, s, H-1); 10.98 (1H, s, OH). **IR (ATR):** (ν_{max}/cm^{-1}) 3384 (N-H); 1619 (C=N); 1574 (C=C).

*3-Methoxysalicylaldehyde hydrazone (**4.3**)*[12]



To a stirred solution of hydrazine hydrate (1.56 ml) in absolute EtOH (70 ml), a solution of 5-chlorosalicylaldehyde (1.03 g, 6.80 mmol) in absolute EtOH (30 ml) was added dropwise over a 2 hour period. After this time, the solvent was reduced to about 2 ml and the solution kept at -20°C for 16 hours. After this time, the precipitate was filtered and washed with water to remove excess hydrazine hydrate. The compound was dried *in vacuo*. Compound **4.3** was obtained as a pale yellow powder (0.326 g, 29%). **^{1}H NMR (399.951 MHz, $CDCl_3$):** (δ, ppm) 3.90 (3H, s, H-8); 5.45 (2H, br s, NH_2); 6.73 (1H, dd, $^4J_{H\text{-}H}$ = 1.83, $^3J_{H\text{-}H}$ = 7.69, H-5); 6.81 (1H, t, $^3J_{H\text{-}H}$ = 7.69, H-4); 6.85 (1H, dd, $^4J_{H\text{-}H}$ = 1.83, $^3J_{H\text{-}H}$ = 7.69, H-3); 7.86 (1H, s, H-1); 11.15 (1H, s, OH). **IR (ATR):** (ν_{max}/cm^{-1}) 3418 (N-H); 1623 (C=N); 1574 (C=C).

*Salicylaldehyde hydrazone (**4.4**)*[12]

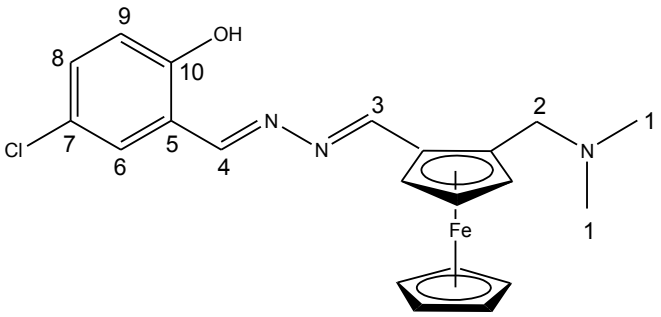


To a stirred solution of hydrazine hydrate (2 ml) in absolute EtOH (70 ml), a solution of salicylaldehyde (0.990 g, 8.11 mmol) in absolute EtOH (15 ml) was added dropwise over a 1 hour period. The solution was stirred for an additional 10 minutes. After this time, the solvent was reduced and the solution kept at -20°C for 16 hours. After this time, a precipitate formed and was filtered. Compound **4.4** was obtained as a white crystalline solid (0.496 g, 45%). **^{1}H NMR (399.951 MHz, $CDCl_3$):** (δ, ppm) 5.43 (2H, br s, NH_2); 6.85 (1H, t, $^3J_{H\text{-}H}$ = 7.69, H-4); 6.93 (1H, d, $^3J_{H\text{-}H}$ = 8.06, H-6); 7.09 (1H, d, $^3J_{H\text{-}H}$ = 7.69, H-3); 7.20 (1H, t, $^3J_{H\text{-}H}$ = 8.79, H-5); 7.86 (1H, s, H-1); 11.02 (1H, s, OH). **IR (ATR):** (ν_{max}/cm^{-1}) 3380 (N-H); 1613 (C=N); 1570 (C=C).

6.4.1.1. General method to prepare ferrocenyl azines 4.5 – 4.7

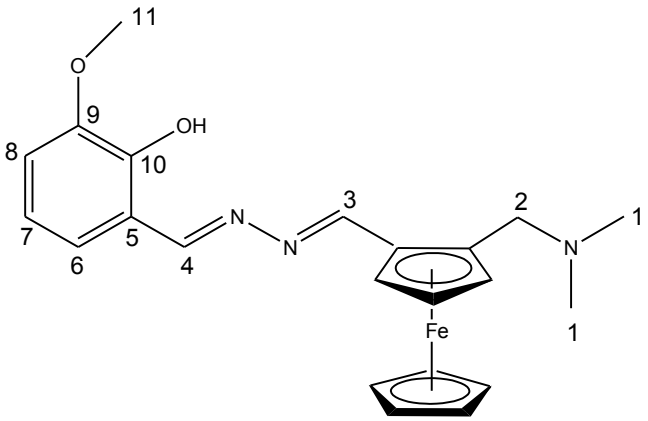
To a stirred solution of the (dimethylamino)methyl ferrocenecarboxaldehyde **4.1** in diethyl ether (20 ml) under argon, the appropriate salicylaldehyde hydrazone was added and the resulting solution stirred for 16 hours at room temperature. As time continued, the solution became dark red in colour. After the 16 hour period, the solvent was removed and the residue purified by silica gel column chromatography. The starting reagents were eluted using 100% diethyl ether. The product was then eluted using a solution of DCM:MeOH (80:20) and 5% Et_3N.

