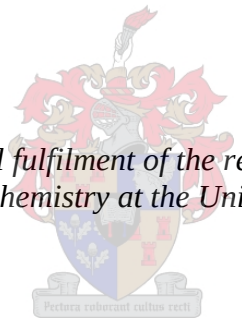
Synthesis and Biological Evaluation of Novel Ferroquine and Phenylequine Analogues

by
Leon Jacobs



Thesis presented in partial fulfilment of the requirements for the degree Master of Science in Chemistry at the University of Stellenbosch

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Declaration

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Abstract

Seven novel series of 4-amino 7-chloroquinolines have been synthesised and fully characterized (A–G). Series A are ferroquine analogues, and have the general formula N^1-(7-chloroquinolin-4-yl)-N^n-(2-((dimethylamino)methyl)ferrocenylmethyl)alkyl-1,*n*-diamine where n = 2 – 6. Series B are phenylequine analogues, and have the general formula N^1-(7-chloroquinolin-4-yl)-N^n-(2-((dimethylamino)methyl)benzyl)alkyl-1,*n*-diamine.

The third and fourth series are ferroquine and phenylequine analogues that contain an oxalamide functional group in the carbon linker. They have the general formulae N^1-(2-(7-chloroquinolin-4-ylamino)alkyl)-N^n-(2-((dimethylamino)methyl)ferrocenylmethyl) oxalamide (Series C) and N^1-(2-(7-chloroquinolin-4-ylamino)alkyl)-N^n-(2-((dimethylamino)methyl)benzyl)oxalamide (Series D) respectively.

Compounds in series A and B contain a reactive secondary amine, which we derivatised further in order to incorporate an ethyl oxamate substituent. These compound have the general formula ethyl-2-((*n*-(7-chloroquinolin-4-ylamino)alkyl)(2((dimethylamino)methyl)ferrocenyl methyl)amino)-2-oxoacetate (Series E) and ethyl-2-((*n*-(7-chloroquinolin-4-ylamino)alkyl)(2-((dimethylamino)methyl)benzyl) amino)-2-oxoacetate (Series F). Finally we substituted a 4-methyl pyridine group in the position of the ferrocenyl and phenyl aromatic groups to obtain Series G.

Excluding Series G, all other compounds were submitted for whole cell testing against the *P. falciparum* chloroquine sensitive strain NF45. The most active compounds **A5** and **D6** returned IC_{50} values of 4.2 nM and 7.4 nM respectively. The results indicate that compounds containing a long alkyl chain (n≥4) show superior efficacy compared to compounds with a short alkyl chain.

β-haematin inhibition assays were carried out on selected compounds with high, medium and poor efficacy to potentially determine whether there is any correlation between the results obtained from whole cell testing and β-haematin inhibition assays.

The results are positive as more than 56% of compounds tested had an IC_{50} values between 10 and 15 nM. The incorporation of the 7-chloro 4-aminoquinoline group is necessary in obtaining low β-haematin inhibition values.

Modeling studies were also carried out on Series D and F to determine whether they could theoretically inhibit the activity of the enzyme *P. falciparum* lactate dehydrogenase. The results suggest that 4-amino 7-chloroquinolines are able to dock in the active site of the enzyme.

Opsomming

Sewe unieke 4-amino 7-chlorokwinolien reekse (A – G) was gesintetiseerd en ten volle gekarakteriseerd. Reeks A bevat *ferroquine* analoë wat die algemene formule N^1-(7-chlorokwinolin-4-yl)-N^n-(2-((dimetielamino)metiel)ferrosenielmetiel)alkiel-1,*n*-diamien bevat, waar *n* = 2 – 6. Reeks B is *phenylequine* analoë, en bevat die algemene formule N^1-(7-chlorokwinolin-4-yl)-N^n-(2-((dimetielamino)metiel)bensiel)alkiel-1,*n*-diamien.

Die derde en vierde reeks is ook *ferroquine* en *phenylequine* analoë, maar bevat ‘n oxalamied funksionele groep in die koolstof ketting. Hierdie reekse het die algemene formule N^1-(2-(7-chlorokwinolin-4-ylamino)alkiel)-N^n-(2-((dimetielamino)metiell) ferrosenielmetiel)oxalamied (Reeks C) en N^1-(2-(7-chlorokwinolin-4-ylamino)alkiel)-N^n-(2-((dimetielamino)metiel) bensiel)oxalamied (Reeks D)

Die verbindings in reeks A en B bevat ‘n reaktiewe sekondere amien. Afgeleides van hierdie twee reekse was gesintetiseerd deur ‘n oxamaat substituent te koppel aan die sekondêre amien. Hierdie reekse het die algemene formule etiel-2-((*n*-(7-chlorokwinolin-4-ylamino)alkiel)(2((dimetielamino)metiel)ferrosenielmetiel)amino)-2-oxoasetaat (Reeks E) en etiel-2-((*n*-(7-chlorokwinolin-4-ylamino)alkiel)(2((dimetielamino)metiel) ferrosenielmetiel)amino)-2-oxoasetaat (Reeks F). Die laaste reeks, Reeks G, is

vergelykbaar met die struktuur van Reeks B, vanwaar die diemtielaminometielbenseen groep vervang is met ‘n 4-metielpiridien aromatiese groep.

Al die reeks, behalwe Reeks G, was ingestuur vir antiparasitiese toetse teenoor die chloroquine sensitiewe lyn van *P. falciparum,* NF54. Die mees aktiewe verbindings, **A5** en **D6**, het IC_{50} waardes van 4.2 nM en 7.4 nM opgelewer. Die resultate het angetoon dat verbindings met langer koolstof ketting skakels (n≥4) het verhoogde aktiwiteit in vergelyking met verbindings met korter ketting lengtes.

β-hematin inhiberings toetse was uitgevoer op verskeie verbindings met hoë, medium en lae antiparasitiese IC_{50} waardes, om ondersoek in te stel of daar ‘n ooreenstemming is met waardes verkry tydens die β-hematin inhiberings toetse. Die β-hematin inhiberings toetse se resultate was gunstig, sedert meer as 56% van al die verbindings wat getoets was IC_{50} waardes tussen 10 and 15 nM vertoon het. Dit bevestig dat die insluiting van die 7-chloro 4-aminokwinolien groep is noodsaaklik vir die inhibering van β-hematin.

Rekenaar modelering was uitgevoer op reekse D en F om ondersoek in te stel of hierdie verbindings die aktiewe setel van die ensiem *P. falciparum* laktaat dehidrogenase kan inhibeer. Die resultate het vasgestel dat die 7-chloro 4-aminokwinolien groep wel in die aktiewe setel kan bind.

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I could not have learned so much and in the same time enjoy my project without the help of my supervisors, lecturers, mentors and friends.

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List of Abbreviations

ITN - insecticide-treated bed nets

QN – quinine

CQ – chloroquine

MQ – mefloquine

AQ – amodiaquine

PQ – phenylequine

FQ – ferroquine

CQS – chloroquine sensitive strain

ACT - artemisinin-combination therapies

Fe(II)PPIX - iron(II) protoporphyrin IX

LDH – lactate dehydrogenase

*pf*LDH – *Plasmodium faciparum* lactate dehydrogenase

DV – digestive vacuole

Cp – cyclopentadienyl

Chapter 1

1.1. Introduction

The aim of this project was to synthesise a novel series of antimalarial compounds and test them for activity against the malaria parasite *Plasmodium falciparum*. The thesis describes the process of compound development, from rational design, to synthesis through the incorporation of different functional groups in each compound. Furthermore biological and β-haematin inhibition testing allowed possible structure-activity relationships to be deducted from the results. Finally, we have been able to discuss a possible mode of action for these compounds.

1.2. History of Malaria

Malaria is a worldwide pandemic disease that has been documented from as early as 2700 BC in China, 2000 BC in Mesopotamia and 1570 BC in Egypt.[1] Advances in agriculture and the migration of humans to colonise new lands resulted in increased frequency of malaria infection[2]. The term malaria originates from the Italian word *mala aria*, meaning “bad air”. The Italian connection to the term malaria comes from the fact that the Roman Empire suffered large numbers of fatalities, which ultimately played a role in the decline of the Roman Empire[3]. Initially malaria was thought to be caused by swamps and marshes, and was commonly referred to as “marsh fever”.[4]

The scientific consensus that microorganisms are the cause of disease, together with the development of Louis Pasteur and Robert Koch’s germ theory, encouraged the search for answers with respect to malaria. Prior to the cause for malaria infection being discovered, it was believed to have been transmitted either by air or water.[1] The causative agent of malaria was however discovered in 1880 by Charles Louis Alphonse Laveran, when he compared infected blood from patients from Algeria with the blood of patients in the Roman Campagna (an area surrounding Rome), and noticed the same pigment within the parasites in both blood samples.[5] After this investigation, Laveran deduced that malaria was in fact caused by a parasite, and later Italian physician Giovanni Battista Grassi confirmed that the mosquito is the vector in

transmitting the disease to humans.[1]. The parasite is transmitted via the female *Anopheles* mosquito, also referred to as the transmission vector, which carries the parasite in its saliva.[6] When the mosquito is feeding on a blood meal, whether it is a human or any other vertebrate, the parasite infects the host, passing directly into the bloodstream. There are 430 species of *Anopheles* and of these, between 30 and 40 species are viable vectors for *Plasmodium* infection.[7]

In most countries in which malaria is predominant, the economical effect that it has is staggering. It was one of the main causes for slow economical growth in the southern part of the USA in the late 19th century.[8] It costs the African continent between 10 and 12 billion dollars annually, as a result of people unable to go to work and children not attending school. This in turn has a serious effect on educational development.[9]

The parasites responsible for malaria infection belong to the genus *Plasmodium*. Eleven species of *Plasmodium* have been found to cause malaria, of which the five species of *Plasmodium, P. falciparum, P. vivax, P. ovale, P. knowlesi* and *P. malariae* are the most widespread disease causing parasites.[10, 11] In particular, *P. falciparum* is considered to be the most prevalent cause of malaria infection, and is known to be the most deadly species.[12]

1.3. Worldwide distribution of malaria

More than 40% of the world is currently at risk of being infected with malaria,[13] and more than 216 million cases of malaria were documented for 2011 according to the World Health Organization's 2011 Malaria report.[13] Almost 3.3 billion people are at risk of malaria, and most of these people reside in tropical and subtropical regions around the world (see **Figure 1**).[13] The highest frequency of malaria infection occurs in developing countries situated in Sub-Saharan Africa, the Americas and Asia. According to the World Health Organization, 81% of documented cases in 2010 originated from African regions, especially Sub-Saharan Africa, and 60% of all deaths are from Nigeria, Mozambique, Ivory Coast, Mali and Burkina Faso.[13]

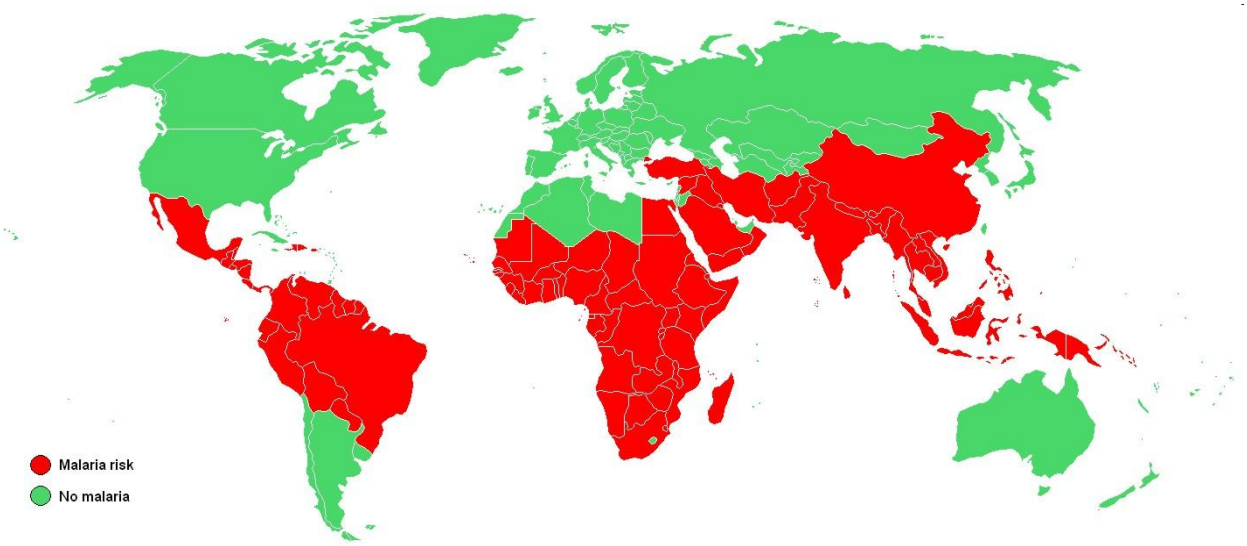


Figure 1: Distribution of malaria infested areas around the world.[14]

Infants and pregnant woman are the most vulnerable to malaria. It is the disease that kills more than 10% of children under the age of fifteen in developing countries, most of which are under the age of five.[9] The disease has a devastating effect on fetal development in the womb, and is associated with more than 200 000 maternal deaths annually in Sub-Saharan Africa.[15] An increase in the production of counterfeit drugs, especially in Cambodia[16] and China[17] has also contributed to the spread of the disease. An analytical study on drug tablets originating from 21 countries in Sub-Saharan Africa indicated that many had failed the required dosage concentration and packaging requirements.[18]

1.4. Life cycle of the parasite

The parasite's life cycle takes place in various stages, including include a primary and secondary host that are essential for its survival (**Figure 2**). The *Anopheles* mosquito, known as the primary host, transfers the motile infective form of the parasite, called a sporozoite, to its human or primate host during a blood meal. The sporozoite resides in the salivary glands of the mosquito prior to infection. When the sporozoite enters the bloodstream of the secondary host, it travels first to the liver, whereupon it enters the hepatocytes. This is the beginning of the exo-erythrocytic cycle. The sporozoite

undergoes asexual reproduction, during which time it actively multiplies to produce the next life stage of the parasite called a merozoite. Finally the infected hepatocyte ruptures, releasing the merozoites into the bloodstream. The erythrocytic phase begins when the merozoites infect red blood cells. Merozoites that infect red blood cells develop into trophozoites.[19]

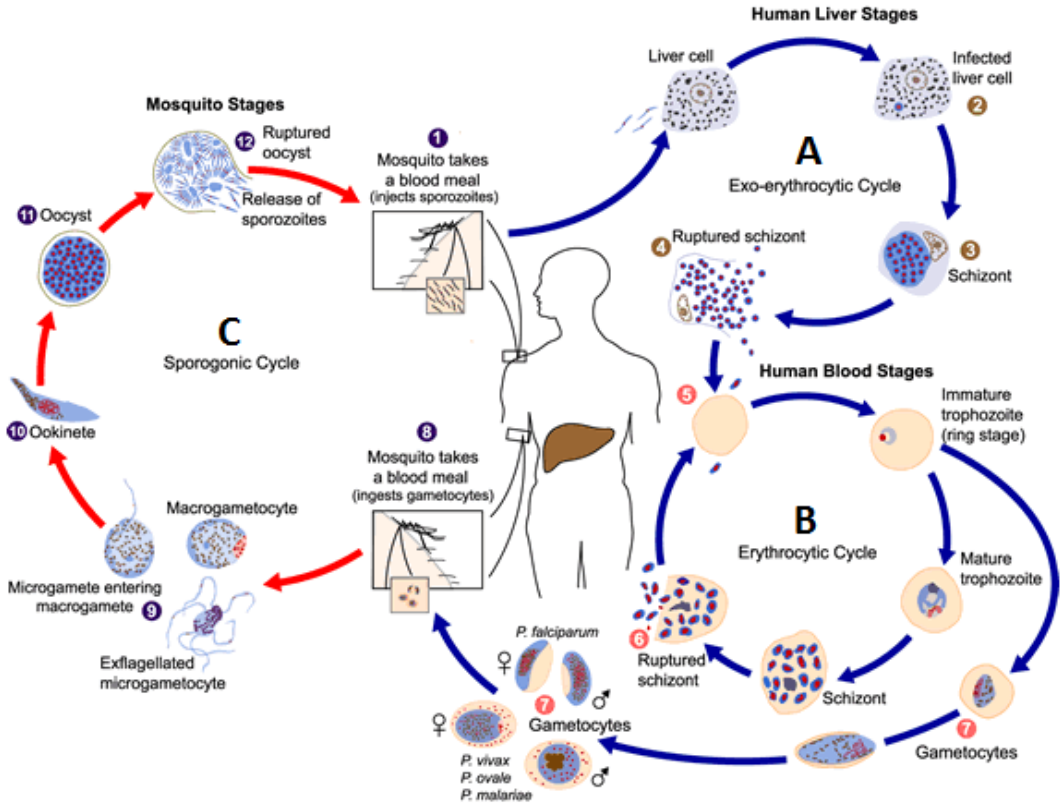


Figure 2*:* Life cycle of *P. falciparum*[20]

Inside the red blood cells, trophozoites initiate an asexual reproductive phase, producing between 8 and 24 new schizonts when fully matured. After a period of 7-14 days, the red blood cells rapture, facilitating the release of newly matured merozoites. This is known as the blood stage or the erythrocytic stage of the parasite and is associated with symptoms like shaking, fever and chills.[19] To complete the life cycle, immature gametes or gametocytes are transferred with the blood meal into the mosquito. This is the sporogonic cycle of the parasite. In the gut of the mosquitoe, the gametes mature into female and male gametocytes, which then undergo fusion to form

zygotes. In turn, these zygotes mature and develop into sporozoites, at which the whole cycle begins anew.[19]

Malaria is known as an acute febrile illness, meaning that it is a disease that has a sudden onset, and is followed by a fever. This fever is the result of the body's rapid immune response to the sudden increase in the amount of merozoites in the bloodstream. People with low immunity can show symptoms of malaria within 7 days, but it usually takes between 10 and 15 days after infection. Early onset of malaria includes symptoms of vomiting, headache, chills and fever, and because these are also symptoms for many other illnesses, diagnosis at first glance can prove to be difficult.[11] Failure to treat malaria caused by *P. falciparum* within 24 hours may result in much more severe symptoms, and even death, as it has a much faster progress towards illness. Children, as previously mentioned, are the age group that is affected the most. Among children infected and suffering from severe malaria, symptoms include severe anemia, cerebral malaria, metabolic acidosis[21] (an increase in acid concentration in bodily fluids) as well as respiratory distress.[13]

Correct medical treatment of malaria will usually result in full recovery. There are however many other cases of relapse of malaria, usually when patients are infected with either *P. vivax* or *P.ovale*.[22] *P. vivax* and *P.ovale* have different developmental stages in their life cycle. It is especially prevalent to *P. vivax* where the sporozoites travel to the hepatocytes (liver cells) where they may lay dormant, only to be reactivated at a later stage, resulting in another attack soon after. The relapse timeframe can either be in short intervals ranging from days between attacks or could take up to 24 weeks.[23] In these cases, special medical treatment is needed to eradicate the parasites and cure the malaria.

1.5. Prevention and treatment of malaria

The lack of awareness regarding malaria is a significant hindrance in the eradication of the disease.[24] The cheapest method for preventing malaria transmission is the distribution of long lasting insecticide-treated bed nets (ITNs), especially in rural African

communities where everyday medical treatment is not available or is too expensive.[13] A study has shown that the distribution of ITNs in some isolated African communities has reduced the mortality rate of children under the age of 5 by as much as 20%.[7] Another method of prevention is vector control, using insecticides and insect repellents to prevent or minimise transmission. Even with this reduction in malaria transmission, infection still occurs and therefore treatment continues to be necessary.

The treatment of malaria has been known since the Inca civilization, when the bark of the *Cinchona* tree was used in malaria therapy. The continuous export of *Cinchona* bark to Europe resulted in the first effective method in combating malaria. The bark was later shown to contain the active compound quinine (QN, **Figure 3**),[25,26] which is widely used even to today in antimalarial chemotherapy.

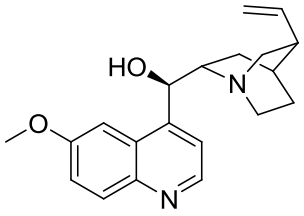


Figure 3: Structure of quinine (QN).

In 1820, the French chemists Pierre Pelletier and Joseph Caventou were the first to isolate QN and this resulted in French physicians using pure QN in the treatment of infected patients years later.[27] *T*he first synthetic antimalarial drug, pamaquine, was synthesised *i*n 1926,[28] and the related compound quinacrine followed in 1932.[29] Pamaquine is based on an 8-amino-quinoline scaffold and quinacrine on a 2-chloro-7-methoxyacridine scaffold.[29] (**Figure 4**)

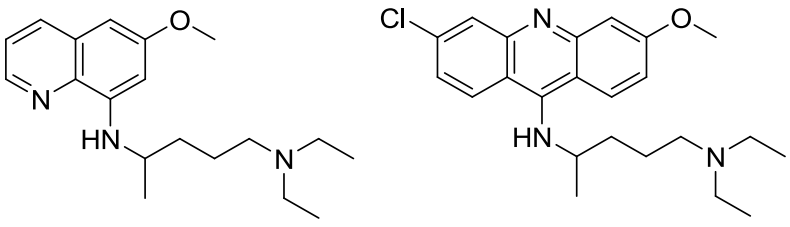


Figure 4: Structure of pamaquine (left) and quinacrine (right).

The first successful full synthesis of QN was completed in 1944 by two American chemists W.E. Doering and R.B. Woodward.[30] This was the antimalarial drug of choice up until the 1940's, after which it was replaced by alternative drugs with fewer side effects. During the Second World War, the supply of *Cinchona* bark was cut off due to the Japanese presence in the South Pacific, which drove the search for more accessible alternatives to QN.[31] Since then, with the advancement of chemical knowledge, many more efficient QN synthetic methods have been achieved. However, when considering cost, the natural source for QN is by far the more economical option. QN is still used today, but mostly reserved for much more severe cases of malaria. Although an effective drug, QN only has an effect on the *merozoite* stage of the parasite and no effect on the gametocyte stage of the parasite, nor does it inhibit the transmission of malaria.[32]

QN belongs to the quinoline family of antimalarials, and is further classified as a quinoline methanol. It is still the most widely used monotherapeutic drug in Africa, due to the higher cost of QN-combination therapies.[33] Until recently, chloroquine (CQ) was one of the most widely used and popular antimalarial drugs. It was synthesised by Hans Andersag in 1934.[7] Reasons for its widespread use included its affordability, efficacy and relatively low toxicity,[34] until the emergence of serious and widespread resistance in *P.falciparum* arose.[35] Mefloquine, CQ, amodiaquine and primaquine all belong to the quinoline family (**Figure 5**). Except for primaquine, these drugs all have a similar mode of action *in vitro*, the inhibition of haemozoin biomineralization[36], and are therefore active during the blood stage of the parasite.

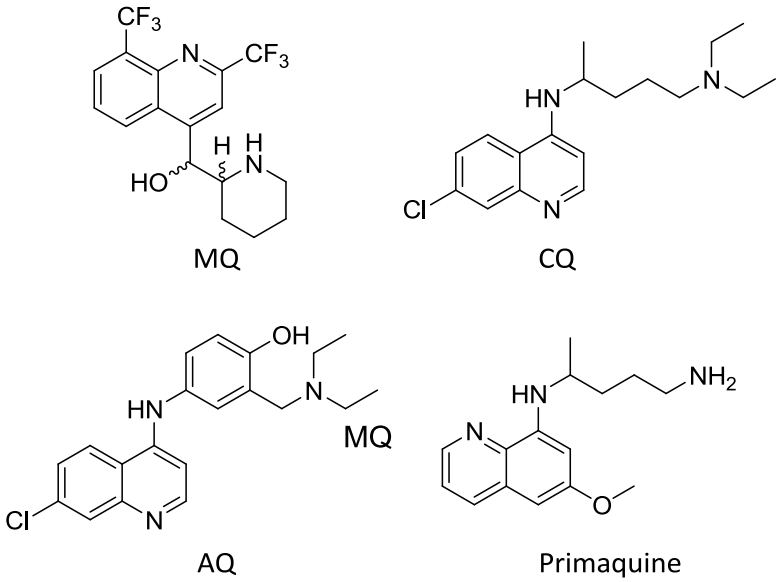


Figure 5: Structures of mefloquine (MQ), chloroquine (CQ), amodiaquine (AQ) and primaquine

Mefloquine is administered for most cases of malaria, in areas where the parasites may have gained some form of resistance towards multiple drugs.[37] Mefloquine is active against *P. falciparum* in both (*R*,*S*)- and (*S*,*R*)-enantiomers.[37]

Studies have shown that amodiaquine exhibits superior efficacy towards CQ resistant strains of *P. falciparum* when compared to CQ,[38] and it is widely used and available in Africa. Primaquine is a drug mainly used to treat *P. vivax* and *P. ovale* infections, as these parasites as previously mentioned have dormant stages in their life cycle and pose a threat for relapse of malaria. This drug has to be administered to the patient immediately in order to prevent such a relapse from occurring.

Primaquine, belonging to the quinoline family, does not inhibit haemozoin formation, and the mode of action is not entirely known. It is active against the parasite's gametocyte phase in *P. falciparum*, the phase where humans infect mosquitoes.[39,40] and the extraerythrocytic forms of *P. vivax* and *P. ovale*, the parasitic forms that cause relapse in malaria symptoms after infection.[7]

For over 2000 years, the Chinese have been using a native herb called Qinghao to treat malaria and fevers. This herb, *Artemisia annua,* contains the antimalarial compound artimicinin.[41] Artemisinin and its derivatives are some of the most effective antimalarials currently available. It is effective against multiple drug resistant strains of *Plasmodium*, and is also active in both the asexual blood stage and the sexual gametocyte stages. The mode of action is still unclear.[42]

They are usually administered together with other antimalarials, also known as artemisinin-combination therapies. Artesunate is the drug of choice when it comes to the treatment of severe malaria in areas where there is low transmission.[43] **Figure 6** shows the structures of artemisinin and artesunate.

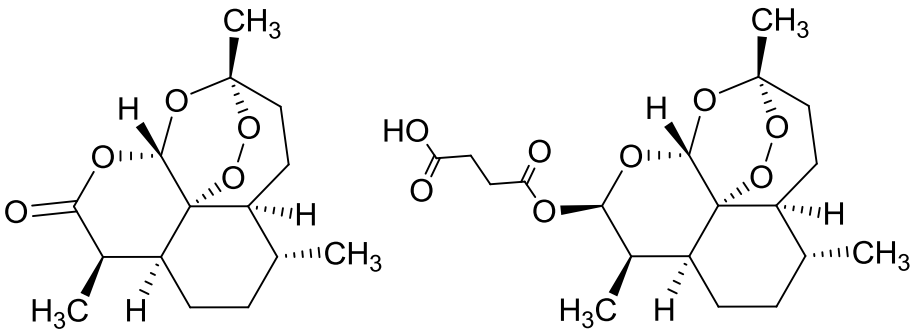


Figure 6: Stucture of artemisinin (left) and artesunate (right)

Antimalarials are also taken as prophylactic drugs, meaning they prevent the onset of malaria infection. It is usually a combination therapy mixture, depending on each country, since each country has its own distribution of different parasites and different resistance patterns.[7]

1.6. Possible targets

The search for a drug molecule which can to act on multiple targets is an attractive concept as it may delay the emergence of resistance.

Target 1: Haemozoin inhibition

As soon as the onset of merozoite reproduction occurs, humans (or any vertebrae that is susceptible to *Plasmodium* infection) start to show symptoms of malaria. The presence of the parasite in the bloodstream results in a sudden immune response causing fever. Malaria also induces anemic side effects.[44] The reason for this is that in order for the parasite to be able to reproduce, it needs a food source rich in protein. The parasite therefore invades the red blood cell that contains haemoglobin, the oxygen-carrying metallo-protein in all living vertebrates. There is a high abundance of protein for the parasite to live on. A dry red blood cell consists of nearly 92% of haemoglobin,[45] and roughly 35% of the total content of blood.[46].

A single healthy red blood cell contains approximately 270 million haemoglobin molecules,[47] with each haemoglobin molecule comprising four globular protein subunits, together forming a tetrahedrally shaped protein.[48] Each protein subunit is connected to a haem prosthetic group, a large iron-containing porphyrin molecule that facilitates the oxygenation and deoxygenation of red blood cells.