*5-Chlorosalicylaldimine ferrocene (**4.5**)*



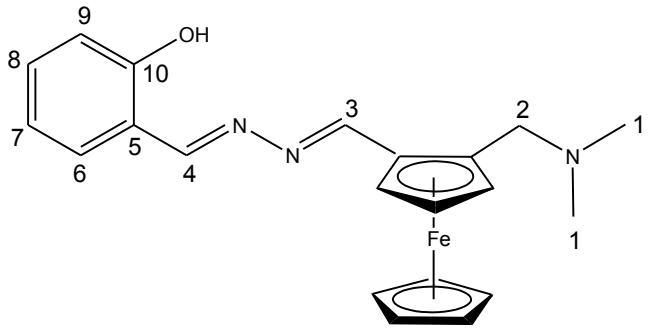
(Dimethylamino)methyl ferrocenecarboxaldehyde **4.1** (0.136 g, 0.499 mmol) and 5-chlorosalicylaldehyde hydrazone **4.2** (0.0849 g, 0.498 mmol) was reacted. The product (**4.5**) was obtained as a red oil (0.186 g, 88%).**^{1}H NMR (399.951 MHz, CDCl$_3$):** (δ, ppm) 2.21 (6H, s, H-1); 3.29 (1H, d, $^{2}J_{H\text{-}H}$ = 13.01, H-2a); 3.73 (1H, d, $^{2}J_{H\text{-}H}$ = 12.92; H-2b); 4.18 (5H, s, Cp$_{unsub}$); 4.51 (1H, t, $^{3}J_{H\text{-}H}$ = 2.57, Cp$_{sub}$); 4.54 (1H, m, Cp$_{sub}$); 4.86 (1H, m, Cp$_{sub}$); 6.96 (1H, d, $^{3}J_{H\text{-}H}$ = 9.48, H-9); 7.27 – 7.30 (1H, m, H-6, H-8); 8.59 (1H, s, H-4); 8.66 (1H, s, H-3); 11.88 (1H, s, OH). **^{13}C{^{1}H} NMR (100.635 MHz, CDCl$_3$):** (δ, ppm) 44.96 (C-1); 57.18 (C-2); 68.42 (Cp$_{sub}$); 70.12 (Cp$_{unsub}$); 70.74 (Cp$_{sub}$); 71.56; 74.33 (Cp$_{sub}$); 76.21; 118.42 (C-9); 119.07; 124.10; 130.91 (C-6); 132.04 (C-8); 158.14; 161.23 (C-3); 163.91 (C-4). **IR (ATR):** (ν_{max}/cm^{-1}) 1618 (C=N); 1582 (C=C). **ESI-MS (HR):** *m/z* 424.0874 (100%, [M+H]$^{+}$, requires 424.0879). **Elemental Analysis** $C_{21}H_{22}ClFeN_3O$ calculated: C 59.53; H 5.23; N 9.92%, found: C 59.61; H 5.50; N 8.40%.

*3-Methoxysalicylaldimine ferrocene (**4.6**)*



(Dimethylamino)methyl ferrocenecarboxaldehyde **4.1** (0.167 g, 0.616 mmol) and 3-methoxysalicylaldehyde hydrazone **4.3** (0.103 g, 0.620 mmol) was reacted. Compound **4.6** was obtained as a red oil (0.171, 66%). **^{1}H NMR (399.951 MHz, $CDCl_3$):** (δ, ppm) 2.22 (6H, s, H-1); 3.32 (1H, d, $^{2}J_{H\text{-}H}$ = 12.61, H-2a); 3.74 (1H, d, $^{2}J_{H\text{-}H}$ = 13.01; H-2b); 3.95 (3H, s, H-11); 4.19 (5H, s, Cp_{unsub}); 4.50 (1H, t, $^{3}J_{H\text{-}H}$ = 2.58, Cp_{sub}); 4.54 (1H, m, Cp_{sub}); 4.87 (1H, m, Cp_{sub}); 6.89 (1H, m, H-7); 6.98 (2H, m, H-6, H-8); 8.66 (1H, s, H-3); 8.67 (1H, s, H-4); 12.18 (1H, s, OH). **^{13}C{^{1}H} NMR (100.635 MHz, $CDCl_3$):** (δ, ppm) 44.87 (C-1); 56.33 (C-11); 57.11 (C-2); 68.40 (Cp_{sub}); 70.09 (Cp_{unsub}); 70.58 (Cp_{sub}); 74.16 (Cp_{sub}); 76.43; 85.71; 114.60 (C-7); 118.18; 119.07 (C-6); 123.73 (C-8); 148.41; 149.64; 162.54 (C-3); 163.22 (C-4). **IR (ATR):** (ν_{max}/cm^{-1}) 1616 (C=N); 1587 (C=C). **ESI-MS (HR):** *m/z* 420.1369 (100%, $[M+H]^+$, requires 420.1374). **Elemental Analysis** $C_{22}H_{25}FeN_3O_2 \cdot H_2O$ calculated: C 60.42; H 6.22; N 9.61%, found: C 60.39; H 5.89; N 8.89%.

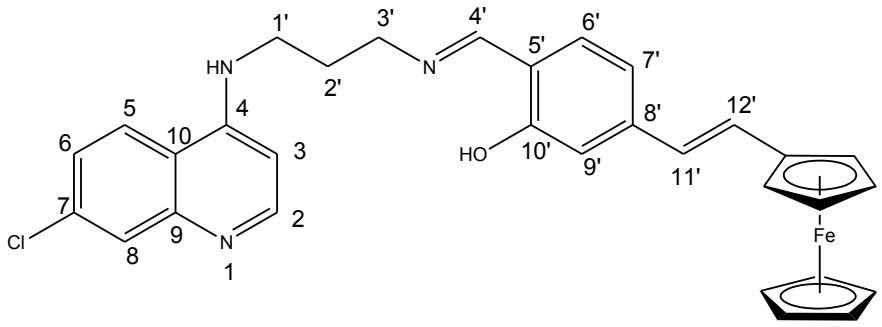
*Salicylaldimine ferrocene (**4.7**)*



(Dimethylamino)methyl ferrocenecarboxaldehyde **4.1** (0.250 g, 0.923 mmol) and salicylaldehyde hydrazone **4.4** (0.126 g, 0.925 mmol) was reacted. Compound **4.7** was obtained as a red oil (0.241, 67%). **^{1}H NMR (399.951 MHz, $CDCl_3$):** (δ, ppm) 2.21 (6H, s, H-1); 3.30 (1H, d, $^{2}J_{H\text{-}H}$ = 12.97, H-2a); 3.72 (1H, d, $^{2}J_{H\text{-}H}$ = 12.96; H-2b); 4.18 (5H, s, Cp_{unsub}); 4.49 (1H, t, $^{3}J_{H\text{-}H}$ = 2.44, Cp_{sub}); 4.52 (1H, m, Cp_{sub}); 4.86 (1H, m, Cp_{sub}); 6.93 (1H, t, $^{3}J_{H\text{-}H}$ = 7.27, H-7); 7.02 (1H, d, $^{3}J_{H\text{-}H}$ = 8.60, H-9); 7.31 - 7.37 (2H, m, H-8, H-6); 8.66 (1H, s, H-3); 8.67 (1H, s, H-4); 11.89 (1H, s, OH). **^{13}C{^{1}H} NMR (100.635 MHz, $CDCl_3$):** (δ, ppm) 45.01 (C-1); 57.18 (C-2); 68.18 (Cp_{sub}); 70.06 (Cp_{unsub}); 70.51 (Cp_{sub}); 74.07 (Cp_{sub}); 76.51; 86.02; 116.91 (C-7); 118.07; 119.44 (C-9); 132.03 (C-6); 132.34 (C-8); 159.61; 162.60 (C-3); 162.99 (C-4). **IR (ATR):** (ν_{max}/cm^{-1}) 1618 (C=N); 1585 (C=C). **ESI-MS (HR):** *m/z* 390.1258 (100%, $[M+H]^+$, requires 390.1269).

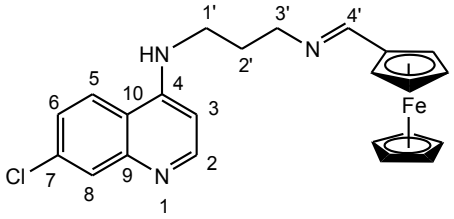
6.4.2. Synthetic methods and characterisation of ferrocenyl quinolines 4.9 – 4.11