When the parasite invades a red blood cell, it starts to digest between 60 and 80% of haemoglobin in its digestive vacuole with the aid of enzymes.[49] In turn it excretes the haem (iron(II) protoporphyrin IX, (Fe(II)PPIX)) as a toxic by product.[50, 51, 52, 53]

Fe(II)PPIX is then excreted to the surrounding aqueous environment where it is oxidised by molecular oxygen to the iron(III) state, the HO^-/H_2O-Fe(III)PPIX (hydroxo-/aqua-ferriprotoporpyrin IX) form.[53] This complex, also referred to as haematin, is toxic to the parasite and poses a threat to the livelihood of the parasite.[54] The iron(III) can potentially catalyze the production of oxygen radicals which may disrupt the parasite's cell membrane. Haematin is soluble in lipids, and can thus cause lipid peroxidation and membrane damage.[5251]The parasite has developed a detoxification mechanism to overcome this problem, by converting free haematin into a micro-crystalline form, known

as haemozoin, via a biomineralization process.[55] This is the non-toxic byproduct of haemoglobin digestion, and accounts for almost 95% of the Fe(III)PPIX released into the digestive vacuole.[50] This process is illustrated in **Figure 7**.

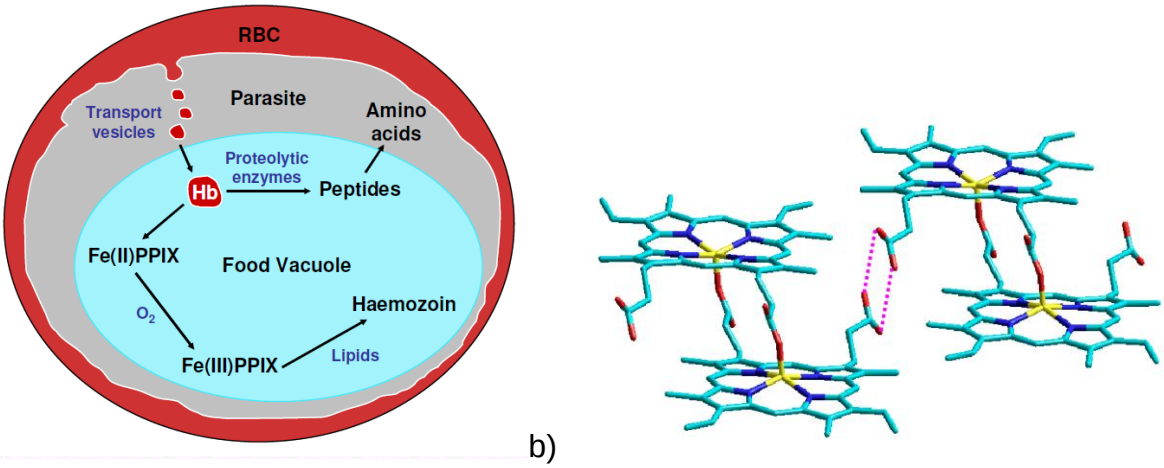


Figure 7: A representation of haemoglobin digestion pathways for haemozoin biomineralization of *P. falciparum*(a)[56] and the molecular structure of β-Haematin, synthetic haemozoin (b)[57]

The use of antimalarials containing quinoline-based scaffolds like QN targets the formation of haemozoin. Chloroquine (CQ) is also a quinoline based drug and in 1964 it was speculated to be targeting the process of haemozoin formation.[58] β-Haematin is the synthetic version of haemozoin, and studies have shown that it consists of discrete dimeric subunits of Fe(III)PPIX.[59]

Compounds containing a 7-chloro-4-aminoquinoline moiety are efficient in inhibiting the formation of β-haematin.[60] Haemozoin formation is neither parasite-encoded, nor is it a similar process present in human hosts, making it a suitable antimalarial target.[61] The inhibition of β-haematin formation with the incorporation of 7-chloro-4-aminoquinolines in our drug synthesis will be one of the main foci in this project.

Target 2: *Plasmodium falciparum* Lactate Dehydrogenase

Lactate dehydrogenase (LDH) is an important enzyme in all living creatures, and is responsible for the catalytic interconversion of pyruvate and L-lactate with the aid of the coenzyme NADH/NAD^+ (**Figure 8**).[62] Anaerobic glycolysis is the major pathway for glucose metabolism in the erythrocytic life stage of *P. falciparum*.[63] Thus in the anaerobic lifestyle of *Plasmodium* it is essential, making the glycolytic pathway imperative for the survival of the parasite.

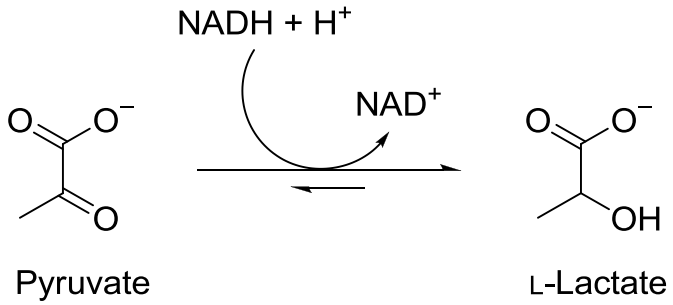


Figure 8: Interconversion of pyruvate and L-lactate through lactate dehydrogenase

When comparing *P. falciparum* Lactate Dehydrogenase (*pf*LDH) with other forms of LDH, one major difference have been observed, that is the enzyme is not inhibited by high lactate concentrations.[64] This implies that the enzyme is constantly active and thus provides scope for targeting by a potential inhibitor. The active site of *pf*LDH contains unique amino acid residues when compared to other variations of LDH. This implies that drug molecules that inhibit *pf*LDH may not necessarily inhibit human LDH.[65] This broadens the scope for docking studies within the active site of *pf*LDH, in order to potentially determine a viable drug molecule.[66] It has been reported that oxamate is a strong competitive inhibitor of LDH showing high substrate affinity for it.[66]

It has been also found that drugs containing an *N*-substituted 2-amino-2-oxoacetic acid functional group have shown superb efficacy in a *pf*LDH inhibition assay,[67] making the incorporation of the oxo-group an attractive concept. Examples of oxamic acids that inhibit *pf*LDH designed by Choi *et al*[66, 67] are shown in **Figure 9**.

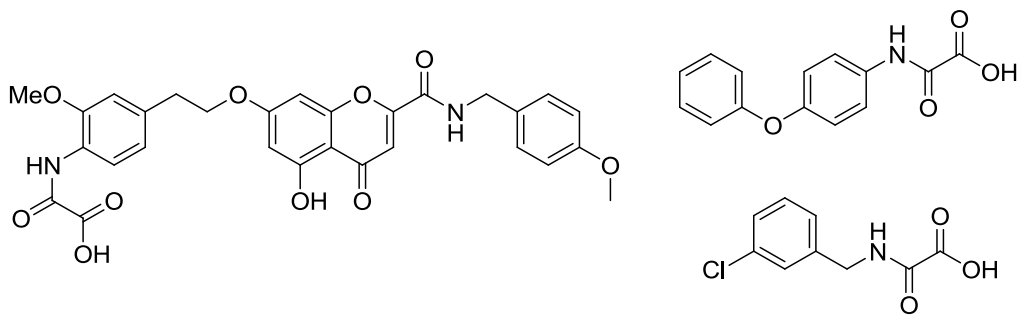


Figure 9: Oxamic acids that exhibit good efficacy against CQ resistant (D6 clone) and MQ resistant (W2 clone) *P. falciparum.*[66, 67]

Menting *et al.*[68] have shown that haematin effectively inhibits *pf*LDH (K_d = 0.25 μM), competing with NADH for the active site. This study was carried out using CQ as the β-haematin inhibition drug. It suggests that equimolar amounts CQ and haematin had a 50% decrease in *pf*LDH inhibition but decreased by more than two orders in the presence of excess CQ. This implied that CQ and haematin forms a complex that binds less strongly to the active site of *pf*LDH. Computational studies by Cortopassi *et al*[69] indicated that chloroquine and other quinoline based drugs binds to haematin via hydrophobic interactions resulting in inhibition of β-haematin formation. The authors propose that this CQ–haematin complex resides in the DV and ultimately leads to parasite death.

1.7. Resistance

The emergence of resistance towards many antimalarial drugs in multiple countries has increased dramatically, particularly in *P. falciparum*, and has contributed to the world wide increase in malaria within the last three decades.[70]

Resistance has its origin on a genetic level. A mutation has occured on the gene *pfcrt* (also referred to as the K76T gene mutation[71]) that encodes for the *Plasmodium falciparum* CQ Resistance Transporter protein. This transporter protein, located in the membrane of the digestive vacuole,[72] has rendered CQ inactive since its mechanism of action is the constant efflux of CQ out of the parasite's digestive vacuole. Polymorphisms of the gene pfmdr1, *Plasmodium falciparum* multidrug resistance 1, have also been linked to lowered efficacy of CQ.[71]

CQ resistance started to appear in the late 1950's on the Thai-Cambodian border.[73] This was followed by a surge in new drugs being discovered. Mefloquine was developed in the 1970's and was introduced as an antimalarial drug in 1984, yet within a period of 6 years, signs of mefloquine resistance started to appear in Thailand, one of the countries suffering from the world's most multi-drug resistant strains of *Plasmodium*.[74] The drug combination Sulfadoxine-Pyrimethamine, once used as a CQ replacement drug, but now administered only during serious cases, has also shown resistance in Indonesia, South America, Africa and Asia.[75] Clinical studies of artemisinin resistance in Cambodia were reported in 2008.[76] The fact of the matter is that different countries are showing serious signs of multi-drug resistance toward multiple species of *Plasmodium* and their respective strains. One of the reasons for the development of drug resistance is the use of monotherapy in controlling malaria.[77] This is when one drug is frequently used as the only means of medication over a widespread area. This implies that the parasite populations are repeatedly exposed to residual drug concentrations that have a slow rate of elimination out of the body.[78]

The use of monotherapy in malaria treatment has been replaced by the combination therapy, the administration of 2 or more antimalarial drugs during treatment. The most widely used combination therapy is artemisinin-combination therapies or ACT's, which is a mixture of Artemisinin or its derivatives plus a companion drug. Companion drugs include those like amodiaquine, sulfadoxine/pyrimethamine, mefloquine, piperaquine and chloroproguanil.[79] The use of combination therapies implies that more than one site is being targeted. The probability that two or more of those targets are affected by genetic mutations is low; hence the use of combination therapy is a much more effective and safer method for treatment than monotherapy. As an alternative to combinational therapy, a dual action drug is a compound that is able to inhibit two or more targets. A series of compounds based on an acridone skeleton has been developed by Kelly *et al.*[61] These have the dual action of targeting haemozoin formation and being attributed to interact with *pf*CRT, blocking its ability to actively pump CQ out of the digestive vacuole. **Figure 10** shows an example of one of these compounds developed.

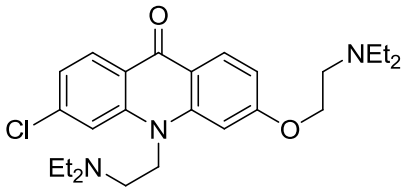


Figure 10: Dual action drug developed by Kelly *et al*[61]

1.8. Rationale for our compound design

In 1997, a ferrocenyl moiety linked to a 4-amino-7-chloroquinoline group was synthesised in a study that focused on substituting ferrocenyl moieties on existing antimalarials used today. This drug is known as Ferroquine (FQ).[80] It showed significant efficacy towards CQ-resistant and -sensitive strains in preclinical *in vitro* and *in vivo* studies.[81] Many other FQ analogues have since been synthesised, and most show good efficacy as well.[82] A detailed study on *in vitro* drug efficacy has shown no signs of potential cross-resistance between FQ and any other drug, including CQ.[83] FQ is a racemate of (+)-FQ and (−)-FQ because it possesses planar chirality. Both enantiomers are equally active in vitro, although the racemate displays slightly better efficacy.[84] Pharmacokinetic profiles have shown that the first-in-human phase 1a trail was favourable, and that FQ has a long drug half life and few side effects with an increase in exposure and doseage.[83] A related compound has also been synthesised that could be classified as phenyl derivative of FQ, it is known as phenylequine (PQ).[85] PQ has shown increased activity towards CQ sensitive and CQ resistant strains of *P. yoelii* and *P. berghei*. **Figure 11** shows the structures of both these compounds.

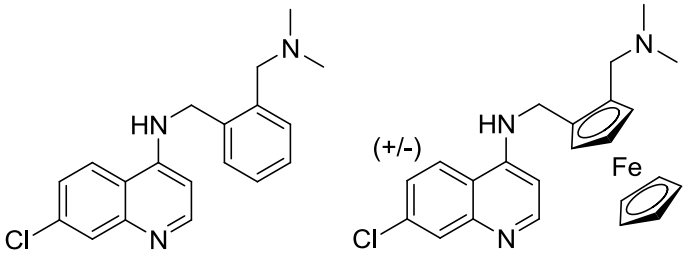


Figure 11: Structures of phenylequine (PQ) and ferroquine (FQ)

The positive results obtained from studies done on FQ have made the idea of synthesizing novel analogues an attractive concept. In our drug design rationale, we combined the information we have about drug resistant strains of *Plasmodium*, the different targets we have identified as major pathways for potential inhibition, and the results published about PQ and FQ.[12]

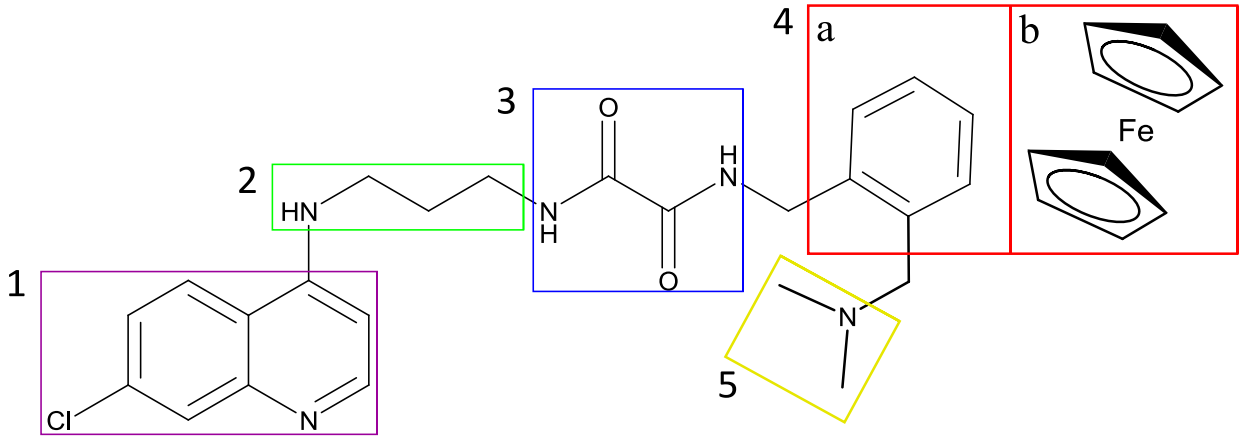


Figure 12: Different functional groups and moieties in target compounds

Figure 12 shows a compound that has the general structure of the analogues we set out to synthesise. In this project we set out to synthesise multiple series of compounds in order to ascertain whether the incorporation of these different moieties into a single molecule has an additive or synergistic effect on antimalarial efficacy.

1. **7-chloro-4-aminoquinoline**: necessary for β-haematin inhibition. This prevents the formation of haemazoin in digestive vacuole of the parasite, and is crucial for the efficacy of this class of antimalarial drug. The 7-chloro group is necessary, since research has proven that absence of the 7-chloro group has a negative effect on drug efficacy.[60]
2. **Methylene spacer**: studies have shown that the length of methylene spacer has an influence on drug efficacy.[86]
3. **Oxalamide**: has a high affinity for *pf*LDH. Adding functionality into a spacer would be an interesting investigation on our drug design, determining if it would

increase or decrease efficacy when comparing results to other PQ and FQ analogues.[66, 12]

4. **Phenyl (a) and ferrocenyl (b) group**: the bulky lipophilic ferrocene moiety, this has proven effectiveness in overcoming *pf*CRT resistance.[87] In addition, ferrocene also has Fe(II) to Fe(III) redox capabilities.[88] We also add the phenyl group as an alternative, as it would be interesting to see the difference in efficacy between phenyl and ferrocenyl analogues.
5. **Tertiary amine**: the digestive vacuole (DV) of *Plasmodium* is acidic of nature, and thus we incorporate a basic amine in order for it to become protonated within the DV, hence making it difficult for the protonated drug to cross over the non-polar hydrophobic membrane. This mechanism is referred to as pH-trapping. It has also been proven that the dimethyl substituted tertiary amine is more effective than other alkyl-substitutuents.[89]

Figure 13 below shows a schematic layout of the different functional groups introduced into our PQ and FQ series that we set out to synthesise. All of these are PQ and FQ analogues, except series G. We know that a basic nitrogen is needed for pH trapping within the DV and the presence of an lipophilic aromatic group on the linker. We decided to add a group that contains both these functionalities.

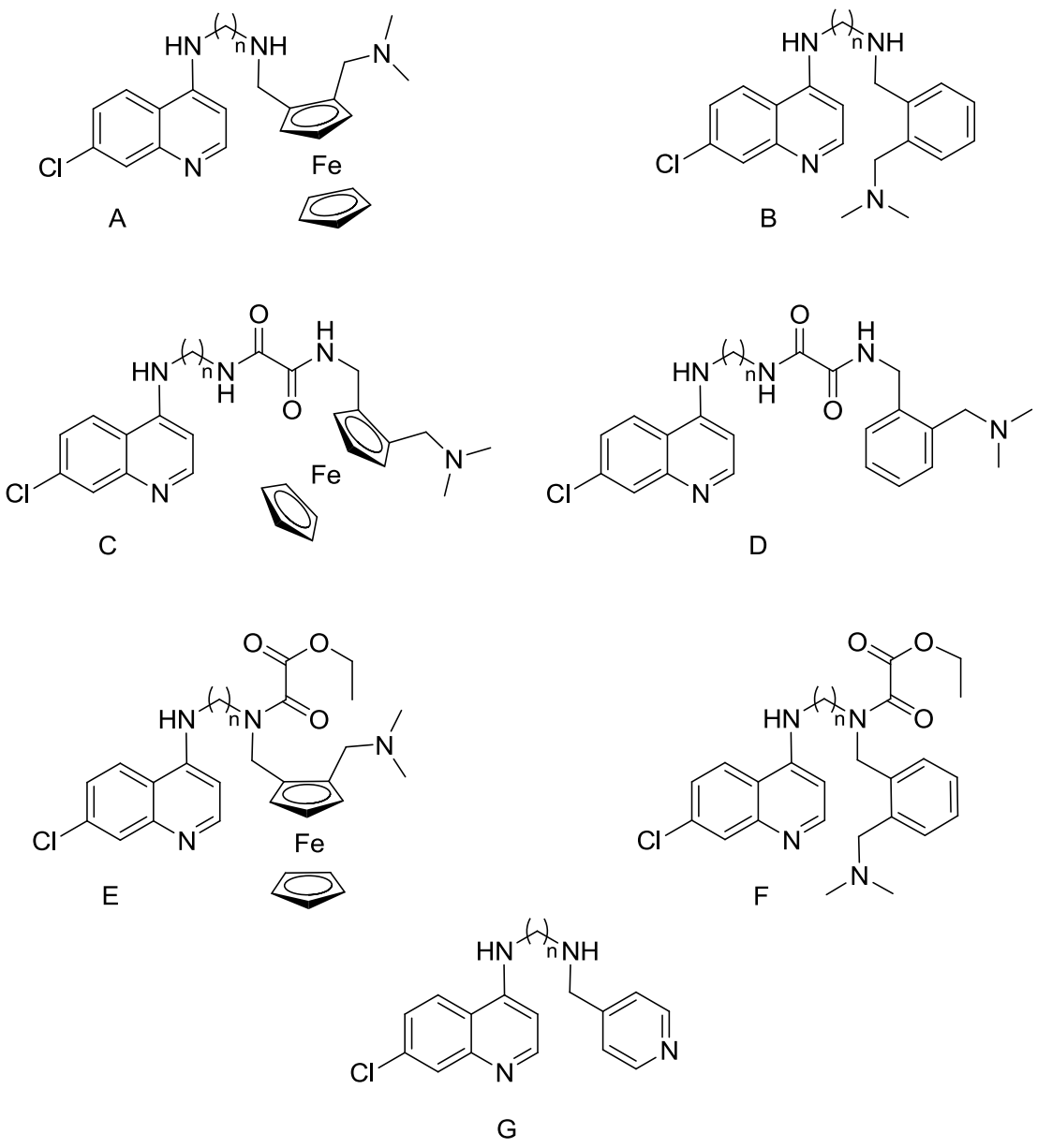


Figure 13: Different series set out to be synthesised. Ferroquine and phenylquine analogues (A, B), oxalamide-ferroquine and –phenylequine analogues (C – D), ethyl oxamate-ferroquine and –pehynlequine analogues (E – F), pyridyl-7-chloroquinolines (G)

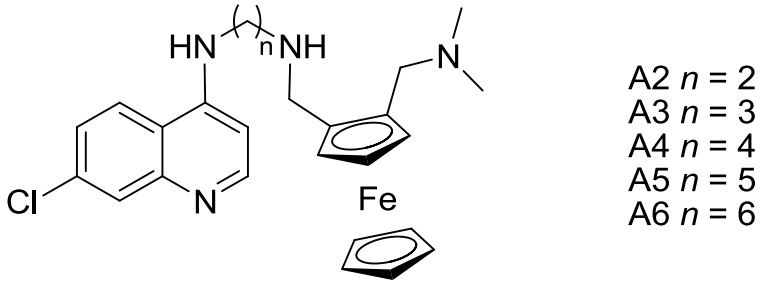
Series A and B are FQ and PQ analogues which contains a diamino alkane linker between the aromatic group (ferrocenyl or phenyl) and the 7-chloroquinoline group.

Series C and D have an oxalamide functional group added to the linker. The oxalamide group is what connects the 7-chloroquinoline bound linker to the aromatic group (ferrocenyl or phenyl).

Series E and F are the original FQ and PQ analogues on which we added an ethyl 2-oxoacetate group on the secondary nitrogen position that links the aromatic group, creating an ethyl oxamate substituent.

Series G are analogous to series B, replacing the *N,N*-dimethylaminomethyl benzene group with a pyridine group.

Figure 14 below describes the numbering of compounds belonging to series A, where **A2** refers to Series A, and the compound has two carbons in the methylene spacer. The same numbering method will be used for compounds belonging to Series B – G.



A2 *n* = 2
A3 *n* = 3
A4 *n* = 4
A5 *n* = 5
A6 *n* = 6

Figure 14: Numbering system for compounds belonging to Series A.

1.9. Objectives

The first objective for this project is the successful synthesis and analytical characterization of series A - G, of which series C - G is completely novel. These series will be sent in for whole cell testing against CQ resistant and sensitive strains of *P. falciparum* followed by β-Haematin inhibition studies to determine their potential efficacy for haemozoin inhibition in vitro. Some docking studies have also been conducted to see if some of these compounds could potentially show an affinity for the enzyme *P. falciparum* Lactate Dehydrogenase. When comparing these results, it is our goal to investigate whether these compounds have a single or multiple modes of action and may possess multi target capabilities, a feature that would potentially increase efficacy against CQ resistant strains of *P. falciparum.*

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Chapter 2

2.1. Introduction

As stated in chapter 1, 4-amino-7-chloroquinoline based drugs show great efficacy towards sensitive strains of *P. falciparum*. In molecules where the side chain is sufficiently different from that of chloroquine (CQ), efficacy towards chloroquine resistant strains is also observed. Ferroquine (FQ) is one such drug, and its phenyl analogue has also been shown to be efficacious. Seven different series of analogues will be synthesized, as show in the **Figure 15** below.

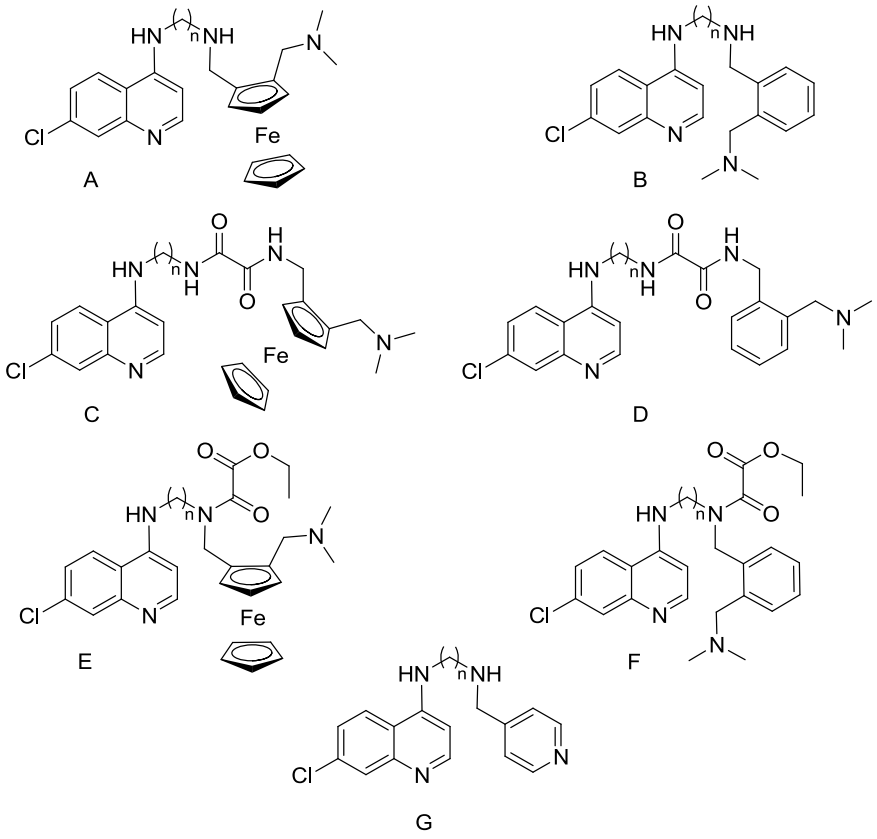
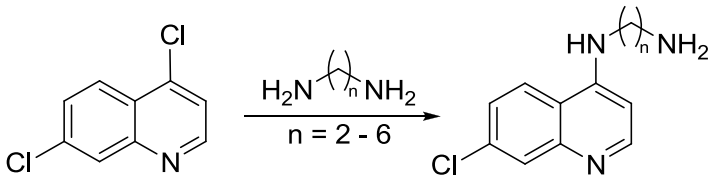


Figure 15: 4-amino 7-chloroquinoline analogues. Series A, C and D are ferroquine analogues, and series B, D and F are phenylequine analogues. Series G is a pyridyl - 7-chloroquinoline analogue of phenylequine.

The search for a drug molecule which can act on multiple targets is an attractive concept as it may delay the emergence of resistance. With this concept in mind, the analogues shown in **Figure 1** have been designed for this purpose. Two key biological processes of *P. falciparum* will be targeted. The first is haemozoin inhibition, of which most 7-chloro-4-amino quinoline based drugs are designed to inhibit. The second pathway is the inhibition of the enzyme *P. falciparum* lactate dehydrogenase (*pf*LDH). We include the oxalamide, a diamide functional group, and ethyl oxamate groups because those functional groups have previously shown a high substrate affinity for this important glycolysis enzyme.[1] The analogues synthesized have a varying 1,*n*-diaminoalkyl linker between the ferrocene/phenyl group and the quinoline group, where *n* ranges between 2 and 6 carbons. It has been found that varying the length of the carbon linker has an effect on the efficacy towards CQ resistant strains of *P. falciparum*.[2]

The first series are phenylequine (PQ) and ferroquine (FQ) analogues, in which the quinoline and ferrocene/phenyl group are linked solely by a carbon chain linker (Series **A** and **B**). The second series will be those containing an additional oxalamide functionality (Series **C** and **D**). The third will be the PQ and FQ analogues containing an ethyl oxamate group, i.e. an amide-ethyl ester functional group (Series **E** and **F**). The final series (**G**) would not be considered a PQ analogue, as the linkage is between a 7-chloroquinoline group and a 4-methyl pyridine group.