*Salicylaldimine ferrocenyl quinoline (**4.9**)*



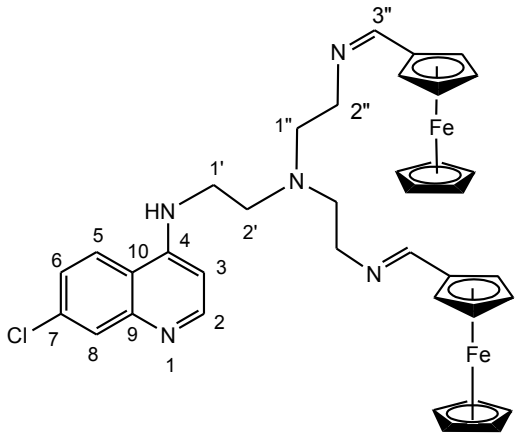
To a solution of ferrocene salicylaldehyde (**4.8**) (30.0 mg g, 0.0903 mmol) in 20 ml of DCM/MeOH (1:1), *N'*-(7-Chloroquinolin-4-yl)-propane-1,3-diamine (**2.1**) (20.4 mg, 0.0865 mmol) was added and the resulting solution stirred for 16 hours at room temperature under argon. The solvent was removed *in vacuo* and the resulting residue washed with cold diethyl ether (2 ml) to remove any unreacted aldehyde. The supernatant was decanted and the resulting red solid dried *in vacuo*. The product (**4.9**) was obtained as a red powder (36.8 mg, 77%). **M.p.** 69 - 72°C. **^{1}H NMR (300.077 MHz, CDCl$_3$):** (δ, ppm) 2.21 (2H, m, H-2'); 3.50 (m, 2H, H-1'); 3.78 (2H, t, $^{3}J_{H\text{-}H}$ = 6.35, H-3'); 4.16 (5H, s, Cp$_{unsub}$); 4.33 (2H, t, $^{3}J_{H\text{-}H}$ = 1.95, Cp$_{sub}$); 4.50 (2H, t, $^{3}J_{H\text{-}H}$ = 1.95, Cp$_{sub}$); 5.10 (1H, m, NH); 6.43 (1H, d, $^{3}J_{H\text{-}H}$ = 5.37, H-3); 6.65 (1H, d, $^{3}J_{H\text{-}H}$ = 16.13, H-11'); 6.94 – 7.06 (3H, m, H-7', H-9', H-12'); 7.16 (1H, d, $^{3}J_{H\text{-}H}$ = 7.81, H-6'); 7.31 (1H, dd, $^{4}J_{H\text{-}H}$ = 1.95, $^{3}J_{H\text{-}H}$ = 8.79, H-6); 7.63 (1H, d, $^{3}J_{H\text{-}H}$ = 8.79, H-5); 7.93 (1H, d, $^{4}J_{H\text{-}H}$ = 1.95, H-8); 8.35 (1H, s, H-4'); 8.54 (1H, d, $^{3}J_{H\text{-}H}$ = 5.37, H-2). **^{13}C{^{1}H} NMR (100.635 MHz, CDCl$_3$):** 30.01 (C-2'); 41.48 (C-1'); 57.40 (C-3'); 67.22 (Cp$_{sub}$); 69.32 (Cp$_{unsub}$); 69.48 (Cp$_{sub}$); 82.61; 99.11 (C-3); 113.59 (C-9'); 116.79 (C-7'); 117.14; 117.31; 120.82 (C-5); 125.12 (C-6); 125.43 (C-11'); 128.81 (C-8); 130.07 (C-12'); 131.60 (C-6'); 134.97; 142.38; 149.09; 149.56; 151.97 (C-2); 161.38; 165.37 (C-4'). **IR (ATR):** (ν_{max}/cm^{-1}) 1611 (C=N); 1578 (C=C). **ESI-MS (HR):** *m/z* 550.1339 (15%, [M+H]$^{+}$, requires 550.1348); 275.5708 (100%, [M+2H]$^{2+}$). **Elemental Analysis** $C_{31}H_{28}ClFeN_3O{\cdot}3H_2O$ calculated: C 61.65; H 5.67; N 6.96%, found: C 62.05; H 5.26; N 5.71%.

*Mono-ferrocenyl quinoline (**4.10**)*



To a suspension of *N'*-(7-Chloroquinolin-4-yl)-propane-1,3-diamine **2.1** (0.203 g, 0.862 mmol) in diethyl ether (30 ml), a solution of ferrocenecarboxaldehyde (0.204 g, 0.952 mmol) in diethyl ether (5 ml) was added and the mixture stirred for 16 hours at room temperature. After this time, an orange precipitate formed and was filtered, washed with diethyl ether to remove excess aldehyde and the solid dried *in vacuo*. Compound **4.10** was obtained as an orange powder (0.310 g, 83%). **M.p.** 83 - 85°C. **^{1}H NMR (399.951 MHz, $CDCl_3$):** (δ, ppm) 2.10 (2H, m, H-2'); 3.47 (2H, m, H-1'); 3.70 (2H, t, $^{3}J_{H\text{-}H}$ = 5.49, H-3'); 4.18 (5H, s, Cp_{unsub}); 4.46 (2H, t, $^{3}J_{H\text{-}H}$ = 1.83, Cp_{sub}); 4.69 (2H, t, $^{3}J_{H\text{-}H}$ = 1.83, Cp_{sub}); 6.36 (1H, d, $^{3}J_{H\text{-}H}$ = 5.49, H-3); 6.76 (1H, m, NH); 7.26 (1H, dd, $^{4}J_{H\text{-}H}$ = 1.83, $^{3}J_{H\text{-}H}$ = 8.79, H-6); 7.80 (1H, d, $^{3}J_{H\text{-}H}$ = 8.79, H-5); 7.93 (1H, d, $^{4}J_{H\text{-}H}$ = 2.19, H-8); 8.18 (1H, s, H-4'); 8.50 (1H, d, $^{3}J_{H\text{-}H}$ = 5.13, H-2). **^{13}C{^{1}H} NMR (100.635 MHz, $CDCl_3$):** (δ, ppm) 29.68 (C-2'); 43.57 (C-1'); 61.32 (C-3'); 68.50 (Cp_{sub}); 69.16 (Cp_{unsub}); 70.78 (Cp_{sub}); 80.31; 98.57 (C-3); 117.49; 122.07 (C-5); 124.83 (C-6); 128.63 (C-8); 134.66; 149.20; 150.27; 152.17 (C-2); 162.00 (C-4). **IR (KBr):** (ν_{max}/cm^{-1}) 3274 (N-H); 1638 (C=N); 1613 (C=N) quinoline. **ESI-MS:** *m/z* 432.0937 (50%, $[M+H]^+$). **Elemental Analysis** $C_{23}H_{22}ClFeN_3 \cdot 2H_2O$ calculated: C 59.06; H 5.60; N 8.98%, found: C 59.21; H 4.92; N 9.09%.