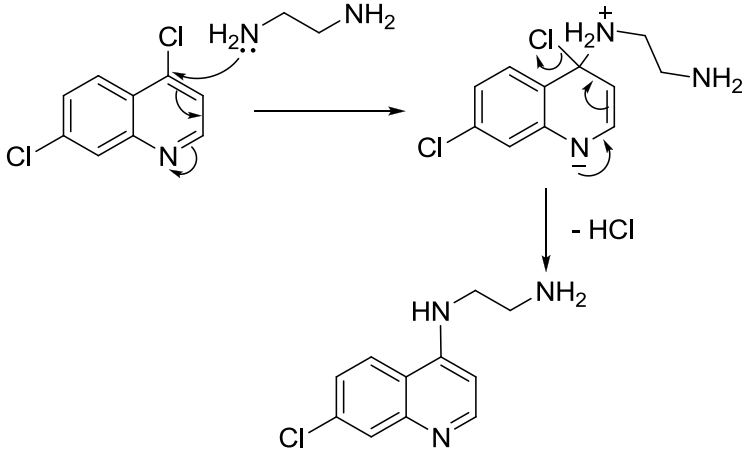
2.2. *Ipso*-nucleophilic substitution reaction



Scheme 1: Reaction of 4,7-dichloroqionoline with various alkyldiamines

A reaction of 4,7-dichloroquinoline with a 1,*n*-diaminoalkane was the first step in the synthesis of all the aforementioned analogues, as shown in **Scheme 1**. The reaction

occurs in a melt of 4,7-dichloroquinoline and 4.5 equivalents of the respective 1,*n*-diaminoalkane. First, the reaction mixture is heated to 80°C for an hour, after which the temperature is increased to 130-140 °C for 3 – 4 hours.[3] 4.5 Equivalents are used to prevent dimerisation of the diaminoalkyl chain with two 4,7-dichloroquinoline groups. The product of these reactions is typically a yellow powder, and yields vary between 75 and 85%. These compounds, numbered **1** – **5**, (*n* = 2 represents compound **1**, *n* = 3 represents compound **2**, etc) form the starting materials for all the subsequent reactions in order to complete the series of analogues. **Scheme 2** shows the mechanism for this reaction.

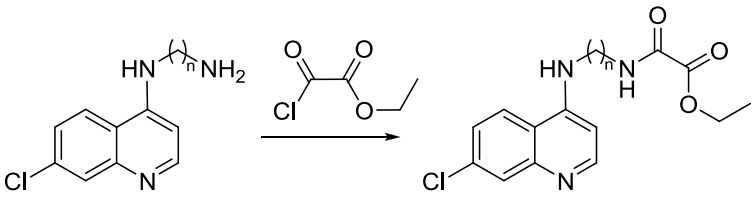


Scheme 2: Ipso-nucleophilic substitution of 4,7-dichloroquinoline and ethandiamine (Compounds 1 – 5)

2.3. Acylation of 7-chloroquinolinalkylamines

In order to obtain the oxalamide linker, it was necessary to proceed via an ethyl oxamate precursor. Compounds **1** – **5** underwent a straight forward acylation reaction with ethyl 2-chloro-2-oxoacetate resulting in the formation of the desired ethyl oxamate precursor.[3] compounds are numbered **6** – **10** respectively (*n* = 2 represents compound **6**, *n* = 3 represents compound **7**, etc). These products are stable. The oxalamide functional group can also be obtained by using oxalyl chloride, however the acid

chloride product would not be isolatable via column chromatography in order to obtain any yield and could also lead to dimerized byproducts of the chloroquinolinalkylamine. The synthetic route followed is illustrated in **Scheme 3**

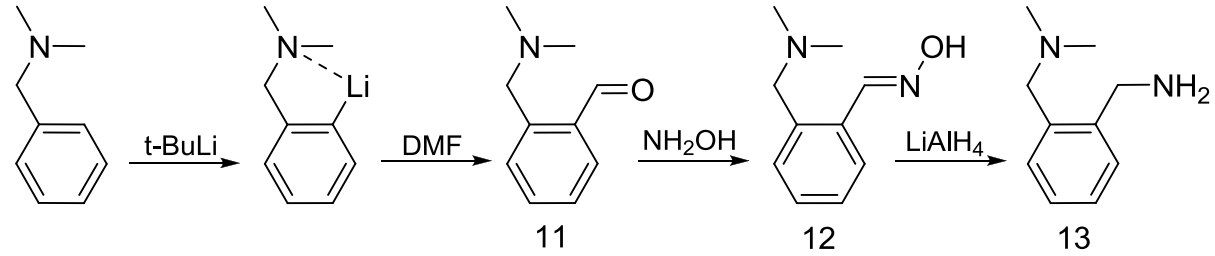


Scheme 3: Acylation of primary amine (Compounds 6 – 10)

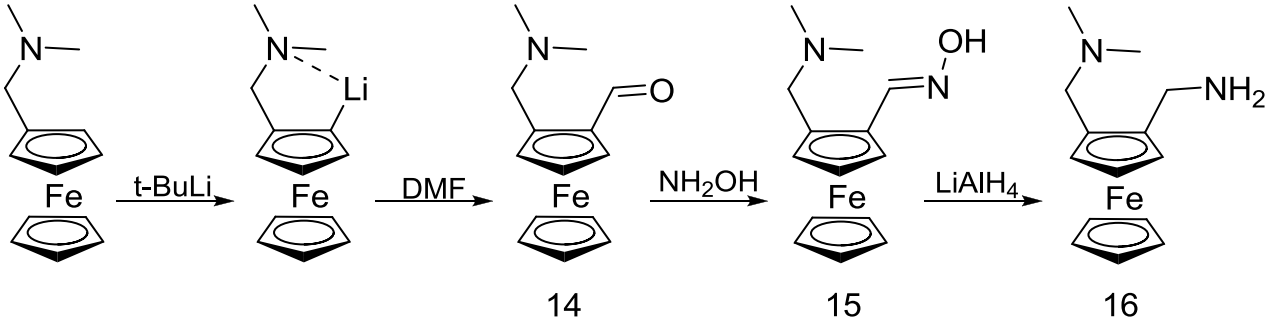
The next reactions illustrate the formation of a phenyl and ferrocenyl primary amine that will be used to form the oxalamide functional group later on.

2.4. Ortholithiation, aldoxime formation and reduction

A straight forward way to introduce an aldehyde is to lithiate a certain species and then react it with *N,N*-dimethyl formamide (DMF). Tertiary butlylithium is a good base for such a reaction since it generates high yields in short times.[4] **Scheme 4** and **Scheme 5** illustrate the formation of the phenyl and ferrocenyl aldehyde, which can be transformed into the desired primary amine in subsequent steps.



Scheme 4: Ortho lithiation, aldoxime formation and reduction to form phenyl primary amine[5]



Scheme 5: Ortho lithiation, aldoxime formation and reduction to form ferrocenyl primary amine[6]

The ortho-lithiation reaction is regioselective, meaning that lithiation will only occur on the carbons *ortho* to the dimethylaminomethyl substituent. On *N,N*-dimethylaminomethyl benzene and *N,N*-dimethylaminomethyl ferrocene, the mono-lithiated species is usually acquired. This was also evident when using *N,N*-dimethylaminomethyl ferrocene as starting material, since lithiation occurred on the top ring (the ring containing the dimethylaminomethyl substituent) and not the bottom ring. Mono-lithiation is also dependent on the solvent used. When using anhydrous tetrahydrofuran (THF), multiple lithiation reactions occur; this is not the case when using anhydrous diethyl ether as solvent.[7]

When doing monolitiation on monosubstituted ferrocenes, the product formed is chiral. When *ortho* lithiating *N,N*-dimethylaminomethylferrocene, the lithiation reaction can occur on either left or the right side of the dimethylaminomethyl group. A racemic mixture of the disubstituted ferrocene would be present in all reactions performed on functional groups on the ferrocene. This can be seen in **Figure 16** below.

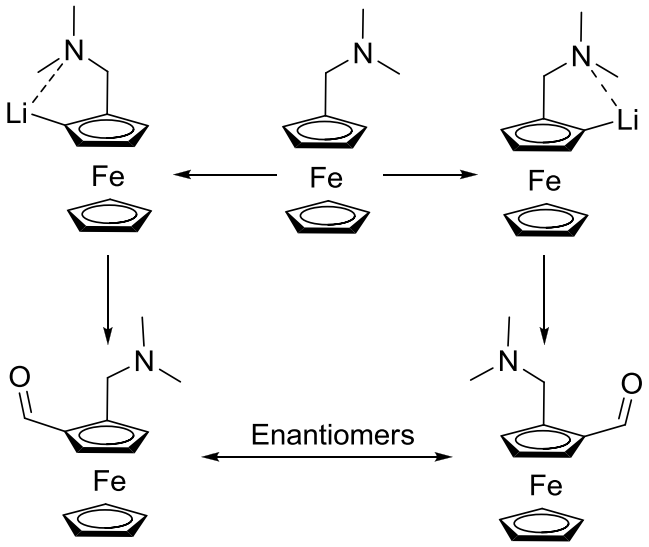


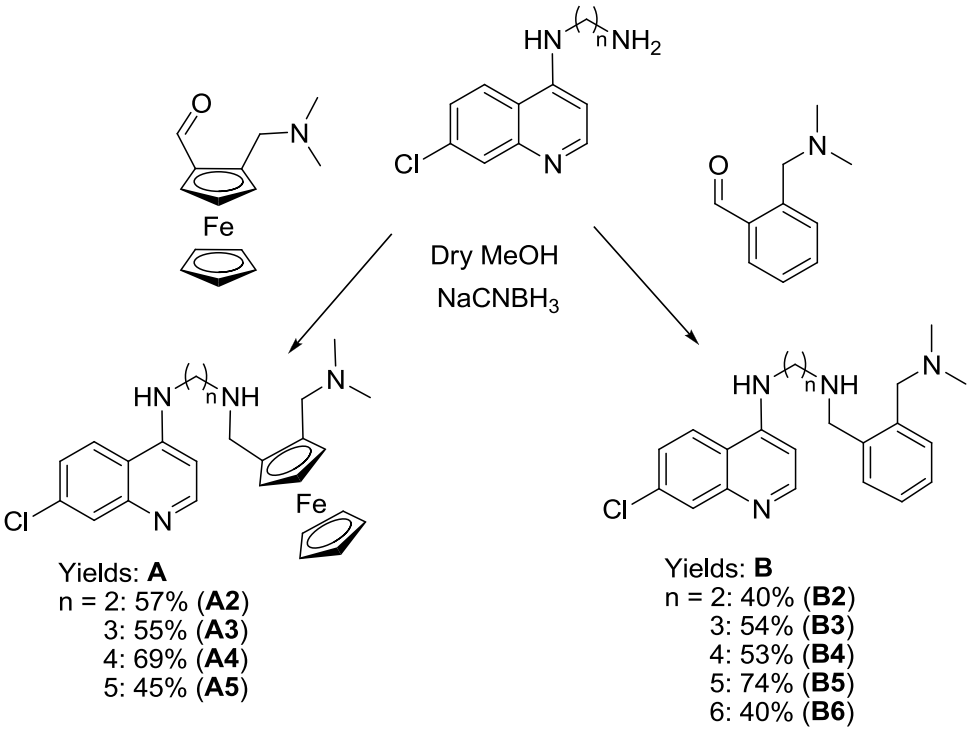
Figure 16: Ortholithiation on *N,N*-dimethylaminomethylferrocene results in a racemic mixture of products.

Stereogenic centers play an important role in drug efficacy. It has been proven that compounds containing enantiomeric ferrocenyl substituents are equipotent.[8] Both enantiomers of ferroquine showed equal IC_{50} values *in vitro*.[8] This means that separation of ferrocenyl enantiomers is not necessary.

Other pathways could have been utilized to form the primary amine, for example a reductive amination of the aldehyde with ammonia[9], or reduction of a nitrile group.[10] Although a lengthy method, the pathway introduced has been chosen since the ferrocenyl and phenyl aldehyde would be used later on in the synthesis of other compounds, and thus it was decided to focus on the development of a single method.

2.5. Reductive amination – synthesis of PQ and FQ analogues

The synthesis of the PQ and FQ analogues, series A and B respectively, is shown below in **Scheme 6**.



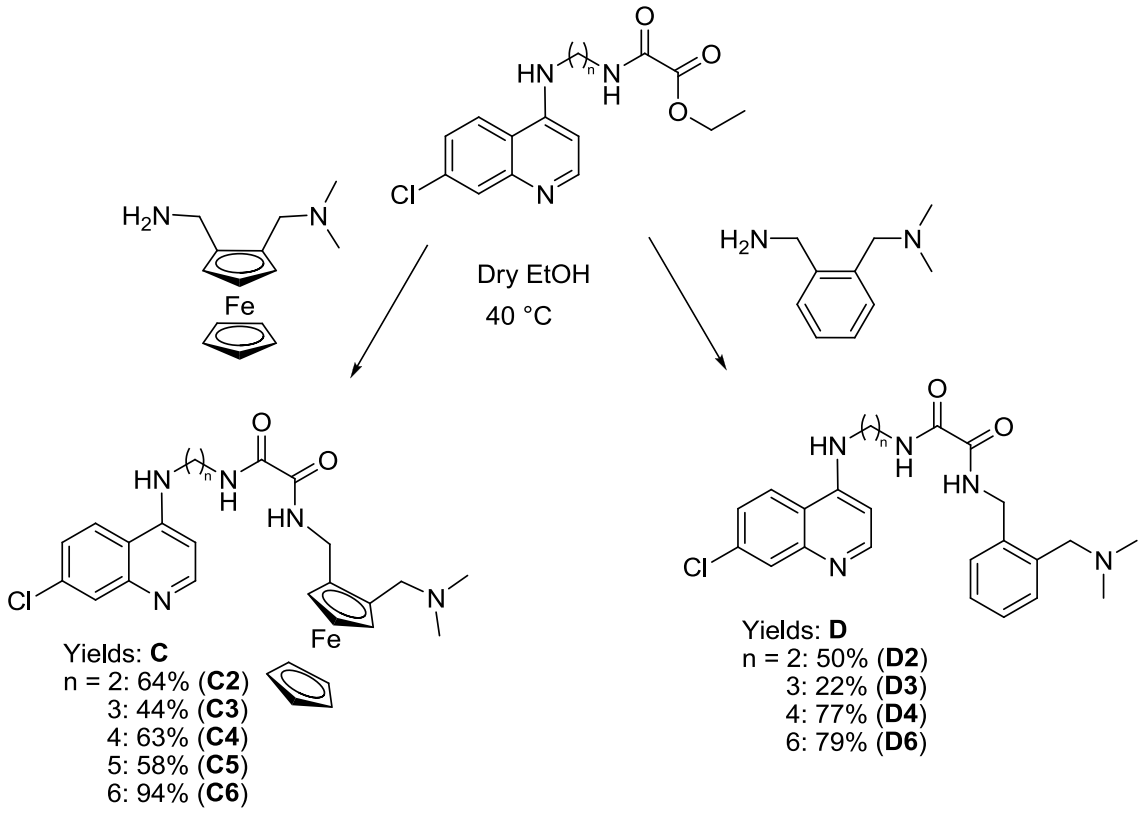
Scheme 6: Synthesis of series A and B and yields obtained.

In the previous section, the *ortho*-lithiation step by which we formed the *ortho*-substituted *N,N*-dimethylaminomethyl benzaldehyde (**11**) as well as the corresponding [(*N*,*N*-dimethyl amino)methyl] ferrocenecarboxaldehyde (**14**) was discussed. These compounds were used to create novel FQ and PQ analogues with compounds **1** – **5** via a reductive amination in dry methanol and sodium cyanoborohydride as the selective imine reducing agent.[11] Typically, yields of between 40% and 74% were obtained. Reasons for low yields could be that the solvent methanol was not totally anhydrous, as well as the fact that water is a byproduct during imine formation. This has an effect on the concentration of the reducing agent in these reactions, since water would quench

$NaCNBH_3$. It was not our intention to optimize the yields for these reactions, but rather to obtain sufficient product to continue with testing and further reactions.

2.6. Oxalamide synthesis

The synthesis of the PQ and FQ oxalamide analogues, series C and D respectively, is shown below in **Scheme 7**.



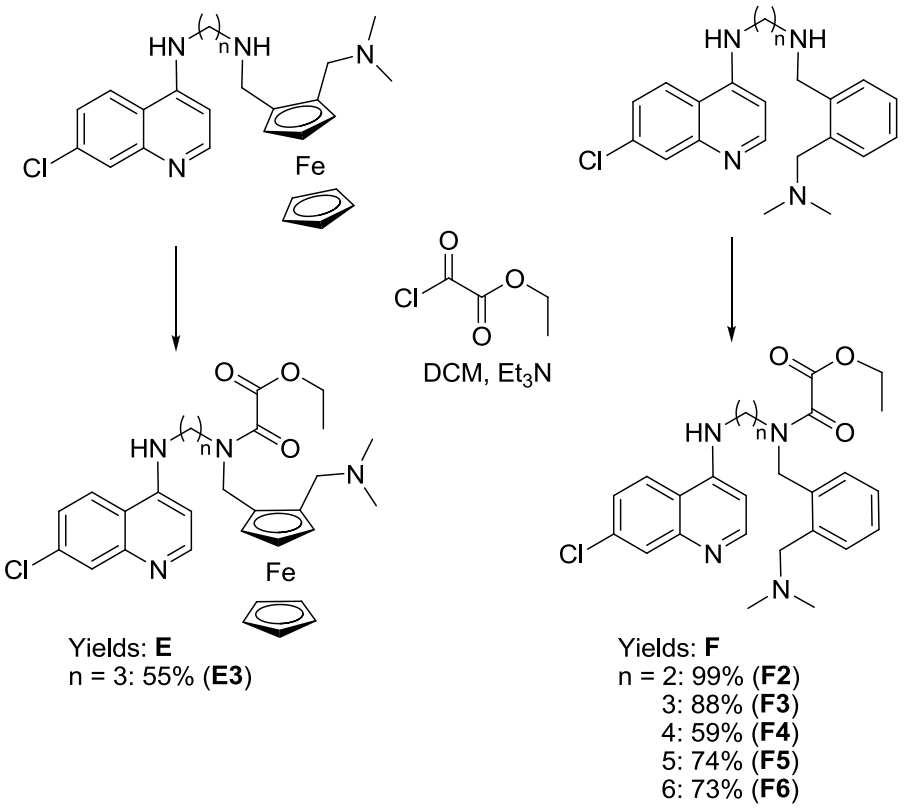
Scheme 7: Synthesis of series C and D and yields obtained.

Series **C** and **D** were synthesized with relative ease. Compounds **6** - **10** were stirred in dry ethanol at 40°C for 12-18 hours with the phenyl and ferrocenyl primary amine. This resulted in the desired compounds, with varying yields between 44% and 95%, with an unusual 22% obtained for one reaction. It was not in our interest to optimize these conditions for maximum yields. The amount of product obtained in after reaction was

enough to submit for testing. The reaction conditions were modified from the initial method, which involved refluxing compounds **6** - **10** and the aromatic amine in a steel bomb in *n*-BuOH at 120 °C for 8 hours[3].

2.7. Acylation of PQ and FQ analogues

The synthesis of the PQ and FQ ethyl oxamate analogues, series E and F respectively, is shown below in **Scheme 8**.



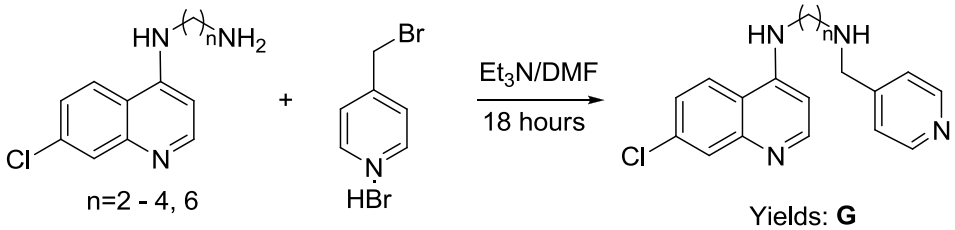
Scheme 8: Synthesis of series E and F and yields obtained

The previous series, synthesised by the reductive amination of the chloroquinolinamines and aromatic aldehyde, underwent an acylation reaction resulting in series **E** and **F.** The reaction is relatively straight forward from a chemistry point of view. Of the two nitrogens in the linker, the 4-amino nitrogen connected to the quinoline group is the less reactive

amine because the lone pair of the nitrogen is delocalized into the quinoline system. The secondary amine connected to the phenyl/ferrocenyl group has a localized lone pair and is thus more nucleophilic in nature making it the preferred site for acylation. The ferrocenyl analogue synthesized produced a yield of 55% and the phenyl analogues delivered yields between 73% and 99%.

2.8. Synthesis of Pyridyl - 7-chloroquinolines

The previous synthesised series all contains a dimethylbenzylamine moiety, necessary for pH trapping in the digestive vacuole of the parasite, and a lipophilic aromatic group to facilitate transfer of the molecule over the liphilic membrane of the digestive vacuole.[12] A pyridine ring was incorporated in order to introduce both functionalities in one step. The synthesis is that of the chloroquinolinalkylamines and 4-(bromomethyl)pyridine in DMF/Et_3N overnight via a substitution reaction to obtain the desired products, portrayed in **Scheme 9** below.



Yields: **G**
n = 2: 46% (**G2**)
3: 22% (**G3**)
4: 36% (**G4**)
5: 23% (**G5**)
6: 33% (**G6**)

Scheme 9: Synthesis of series G and yields obtained

The yields obtained were low in these reactions. Possible reasons for these lowered yields are that a stronger base could have been used in order to deprotonate the primary amine to increase the nucleophilicity of the amine. **Scheme 9** shows the general reaction and yields.

2.9. Characterisation data

2.9.1. Acylation of chloroquinolinalkylamine with ethyl-2-chloro-2-oxoacetate

Figure 17 shows that the ethyl oxamate group has been added to compound **10**

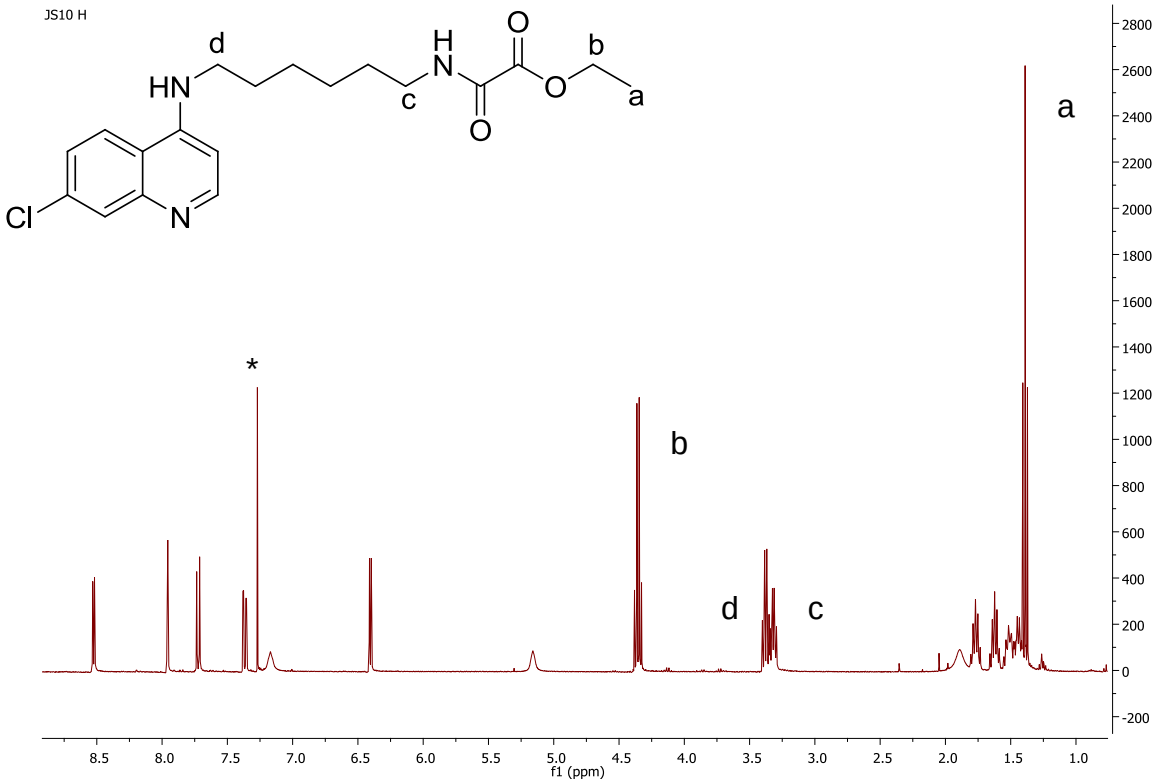


Figure 17: ^{1}H-NMR of 10 after acylation with of 5 with ethyl 2-chloro-2-oxoacetate. Solvent peaks are indicated with an asterisk (*)

This proton NMR shows that the ethyl oxamate group has been added to compound **10**. The protons of the ethyl oxamate group occur at 1.39 ppm and 4.35 ppm respectively. The signals between 3.29 ppm and 3.40 ppm show that the product has been synthesized. These are the methylene carbons adjacent to the ethyl oxamate and the 7-

chloroquinoline group. The signals would have been significantly more upfield if the nitrogens were not connected to either the 7-chloroquinoline and ethyl oxamate group.

2.9.2. Ortholithiation of *N,N*-dimentylaminomethyl ferrocene

The ^{1}H-NMR spectrum of compound **14** shown (**Figure 18**) clearly shows the aldehyde peak at 10.10 ppm, the unsubstituted cyclopentadienyl ring at 4.22 ppm and the three protons on the *ortho*-substituted cyclopentadienyl ring between 4.55 ppm and 4.78 ppm.

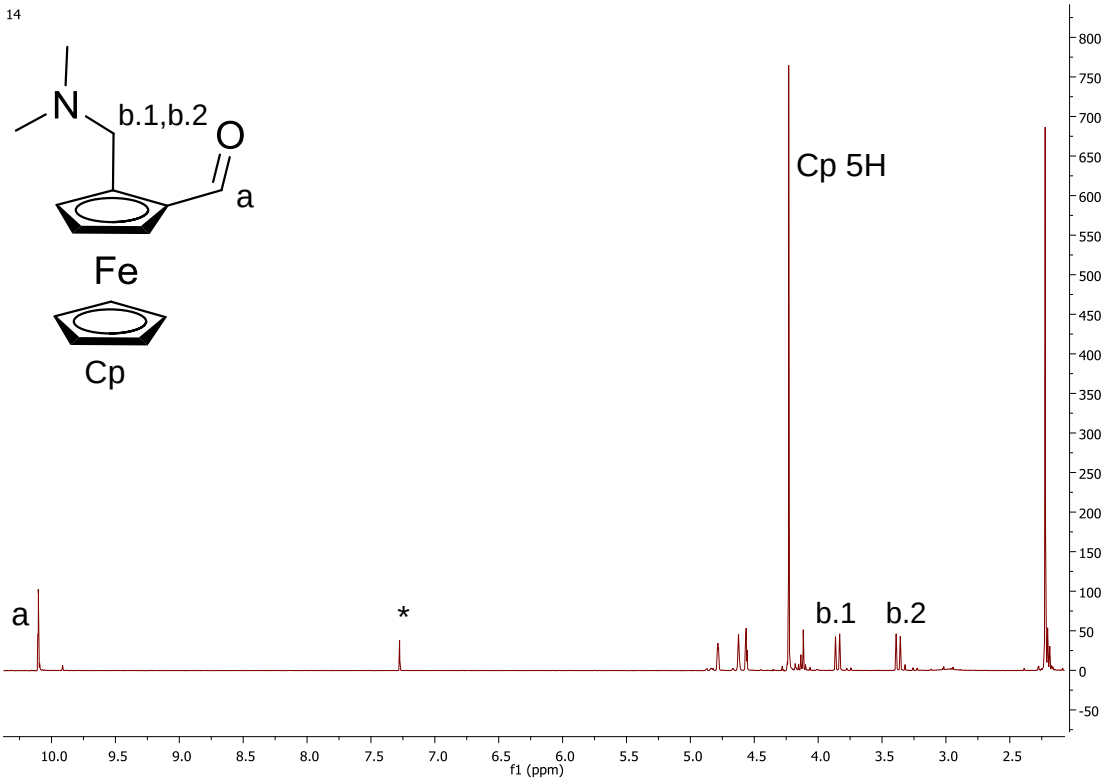


Figure 18: ^{1}H-NMR of 14 after ortholithiating and quenching with DMF. Solvent peaks are indicated with an asterisk (*)

This confirms that monolithiation occurred before quenching with DMF to form the aldehyde functional group. The splitting of the methylene protons is a clear indication that the 1, 2-disubstituted ferrocenyl products are chiral. The chemical environment on the substituted cyclopentadienyl (Cp) ring is different from the environment containing

the iron(II) ion and bottom Cp ring. Hence each methylene proton would be in a different chemical environment and thus undergoes splitting from each other creating the distinctive doublets at 3.83 and 3.35 ppm with coupling constants ranging between 12 - 13 Hz (protons b.1, b.2, **Figure 4**). This is illustrated in **Figure 19**, indicating two methylene protons in blue and red.