*Bis-ferrocenyl quinoline (**4.11**)*



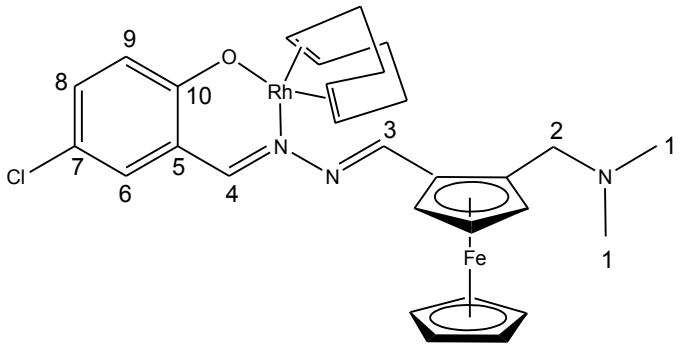
N-(7-Chloroquinolin-4-yl)-tris(2-aminoethyl)amine (**3.1**) (0.144 g, 0.469 mmol) was dissolved in EtOH (25 ml). To this, ferrocenecarboxaldehyde (0.200 g, 0.935 mmol) in EtOH (5 ml) was added and the resulting solution stirred at room temperature for 16 hours. The solvent was removed *in vacuo* and the residue washed continuously with pentane. The product (**4.11**) was isolated as a orange-brown powder (0.102 g, 31%). **M.p.** 67 - 70°C. **^{1}H NMR (399.951 MHz, CDCl$_3$):** (δ, ppm) 2.91 (4H, t, $^{3}J_{H\text{-}H}$ = 5.49, H-1"); 2.99 (2H, t, $^{3}J_{H\text{-}H}$ = 5.13, H-2'); 3.31 (2H, m, H-1'); 3.59 (4H, t, $^{3}J_{H\text{-}H}$ = 5.86, H-2"); 4.31 (10H, s, Cp$_{unsub}$); 4.29 (4H, m, Cp$_{sub}$); 4.50 (4H, m, Cp$_{sub}$); 6.33 (1H, d, $^{3}J_{H\text{-}H}$ = 5.13, H-3); 6.66 (1H, m, NH); 7.07 (1H, d, $^{3}J_{H\text{-}H}$ = 8.42, H-6); 7.70 (1H, d, $^{3}J_{H\text{-}H}$ = 8.79, H-5); 7.89 (1H, s, H-8); 8.10 (2H, s, H-3"); 8.48 (1H, d, $^{3}J_{H\text{-}H}$ = 5.13, H-2). **^{13}C{^{1}H} NMR (100.635 MHz, CDCl$_3$):** (δ, ppm). 41.11 (C-2'); 53.19 (C-1'); 55.95 (C-1"); 60.89 (C-2"); 68.48 (Cp$_{sub}$); 69.05 (Cp$_{unsub}$); 70.47 (Cp$_{sub}$) 80.31; 99.01 (C-3); 117.78; 122.35 (C-5); 125.18 (C-6); 128.31 (C-8); 134.54; 149.22; 150.37; 151.95 (C-2); 162.04 (C-3"). **IR (KBr):** (ν_{max}/cm^{-1}) 3267 (N-H); 1639 (C=N); 1611 (C=N) quinoline. **ESI-MS (HR):** *m/z* 700.1611 (100%, [M+H]$^{+}$, requires 700.1593). **Elemental Analysis** $C_{37}H_{38}ClFe_2N_5 \cdot 4H_2O$ calculated: C 57.57; H 6.01; N 9.07%, found: C 57.46; H 4.54; N 9.00%.

6.4.3. Synthetic methods and characterisation of ferrocenyl salicylaldimine Rh(I) complexes 4.12 – 4.14

6.4.3.1. General method to prepare complexes 4.12 – 4.14

To a solution of the appropriate ligand in DCM (30 ml), NaH was added. The mixture was stirred at room temperature under argon for 1.5 hours. After this time, a solution of $[RhCl(COD)]_2$ was added and the mixture stirred for a further 3 hours at room temperature. The resulting mixture was washed with water (2 x 20 ml) and the organic layer collected. The organic layer was dried over Na_2SO_4 and the drying agent removed by filtration. The filtrate was collected, the solvent removed *in vacuo* and the resulting solid dried.