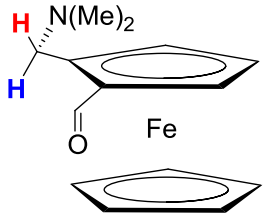


Figure 19: Methylene protons of compound 14 in different chemical environments.

2.9.3. Reductive amination

Figure 20 shows the proton NMR spectrum of compound **A3**. When observing the aromatic region, it is possible to see the 7-chloroquinoline proton signals as well as the protons on the phenyl group. However, if we did not manage to synthesise the reduced amine product but rather the imine, we would have observed a singlet at the region of 8.30 – 8.90 ppm, easily distinguishable from 7-chloroquinoline signals that all exhibit multiplicity.[13] The formation of series **C** and **D** was confirmed by ^{1}H-NMR

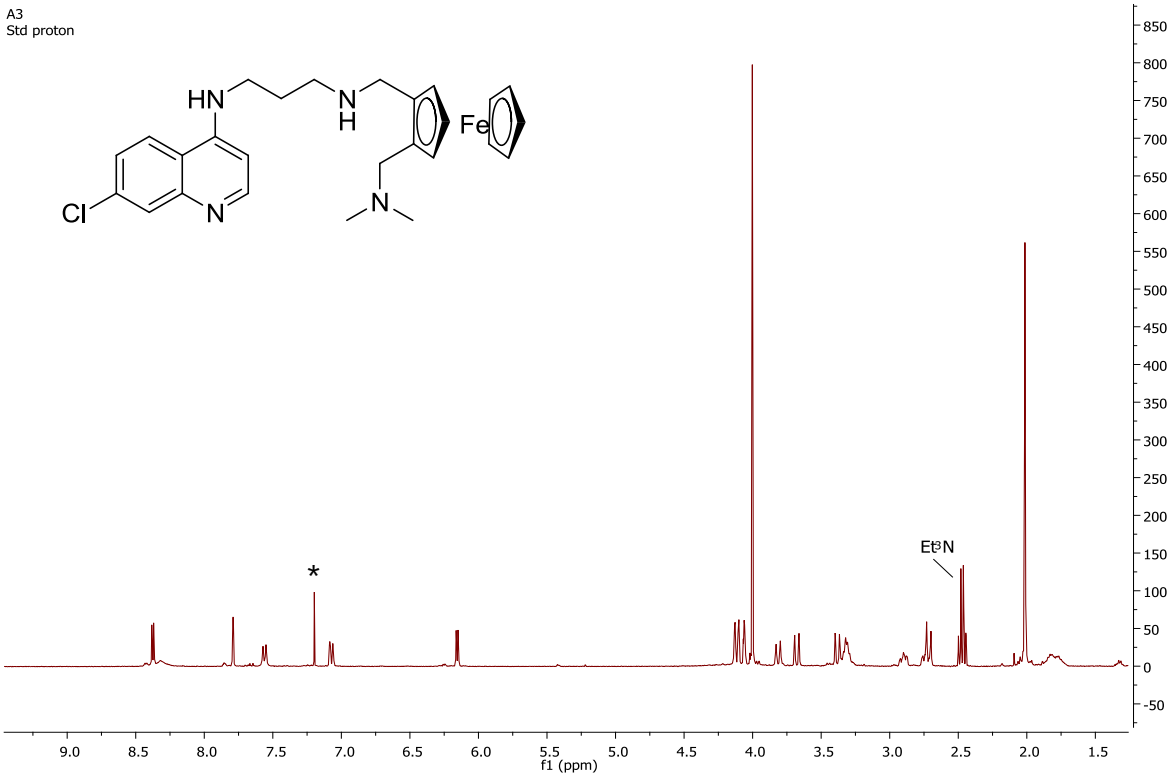


Figure 20: ^{1}H-NMR spectrum of A3 showing complete reduction of the imine precursor.

2.9.4. Oxalamide synthesis

The ^{13}C-NMR spectrum shows distinctive signals in the downfield region. When observing **Figure 21**, the proton decoupled carbon spectrum of compound **D6**, the two signals at 160 ppm and 159 ppm are a clear indication that the oxalamide functional group has been formed.

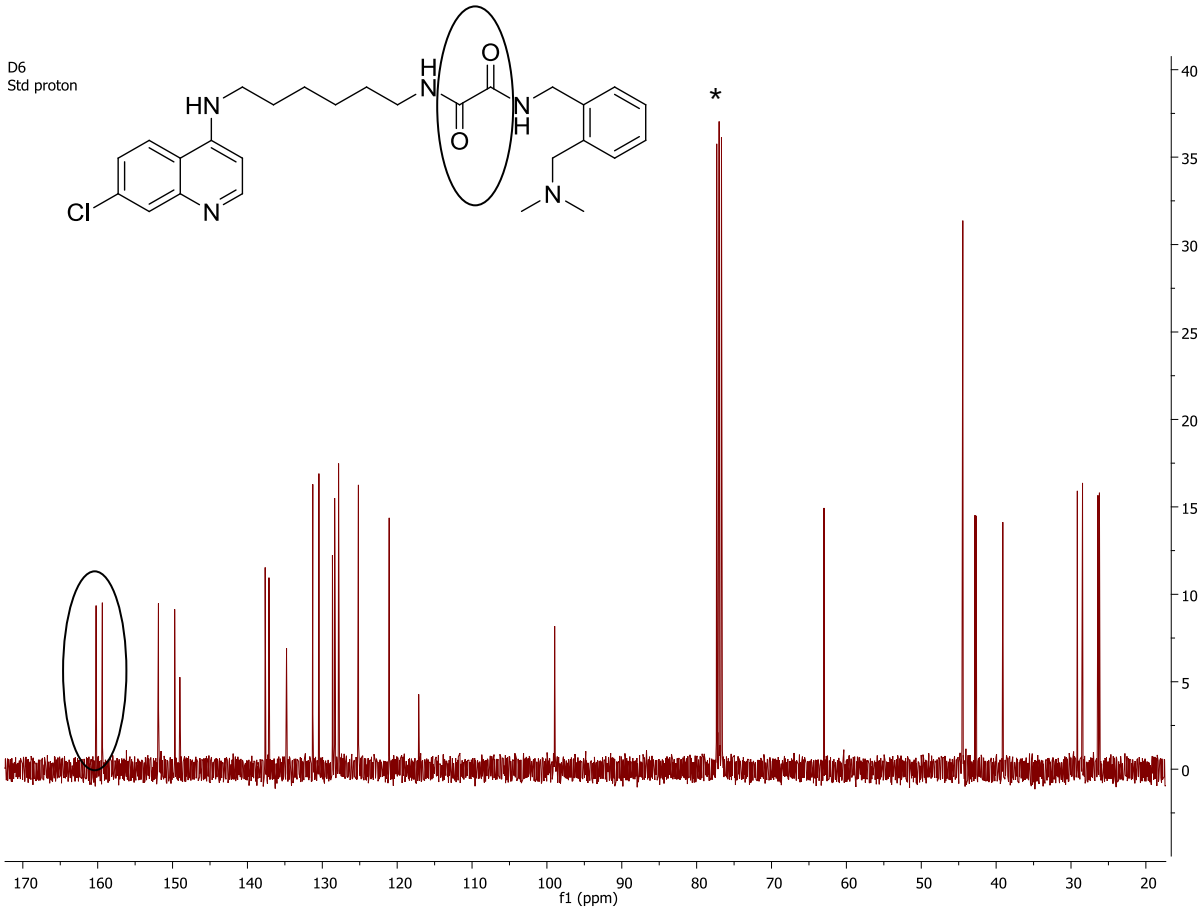


Figure 21: $^{13}C\{H\}$-NMR spectrum of compound D6 indicating characteristic oxalamide signals

When examining the IR spectrum of all the compounds in these two series (**C** and **D**), a strong absorption band at 1655 cm^{-1} is visible, indicating the amide characteristics of the oxalamide moiety. This is shown in **Figure 22** below, the IR spectrum of **D6.**

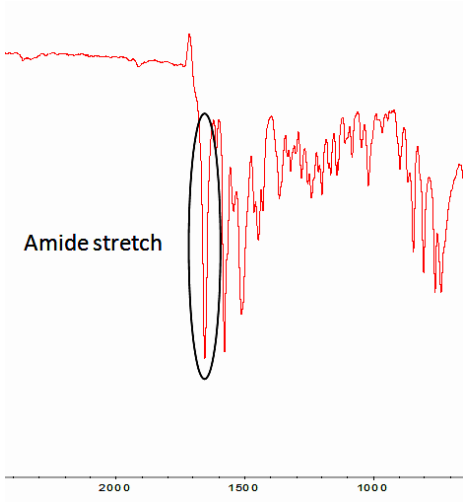


Figure 22: Infra red spectrum of compound D6 indicating the amide stretch characteristic of the oxalamide functional group

A single crystal X-ray diffraction structure (**Figure 23**) has been obtained for compound **D2,** providing further proof that the method used to synthesise series **C** and **D** is correct. An intramolecular hydrogen bond is observed between the oxalamide proton (on nitrogen N4) and the tertiary benzyl amine (shown as N5)

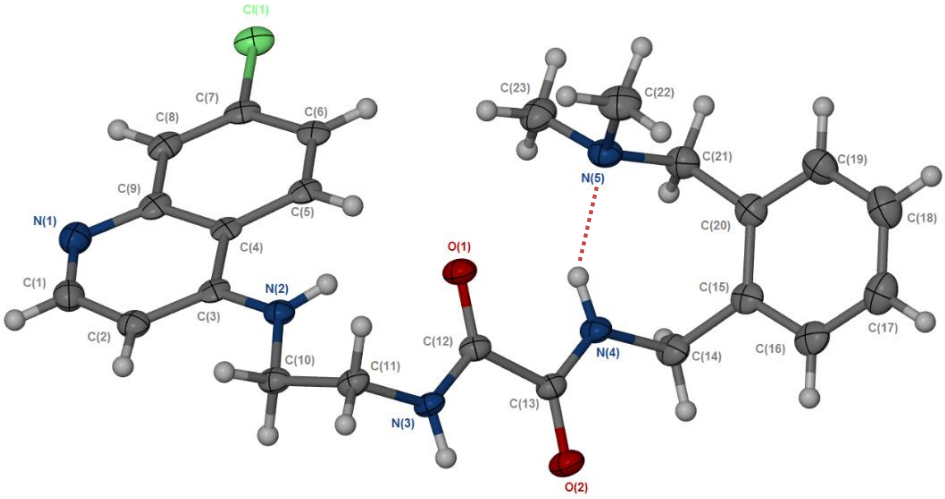


Figure 23: Molecular structure of compound D2. Thermal ellipsoids are shown at 50% probability. The intramolecular hydrogen bond is indicated as a dashed red line. (N4-H4---N5, 2.04 Å, 148.00 °)

The packing arrangement of **D2** show that the 7-chloroquinoline group exhibiting a stacking interaction with neighboring 7-chloroquinoline groups, shown in **Figure 24**

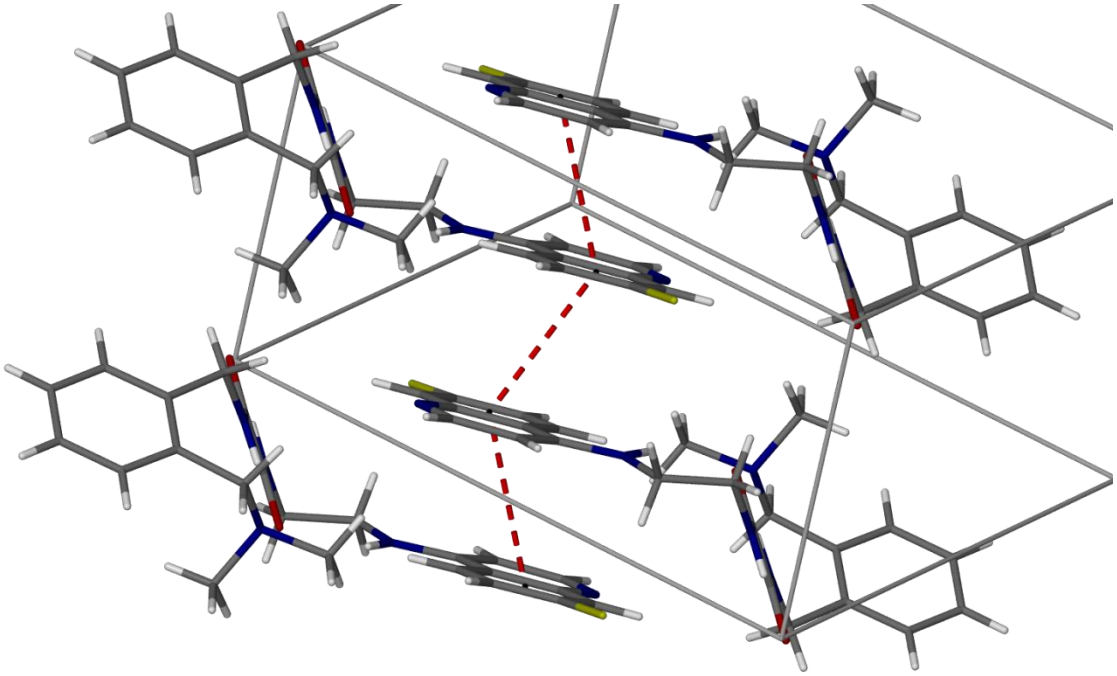


Figure 24 : Stacking of the aromatic 7-chloroquinoline groups of compound D2

2.9.5. Acylation of PQ and FQ analogues with ethyl oxamate as substituent

The amide part of the ethyl oxamate group has an interesting effect on the proton and carbon NMR spectra. The delocalization of the lone pair electrons on the amide nitrogen into the carbonyl group results in a partial double bond character between the nitrogen and carbon thus rotation around that bond is restricted at room temperature. The result was that two sets of peaks were observed in the ^{1}H and ^{13}C-NMR spectra collected at room temperature. To overcome this problem, we focused on variable temperature NMR. At a sufficient high temperature, the rotational energy barrier of the amide group would be surpassed and the related peaks would merge into one. **Figure 25** shows the two rotamers that exist at room temperature.

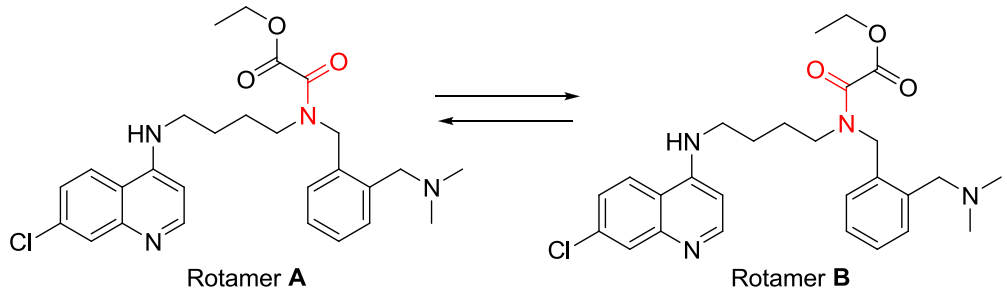


Figure 25: Different amide conformations of F4.

Figure 26 shows the double peak effect caused by the rotamers in solution of the above compound **F4** where the signals are doubled (bottom spectrum, indicated in red) and where they converge (top spectrum, indicated in blue)

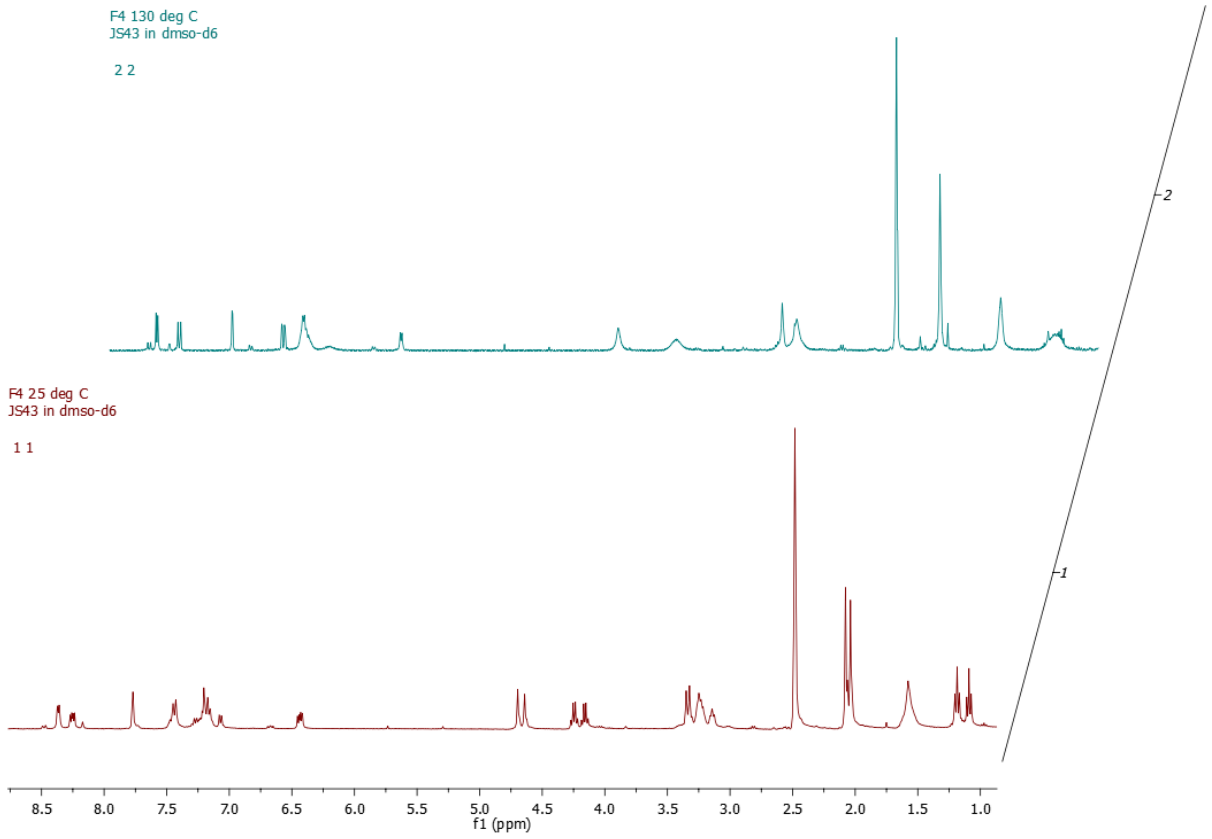


Figure 26: The ^{1}H-NMR spectrum of compound F4 recorded at 25 °C (bottom) and 130 °C (top) in d_6-DMSO

When investigating the ^{1}H-NMR spectrum recorded at 25 °C, the two sets of signals are easily observed especially in the 3ppm - 5ppm region of the spectrum. The variable temperature experiment was carried out by using deuterated DMSO as the solvent. The NMR probe was initially set at 25 °C, after which it was raised to 50 °C, 80 °C, 100 °C, 120 °C and then 130 °C where the rotational barrier was overcome and the signals finally converged.

2.9.6. Pyridyl - 7-chloroquinolines

7-chloroquinolinalkylamine-pyridyl compounds belonging to series G were easily charaterised by recognising distinctive peaks in the aromatic region, as seen in **Figure 27** below.

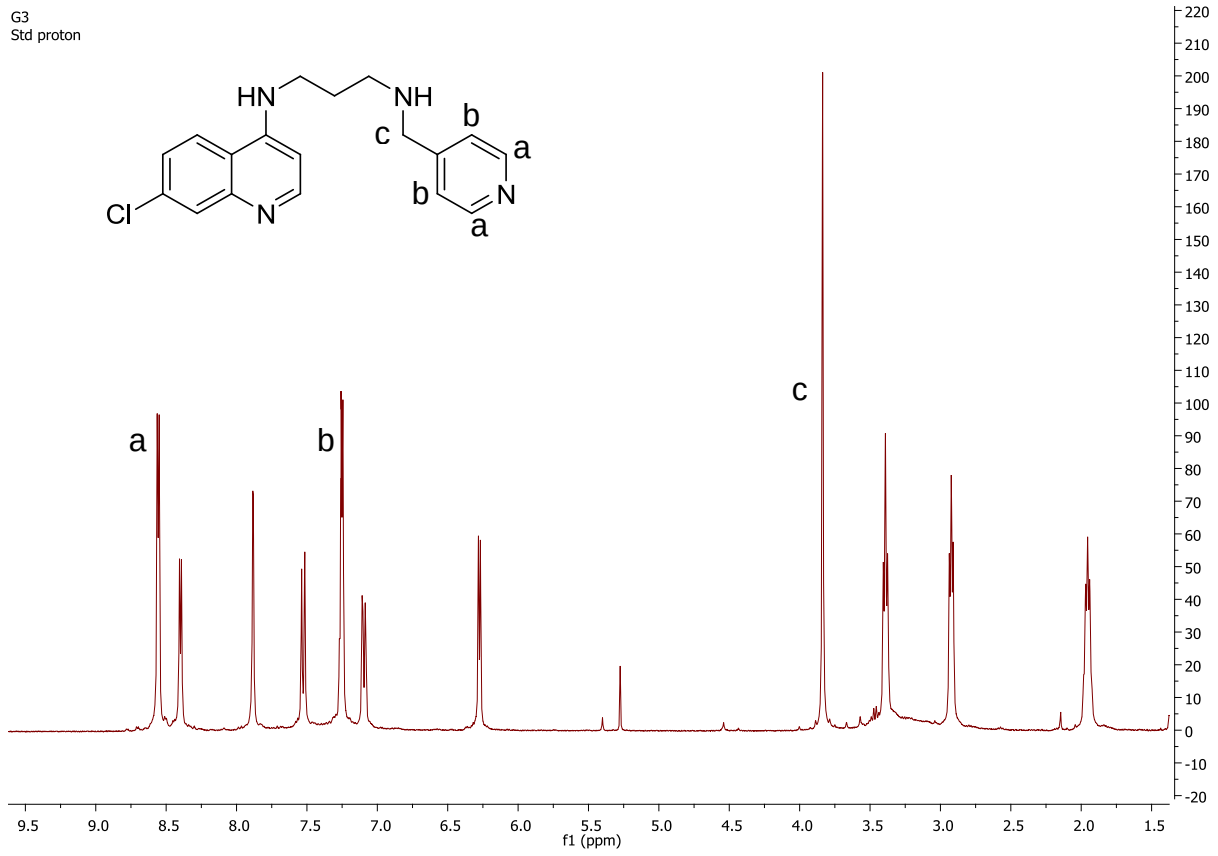


Figure 27: ^{1}H-NMR of G3 indicating chemically equivalent pyridine protons (a, b)

The 4-methylpyridyl substituent is symmetric, and this is visible in the aromatic region at 8.55 ppm, the protons *ortho* to the pyridine nitrogen, and 7.25 ppm, the protons *meta* to the pyridine nitrogen where the chemically equivalent proton signals appear.

2.10. Concluding remarks

Each series of compounds were successfully synthesised, isolated and fully characterised using ^{1}H-NMR, ^{13}C-NMR, Infra red spectroscopy and mass spectrometry. A crystal structure has been acquired that confirms the proposed molecular structure of compound **D2**. This implies that the method used to synthesise both these series was correct.

2.11. References

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Chapter 3

Biological and Biophysical Results

3.1. Introduction

In this chapter, the biological evaluation of all novel compounds synthesized in series A – F is reported. Series G was not submitted for whole cell testing, however the β-haematin inhibition assay was performed for three compounds belonging to this series.

The aim of this chapter is to attempt to rationalise the observed activity in both whole cell and β-haematin inhibition assays. Series A - F include either a hydrophobic phenyl or ferrocenyl aromatic group, and each has a carbon methylene spacer ranging from 2 – 6 carbons, which connects the 7-chloroquinoline with the respective aromatic group. Introducing an oxalamide functional group within the methylene spacer (series C and D), we looked to compare the activity of these two series to compounds not having this functional group. An ethyl-oxamate group has also been added to the spacer (series E and F), giving us scope to compare whether the oxalamide or ethyl oxamate group has a greater influence on activity. A less hydrophobic aromatic pyridyl group was incorporated in series G.

Based on these results, we can gain a better understanding on the importance of certain moieties, and insight into whether a specific compound has a target, or whether there is the possibility of more than one target.

3.2. Whole cell testing

The whole cell assay was only conducted on the chloroquine sensitive (CQS) NF54 strain of *P. falciparum*. The observed IC_{50} values for each compound are reported in Table 1.

Table 1: *In vitro* activity of series A - F, compared to chloroquine and artesunate.

Series	Compound	NF54 IC_{50} (nM)	Efficacy relative to chloroquine
A	**A2**	14.1	1.57
	A3	34.8	0.64
	A4	25.4	0.87
	A5	4.2	5.26
B	**B2**	19	1.16
	B3	19.6	1.13
	B4	32.7	0.68
	B5	22.9	0.97
	B6	35.1	0.63
C	**C2**	14.5	1.52
	C3	9.4	2.35
	C4	14.2	1.56
	C5	10.8	2.05
	C6	14.2	1.56
D	**D2**	21.6	1.02
	D3	14.3	1.55
	D4	9.8	2.26
	D6	7.4	2.99
E	**E3**	12.3	1.80
F	**F2**	185.5	0.12
	F3	45.8	0.48
	F4	22.7	0.97
	F5	8.7	2.54
	F6	14.1	1.57
	Chloroquine	22.1	1
	Artesunate	11.4	

When investigating the results, it is evident that 17 compounds are more active than cloroquine. It is noteworthy that the most active compounds, **A5**, **C3**, **C5**, **D4**, **D6** and **F5** have longer methylene spacers (n≥4) with the exception of compound **C3** which has a spacer of 3 carbons. This implies that, regardless of whether the compound contains the oxalamide functional group (**C3**, **C5**, **D4**, **D6**), an ethyl oxamate substituent (**F5**) or is

a ferroquine analogue (**A5**), the length of the spacer appears to influence the activity of the compound. It is possible to speculate that increased hydrophobicity of the compound when increasing the chain length is responsible for the observation. In order for a drug to pass through a liphophilic cell membrane it has to have a certain degree of lipophilicity as well, which could explain the increased activity in these compounds.[1] However, steric factors may also play a role

Figure 28 illustrates the IC_{50} values relative to chain length with respect to each series.

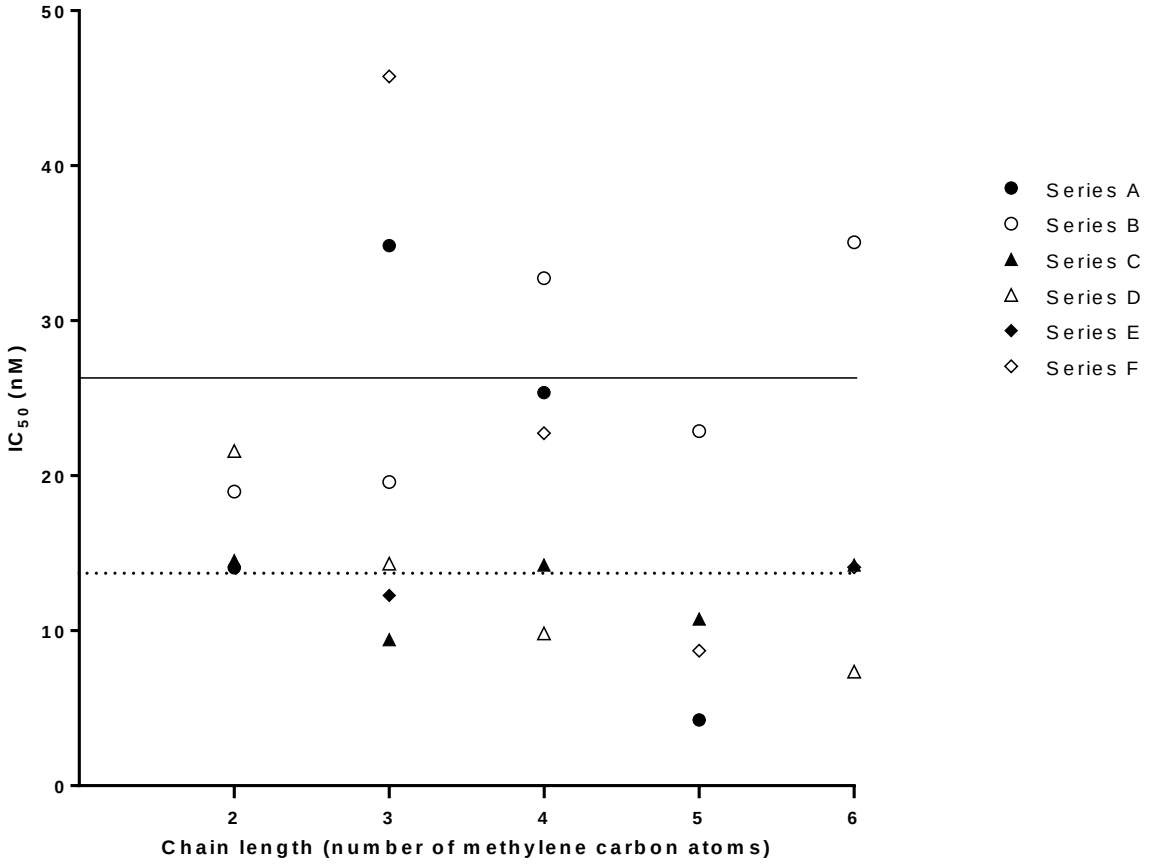

Figure 28: The variation in IC_{50} value determined against the chloroquine sensitive NF54 strain of *P. falciparim*, as a function of the length of the methylene spacer. The dashed line indicates the activity af artesunate, and the solid line the activity of chloroquine. Solid symbols refer to series containg the ferrocenyl group, while open symbols refer to series containing the phenyl aromatic group.

Series A, C and E have compounds containing the *N,N*-dimethylaminomethyl ferrocenyl group, while series B, D and F are the equivalent phenyl analogues. When investigating **Figure 1**, it is evident that below an IC_{50} value of 15 nM, compounds containing the ferrocenyl moiety are much more active than those with the phenyl group. Eight ferrocenyl compounds show good activity below 15 nM compared to 5 compounds having the phenyl group. The ferrocenyl and phenyl groups are both hydrophobic/lypophilic, thus facilitating the diffusion of these compounds accross the DV membrane. It also implies that, regardless of any differences in lipophilicity, the proposed redox capabilities that ferrocenyl compounds have[2] may contribute significantly to their efficacy. **Figure 29** shows the difference in the activity when comparing series A and B, the ferroquine and phenylequine analoges, with their respective oxalamide (C and D) and ethyl oxamate (E and F) coupled analogues.

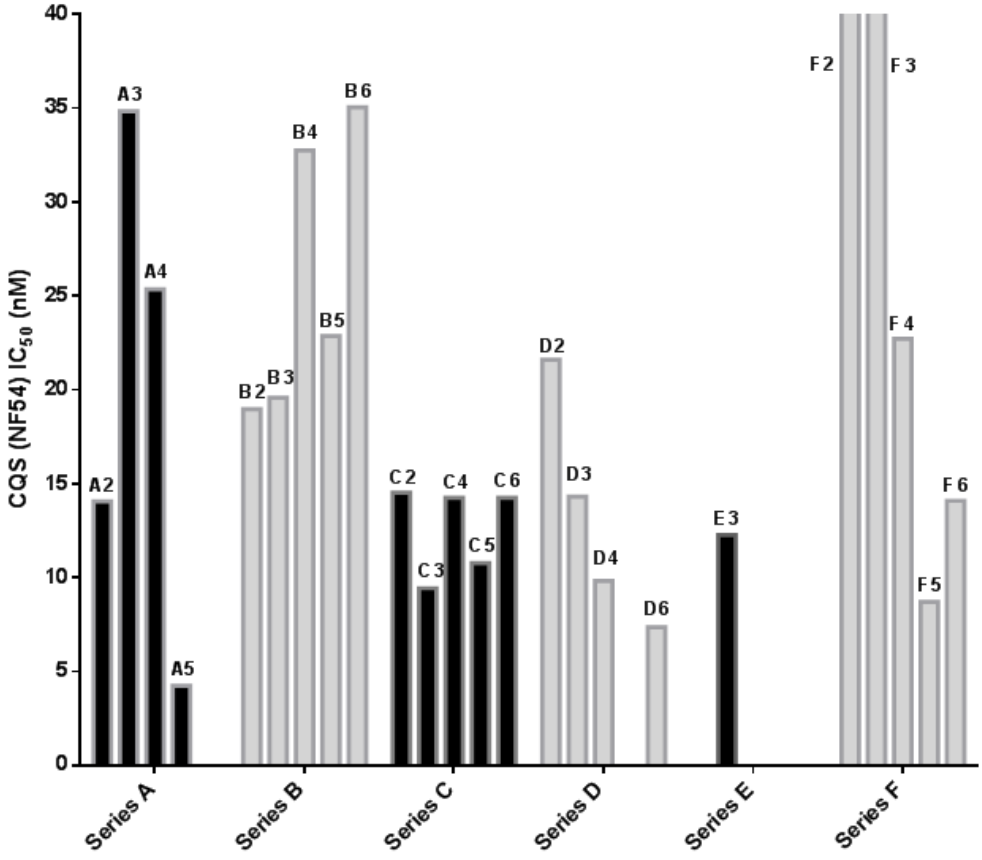

Figure 29: Series A – F, showing variation in activity in each series while illustrating change in efficacy for each individual series with increase in chain length.

The data of series C shows that the IC_{50} values does not vary significantly, ranging between 9 and 15 nM, and the ferroquine analogues of series A have a lowered efficacy on average. We do observe an increase in efficacy when comparing compound **A5** with series C.

When comparing Series B and D, it is evident that the oxalamide functional group does play a large part in the efficacy, showing a structure activity related to chain length versus efficacy. Series B does show a trend in decreased activity with increase in chain length, with a large decrease in activity for compound **B4**. The data thus show that incorporation of an oxalamide functional group in FQ and PQ analogues does induce an increased efficacy.

Series E and F are the PQ and FQ ethyl-oxamate analogues. Compound **E3**, the FQ ethyl oxamate analogue to compound **A3**, shows a large incease in efficacy when introducing an ethyl-oxamate substituent to the carbon linker. This however is almost the reverse effect when comparing series B with series F. We could rationalise that with increase in the carbon chain spacer length, efficacy decreases in series B, and increases series E.

3.3. β-haematin inhibition

Each of the compounds tested contained the necessary 7-chloroquinoline group, essential for the inhibition of haemozoin in *P. falciparum*.[4] As previously stated, drugs containing the quinoline moiety are thought to have one main mode of action, which is the inhibition of β-haematin formation. From the results of the β-haematin inhibition assay, we can anticipate one or more of the following:

Firstly, compounds that show mediocre efficacy against CQS *P. falciparum*, but which also exhibits β-haematin inhibition, have a high probability of having only β-haematin inhibition as their target. Secondly, compounds that show significant efficacy *in vitro* and also have good β-haematin inhibition values, may either be good β-haematin inhibitiors or may also have another target within the parasite. Thirdly, mediocre β-haematin

inhibition together with good *in vitro* efficacy would strongly suggest that, although β-haematin inhibition is a target, some other target may likely be inhibited or targeted by the drug. Lastly, the compound may also not be absorbed into the DV, so that while it may shows good β-haematin inhibition, it shows poor *in vitro* activity.

The following graph (**Figure 30**) shows the observed correlation between the IC_{50} values for β-haematin inhibition and the *in vitro* assays for compounds tested. When compound **F2** is included in the correlation, a best fit linear regression (R^2 = 0.81) is obtained.

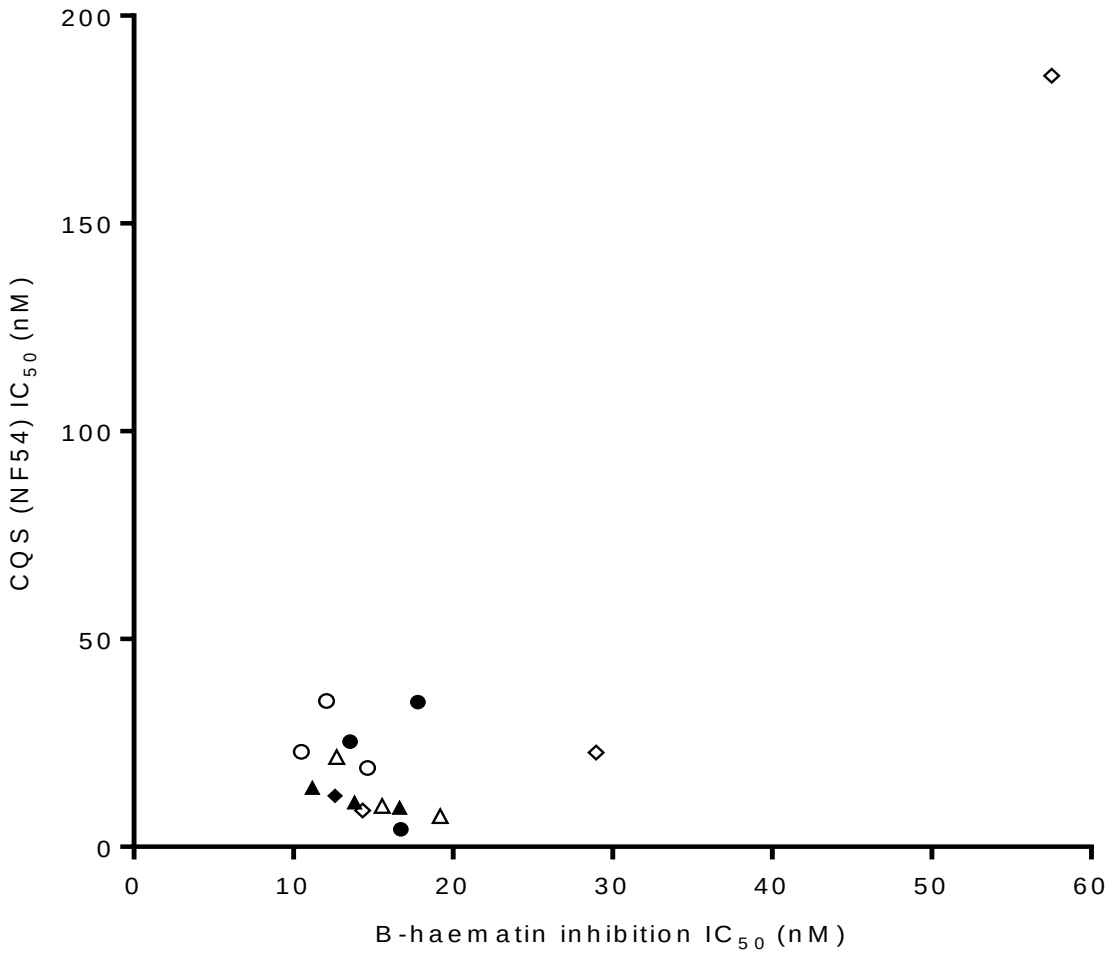

Figure 30: Correlation between whole cell inhibition IC_{50} values and β-haematin inhibition

Inspection of this graph shows a conglomerate of compounds in the range of 10 to 20 nM that shows good β-haematin inhibition, except two outliers, compound **F4** (28.97 nM β-H; 22.74 nM CQS) and **F2** (57.53 nM β-H; 185.51 nM CQS). Excluding these two outliers, it is apparent that there is no significant correlation between β-haematin inhibition IC_{50} values and *in vitro* activity. (**Figure 31**) However when excluding **A3** as an outlier, there does appear to be a correlation, compounds showing good in vitro activity have poorer β-haematin inhibition IC_{50} values, and vice versa.

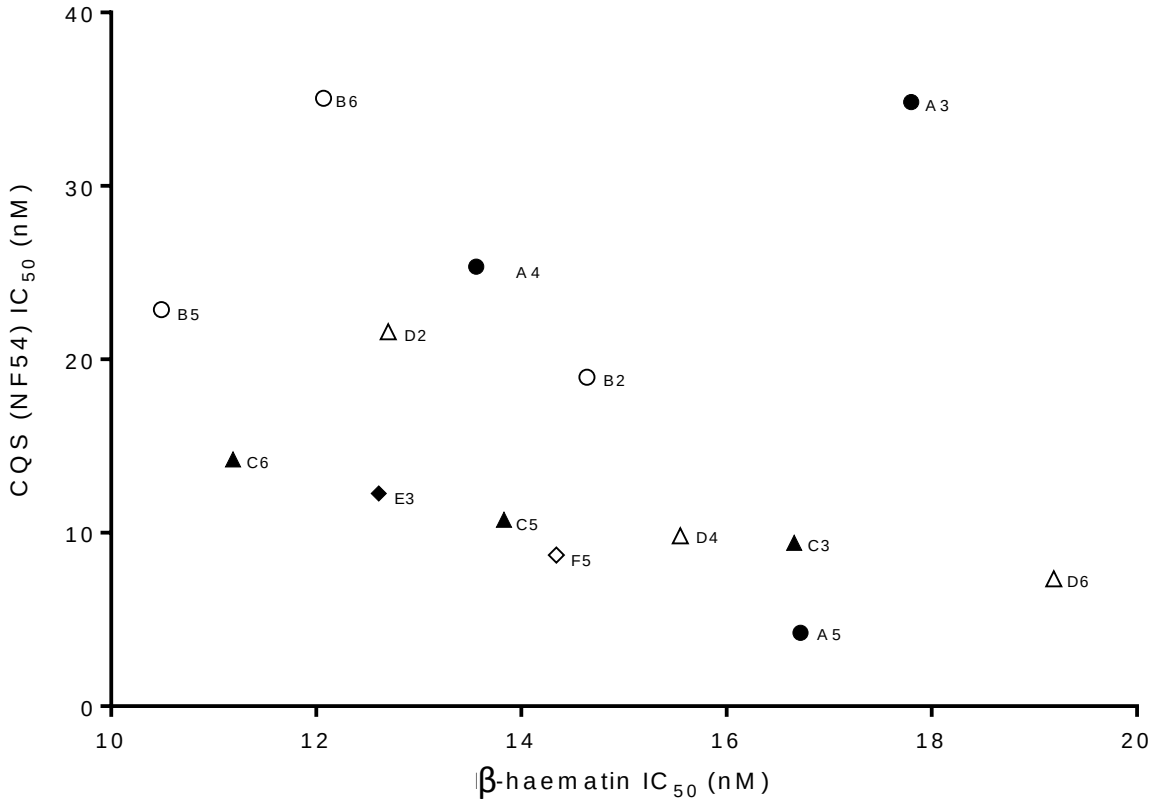

Figure 31: Whole cell inhibition IC_{50} values compared to β-haematin inhibition IC_{50} values, excluding compounds **F2** and **F4**.

The compounds shown here all have β-haematin inhibition IC_{50} values that range between 10.40 nM and 19.19 nM. This is a narrow interval of values, meaning these selected compounds all have good β-haematin inhibition abilities. However, it is interesting to see how the whole cell IC_{50} data is distributed over a much wider range, between 4.03 nM and 35.06 nM. This shows that even while most of these compounds

do inhibit β-haematin formation, it is attributable to the chloroquinoline group present in all structures. *In vitro* however, it may be possible that there is another target that could explain the increase in efficacy.

3.4. Docking studies: *Plasmodium falciparum* lactate dehydrogenase (*pf*LDH)

Using the program Accelrys Discovery Studio, a modeling study has been carried out on the docking capabilities of some of our synthesized compounds on the active site of *P. falciparum* lactate dehydrogenase. A co-crystalised structure of chloroquine and *pf*LDH as been published which shows that chloroquine binds in the NADH co-factor binding site of *pf*LDH, an important glycolytic enzyme, and acts as a competitive inhibitor of this binding site.[5] **Figure 32** shows two superimposed *pf*LDH crystal structures, one containing NADH and the other containing chloroquine in the active site.

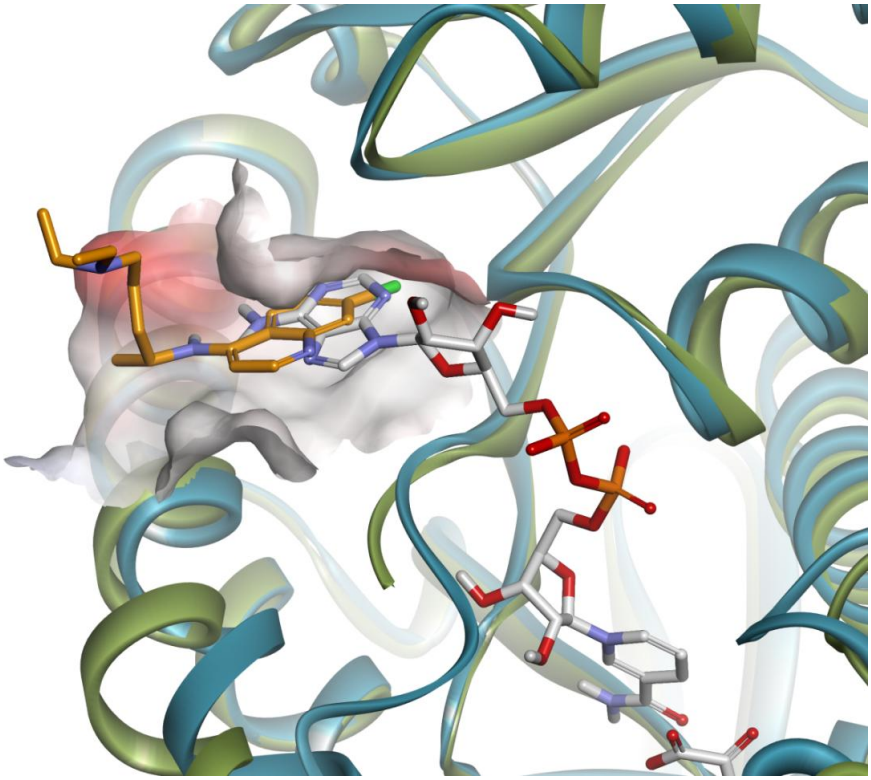

Figure 32: Overlay of two *pf*LDH crystal structures containing chloroquine and NADH, chloroquine shown in orange at the top left of the active site.

The crystal structure in which chloroquine acts as the ligand was utilized to develop a pharmacophore model to facilitate the similar placement of the structures synthesised in the current study within the binding site. The pharmacophore feature, shown in **Figure 33**, describes the 4-amino nitrogen with a proton donor feature (shown in purple), the quinoline nitrogen with a proton acceptor feature (shown in green) and two aromatic hydrophobic features for the quinoline portion of the molecule (shown in blue).

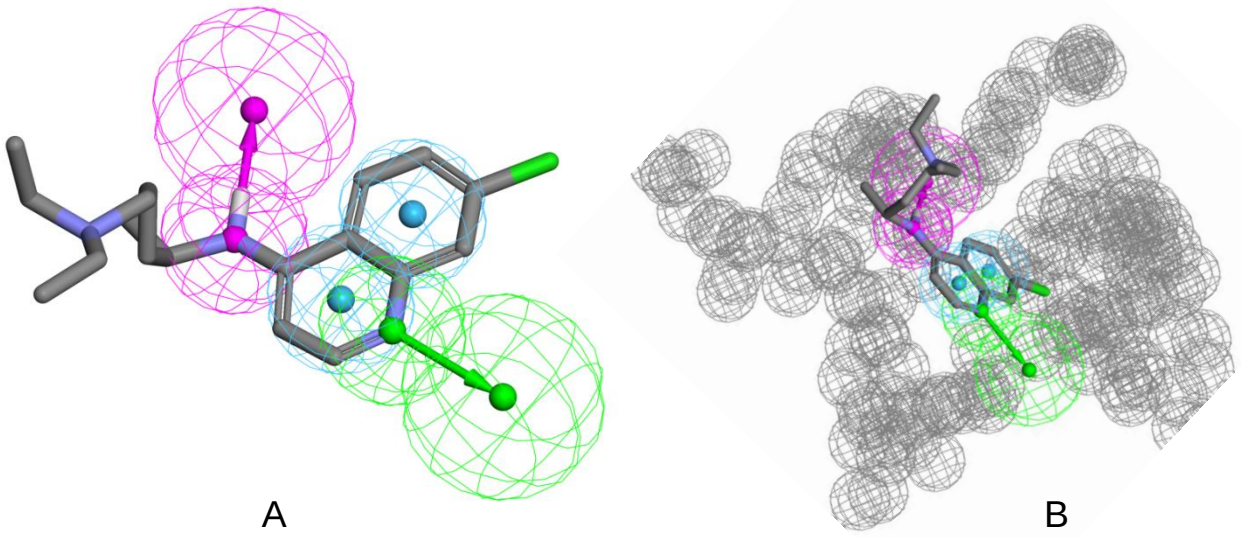

Figure 33: Pharmacophore model of chloroquine (A) and excluded volumes around pharmacophore (B)

The position of the original ligand (NADH) in the binding site was retained, and the entire pharmacophore was positioned in the binding site of the enzyme. The amino acid residues around the active site had to be taken into account in order to avoid potential steric clashes, therefore all the amino acid residues within a 5 Å radius of the original bound chloroquine were selected to be excluded volumes. These are the spaces within the active site that will not have any interaction with the ligands. This is shown in **Figure 6**. We have created a pharmacophore model, and our compounds have been screened for their ability to fit into this model. Multiple conformations of each compound were generated (up to 255 conformers) to search for the best conformation that fits the pharmacophore model. In order for a compound to be considered a hit, all the features of the model must match, i.e. the proton donors and acceptors must have an interaction with the compound, and no violations of excluded volumes are permitted. The

compounds screened were those of series D (containing an oxalamide functional group) and F (containing an ethyl oxamate substituent). Docking studies with ferrocenyl compounds proved to be difficult because the ferrocenyl moiety falls outside the standard parameters. As a result we decided to focus on phenylequine analogues for the interest of seeing if we could obtain usable data. Nine compounds were screened, and all of them met the requirements for the model, with some having a better fit value than others. Compounds that fit the requirements for the pharmacophore model the best are those that show the best fit value, and that fit value is solely related to the model. There is no interaction with the protein and the fitted ligands that influences this value.

The subsequent step was to calculate the interaction that the protein has with the ligand, i.e. what affect the surrounding residues have on the ligand. We therefore fitted each compound in the active site, with the aid of exclusion volumes and our pharmacophore model. Calculations were run to determine the binding energy of each ligand (Table 2). These are far more computationally expensive than the previous pharmacophore fitting model, as this procedure enables the minimization of the ligand and the residues within 5 Å radius of the docked ligand. This is done with a solvation model, which represents the docking of the ligand in an aqueous environment. In the absence of a solvation model, it gives results as if the docking is modeled in vacuum.

Table 2: Fit values and binding energy of series D and F

compound	FitValue	Binding Energy (kcal/mol)	Total Binding Energy (kcal/mol)	Ligand Energy (kcal/mol)
D2	3.88885	6.6819	13.241	-33.74008
D3	3.91788	3.64638	9.6048	-29.92875
D4	3.90527	-0.20755	8.6643	-29.02135
D6	3.87538	8.6568	16.321	-30.56801
F2	3.9123	3.14745	11.447	-30.92394
F3	3.92695	1.25777	7.0698	-51.97665
F4	3.88754	4.25799	9.3996	-54.48959
F5	3.91667	9.18081	14.447	-59.81569
F6	3.89612	-3.32481	2.9212	-53.8668

Compound **F6** shows the most favorable binding energy with the protein, as it has a hydrogen bond (originating from the secondary amine bonded to the quinoline) close by the glutamate residue (GLU122). Not all the structures exhibit this particular hydrogen bonding interaction. Comparing the fit values, it is evident that the deviation in these values are small, thus the pharmacophore model works well with the use of the 7-chloroquinoline group in the active site. The deviation of the binding energies are large, meaning the chain length, oxalamide and ethyl oxamate functional groups now come in to play influencing the binding energy.

3.5. Concluding remarks

The results show that the majority of the compounds synthesised in this project exhibit good efficacy against the NF54 CQS strain of *P. falciparum*. It is evident that the length of the methylene spacer has an influence on activity, longer spacers showing increased efficacy over shorter spacers. The results also further indicate that compounds containing the ferrocenyl moiety exhibits an increased efficacy compared to the phenyl analogues. The β-haematin inhibition assay concluded that all compounds except **F2** and **F4** had acceptable IC_{50} values in a narrow interval, ranging from 10 – 19 nM. There appears to be no correlation between β-haematin inhibition and whole cell inhibition. After we managed to acquire some modeling data with *pf*LDH, it must be noted that this data only theoretically predicts which compound could be a better inhibitor of *pf*LDH, and can only be validated if a *P. falciparum* lactate dehydrogenase inhibition assay is carried out to compare the results.

3.6. References

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(2) Stone, D. L.; Smith, D. K.; McGrail, P. T. *J. Am. Chem. Soc.* **2002**, *124*, 856-864.

(3) Biot, C.; Chavain, N.; Dubar, F.; Pradines, B.; Trivelli, X.; Brocard, J.; Forfar, I.; Dive, D. *J. Organomet. Chem*. **2009**, *694*, 845–854

(4) Egan, T. J.; Hunter, R.; Kaschula, C. H.; Marques, H. M.; Misplon, A.; Walden, J. *J. Med. Chem.* **2000**, *43*, 283-291.

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Chapter 4

4.1. Conclusions

The aim of this project was to synthesise seven series of novel ferroquine and phenylequine analogues, and to fully characterise each compound using ^{1}H-NMR and ^{13}C-NMR spectroscopy, infra red spectroscopy and mass spectrometry.
Following synthesis, compounds were also submitted for whole cell and β-haematin inhibition assays in order to probe their mechanism of action. With this data, we set out to rationalise whether each series show structure-activity relationships when comparing differences in diaminoalkyl chain lenth, aromatic groups, and functional groups.

These compounds were tested *in vitro* for antiparasitic activity against the *P. falciparum* sensititive strain NF54. All compounds that were tested had good to medium efficacy, ranging from 4.2 – 34.8 nM, except for two compounds that were considered to be outliers due to much lower efficacy, in comparison.

The first two series, the ferroquine and phenylequine analogues, showed efficacy between 0.5 and 1.5 times that of chloroquine, with the exception of one compound, a ferroquine analogue with a 5 carbon methylene spacer, that was shown to be 5.3 times more efficacious than chloroquine. The phenylequine series *in vitro* results concluded that the activity decreases with an increase in methylene spacer length. When comparing the *in vitro* results of these two series, it was evident that inclusion of a ferrocenyl group rather than a phenyl group had better efficacy.

The third and fourth series are ferroquine and phenylequine analogues that contain an oxalamide functional group. Inclusion of this oxalamide functional group had a significant effect on efficacy, as evidenced by the increased efficacy when compared to the activity of the first two series. The most effective compound of the third series was 2.4 times more active than chloroquine, and 1.2 times more active than artesunate. In

the fourth series, the most active compound had an *in vitro* activity of 7.4 nM, 3 times more active than chloroquine and 1.5 times more active than artesunate.

The fifth and sixth series are ferroquine and phenylequine analogues that contain an ethyl oxamate substituent. It is interesting to note that *in vitro* activity of the phenylequine ethyl oxamate compounds increase with increase in the methylene spacer length, of which the most active compound belonging to this series has an activity of 8.7 nM, 2.5 times that of chloroquine. The ferroquine analogue showed an activity of 12.3 nM, 1.8 times more active than chloroquine. It appears that inclusion of the ethyl oxamate substituent with compounds containing a longer methylene spacer had a positive effect on efficacy.

β-haematin inhinbition studies have been performed on selected compounds in each series showing good, medium and poor *in vitro* efficacy. The majority of compounds in the assay showed a narrow range of IC_{50} values for β-haematin inhibition, between 10.4 nM and 19.2 nM. This assay was done to potentially correlate *in vitro* activity with β-haematin inhibition. However, we did not observe any direct correlation for each series. However, a trend has been observed when comparing both β-haematin inhibition and *in vitro* IC_{50} values. Compounds that show good *in vitro* efficacy would show poor β-haematin inhibition, and vice versa. This appears evident for most compounds tested except for an outlier **A3** that exhibits poor *in vitro* and β-haematin inhibition IC_{50} values. This suggests that these compounds could potentially act on a different target before reaching the digestive vacuole.