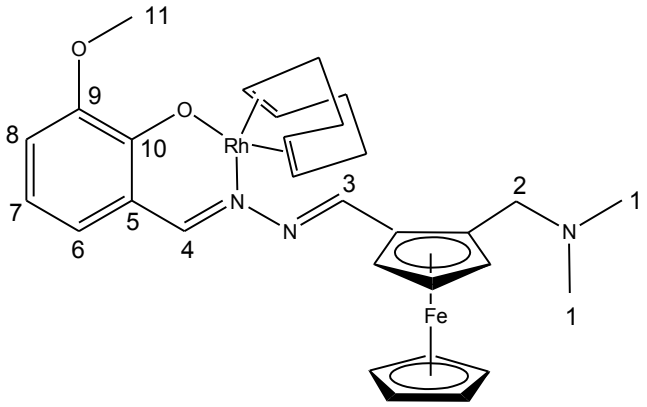
*5-Chlorosalicylaldimine ferrocene Rh(I) complex (**4.12**)*



Compound **4.5** (59.1 mg, 0.139 mmol), NaH (13.4 mg, 0.558 mmol) and $[RhCl(COD)]_2$ (34.7 mg, 0.0704 mmol) were reacted. Compound **4.12** was obtained as an orange solid (86.2 mg, 97%). **M.p.** 73 - 76°C. **^{1}H NMR (399.951 MHz, $CDCl_3$):** (δ, ppm) 1.95 (4H, m, $(COD)_{exo}$); 2.19 (6H, s, H-1); 2.46 (4H, m, $(COD)_{endo}$); 3.24 (1H, d, $^2J_{H\text{-}H}$ = 12.99, H-2a); 3.59 (1H, d, $^2J_{H\text{-}H}$ = 13.03, H-2b); 3.98 (2H, m, C*H*=CH (COD)); 4.20 (5H, s, Cp_{unsub}); 4.45 (1H, t, $^3J_{H\text{-}H}$ = 2.54, Cp_{sub}); 4.50 (1H, m, Cp_{sub}); 4.60 (2H, m, CH=C*H* (COD)); 4.70 (1H, m, Cp_{sub}); 6.84 (1H, d, $^3J_{H\text{-}H}$ = 9.08, H-9); 7.12 (1H, d, $^4J_{H\text{-}H}$ = 2.75, H-6); 7.22 (1H, dd, $^4J_{H\text{-}H}$ = 2.77, $^3J_{H\text{-}H}$ = 9.09, H-8); 7.58 (1H, s, H-4); 7.98 (1H, s, H-3). **^{13}C{^{1}H} NMR (100.635 MHz, $CDCl_3$):** (δ, ppm) 29.17 (d, $^2J_{Rh\text{-}C}$ = 6.73, COD); 31.48 (d, $^2J_{Rh\text{-}C}$ = 6.73, COD); 45.04 (C-1); 57.22 (C-2); 67.92 (Cp_{sub}); 70.03 (Cp_{unsub}); 70.27 (Cp_{sub}); 73.38 (d, $^1J_{Rh\text{-}C}$ = 13.36, COD); 73.77 (Cp_{sub}); 76.05; 85.43 (d, $^1J_{Rh\text{-}C}$ = 12.12, COD); 85.95; 118.03; 118.42; 123.36 (C-9); 132.21 (C-6);

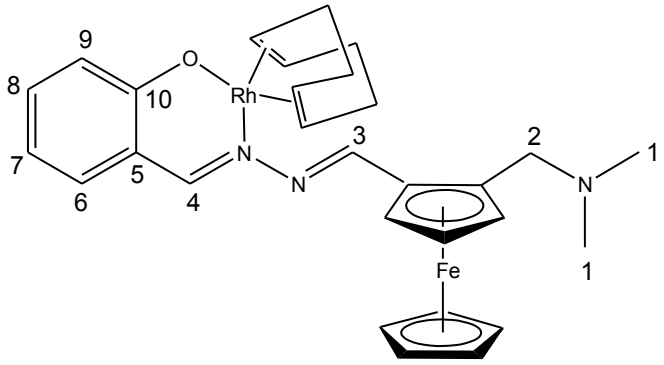
133.95 (C-8); 152.06 (C-4); 157.65 (C-3); 164.94. **IR (ATR):** (ν_{max}/cm^{-1}) 1600 (C=N); 1574 (C=C). **ESI-MS (HR):** *m/z* 634.0776 (20%, $[M+H]^+$, requires 634.0795). **Elemental Analysis** $C_{29}H_{33}ClFeN_3ORh$ calculated: C 54.96; H 5.25; N 6.63%, found: C 55.85; H 5.91; N 5.44%.