Docking studies have shown that the compounds synthesised in this study do have an inhibitory effect on the enzyme *P. falciparum* lactate dehydrogenase. A crystal structure of *pf*LDH with chloroquine has been published, indicating that the 4-amino 7-chloroquine moiety is a good pharmacophore to inhibit the active site of the enzyme. Since all compounds synthesised in this study are based on a 4-amino 7-chloroquine scaffold, this is likely the reason fot the modeling displaying good results. However, *in*

*vitro pf*LDH testing is still required before it would be possible to correlate any results obtained from computational modeling.

4.2. Future work

It is evident that compounds containing the oxalamide functional group are the most potent compounds we have synthesised. Whole cell testing results against chloroquine resistant strains of *P. falciparum* would be interesting in order to compare with results we have already obtained. It would be noteworthy to observe changes in *in vitro* efficacy when further changes are made to the general molecular structure. The original diaminoalkyl spacer could be substituted for a dithiolalkyl or a dihydroxyalkyl chain instead. This could bring forth better understanding on what functional group would be more efficacious, and whether it is the oxo group that induces a larger effect on efficacy or the oxalamide moiety itself.

The inclusion of an ethyl oxamate substituent did prove to have good *in vitro* results, thus it would be interesting to see changes in activity when derivitising the ethyl oxamate group to an oxalic acid substituent. Furthermore, compounds that include a ferrocenyl moiety are known to possess increased efficacy compared to phenyl analogues. Thus introducing this moiety to novel compounds that could potentially be a hit, would bring forth some understanding towards the role that the ferrocenyl moiety contributes.

Finally, the computational modeling results point out to the the inhibition of *pf*LDH as a potential target, since many antimalarial compounds to date have also been shown to inhibit β-haematin formation, this result is certainly encouraged. In the future, compounds with high affinity for the active site of *pf*LDH may prove to be effective, and more importantly novel (given resistance), antimalarials. It has been proven that haem has a high affinity for the binding site of the *pf*LDH. Derivitisation of haem and its backbone structure, porphine, may potentially lead to increased binding strength in the active site of the enzyme.

Chapter 5

5.1. General Information regarding to synthesis and characterization

All chemicals used were bought from Merck, Fluka and Aldrich. Tetrahydrofuran (THF) and diethylether (Et_2O) were dried over sodium wire/sand and distilled under nitrogen with benzophenone as an indicator. Dichloromethane (DCM) was distilled over calcium hydride under nitrogen. Other solvents e.g. ethyl acetate, hexane and tri-ethylamine were purified according to standard procedures.[1] The molarity of *n*Bu-Li was determined using a method as described in the literature.[2] Reactions requiring anhydrous conditions were performed under nitrogen atmosphere. All ^{1}H and ^{13}C nuclear magnetic resonance spectra were obtained using a 300 MHz Varian VNMRS (75 MHz for ^{13}C), a 400 MHz Varian Unity Inova (100 MHz for ^{13}C) and a 600MHz Varain Unity Inova (150 MHz for ^{13}C) *d*-Chloroform was used as standard solvent. Variable temperature NMR was carried out on a 400 MHz Varian Unity Inova (100 MHz for ^{13}C) and 600MHz Varain Unity Inova (150 MHz for ^{13}C) using DMSO-d_6 as solvent. Chemical shifts (δ) were recorded using residual chloroform peaks at δ 7.26 in ^{1}H NMR and δ 77.0 in ^{13}C NMR, and residual DMSO peaks at δ 2.50 in ^{1}H NMR and δ 39.51 in ^{13}C NMR. All chemical shifts are reported in ppm and coupling constants, *J*, are given in Hertz (Hz). All spectra were obtained at 25 °C. Variable temperature NMR spectra were obtained at 130 °C. All NMR spectroscopy and mass spectrometry was performed by the CAF (Central Analytical Facility) Institute at Stellenbosch University. Infrared spectra were obtained using a Nexus Thermo-Nicolet FT-IR using the ATR.

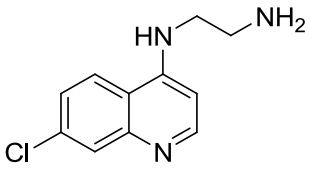
All chromatography was performed using either (or a combination of) ethyl acetate, methanol, ethanol, triethylamine and dichloromethane. Thin layer chromatography (tlc) was carried out on aluminium backed Merck silica gel 60 F_{254} plates. Visualization was achieved with a UV lamp, iodine vapour or by spraying with a Cerium Ammonium

Molybdate solution (CAM) or a ninhydrin solution and then heating. All column chromatography was carried out with Merck silica gel 60 (particle size 0.040-0.063 mm).

Beta-haematin testing was carried out at the University of Cape Town under supervision of Professor Timothy Egan. Haemin (≥98%, Fluka), amodiaquine, dimethyl sulfoxide (DMSO), acetone, acetic acid, sodium acetate trihydrate, HEPES and pyridine were purchased from Sigma-Aldrich. NP-40 detergent was obtained from Pierce Biotechnology, Rockford, IL. 96-well plates were purchased from Greiner Bio One (catalogue number 655 180; Cellstar)

5.2. Experimental Details pertaining to Synthesis

N^1-(7-chloroquinolin-4-yl)ethane-1,2-diamine [1]



4,7-dichloroquinoline (25.3 mmol, 5 g) was placed in a round bottomed flask with 4.5 equivalents of ethylene diamine (6.92 g, 7.71 mL, 113.6 mmol) and 1.2 equivalents of dry Et_3N (2.44 mL). The reaction took place under nitrogen and under reflux. The mixture was stirred at 80 °C for an hour after which the temperature was increased to 135-140 °C for a further 4 hours. The initial yellow reaction mixture turned into a light cream as the reaction proceeded. The reaction mixture was then added to a separating funnel, diluted with ethyl acetate (100 mL) and washed with water (50 mL). The water layer was washed with ethyl acetate (100 mL), the organic fractions was combined, dried over Na_2SO_4 and collected as a yellow powder in 66% yield following solvent removal *in vacuo.*

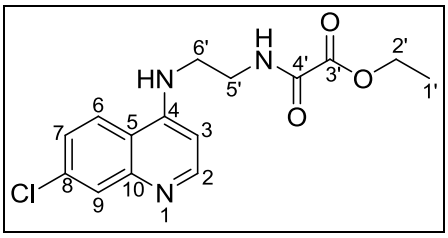
N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine [2]

N^1-(7-chloroquinolin-4-yl)butane-1,4-diamine [3]

N^1-(7-chloroquinolin-4-yl)pentane-1,5-diamine [4]

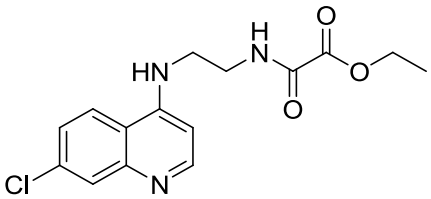
N^1-(7-chloroquinolin-4-yl)hexane-1,6-diamine [5]

The method to synthesize **2, 3, 4** and **5** was the same as that of **1**. The resulting products were obtained as pale yellow powders with yields of 93% [**2],** 60% [**3**], 54% [**4**] and 82% [**5**]. This is a well known reaction and product was identified by tlc.[3]



The assignment of NMR chemical shifts will follow the code layout of the above structure. Carbons will we assigned " C6' " and protons " H2 ".

Ethyl 2-(2-(7-chloroquinolin-4-ylamino)ethylamino)-2-oxoacetate [6][4]



1 (1.67 g, 7.53 mmol) was added to a 2-neck round bottomed flask with DCM (40 mL) and Et_3N (1.1 mL). The mixture was cooled to 0 °C, and 1.5 equivalents ethylchlorooxoacetate was added dropwise under nitrogen. The mixture was allowed to warm to room temperature and stirred for 2 hours. The yellow reaction mixture turned to a light cream color. The reaction mixture was diluted with deionised water (50 mL), and the product extracted with DCM (2x100 mL). The organic fractions were collected, combined, dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure. The product was purified by column chromatography over silica gel (90% EtOAc, 5% Et_3N, 5%EtOH). (Yield: 0.65 g, 27%). R_f: 0.6 (80% EtOAc, 15%, Et_3N, 5% EtOH)

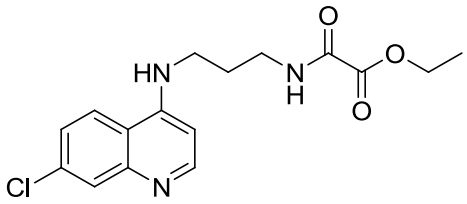
^{1}H NMR (300 MHz, CHLOROFORM-*d*) *δ* ppm 1.37 - 1.45 (m, 3 H, H1') 3.48 - 3.55 (m, 2 H, H5') 3.78 - 3.86 (m, 2 H, H6') 4.39 (q, *J*=7.09 Hz, 2 H, H2') 6.36 (d, *J*=5.43 Hz, 1 H, H3) 7.41 (dd, *J*=8.95, 2.05 Hz, 1 H, H6) 7.76 (d, *J*=8.80 Hz, 1 H, H8) 7.96 (d, *J*=2.05 Hz, 1 H, H4) 8.53 (d, *J*=5.28 Hz, 1 H, H2),

^{13}C{H} NMR (75 MHz, CHLOROFORM-*d*) *δ* ppm 14.0 (1'), 39.3 (C5'), 44.9 (C6'), 63.7 (C2'), 98.5 (C3), 117.0 (C5), 121.6 (C7), 125.8 (C6), 128.5 (C9), 135.1(C8), 148.9 (C10), 149.7 (C2), 151.8 (C4), 159.0 (C4'), 160.2 (C3'),

IR ATR: 3382 br m (NH), 2936 w, 1673 s (O=C-OEt) 1640 (O=C-N) vs, 1612 s (7-chloroquinoline), 1454 m, 1218 w, 759 w.

Compounds **7**, **8**, **9** and **10** had the same workup procedure as that of **6**

Ethyl 2-(2-(7-chloroquinolin-4-ylamino)propylamino)-2-oxoacetate [7]



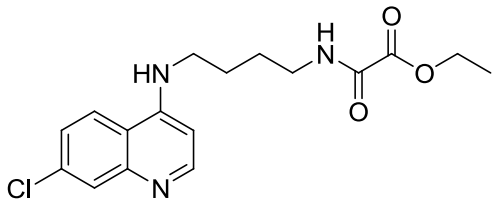
In a round bottom flask **2** (2 g, 8,48 mmol) was mixed with DCM (40 mL) and Et_3N (1.4 mL). The mixture was cooled to 0 ^{0}C, whereby 1.5 equivalents ethylchlorooxoacetate (1.42 mL, 12.7 mmol) was added dropwise under nitrogen and stirred for 2 hours. The product was purified by column chromatography over silica gel (90% EtOAc, 5% Et_3N, 5%EtOH). (Yield: 2.34 g, 82%). R_f: 0.47 (90% EtOAc,%5 Et_3N, 5% EtOH)

^{1}H NMR (400 MHz, CHLOROFORM-*d*) *δ* ppm 1.43 (t, *J*=1.00 Hz, 3 H, H1'), 1.93 (m, 2 H, H6'), 3.43 (m, 2 H, H5'), 3.52 (m, 2 H, H7'), 4.40 (q, *J*=1.00 Hz, 2 H, H2'), 6.40 (d, *J*=5.47 Hz, 1 H, H3), 7.38 (dd, *J*=8.98, 2.15 Hz, 1 H, H7), 7.89 (d, *J*=8.98 Hz, 1 H, H9), 7.95 (d, *J*=2.15 Hz, 1 H, H6), 8.51 (d, *J*=5.27 Hz, 1 H, H2),

^{13}C{H} NMR (101 MHz, CHLOROFORM-*d*) *δ* ppm 14.0 (C1'), 27.7 (C6'), 36.8 (C5'), 39.0 (C7'), 63.7 (C2'), 98.5 (3), 117.4 (5), 121.6 (C7), 125.5 (C6), 128.5 (C9), 135.0 (C8), 149.2 (C10), 149.6 (C2), 151.8 (C4), 158.0 (C4'), 160.4 (C3'),

IR ATR; 3373br w (NH), 1726 w (O-C=O), 1631 vs (N-C=O), 1610 (7-chloroquinoline) vs, 1452 m, 1210 m, 758 m.

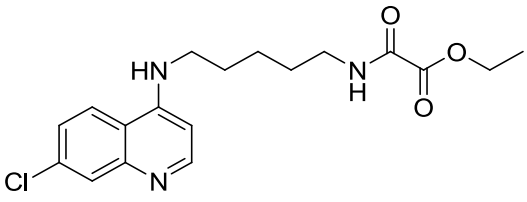
Ethyl 2-(4-(7-chloroquinolin-4-ylamino)butylamino)-2-oxoacetate [8]



In a round bottom flask **3** (2 g, 8,01 mmol) was mixed with DCM (40 mL), Et_3N (1.4 mL) and ethyl chlorooxoacetate (1.42 mL, 12.7 mmol) was added dropwise at 0 °C. The mixture was allowed to warm to room temperature and stirred under nitrogen for 2 hours. The product was purified by column chromatography over silica gel (90% EtOAc, 5% Et_3N, 5%EtOH). (Yield: 1.67 g, 60%). R_f: 0.17 (90% DCM, 5% Et_3N, 5% MeOH)

^{1}H NMR (400 MHz, CHLOROFORM-*d*) *δ* ppm 1.40 (t, *J*=1.00 Hz, 3 H, H1'), 1.73 - 1.87 (m, 4 H, H6', H7'), 3.38 (q, *J*=1.00 Hz, 2 H, H5'), 3.46 (q, *J*=6.70 Hz, 2 H, H8'), 4.36 (q, *J*=1.00 Hz, 2 H, H2'), 6.41 (d, *J*=5.47 Hz, 1 H, H3), 7.38 (dd, *J*=8.98, 2.15 Hz, 1 H, H7), 7.74 (d, *J*=8.98 Hz, 1 H, H9), 7.96 (d, *J*=2.15 Hz, 1 H H6), 8.54 (d, *J*=5.47 Hz, 1 H, H2),
^{13}C{H} NMR (CHLOROFORM-d, 101MHz): *δ* ppm 160.7 (C3'), 156.9 (C4'), 151.9 (C4), 149.6 (C2), 149.1 (C10), 134.9 (C8), 128.8 (C9), 125.4 (C6), 121.0 (C7), 117.2 (C5), 99.1 (C3), 63.4 (C2'), 42.9 (C8'), 39.4 (C5'), 27.2 (C6'), 25.7 (C7'), 14.0 (C1'),
IR ATR: 3377br w (NH), 1753 w (O-C=O), 1672 m (N-C=O), 1611 vs (7-chloroquinoline), 1449 m, 1211 s, 760 w

Ethyl 2-(5-(7-chloroquinolin-4-ylamino)pentylamino)-2-oxoacetate [9]



In a round bottom flask 4 (2 g, 7.58 mmol) was mixed with DCM (40 mL), Et_3N (1.4 mL) and ethyl chloro oxo acetate (1.42 mL, 12.7 mmol) was added drop wise at 0°C. The mixture was allowed to warm to room temperature and stirred under nitrogen

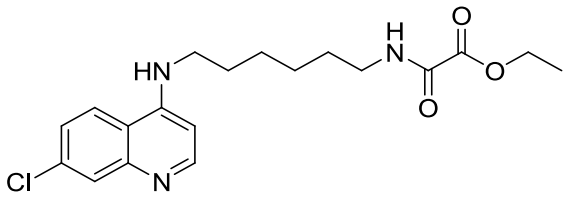
for 2 hours. The product was purified by column chromatography over silica gel (90% EtOAc, 5% Et3N, 5%EtOH). (Yield: 1.76 g, 64%). R_f: 0.35 (97.5% EtOAc, 2.5% Et_3N)

^{1}H NMR (400 MHz, CHLOROFORM-d) δ ppm 1.37 (t, *J*=1.00 Hz, 3 H, H1'), 1.50 (quin, *J*=1.00 Hz, 2 H, H7'), 1.65 (m, 2 H, H6'), 1.81 (m, 2 H, H8'), 3.26 - 3.34 (m, 2 H, H5'), 3.39 (q, *J*=6.74 Hz, 2 H, H9'), 4.34 (q, *J*=1.00 Hz, 2 H, H2'), 6.57 (dd, *J*=1.00 Hz, 1 H, H3), 7.33 (dd, *J*=1.00 Hz, 1 H, H7), 7.88 - 7.94 (m, 2 H, H6, H9), 8.45 (d, *J*=5.57 Hz, 1 H, H2),

^{13}C{H} NMR (CHLOROFORM-d) δ ppm 14.0 (C1'), 24.0 (C7'), 27.8 (C6'), 29.0 (C8'), 39.2 (C5'), 43.1 (C9'), 63.3 (C2'), 98.7 (C3), 117.0 (C5), 121.9 (C7), 125.4 (C6), 127.5 (C9), 135.2 (C8), 147.9 (C10), 150.4 (C2), 150.8 (C4), 157.0 (C4'), 160.6 (C3'),

IR ATR: 3272br w (NH), 1730 w (O-C=O), 1644 vs (N-C=O), 1614 vs (7-chloroquinoline), 1455 m, 1208 s, 763 s,

Ethyl 2-(6-(7-chloroquinolin-4-ylamino)hexylamino)-2-oxoacetate [10]



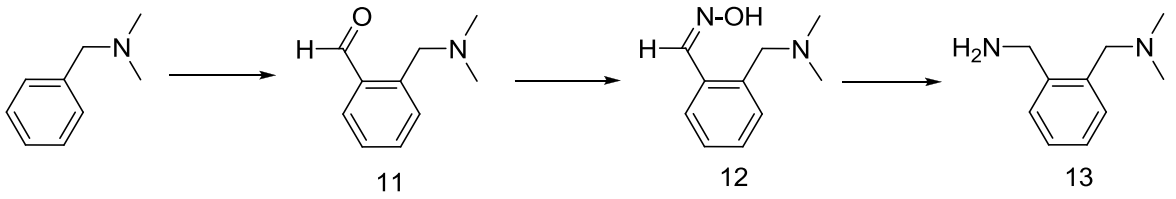
In a round bottom flask **5** (2 g, 7.58 mmol) was mixed with DCM (40 mL), Et_3N (1.4 mL) and ethyl chlorooxoacetate (1.42 mL, 12.7 mmol) was added dropwise at 0°C. The mixture was allowed to warm to room temperature and stirred under nitrogen for 2 hours. The product was purified by column chromatography over silica gel (90% EtOAc, 5% Et_3N, 5% EtOH). (Yield: 1.77 g, 65%). R_f: 0.68 (90% EtOAc, 5% EtOH, 5% Et_3N)

^{1}H NMR (CHLOROFORM-d) δ ppm 1.39 (t, *J*=1.00 Hz 3 H, H1'), 1.41 - 1.48 (m, 2H, H7'), 1.48 - 1.56 (m, 2H, H8'), 1.62 (m, H6'), 1.77 (m, H9'), 3.32 (m, H5'), 3.38 (m, H10'), 4.35 (q, *J*=1.00 Hz, H2'), 5.16 (br. s., NH), 6.40 (d, *J*=5.47 Hz, H3), 7.17 (br. s. NH), 7.37 (dd, *J*=8.98, 2.15 Hz, H7), 7.72 (d, *J*=8.79 Hz, H9), 7.96 (d, *J*=2.15 Hz H6), 8.52 (d, *J*=5.27 Hz 1 H, H2),

^{13}C{H} NMR (CHLOROFORM-d) δ ppm 14.0 (C1'), 26.3 (C7'), 26.5 (C8') , 28.6 (C6'), 29.1 (C9'), 39.5 (C5'), 43.0 (C10'), 63.3 (C2'), 99.0 (C3), 117.1 (C5), 121.0 (C7), 125.3 (C6), 128.6 (C9), 134.9 (C8), 148.9 (C10), 149.8 (C2), 151.8 (C4), 156.7 (C4'), 160.8 (C3'),

IR ATR; 3360 w (NH), 1729 m (O-C=O), 1675 vs (N-C=O), 1609 m (7-chloroquinoline), 1456 m, 1209 s, 737 m

Synthesis of compounds 11 - 13[5]



11 12 13

2-((dimethylamino)methyl)benzaldehyde [11]

N,N-dimethylaminomethylbenzene (4.06 g, 4.5 mL, 30 mmol) was added to dry diethyl (90 mL) ether in a pre-dried two-neck round bottomed flask. The mixture was stirred under nitrogen, after which *t*-BuLi (1,48 M, 40 mL, 41.5 mmol) was added to the mixture in a dropwise fashion in an ice bath. The mixture turned into a light yellow suspension, and was stirred for 1 hour under nitrogen at room temperature. Anhydrous dimethylformamide (2.7 mL, 35 mmol) was then added to the light yellow suspension, and the reaction mixture turned orange. After stirring for 1 hour, the mixture was quenched with deionised water (40 mL). The water layer washed with DCM (2 x 50 mL). The organic fractions were combined, dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure. No purification was necessary since the yield was quantitative. The product is a light orange liquid. (4.85 g, quantitative)

^{1}H NMR (CHLOROFORM-d) δ ppm 2.20 (s, 6 H, $N(Me)_2$), 3.70 (s, 2 H, $-CH_2-$), 7.32 - 7.35 (m, 1 H, Ar), 7.38 (t, *J*=1.00 Hz, 1 H, Ar), 7.47 (m, 1 H, Ar), 7.84 (dd, *J*=7.62, 1.56 Hz, 1 H, Ar), 10.38 (s, 1 H, CHO),

^{13}C{H} NMR (CHLOROFORM-d) δ ppm 45.0 ($N(Me)_2$), 60.8 ($-CH_2-$), 127.6 (Ar), 129.3 (Ar), 130.3 (Ar), 133.0 (Ar), 134.9 (Ar), 141.6 (Ar), 192.0 (CHO),

2-((dimethylamino)methyl)benzaldehyde oxime [12]

11 (1.92 g, 11.8 mmol) was added together with 2M NaOH (36 mL), 2.21 g hydroxylamine hydrochloride and ethanol (126 mL), and stirred under reflux for 14 hours at 90 °C. The previously light yellow solution turned black. The reaction mixture was cooled down to room temperature, and neutralised by the addition of solid CO_2. The mixture was diluted with water and the product extracted with DCM. The organic fractions were combined, dried over Na_2SO_4, and the solvent removed under reduced pressure. The yield was quantitative, 2.07 g.

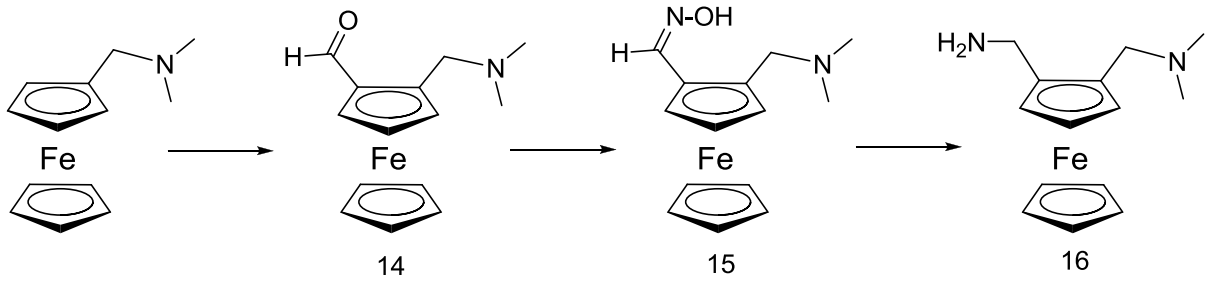
^{1}H NMR (CHLOROFORM-d) δ ppm 2.22 (s, 6 H, N(Me)$_2$), 3.52 (s, 2 H, -C*H*$_2$-), 7.19 - 7.30 (m, 3 H, Ar), 7.74 - 7.77 (m, 1 H, Ar), 8.51 - 8.54 (m, 1 H, -C*H*=NOH),
^{13}C{H} NMR (101 MHz, (CHLOROFORM-d) δ ppm 45.1 (N(Me)$_2$), 61.2 (-C*H*$_2$-), 126.5 (Ar), 127.4 (Ar), 128.8 (Ar), 130.7 (Ar), 132.2 (Ar), 136.4 (Ar), 147.6 (C=NOH)

1-(2-(aminomethyl)phenyl)-*N,N*-dimethylmethanamine [13]

12 (2.07 g, 11.6 mmol) was added in a pre-dried 3-neck round bottomed flask, and mixed with dry THF (120 mL). $LiAlH_4$ (2.64 g, 6 equivalents) was added to the solution and was refluxed under nitrogen at 70 °C overnight. The dark grey reaction mixture was then diluted with diethyl ether (50 mL) and quenched with the dropwise addition of brine. The organic layer was separated, and the aqueous phase washed with diethyl ether (2 x 60 mL). The organic fractions were combined, dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was recovered as a yellow oil. Yield was quantitative, 1.9 g.

^{1}H NMR (CHLOROFORM-d) δ ppm 2.27 (s, 6 H, N(Me)$_2$), 3.50 (s, 2 H, -C*H*$_2$-N(Me)$_2$), 3.90 (s, 2 H, -C*H*$_2$-NH$_2$), 7.25 - 7.38 (m, 4 H, Ar),
^{13}C{H} NMR (CHLOROFORM-d) δ ppm 44.7 (N(Me)$_2$), 57.0 (CH$_2$-NH$_2$), 62.4 (CH$_2$-N(Me)$_2$), 126.7 (Ar), 127.9 (Ar), 129.0 (Ar), 130.7 (Ar), 136.6 (Ar), 141.7 (Ar)

Synthesis of compounds 14 - 16[6]



[(2-Dimethylamino)methyl]ferrocenecarboxaldehyde [14]

[(2-Dimethylamino)methyl]ferrocene (5 g, 20.56 mmol, 4.07 mL) and dry diethyl ether (60 mL) were added together in a pre-dried 3-neck round bottomed flask. *t*BuLi (1.6 M, 19.3 mL, 1.5 equivalents) was added to the mixture in a dropwise fashion, and the reaction mixture was stirred for 1 hour under nitrogen. After 1 hour a bright orange suspension formed. Anhydrous dimethylformamide (23.1 mmol, 1.79 mL, 1.2 equivalents) was added to the mixture, which was then stirred for another hour under nitrogen. The mixture was quenched with the addition of deionised water (30 mL), after which the solution turned dark red. The layers were separated and the aqueous phase washed with DCM (3 x 80 mL). The organic fractions were combined, dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was not purified since the yield was quantitative.

^{1}H NMR (400 MHz, CHLOROFORM-d) δ ppm 2.21 (s, 6 H, NMe_2), 3.34 (d, *J*=12.89 Hz, 1 H, C*H*-NMe_2), 3.83 (d, *J*=13.08 Hz, 1 H, C*H*-NMe_2), 4.22 (s, 5 H, Cp'), 4.56 (br. s., 1 H, Cp*H*-αCH_2NMe_2), 4.61 (s, 1 H, Cp*H*-β CHO), 4.78 (br. s., 1 H, Cp*H*-α CHO), 10.10 (s, 1 H, C*H*O). ^{13}C{H} NMR (101 MHz, CHLOROFORM-d) δ ppm 44.8 (-NMe_2), 56.6 (*C*H_2), 70.2 (5C, Cp), 70.3 (Cp*C*-α CHO), 71.8 (Cp*C*-α CH-NMe_2), 75.8 (Cp), 77.8 (C^{IV} Cp), 86.7 (C^{IV} Cp) 193.2 (CHO)

[(2-Dimethylamino)methyl]ferrocenecarboxaldehyde oxime [15]

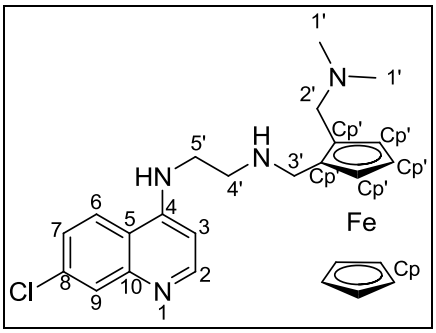
14 (5.08g, 17.74 mmol) was mixed with ethanol (190 mL) in a two neck round bottomed flask. Hydroxylamine hydrochloride (3.33 g, 2.7 equivalents) was added to the solution together with aqueous 2M NaOH (51 mL). The reaction mixture was stirred under reflux at 90 °C overnight, then cooled down to room temperature. The mixture was neutralized by the addition of solid CO_2, after which the mixture was diluted with water and the product extracted with DCM. The organic fractions were combined, dried over Na_2SO_4, and the solvent removed under reduced pressure. The product was recovered as a dark red oil. (Yield: 4.43 g, 82.7%)

^{1}H (300 MHz, CHLOROFORM-*d*) *δ* ppm 2.21 (br. s., 1 H, CH=NO*H*), 2.43 (br. s., 6 H, N(Me)$_2$), 3.46 - 3.55 (m, 1 H, C*H*-N(Me)$_2$), 3.99 (m, 1 H, C*H*-N(Me)$_2$) 4.13 - 4.23 (m, 5 H, Cp), 4.42 (br. s., 1 H, Cp'), 4.55 (br. s., 1 H, Cp'), 8.05 (br. s., 1 H, -C*H*=NOH)

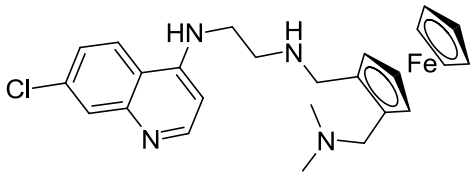
[(2-Dimethylamino)methyl]ferrocenemethylamine [16]

15 (4.43 g, 15.6mmol) was added to anhydrous THF (100 mL) in a pre-dried round bottomed flask, followed by the addition of 1.95 g $LiAlH_4$ (51.5mmol). The reaction was stirred over night under reflux at 70 °C and under nitrogen. The reaction mixture was then diluted with diethyl ether, after which it was quenched by adding brine solution (60 mL). The layers were separated and the aqueous phase washed with diethyl ether (2 x 100 mL). The organic fractions were combined, dried over Na_2SO_4, and reduced under reduced pressure. The product was purified by column chromatography over silica gel. (80% DCM, 10% Et_3N, 10% EtOH) recovered as a dark red oil. (Yield: 2.69 g, 64%)

^{1}H NMR (300 MHz, CHLOROFORM-*d*) *δ* ppm 2.03 (s, 6 H, N(Me)$_2$), 2.75 (d, *J*=12.47 Hz, 1 H, CH2-NH$_2$), 3.05 (br. s., 2 H, NH$_2$), 3.37 (d, *J*=13.50 Hz, 1 H, CH2-N(Me)$_2$), 3.52 (d, *J*=12.62 Hz, 1 H, CH2-NH$_2$), 3.59 (d, *J*=13.79 Hz, 1 H, CH2-N(Me)$_2$), 3.91 (br. s., 1 H, Cp'), 3.94 (s, 5 H, Cp), 4.00 (br. s., 1 H, Cp'), 4.04 (br. s., 1 H, Cp')
^{13}C NMR (75 MHz, CHLOROFORM-*d*) *δ* ppm 39.8 (CH$_2$-NH$_2$), 44.5 (N(Me)$_2$), 57.6 (CH$_2$-N(Me)$_2$), 65.5 (Cp'), 68.2 (Cp'), 68.5 (Cp), 70.5 (Cp'), 82.8 (C^{IV} Cp'), 88.8 (C^{IV} Cp')



N^1-(7-chloroquinolin-4-yl)-N^2-(2-((dimethylamino)methyl)ferrocenylmethyl)-ethan-1,2-diamine [A2]



200 mg of **14** (0.823 mmol, 1 equivalent)**,** 273 mg of **1** (1.234 mmol, 1.5 equivalents), 103.4 mg $NaCNBH_3$ (2 equivalents) and 17 mg p-toluenesulfonic acid was mixed in methanol (6 mL) in a two-neck round bottomed flask, and stirred for 18 hours at room temperature under nitrogen. Deionized water (30 mL) was added to the reaction mixture. The product was extracted with DCM (2 x 50 mL). The product was purified by column chromatography over silica gel. (95% DCM, 2.5% Et_3N, 2.5% EtOH) resulting in a light orange solid as the product. (Yield: 147 mg, 38%). R_f: 0.20 (7% Et_3N, 93% DCM),

^{1}H NMR (400 MHz, CHLOROFORM-*d*) *δ* ppm 2.04 (s, 6 H, H1'), 2.80 (d, *J*=12.69 Hz, 1 H, H2'b), 2.94 (d, *J*=5.08 Hz, 2 H, H4'), 3.35 (m, 2 H, H5'), 3.44 (d, *J*=13.28 Hz, 1 H, H3'b), 3.70 (d, *J*=12.69 Hz, 1 H, H2'a), 3.92 (d, *J*=13.08 Hz, 1 H, H3'a), 4.06 (s, 5 H, Cp), 4.07 (m, 1 H, Cp'), 4.12 (m, 1 H, Cp'), 4.17 (d, *J*=2.15 Hz, 1 H, Cp'), 6.34 (d, *J*=5.27 Hz, 1 H, H3), 7.35 (dd, *J*=8.79, 2.15 Hz, 1 H, H7), 7.86 (d, *J*=8.98 Hz, 1 H, H9), 7.93 (d, *J*=2.15 Hz, 1 H, H6), 8.50 (d, *J*=5.47 Hz, 1 H, H2)

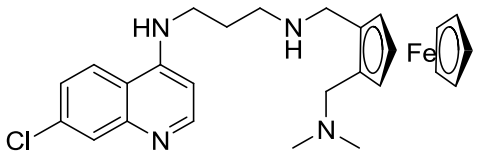
^{13}C{H} NMR (101 MHz, CHLOROFORM-*d*) *δ* ppm 43.6 (C5'), 44.7 (C1'), 46.2 (C3'), 48.0 (C4'), 58.1 (C2'), 66.0 (Cp'), 69.1 (Cp), 70.5 (Cp'), 71.3 (Cp'), 84.0 (Cp'), 97.8 (C3),

117.7 (C5), 123.0 (C7), 124.9 (C6), 128.1 (C9), 134.4 (C8), 149.1 (C10), 150.5 (C2), 151.9 (C4)

IR ATR; 2944br w (NH), 1610 w (7-chloroquinoline), 1139 w, 1104 w (ferrocene)

HRMS (EI) *m/z* [M^+]+1 477.1519 [M^++1, $C_{25}H_{30}ClFeN_4$ requires 477.1508]

N^1-(7-chloroquinolin-4-yl)-N^3-(2-((dimethylamino)methyl)ferrocenylmethyl)-propan-1,3-diamine [A3]



200 mg of **14** (0.823 mmol, 1 equivalent)**,** 291 mg of **2** (1.234 mmol, 1.5 equivalent), 103.4 mg $NaCNBH_3$ (2 equivalents)and 17 mg p-toluenesulfonic acid was mixed in methanol (6 mL) in a two-neck round bottomed flask, and stirred for 18 hours at room temperature under nitrogen. The workup procedure and purification method is the same as that of **A2.** (Yield: 112 mg, 28%). R_f: 0.25 (3% Et_3N, 5% MeOH, 92% DCM)

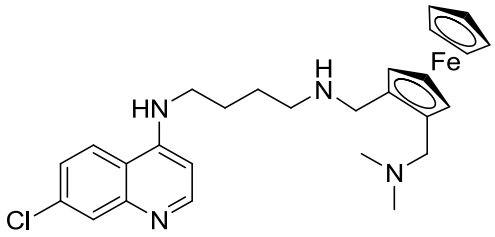
^{1}H NMR (400 MHz, CHLOROFORM-*d*) *δ* ppm 1.78 - 1.96 (m, 2 H, H5'), 2.07 - 2.10 (m, 6 H, H1'), 2.79 (d, *J*=12.69 Hz, 1 H, H2'b), 2.97 (br. s., 2 H, H'4), 3.35 - 3.42 (m, 2 H, H6'), 3.46 (d, *J*=12.50 Hz, 1 H, H3'b), 3.75 (d, *J*=12.69 Hz, 1 H, H2'a), 3.89 (d, *J*=12.50 Hz, 1 H, H3'a), 4.06 - 4.08 (m, 5 H, Cp), 4.12 - 4.14 (m, 1 H, Cp'), 4.17 (d, *J*=0.98 Hz, 1 H, Cp'), 4.19 - 4.22 (m, 1 H, Cp'), 6.23 (dd, *J*=5.37, 1.27 Hz, 1 H, H3), 7.12 - 7.17 (m, 1 H, H7), 7.63 (d, *J*=8.59 Hz, 1 H, H9), 7.85 - 7.88 (m, 1 H, H6), 8.45 (dd, *J*=5.37, 1.27 Hz, 1 H, H2)

^{13}C{H} NMR (101 MHz, CHLOROFORM-*d*) *δ* ppm 26.5 (C5'), 43.6 (C6'), 44.7 (C1'), 46.2 (C3'), 48.0 (C4'), 58.1 (C2'), 66.0 (Cp'), 69.1 (Cp), 70.5 (Cp'), 71.3 (Cp'), 84.0 (Cp'), 97.8 (C3), 117.7 (C5), 123.0 (C7), 124.9 (C6), 128.1 (C9), 134.4 (C8), 149.1 (C10), 150.6 (C2), 151.94 (C4)

IR ATR; 3365br w (NH), 1610 w (7-chloroquinoline), 1132 w , 1104 w (ferrocene)

HRMS (EI) *m/z* [M^+]+2 492.4088 [M^++2, $C_{26}H_{33}ClFeN_4$ requires 492.1745], 464.2778 [M+K^+]

N^1-(7-chloroquinolin-4-yl)-N^4-(2-((dimethylamino)methyl)ferrocenylmethyl)-butane-1,4-diamine [A4]



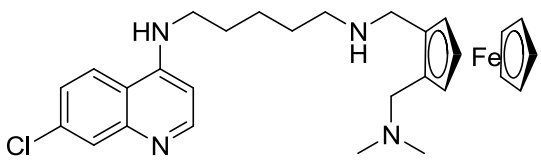
326 mg of **14** (1.20 mmol, 1.5 equivalent)**,** 200 mg of **3** (0.8 mmol, 1 equivalent), 176 mg $NaCNBH_3$ (3.5 equivalents) and 0.5 mol% acetic acid was mixed in methanol (3 mL) in a two-neck round bottomed flask, and stirred for 18 hours at room temperature under nitrogen. The workup procedure and purification method is the same as that of **A2.** R_f: 0.16 (5% Et_3N, 5% EtOH, 90% DCM)

^{1}H NMR (300 MHz, CHLOROFORM-*d*) *δ* ppm 1.70 (br. s., 2 H, H5'), 1.80 (d, *J*=5.43 Hz, 2 H, H6'), 2.14 (m, 6 H, H1'), 2.66 (br. s., 2 H, H4'), 2.80 (d, *J*=12.77 Hz, 1 H, H2'b), 3.27 (m, 2 H, H7'), 3.46 (d, *J*=12.62 Hz, 1 H, H3'b), 3.74 (d, *J*=12.91 Hz, 1 H, H2'a), 3.99 (d, *J*=3.67 Hz, 1 H, H3'a), 4.05 (s, 5 H, Cp), 4.12 (s, 1 H, Cp'), 4.16 (br. s., 1 H, Cp'), 6.29 (d, *J*=5.28 Hz, 1 H, H3), 7.27 (d, *J*=6.75 Hz, 1 H, H7), 7.88 (s, 1 H, H9), 8.00 (d, *J*=8.22 Hz, 1 H, H6), 8.43 (d, *J*=5.43 Hz, 1 H, H2)

^{13}C NMR (75 MHz, CHLOROFORM-*d*) *δ* ppm 25.7 (C5'), 26.4 (C6'), 42.8 (C7'), 44.9 (C1'), 46.7 (C3'), 47.4 (C4'), 58.1 (C2'), 66.7 (Cp'), 69.4 (Cp), 71.0 (Cp'), 71.8 (Cp'), 82.7 (Cp'), 84.1 (Cp'), 98.7 (C3), 117.8 (C5), 123.1 (C7), 125.3 (C6), 128.3 (C9), 134.8 (C8), 149.3 (C10), 150.6 (C2), 152.2 (C4)

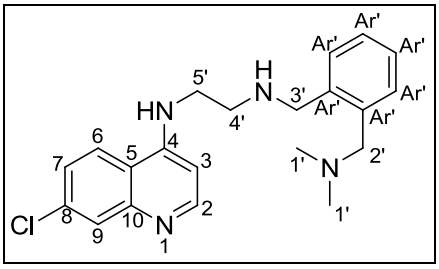
IR ATR; 3278br w (NH), 1609 w (7-chloroquinoline), 1137 w , 1104 w (ferrocene)

N^1-(7-chloroquinolin-4-yl)-N^5-(2-((dimethylamino)methyl)ferrocenyl)-pentan-1,5-diamine [A5]

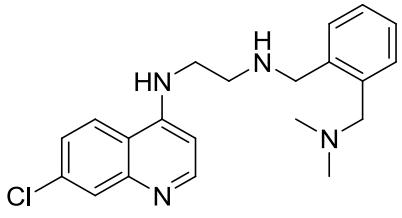


308.4 mg of **14** (1.14 mmol, 1.5 equivalents)**,** 200 mg of **4** (0.76 mmol, 1 equivalent), 167 mg $NaCNBH_3$ (3, 5 equivalents) and 0.5 mol % acetic acid was mixed in methanol (3 mL) in a two-neck round bottomed flask, and stirred for 18 hours at room temperature under nitrogen. The workup procedure and purification method is the same as that of **A2.** (Yield: 207.9 mg, 53%) R_f: 0.24 (92% DCM, 4% MeOH, 4% Et_3N)

^{1}H NMR (400 MHz, CHLOROFORM-*d*) d ppm 1.58 (br. s., 2 H, H6'), 1.70 (d, *J*=5.47 Hz, 2 H, H7'), 1.80 (br. s., 2 H, H5'), 2.08 (s, 6 H, H1'), 2.61 - 2.77 (m, 2 H, H4'), 2.84 (d, *J*=12.89 Hz, 1 H, H2'b), 3.40 (br. s., 2 H, H8'), 3.83 (d, *J*=12.89 Hz, 1 H, H3'b), 4.12 (s, 5 H, Cp), 4.14 - 4.17 (m, 1 H, Cp'), 4.19 (br. s., 1 H, Cp'), 4.27 (br. s., 1 H, Cp'), 4.37 (d, *J*=13.28 Hz, 1 H, H3'a), 6.33 (d, *J*=5.47 Hz, 1 H, H3), 7.36 (d, *J*=7.42 Hz, 1 H, H7), 7.86 - 7.92 (m, 1 H, H9), 8.37 (d, *J*=8.98 Hz, 1 H, H6), 8.46 (d, *J*=5.08 Hz, 1 H, H2)
^{13}C NMR (151 MHz, CHLOROFORM-*d*) *δ* ppm 23.31 (C6'), 26.08 (C7'), 26.85 (C5'), 41.88 (C8'), 44.24 (C1'), 45.22 (C3'), 46.59 (C4'), 57.72 (C2'), 66.94 (Cp'), 69.29 (Cp'), 69.55 (Cp), 71.30 (Cp'), 71.74 (Cp'), 83.83 (Cp'), 98.44 (C3), 117.59 (C5), 123.35 (C7), 125.03 (C6), 128.04 (C9), 134.77 (C8), 149.14 (C10), 150.40 (C2), 151.63 (C4)
IR ATR: 3273br w (NH), 1609 w (7-chloroquinoline), 1137 w , 1104 w (ferrocene)



***N*^{1}-(7-chloroquinolin-4-yl)-*N*^{2}-(2-((dimethylamino)methyl)benzyl)ethan-1,2-diamine [B2]^5**



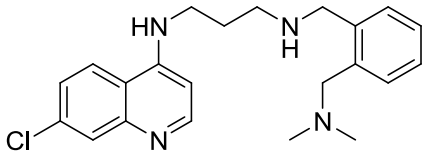
200 mg of **11** (1.1 equivalent)**,** 247 mg of **1** (1 equivalent), 140 mg $NaCNBH_3$ (2 equivalents) and 21 mg *p*-toluenesulfonic acid was mixed in anhydrous methanol (6 mL) in a two-neck round bottomed flask, and stirred for 18 hours at room temperature under nitrogen. Deionized water (30 mL) was added to the reaction mixture. The product was extracted with DCM (2 x 50 mL). The product was purified by column chromatography over silica gel. (95% DCM, 2.5% Et_3N, 2.5% EtOH) resulting in white crystals as the product. (Yield: 135 mg, 40%). R_f: 0.29 (6% Et_3N, 94% DCM)

^{1}H NMR (400 MHz, CHLOROFORM-d) δ ppm 2.18 (s, 6 H, H1'), 3.04 (t, *J*=1.00 Hz, 2 H, H4'), 3.53 (t, *J*=5.47 Hz, 2 H, H5'), 3.58 (s, 2 H, H3'), 3.99 (s, 2 H, H2'), 6.34 (d, *J*=5.47 Hz, 1 H, H3), 7.22 - 7.26 (m, 1 H, H7), 7.28 - 7.38 (m, 4 H, Ar'), 7.88 - 7.93 (m, 2 H, H6, H9), 8.47 (d, *J*=5.27 Hz, 1 H, H2)
^{13}C{H} NMR (101 MHz, CHLOROFORM-d) δ ppm 41.5 (2C, C1'), 44.2 (C4'), 46.2 (C5'), 51.8 (C3'), 62.5 (C2'), 98.7 (C3), 117.3 (C5), 122.1 (C7), 125.6 (Ar'), 128.4 (Ar'), 128.5 (C6), 128.9 (Ar'), 131.2 (Ar'), 131.6 (C9), 135.1 (Ar'), 135.7 (Ar'), 136.6 (C8), 149.0 (C10), 149.9 (C2), 151.8 (C4)
IR ATR; 3252br w (NH), 1610 w (7-chloroquinoline), 1577 vs (aromatic C-C),
HRMS (EI) *m/z* [M^+]+1 369.1852 [M^++1, $C_{21}H_{25}ClN_4$ requires 368.1846], 391.2847 [$M+Na^+$]

The synthetic method for compounds **B3** to **B6** was the same as that of **B2.**

For the synthesis of compounds **B3** – **B6**, 200 mg of **11** (1.1 equivalents) was used in each reaction with the corresponding mass for the chloroquinoline diamine (1 equivalent). The resulting products were all purified by column chromatography over silica gel. (95% DCM, 2.5% Et_3N, 2.5% EtOH) and were all obtained as pale yellow semi-solids with yields of 54% (**B3),** 52% (**B4**), 74% **(B5)** and 40% (**B6**).

N^1-(7-chloroquinolin-4-yl)-N^3-(2-((dimethylamino)methyl)benzyl)propan-1,3-diamine [B3]



R_f: 0.47 (6% Et_3N, 94% DCM)

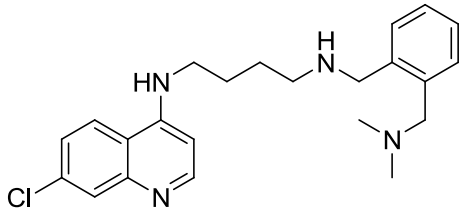
^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 1.93 (m, 2 H, H5'), 2.20 (s, 6 H, H1'), 2.97 (t, *J*=1.00 Hz, 2 H, H4'), 3.38 - 3.43 (m, 2 H, H6'), 3.47 (s, 2 H, H3'), 3.84 (s, 2 H, H2'), 6.26 (d, *J*=5.47 Hz, 1 H, H3), 6.96 (dd, *J*=8.98, 2.15 Hz, 1 H, H7), 7.28 - 7.38 (m, 4 H, Ar'), 7.42 (d, *J*=9.18 Hz, 1 H, H9), 7.87 (d, *J*=2.15 Hz, 1 H, H6), 8.47 (d, *J*=5.47 Hz, 1 H, H2),

^{13}C{H} NMR (101 MHz, CHLOROFORM-*d*) δ ppm 26.9 (C5'), 43.9 (C6'), 44.9 (C7'), 49.0 (C1'), 52.9 (C3'), 62.8 (C2'), 97.9 (C3), 117.6 (C5), 122.7 (C7), 124.7 (Ar'), 127.5 (Ar'), 128.2 (2C, Ar', C6), 131.0 (Ar'), 131.5 (C9), 134.4 (Ar'), 137.5 (Ar'), 138.7 (C8), 149.1 (C10), 150.6 (C2), 152.0 (C4)

IR ATR: 3264br w (NH), 1610 w (7-chloroquinoline), 1585 vs (aromatic C-C),

HRMS (EI) *m/z* [M^+]+1 383.2003 [M^++1, $C_{22}H_{27}ClN_4$ requires 383.2002].

N^1-(7-chloroquinolin-4-yl)-N^4-(2-((dimethylamino)methyl)benzyl)butan-1,4-diamine [B4]



R_f: 0.40 (4% Et_3N, 5% MeOH, 91% DCM)

^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 1.73 (m, 2 H, H5'), 1.90 (m, 2 H, H6'), 2.21 (s, 6 H, H1'), 2.72 (t, *J*=6.44 Hz, 2 H, H4'), 3.31 (t, *J*=6.15 Hz, 2 H, H7'), 3.46 (s, 2 H, H2'), 3.87 (s, 2 H, H3'), 6.36 (d, *J*=5.47 Hz, 1 H, H3), 7.17 (dd, *J*=8.98, 2.15 Hz, 1 H,

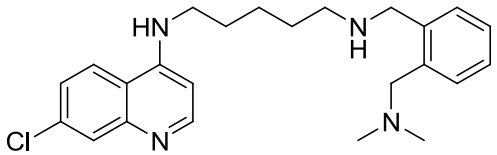
H7), 7.25 - 7.35 (m, 4 H, Ar'), 7.79 (d, *J*=8.98 Hz, 1 H, H9), 7.94 (d, *J*=2.15 Hz, 1 H, H6), 8.52 (d, *J*=5.47 Hz, 1 H, H2),

^{13}C{H} NMR (101 MHz, CHLOROFORM-*d*) *δ* ppm 26.4 (C5'), 27.7 (C6'), 43.3 (C1'), 45.2 (C4'), 48.3 (C7'), 52.5 (C3'), 62.9 (C2'), 98.8 (C3), 117.7 (C5), 122.5 (C7), 124.9 (Ar'), 127.4 (Ar'), 127.9 (C6), 128.5 (Ar'), 130.7 (Ar'), 131.5 (C9), 134.7 (Ar'), 137.6 (Ar'), 139.1 (C8), 149.4 (C10), 150.5 (C2), 152.2 (C4),

IR ATR: 3303br w (NH), 1610 w (7-chloroquinoline), 1581 vs (aromatic C-C),

HRMS (EI) *m/z* [M$^+$]+1 397.2142 [M$^+$+1, $C_{23}H_{29}ClN_4$ requires 397.2159], 436.2434 [M$^+$+K]

N^1-(7-chloroquinolin-4-yl)-N^5-(2-((dimethylamino)methyl)benzyl)pentan-1,5-diamine [B5]



Rf: 0.22 (7% Et_3N, 93% DCM)

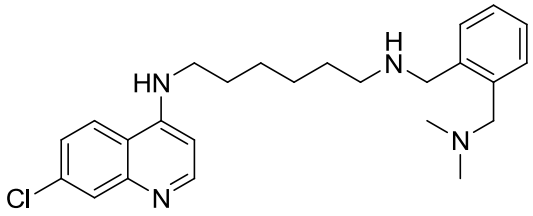
^{1}H NMR (400 MHz, CHLOROFORM-*d*) *δ* ppm 1.41 - 1.50 (m, 2 H, H6'), 1.64 (m, 2 H, H5'), 1.72 (m, 2 H, H7'), 2.10 (s, 6 H, H1'), 2.63 (t, *J*=6.64 Hz, 2 H, H4'), 3.30 (q, *J*=6.38 Hz, 2 H, H8'), 3.41 (s, 2 H, H2'), 3.89 (s, 2 H, H3'), 6.29 (d, *J*=5.47 Hz, 1 H, H3), 7.15 - 7.19 (m, 1 H, H7), 7.22 - 7.30 (m, 4 H, Ar'), 7.85 (d, *J*=2.15 Hz, 1 H, H9), 7.94 (d, *J*=8.79 Hz, 1 H, H6), 8.42 (d, *J*=5.47 Hz, 1 H, H2)

^{13}C{H} NMR (101 MHz, CHLOROFORM-*d*) *δ* ppm 24.0 (C6'), 27.7 (C5'), 42.3 (C7'), 44.7 (C1'), 51.7 (C3'), 62.5 (C2'), 98.7 (C3), 117.4 (C5), 122.1 (C7), 125.1 (C6), 128.4 (Ar'), 128.5 (Ar'), 131.3 (C9), 131.5 (Ar'), 134.7 (Ar'), 137.1 (C8), 149.2 (C10), 150.0 (C2), 151.9 (C4)

IR ATR: 3231br w (NH), 1609 w (7-chloroquinoline), 1577 vs (aromatic C-C),

HRMS (EI) *m/z* M$^+$+1 411.2315 [M$^+$+1, $C_{24}H_{31}ClN_4$ requires 411.2315]

N^1-(7-chloroquinolin-4-yl)-N^6-(2-((dimethylamino)methyl)benzyl)hexan-1,6-diamine [B6]



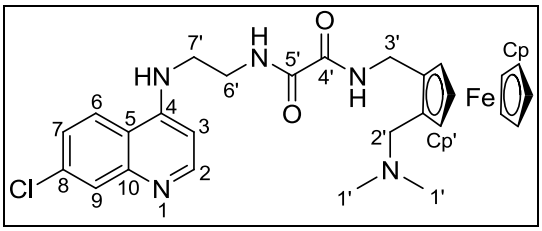
R_f: 0.39 (3% Et_3N, 5% MeOH, 91% DCM)

^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 1.42 (m, 4 H, H6', H7'), 1.59 (m, 2 H, H5'), 1.74 (m, 2 H, H8'), 2.27 (s, 6 H, H1'), 2.72 (t, *J*=7.23 Hz, 2 H, H4'), 3.28 (t, *J*=6.93 Hz, 2 H, H9'), 3.53 (s, 2 H, H2'), 3.95 (s, 2 H, H3'), 6.36 (d, *J*=5.47 Hz, 1 H, H3), 7.24 - 7.27 (m, 1 H, H7), 7.29 - 7.38 (m, 4 H, Ar'), 7.89 (d, *J*=2.15 Hz, 1 H, H9), 7.96 (d, *J*=8.98 Hz, 1 H, H6), 8.46 (d, *J*=5.47 Hz, 1H, H2)

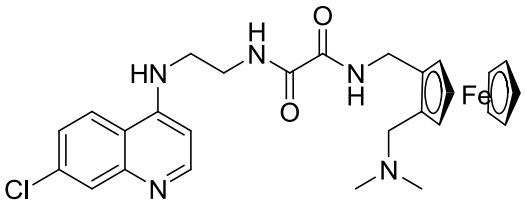
^{13}C{H} NMR (101 MHz, CHLOROFORM-*d*) δ ppm 26.5 (C6'), 28.1 (C7'), 28.4 (C5'), 42.7 (C8'), 44.3 (C4'), 45.9 (C9'), 47.7 (C1'), 51.4 (C3'), 62.2 (C2'), 98.5 (C3), 117.1 (C5), 122.1 (C7), 124.7 (Ar'), 127.6 (Ar'), 127.9 (C6), 128.1 (Ar'), 130.9 (Ar'), 131.1 (C9), 134.45 (Ar'), 136.10 (Ar'), 136.64 (C8), 148.59 (C10), 150.07 (C2), 151.43 (C4)

IR ATR; 3229br w (NH), 1609 w (7-chloroquinoline), 1576 vs (aromatic C-C)

HRMS (EI) *m/z* [M^+]+1 425.2478 [M^++1, $C_{25}H_{33}ClN_4$ requires 425.2472], 464.2778 [$M+K^+$]



N^1-(2-(7-chloroquinolin-4-ylamino)ethyl)-N^2-(2-((dimethylamino)methyl)ferrocenyl methyl)oxalamide [C2]



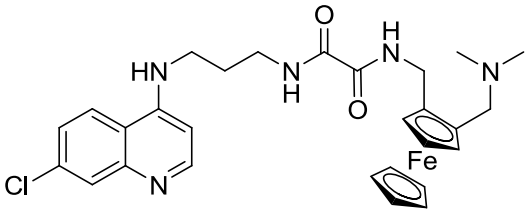
120 mg (0.373 mmol) **6** and 203 mg (0.746 mmol) **16** was dissolved in dry ethanol (3 mL), and stirred at 40 °C for 18 hours in a dry round bottomed flask under nitrogen. After the reaction time the solvent was removed and the resulting residue was dissolved in DCM. The product was purified by column chromatography over silica gel. (88% EtOAc, 2% Et_3N, 10% EtOH) resulting in a light orange powder as the product. (Yield: 130 mg, 63.6%). R_f: 0.40 (5% Et_3N, 5% EtOH, 90% EtOAc)