*3-Methoxysalicylaldimine ferrocene Rh(I) complex (**4.13**)*



Compound **4.6** (56.9 mg, 0.136 mmol), NaH (13.0 mg, 0.543 mmol) and $[RhCl(COD)]_2$ (33.6 mg, 0.0681 mmol) were reacted. Compound **4.13** was obtained as a red solid (83.3 mg, 98%). **M.p.** 79 - 82°C. **^{1}H NMR (300.077 MHz, $CDCl_3$):** (δ, ppm) 1.95 (4H, m, $(COD)_{exo}$); 2.20 (6H, s, H-1); 2.47 (4H, m, $(COD)_{endo}$); 3.28 (1H, d, $^2J_{H\text{-}H}$ = 13.67, H-2a); 3.60 (1H, d, $^2J_{H\text{-}H}$ = 12.69, H-2b); 3.84 (3H, s, H-11); 3.98 (2H, m, C*H*=CH (COD)); 4.20 (5H, s, Cp_{unsub}); 4.44 (1H, t, $^3J_{H\text{-}H}$ = 2.44, Cp_{sub}); 4.50 (1H, m, Cp_{sub}); 4.70 (3H, m, Cp_{sub}, CH=C*H* (COD)); 6.51 (1H, t, $^3J_{H\text{-}H}$ = 8.30, H-7); 6.82 (1H, d, $^3J_{H\text{-}H}$ = 8.30, H-8); 6.88 (1H, d, $^3J_{H\text{-}H}$ = 7.32, H-6); 7.68 (1H, s, H-4); 8.00 (1H, s, H-3). **^{13}C{^{1}H} NMR (100.635 MHz, $CDCl_3$):** (δ, ppm) 29.16 (d, $^2J_{Rh\text{-}C}$ = 9.42, COD); 31.52 (d, $^2J_{Rh\text{-}C}$ = 10.77, COD); 44.98 (C-1); 56.96 (C-11); 57.22 (C-2); 67.93 (Cp_{sub}); 70.00 (Cp_{unsub}); 70.15 (Cp_{sub}); 72.77 - 73.35 (m, COD); 73.62 (Cp_{sub}); 76.29; 84.86 (d, $^1J_{Rh\text{-}C}$ = 10.71, COD); 85.78; 113.65 (C-7); 115.79 (C-6); 117.51; 126.50 (C-8); 151.34; 152.99 (C-4); 157.55 (C-3); 158.11. **IR (ATR):** (ν_{max}/cm^{-1}) 1600 (C=N); 1576 (C=C). **ESI-MS (HR):** *m/z* 630.1291 (35%, $[M+H]^+$, requires 630.1290). **Elemental Analysis** $C_{30}H_{36}FeN_3O_2Rh$ calculated: C 57.25; H 5.77; N 6.68%, found: C 57.42; H 6.09; N 6.69%.

*Salicylaldimine ferrocene Rh(I) complex (**4.14**)*



Compound **4.7** (92.5 mg, 0.238 mmol), NaH (23 mg, 0.950 mmol) and $[RhCl(COD)]_2$ (58.3 mg, 0.118 mmol) were reacted. Compound **4.14** was obtained as a red solid (0.133 g, 94%). **M.p.** 75 - 78°C. **^{1}H NMR (399.951 MHz, $CDCl_3$):** (δ, ppm) 1.95 (4H, m, $(COD)_{exo}$); 2.20 (6H, s, H-1); 2.48 (4H, m, $(COD)_{endo}$); 3.27 (1H, d, $^2J_{H\text{-}H}$ = 13.08, H-2a); 3.59 (1H, d, $^2J_{H\text{-}H}$ = 13.32, H-2b); 3.98 (2H, m, C*H*=CH (COD)); 4.21 (5H, s, Cp_{unsub}); 4.44 (1H, t, $^3J_{H\text{-}H}$ = 2.29, Cp_{sub}); 4.49 (1H, m, Cp_{sub}); 4.62 (2H, m, CH=C*H* (COD)); 4.71 (1H, m, Cp_{sub}); 6.58 (1H, t, $^3J_{H\text{-}H}$ = 6.79, H-7); 6.90 (1H, d, $^3J_{H\text{-}H}$ = 8.11, H-9); 7.17 (1H, d, $^3J_{H\text{-}H}$ = 7.82, H-6); 7.31 (1H, m, H-8); 7.67 (1H, s, H-4); 8.01 (1H, s, H-3). **^{13}C{^{1}H} NMR (100.635 MHz, $CDCl_3$):** (δ, ppm) 29.20 (d, $^2J_{Rh\text{-}C}$ = 7.40, COD); 31.51 (d, $^2J_{Rh\text{-}C}$ = 8.08, COD); 45.02 (C-1); 57.20 (C-2); 67.88 (Cp_{sub}); 69.99 (Cp_{unsub}); 70.13 (Cp_{sub}); 72.86 (d, $^1J_{Rh\text{-}C}$ = 13.46, COD); 73.61 (Cp_{sub}); 76.29; 85.02 (d, $^1J_{Rh\text{-}C}$ = 12.12, COD); 85.91; 114.58 (C-7); 117.39; 121.80 (C-9); 134.12 (C-6); 134.22 (C-8); 153.12 (C-4); 157.57 (C-3); 166.38. **IR (ATR):** (ν_{max}/cm^{-1}) 1601 (C=N); 1574 (C=C). **ESI-MS (HR):** *m/z* 600.1182 (20%, $[M+H]^+$, requires 600.1184). **Elemental Analysis** $C_{29}H_{34}FeN_3ORh{\cdot}0.5H_2O$ calculated: C 57.25; H 5.80; N 6.91%, found: C 57.40; H 6.10; N 5.20%.

6.5. *In vitro* antiplasmodial assay

The test samples were tested in triplicate on one occasion against the chloroquine-sensitive NF54 strain and chloroquine-resistant Dd2 or K1 strains of *Plasmodium falciparum*. Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen.[13] Quantitative assessment of antiplasmodial activity *in vitro* was determined via the parasite lactate dehydrogenase assay using a modified method described by Makler *et al*.[14] The test samples were prepared as a 20 mg/ml stock solution using DMSO and sonicated to enhance solubility. Samples were tested as a suspension if not completely dissolved. Stock solutions were stored at -20°C. Further dilutions were prepared on the day of the experiment. Chloroquine was used as the reference drug in all experiments. A full dose-response measurement was performed for all compounds to determine the concentration inhibiting 50% of parasite growth (IC_{50} value). Test samples were tested at a starting concentration of 1000 ng/ml, which was then serially diluted 2-fold in complete medium to give 10 concentrations; with the lowest concentration being 2 ng/ml. The same dilution technique was used for all samples. The highest concentration of solvent to which the parasites were exposed to had no measurable effect on the parasite viability. The IC_{50} values were obtained using a non-linear dose-response curve fitting analysis via Graph Pad Prism v.4.0 software. Evaluation is based on means from three independent experiments.

6.6. β-Haematin inhibition assay

The β-haematin formation assay was adapted from the method described by Wright and co-workers.[15] Test compounds were prepared as a 10mM stock solution in 100% DMSO. Test samples were tested at a starting concentration of 500μM and the lowest drug concentration being 5 μM. The stock solution was serially diluted to give 12 concentrations in a 96 well flat-bottom assay plate. NP-40 detergent was then added to mediate the formation of β-haematin (30.55 μM, final concentration). A 25 mM stock solution of haematin was prepared by dissolving haemin (16.3 mg) in dimethyl sulfoxide (1 ml). A 177.76 μl aliquot of haematin stock was suspended in 20 ml of a 2 M acetate buffer, pH 4.7. The haematin suspension was then added to the plate to give a final haematin concentration of 100 μM. The plate was then incubated for 16 hours at 37°C. The assay was analysed using the pyridine-ferrochrome method developed by Ncokazi and Egan.[16] 32 μl of a solution of 50% pyridine,

20% acetone, 20% water, and 10% 2M HEPES buffer (pH 7.4) was added to each well. To this, 60 µl acetone was then added to each well and mixed. The absorbance of the resulting complex was measured at 405 nm on a SpectraMax 340PC plate reader. The IC_{50} values were obtained using a non-linear dose-response curve fitting analysis via Graph Pad Prism v.5.0 software.

6.7. *In vitro* cytotoxicity (MTT) assay

The oesophageal cancer cell line WHCO1, derived from a primary oesophageal squamous cell carcinoma, was provided by Professor Rob Veale (University of the Witwatersrand, Johannesburg, South Africa). IC_{50} determinations were carried out using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.[17] 3000 cells were seeded per well in 96-well plates. Cells were incubated at 37 °C under 5% CO_2 (24 hours), after which aqueous DMSO solutions of each compound (10 µL, with a constant final concentration of DMSO of 0.2%) were plated at various concentrations. After 48 hours incubation, observations were made, and MTT (10 µL) solution added to each well. After 4 hours of incubation, solubilisation solution (100 µL) was added to each well, and incubated overnight. Plates were read at 595 nm on a BioTek microplate reader, and IC_{50} values calculated using GraphPad Prism 4.00 Package of GraphPad Software, San Diego, USA.

6.8. *In vitro* cytotoxicity (WST1) assay

KMST-6 cell lines were obtained from Sigma Aldrich. Cell proliferation was determined using the WST1 (4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) assay in which cells were trypsinized and cell counts of 5.0×10^4 (for all cell lines) were prepared in 15 ml cell culture tubes. 100 µl of this was withdrawn and seeded into wells of 96 well plates and were allowed to grow. The culture medium was then removed and replaced with 100 µl of fresh medium with or without various concentrations of the test compounds. Triplicate wells were carried out for each concentration and the cells were incubated for 24 hours at 37°C in a humidified CO_2 incubator. WST1 reagent (10 µl solution) was added to each well and the plates were incubated for 4 hours at 37°C in a humidified CO_2 incubator. At the end of the incubation period the plate was shaken on a rotating shaker for 10

minutes before taking readings at 450 nm and 600 nm using a microplate reader. Assays were done in triplicate to ensure reproducibility.

6.9. *In vitro T. vaginalis* assay

Cultures of *T. vaginalis* G3 strain were grown in 5 ml complete TYM Diamond's media in a 37ºC incubator for 24 hours. 10 mM stocks of the compounds were made by dissolving in DMSO and were screened against G3 stain of *T. vaginalis*. Cells untreated and inoculated with 5 µl DMSO are used as controls. 5 µl of 10 mM stocks of compound library were inoculated for a final concentration of 10 µM. Results were calculated based on counts utilising a haemocytometer after 24 hours.

6.10. References

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