^{1}H NMR (300 MHz, CHLOROFORM-*d*) *δ* ppm 2.33 (s, 6 H, H1'), 2.98 (d, *J*=12.76 Hz, 1 H, H3'b), 3.07 (s, 1 H, H2'b), 3.16 (s, 1 H, H2'a), 3.47 (m, 2 H, H6'), 3.75 (m, 2 H, H7'), 3.88 (d, *J*=12.47 Hz, 2 H, H3'a), 4.06 (t, *J*=2.49 Hz, 1 H, Cp'), 4.11 (s, 5 H, Cp), 4.14 - 4.17 (m, 1 H, Cp'), 4.19 (m, *J*=2.10 Hz, 1 H, Cp'), 6.32 (d, *J*=5.43 Hz, 1 H, H3), 7.25 (d, *J*=2.05 Hz, 1 H, H7), 7.83 (d, *J*=8.80 Hz, 1 H, H9), 7.96 (d, *J*=2.05 Hz, 1 H, H6), 8.49 (d, *J*=5.43 Hz, 1 H, H2)

^{13}C{H} NMR (75 MHz, CHLOROFORM-d) δ ppm 38.3 (H6'), 38.9 (H3'), 44.2 (H7'), 45.0 (H1'), 57.8 (C2'), 66.3 (Cp'), 69.2 (Cp), 70.0 (Cp'), 71.2 (Cp'), 83.4 (Cp'), 98.3 (C3), 117.1 (C5), 122.1 (C7), 125.4 (C6), 127.9 (C9), 135.2 (C8), 150.3 (C10), 151.2 (C2), 148.2 (C4) 158.5 (C5'), 162.5 (C4') IR ATR; 3292br w, 1659 s (N-C=O), 1611 w (7-

chloroquinoline), 1579 s (7-chloroquinoline), 1505 s (N-C=O), 1141 w, 1001 w (ferrocene),

N^1-(3-(7-chloroquinolin-4-ylamino)propyl)-N^3-(2-((dimethylamino)methyl)ferrocenyl methyl)oxalamide [C3]



125 mg (0.373 mmol) **7** and 203 mg (0.746 mmol) **16** was dissolved in dry ethanol (3 mL), and stirred at 40 °C for 18 hours in a dry round bottomed flask under nitrogen. After the reaction time the solvent was removed and the resulting residue was dissolved in DCM. The product was purified by column chromatography over silica gel. (88% EtOAc, 2% Et_3N, 10% EtOH) resulting in a yellow powder as the product. (Yield: 92 mg, 44%). R_f: 0.42 (5% Et_3N, 5% EtOH, 90% EtOAc)

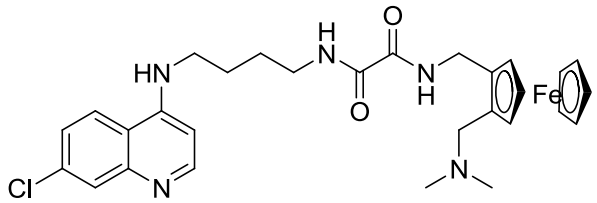
^{1}H NMR (400 MHz, CHLOROFORM-*d*) *δ* ppm 1.85 - 1.93 (m, 2 H, H7'), 2.24 (s, 6 H, H1'), 2.88 (d, *J*=12.69 Hz, 1 H, H2'b), 3.37 (d, *J*=5.47 Hz, 2 H, H6'), 3.42 - 3.50 (m, 2 H, H8'), 3.81 (d, *J*=12.50 Hz, 1 H, H2'a), 4.05 (t, *J*=2.44 Hz, 1 H, H3'b), 4.11 (s, 5 H, Cp'), 4.12 - 4.17 (m, 2 H, Cp'), 4.20 (s, 1 H, Cp'), 4.53 (dd, *J*=14.26, 8.59 Hz, 1 H, H3'a), 6.39 (d, *J*=5.50 Hz, 1 H, H3), 7.32 (dd, *J*=8.89, 2.05 Hz, 1 H, H7), 7.88 (d, *J*=8.98 Hz, 1 H, H9), 7.95 (d, *J*=1.95 Hz, 1 H, H6), 8.50 (d, *J*=5.50 Hz, 1 H, H2)

^{13}C{H} (101 MHz, CHLOROFORM-*d*) *δ* ppm 27.8 (C7'), 36.4 (C6'), 38.4 (C3'), 38.8 (C8'), 44.4 (C1'), 57.9 (C2'), 65.9 (Cp'), 69.2 (Cp), 69.8 (Cp'), 71.2 (Cp'), 83.4 (Cp'), 84.3 (Cp'), 98.5 (C3), 117.6 (C5), 121.6 (C7), 125.1 (C6), 128.7 (C9), 134.8 (C8), 149.8 (C10), 151.9 (C2), 158.5 (C4), 161.5 (C5'), 165.5 (C4')

IR ATR; 3304br w (NH), 1659 s (N-C=O), 1610 w, 1578 s (7-cholorquinoline), 1505 s (N-C=O), 1138 w, 1104 w (ferrocene)

HRMS (EI) *m/z* [M^+]+1 562.1675 [M^++1, $C_{28}H_{33}ClFeN_5O_2$ requires 562.1672]

N^1-(4-(7-chloroquinolin-4-ylamino)butyl)-N^4-(2-((dimethylamino)methyl)ferrocenyl methyl)oxalamide [C4]



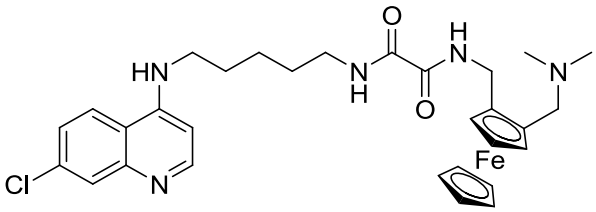
136 mg (0.389 mmol) **8** and 212 mg (0.778 mmol) **16** was dissolved in dry ethanol (3 mL), and stirred at 40 °C for 18 hours in a dry round bottomed flask under nitrogen. After the reaction time the solvent was removed and the resulting residue was dissolved in DCM. The product was purified by column chromatography over silica gel. (88% EtOAc, 2% Et_3N, 10% EtOH) resulting in a light orange powder as the product. (Yield: 142 mg, 63.4%). Rf: 0.44 (5% Et_3N, 5% EtOH, 90% EtOAc)

^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 1.71 - 1.76 (m, 2 H, H7'), 1.77 - 1.83 (m, 2 H, H8'), 2.19 (s, 6 H, H1'), 2.85 (d, *J*=12.69 Hz, 1 H, H2'a), 3.32 - 3.38 (m, 2 H, H6'), 3.41 (d, *J*=6.83 Hz, 2 H, H9'), 3.77 (d, *J*=12.69 Hz, 1 H, H2'b), 4.03 (t, *J*=2.44 Hz, 1 H, H3'a), 4.10 (s, 5 H, Cp), 4.12 (d, *J*=2.15 Hz, 2 H, Cp'), 4.19 (s, 1 H, Cp'), 4.51 (dd, *J*=14.35, 8.49 Hz, 1 H, H3'b), 6.40 (d, *J*=5.47 Hz, 1 H, H3), 7.35 (dd, *J*=8.89, 2.25 Hz, 1 H, H7), 7.75 (d, *J*=8.98 Hz, 1 H, H9), 7.96 (d, *J*=2.15 Hz, 1 H, H6), 8.53 (d, *J*=5.47 Hz, 1 H, H2)

^{13}C{H} NMR (101 MHz, CHLOROFORM-*d*) δ ppm 25.6 (C7'), 27.6 (C8'), 38.2 (C6'), 39.0 (C3'), 43.2 (C9'), 44.4 (C1'), 57.9 (C2'), 65.9 (Cp'), 69.1 (Cp), 69.7 (Cp'), 71.1 (Cp'), 83.6 (Cp'), 84.3 (Cp'), 99.1 (C3), 117.2 (C5), 121.1 (C7), 125.3 (C6), 128.8 (C9), 134.8 (C8), 149.1 (C10), 149.7 (C2), 152.0 (C4), 158.9 (C5'), 160.5 (C4')

IR ATR; 3303br w (NH), 1659 s (N-C=O), 1610 w, 1578 s (7-chloroquinoline), 1505 s (N-C=O), 1032 w, 1001 w (ferrocene)

N^1-(5-(7-chloroquinolin-4-ylamino)pentyl)-N^5-(2-((dimethylamino)methyl)ferrocenyl methyl)oxalamide [C5]



95 mg (0.261 mmol) **9** and 150 mg (0.550 mmol) **16** was dissolved in dry ethanol (3 mL), and stirred at 40 °C for 18 hours in a dry round bottomed flask under nitrogen. After the reaction time the solvent was removed and the resulting residue was dissolved in DCM. The product was purified by column chromatography over silica gel. (88% EtOAc, 2% Et_3N, 10% EtOH) resulting in a light orange powder as the product. (Yield: 90 mg, 58.4%). R_f: 0.49 (5% Et_3N, 5% EtOH, 90% EtOAc)

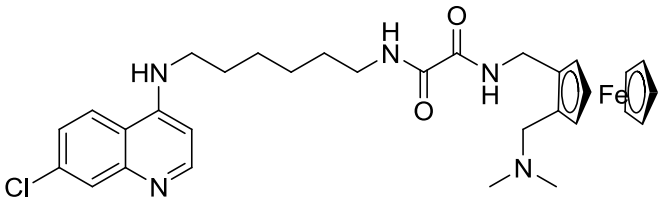
^{1}H NMR (400 MHz, CHLOROFORM-*d*) *δ* ppm 1.52 (m, *J*=7.00 Hz, 2 H, H8'), 1.61 - 1.70 (m, 2 H, H7'), 1.80 (m, 2 H, H9'), 2.19 (s, 6 H, H1'), 2.86 (d, *J*=12.50 Hz, 1 H, H2'a), 3.28 - 3.32 (m, 2 H, H6'), 3.33 - 3.37 (m, 2 H, H10'), 3.76 (d, *J*=12.69 Hz, 1 H, H2'b), 4.04 (t, *J*=2.54 Hz, 1 H, H3'a), 4.10 (s, 5 H, Cp), 4.11 - 4.13 (m, 2 H, Cp'), 4.19 (s, 1 H, Cp'), 4.50 (dd, *J*=14.26, 8.59 Hz, 1 H, H3'b), 6.40 (d, *J*=5.50 Hz, 1 H, H3), 7.35 (dd, *J*=8.79, 2.15 Hz, 1 H, H7), 7.67 (d, *J*=8.98 Hz, 1 H, H9), 7.96 (d, *J*=2.15 Hz, 1 H, H6), 8.54 (d, *J*=5.50 Hz, 1 H, H2)

^{13}C{H} NMR (75 MHz, CHLOROFORM-*d*) *δ* ppm 24.3 (C8'), 28.4 (C7'), 29.1 (C9'), 38.2 (C6'), 39.1 (C3'), 43.1 (C10'), 44.4 (C1'), 58.0 (C2'), 66.0 (Cp'), 69.2 (Cp), 69.7 (Cp'), 71.1 (Cp'), 83.6 (Cp'), 84.2 (Cp'), 99.1 (C3), 117.1 (C5), 120.9 (C7), 125.3 (C6), 128.8 (C9), 134.9 (C8), 149.1 (C10), 149.7 (C2), 152.0 (C4), 159.0 (C5'), 160.3 (C4')

IR ATR; 3305br w (NH), 1659 s (N-C=O), 1609 w, 1578 s (7-chloroquinoline) 1505 s (N-C=O), 1033 w, 1001 w (ferrocene),

HRMS (EI) *m/z* [M^+]+1 590.1992 [M^++1, $C_{30}H_{37}ClFeN_5O_2$ requires 590.1985] 545.1419 [M^+ – $N(Me)_2$]

N^1-(6-(7-chloroquinolin-4-ylamino)hexyl)-N^6-(2-((dimethylamino)methyl)ferrocenyl methyl)oxalamide [C6]



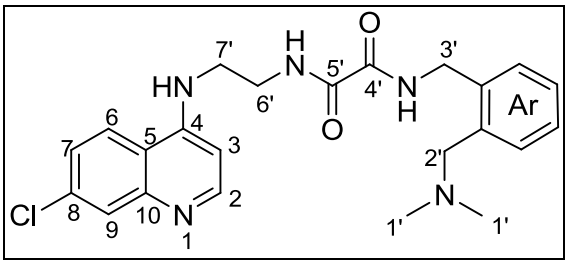
140 mg (0.373 mmol) **10** and 203 mg (0.746 mmol) **16** was dissolved in dry ethanol (3 mL) and stirred at 40 °C for 18 hours in a dry round bottomed flask under nitrogen. After the reaction time the solvent was removed and the resulting residue was dissolved in DCM. The product was purified by column chromatography over silica gel. (88% EtOAc, 2% Et_3N, 10% EtOH) resulting in a light orange powder as the product. (Yield: 239 mg, 94%). R_f: 0.51 (5% Et_3N, 5% EtOH, 90% EtOAc)

^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 1.38 - 1.46 (m, 2 H, H8'), 1.49 (br. s., 2 H, H9'), 1.54 - 1.63 (m, 2 H, H7'), 1.75 (t, *J*=7.03 Hz, 2 H, H10'), 2.17 (s, 6 H, H1'), 2.84 (d, *J*=12.69 Hz, 1 H, H2'b), 3.26 - 3.35 (m, 4 H, H11', H6'), 3.75 (d, *J*=12.69 Hz, 1 H, H2'a), 4.02 (t, *J*=2.44 Hz, 1 H, H3'b), 4.10 (s, 5 H, Cp), 4.10 - 4.12 (m, 2 H, Cp'), 4.17 - 4.19 (m, 1 H, Cp'), 4.48 (d, *J*=8.59 Hz, 1 H, H3'a), 6.40 (d, *J*=5.50 Hz, 1 H, H3), 7.36 (dd, *J*=8.98, 2.15 Hz, 1 H, H7), 7.70 (d, *J*=8.98 Hz, 1 H, H9), 7.96 (d, *J*=2.15 Hz, 1 H, H6), 8.53 (d, *J*=5.50 Hz, 1 H, H2)

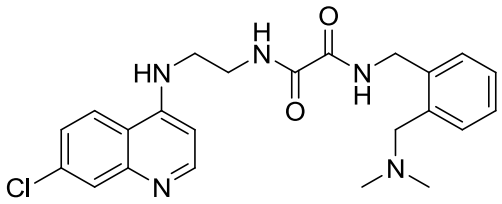
^{13}C{H} NMR (101 MHz, CHLOROFORM-*d*) δ ppm 26.4 (C8'), 28.6 (C9'), 29.2 (C7'), 30.9 (C10'), 38.1 (C6'), 39.1 (C3'), 42.9 (C11'), 44.4 (C1'), 58.0 (C2'), 65.9 (Cp'), 69.1 (Cp), 69.6 (Cp'), 71.0 (Cp'), 83.6 (Cp'), 84.4 (Cp'), 99.1 (C3), 117.1 (C5), 120.9 (C7), 125.2 (C6), 128.9 (C9), 134.8 (C8), 149.1 (C10), 149.6 (C2), 152.1 (C4), 159.0 (C5'), 160.2 (C4')

IR ATR; 3303br w, 2932 w (NH), 1659 s (N-C=O), 1610 w, 1578 s (7-chloroquinoline), 1504 s (N-C=O), 1032 w, 1011 w (ferrocene),

HRMS (EI) *m/z* [M^+]+1 604.2128 [M^++1, $C_{31}H_{39}ClFeN_5O_2$ requires 604.2142]



N^1-(2-(7-chloroquinolin-4-ylamino)ethyl)-N^2-(2-((dimethylamino)methyl)benzyl) oxalamide [D2]



70 mg (0.217 mmol) of **6** and 70.6 mg (0.43 mmol, 2 equivalents) **13** was dissolved in dry ethanol (3 mL), and stirred at 40 °C for 18 hours in a dry round bottomed flask under nitrogen. After the reaction time the solvent was removed and the resulting residue was dissolved in DCM. The product was purified by column chromatography over silica gel. (88% EtOAc, 2% Et_3N, 10% EtOH) resulting in a white powder as the product. (Yield: 47.9 mg, 50%). R_f: 0.36 (5% Et_3N, 5% EtOH, 90% EtOAc)

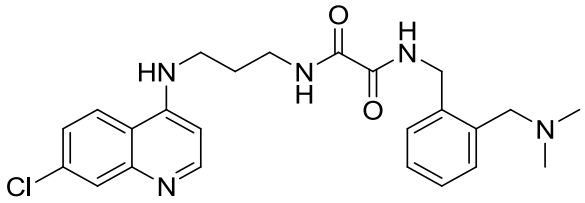
^{1}H NMR (300 MHz, CHLOROFORM-*d*) *δ* ppm 2.35 (s, 6 H, H1'), 3.43 - 3.51 (m, 2 H, H6'), 3.53 (s, 2 H, H2'), 3.73 - 3.82 (m, 2 H, H7'), 4.49 (d, *J*=5.87 Hz, 2 H, H3'), 6.31 (d, *J*=5.58 Hz, 1 H, H3), 7.21 - 7.29 (m, 4 H, Ar), 7.32 - 7.36 (m, 1 H, H7), 7.82 (d, *J*=8.95 Hz, 1 H, H9), 7.97 (d, *J*=2.05 Hz, 1 H, H6), 8.47 (d, *J*=5.43 Hz, 1 H, H2)

^{13}C{H} NMR (75 MHz, CHLOROFORM-*d*) 38.9 (C6'), 43.0 (C3'), 44.4 (C7'), 45.2 (C1'), 62.9 (C2'), 98.2 (C3), 116.9 (C5), 122.1 (C7), 125.4 (Ar), 127.5 (Ar), 127.5 (C6), 128.0 (Ar), 128.5 (Ar), 130.5 (C9), 131.5 (Ar), 135.4 (C8), 137.0 (Ar), 137.5 (C10), 150.6 (C2), 150.7 (C4), 158.6 (C5'), 162.8 (C4')

IR ATR 3295br w, 2924 w (NH), 1656 s (N-C=O), 1610 w, 1582 s (7-chloroquinoline) 1537 s (N-C=O), 1514 s (aromatic C=C)

HRMS (EI) *m/z* [M^+]+1 440.1853 [M^++1, $C_{23}H_{27}ClFeN_5O_2$ requires 440.1842]

N^1-(3-(7-chloroquinolin-4-ylamino)propyl)-N^3-(2-((dimethylamino)methyl)benzyl) oxalamide [D3]



125 mg (0.373 mmol) of **7** and 123 mg (0.746 mmol, 2 equivalents) **13** was dissolved in dry ethanol (3 mL), and stirred at 40 °C for 18 hours in a dry round bottomed flask under nitrogen. After the reaction time the solvent was removed and the resulting residue was dissolved in DCM. The product was purified by column chromatography over silica gel. (88% EtOAc, 2% Et_3N, 10% EtOH) resulting in a white powder as the product. (Yield: 37 mg, 22%). R_f: 0.37 (5% Et_3N, 5% EtOH, 90% EtOAc)

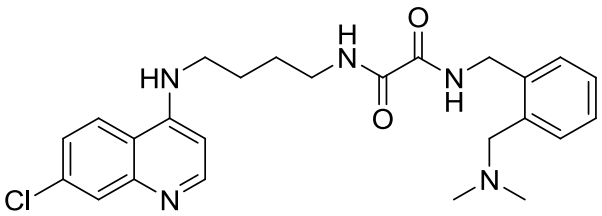
^{1}H NMR (400 MHz, CHLOROFORM-*d*) *δ* ppm 1.93 (m, 2 H, H7'), 2.29 - 2.33 (m, 6 H, H1'), 3.40 - 3.45 (m, 2 H, H6'), 3.45 - 3.50 (m, 2 H, H8'), 3.52 (s, 2 H, H2'), 4.51 (d, *J*=5.86 Hz, 2 H, H3'), 6.41 (d, *J*=6.05 Hz, 1 H, H3), 7.23 - 7.25 (m, 1 H, H7), 7.27 - 7.38 (m, 4 H, Ar), 8.03 (d, *J*=2.15 Hz, 1 H, H9), 8.07 (d, *J*=8.98 Hz, 1 H, H6), 8.41 (d, *J*=5.86 Hz, 1 H, H2)

^{13}C{H} NMR (101 MHz, CHLOROFORM-*d*) *δ* ppm 30.1 (C7), 36.7 (C6'), 39.5 (C3'), 43.2 (C8'), 44.7 (C1'), 63.0 (C2'), 98.3 (C3), 117.2 (C5), 122.7 (C7), 126.1 (Ar), 126.3 (Ar), 128.3 (C6), 128.8 (Ar), 130.8 (Ar), 131.7 (C9), 136.5 (Ar), 137.2 (C8), 137.7 (Ar), 149.1 (C10), 151.8 (C2), 153.5 (C4), 159.1 (C5'), 161.8 (C4')

IR ATR 3309br w, 2935 w (NH), 1650 s (N-C=O), 1611 w, 1578 s (7-chloroquinoline), 1544 s (N-C=O), 1510 s (aromatic C=C)

HRMS (EI) *m/z* [M^+]+1 454.2006 [M^++1, $C_{24}H_{29}ClFeN_5O_2$ requires 454.2010]

N^1-(4-(7-chloroquinolin-4-ylamino)butyl)-N^4-(2-((dimethylamino)methyl)benzyl) oxalamide [D4]



130 mg (0.373 mmol) **8** and 123 mg (0.746 mmol) **13** was dissolved in dry ethanol (3 mL) and stirred at 40 °C for 18 hours in a dry round bottomed flask under nitrogen. After the reaction time the solvent was removed and the resulting residue was dissolved in DCM. The product was purified by column chromatography over silica gel. (88% EtOAc, 2% Et_3N, 1% EtOH) resulting in a white powder as the product. (Yield: 133.6 mg, 76%). R_f: 0.38 37 (5% Et_3N, 5% EtOH, 90% EtOAc)

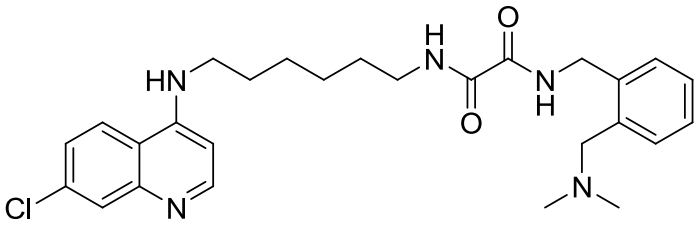
^{1}H NMR (400 MHz, CHLOROFORM-*d*) *δ* ppm 1.71 - 1.76 (m, 2 H, H7'), 1.76 - 1.81 (m, 2 H, H8'), 2.27 (s, 6 H, H1'), 3.32 - 3.38 (m, 2 H, H6'), 3.41 (q, *J*=6.51 Hz, 2 H, H9'), 3.50 (s, 2 H, H2'), 4.50 (d, *J*=5.86 Hz, 2 H, H3'), 6.39 (d, *J*=5.47 Hz, 1 H, H3), 7.13 - 7.18 (m, 1 H, Ar), 7.21 - 7.24 (m, 2 H, Ar), 7.28 - 7.30 (m, 1 H, Ar), 7.34 (dd, *J*=8.98, 2.15 Hz, 1 H, H7), 7.36 - 7.39 (m, 1 H, Ar), 7.77 (d, *J*=8.98 Hz, 1 H, H9), 7.96 (d, *J*=2.15 Hz, 1 H, H6), 8.52 (d, *J*=5.47 Hz, 1 H, H2)

^{13}C{H} NMR (101 MHz, CHLOROFORM-*d*) *δ* ppm 25.9 (C7'), 27.9 (C8'), 39.4 (C6'), 43.1 (C3'), 43.5 (C9), 44.8 (C1'), 63.3 (C2'), 99.4 (C3), 117.5 (C5), 121.5 (C7), 125.7 (Ar), 128.2 (Ar), 128.8 (C6), 129.0 (Ar), 130.9 (Ar), 131.7 (C9), 135.2 (Ar), 137.4 (C8), 138.0 (Ar), 150.1 (C10), 152.2 (C2), 159.6 (C5'), 160.9 (C4')

IR ATR 3285br w, 2937 w (NH), 1652 s (N-C=O), 1610 w, 1577 s (7-chloroquinoline), 1505 s (aromatic C=C)

HRMS (EI) *m/z* [M^+]+1 468.2156 [M^++1, $C_{25}H_{31}ClFeN_5O_2$ requires 468.2166]

N^1-(6-(7-chloroquinolin-4-ylamino)hexyl)-N^6-(2-((dimethylamino)methyl)benzyl) oxalamide [D6]



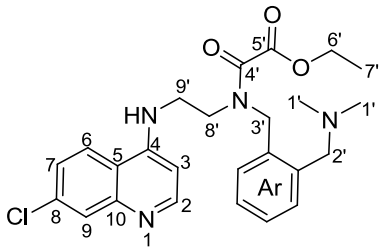
140 mg (0.373 mmol) **10** and 122.5 mg (0.746 mmol) **13** was dissolved in dry ethanol (3 mL), and stirred at 40 °C for 18 hours in a dry round bottomed flask under nitrogen. After the reaction time the solvent was removed and the resulting residue was dissolved in DCM. The product was purified by column chromatography over silica gel. (88% EtOAc, 2% Et_3N, 10% EtOH) resulting in a light orange powder as the product. (Yield: 146.6 mg, 79%). R_f: 0.57 (5% Et_3N, 5% EtOH, 90% EtOAc)

^{1}H NMR (400 MHz, CHLOROFORM-*d*) *δ* ppm 1.36 - 1.43 (m, 2 H, H8'), 1.43 - 1.50 (m, 2 H, H9'), 1.56 (m, 2 H, H7'), 1.72 (m, 2 H, H10'), 2.25 (s, 6 H, H1'), 3.29 (m, 4 H, H6', H11'), 3.47 (s, 2 H, H2'), 4.49 (d, *J*=6.05 Hz, 2 H, H3'), 6.38 (d, *J*=5.47 Hz, 1 H, H3), 7.19 - 7.22 (m, 1 H, Ar), 7.24 - 7.28 (m, 2 H, Ar), 7.33 (dd, *J*=8.98, 2.15 Hz, 1 H, Ar), 7.35 - 7.38 (m, 1 H, H7), 7.73 (d, *J*=8.98 Hz, 1 H, H9), 7.95 (d, *J*=1.95 Hz, 1 H, H6), 8.51 (d, *J*=5.47 Hz, 1 H, H2)

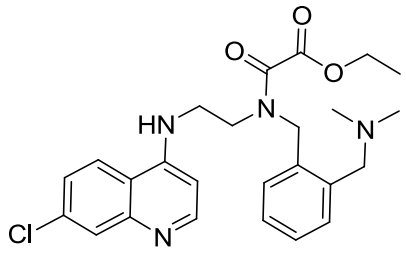
^{13}C{H} NMR (101 MHz, CHLOROFORM-*d*) *δ* ppm 26.2 (C8'), 26.4 (C9'), 28.46 (C7'), 29.1 (C10'), 39.1 (C6'), 42.7 (C3'), 42.8 (C11'), 44.5 (C1'), 63.0 (C2'), 99.0 (C3), 117.1 (C5), 121.1 (C7), 125.2 (Ar), 127.8 (Ar), 128.4 (C6), 128.7 (Ar), 130.5 (Ar), 131.3 (C9), 134.8 (Ar), 137.1 (C8), 137.6 (Ar), 149.0 (C10), 149.7 (C2), 151.9 (C4), 159.4 (C5'), 160.2 (C4')

IR ATR 3253br w, 2928 w (NH), 1655 s (N-*C*=*O*), 1612 w, 1577 s (7-chloroquinoline), 1543 s (N-*C*=*O*), 1512 (aromatic C=C)

HRMS (EI) *m/z* [M^+]+1 496.2465 [M^++1, $C_{27}H_{35}ClFeN_5O_2$ requires 496.2479]



Ethyl2-((2-(7-chloroquinolin-4-ylamino)ethyl)(2-((dimethylamino)methyl)benzyl) amino)-2-oxoacetate [F2][4]



100 mg (0.27 mmol) of **B2** was dissolved in a mixture of dry DCM (3 mL) and Et_3N (2 mL), followed by the dropwise addition of ethyl 2-chloro-2-oxoacetate (45 µL, 0.4 mmol, 1.5 equivalents) at 0 °C for 2 hours under nitrogen. The product was purified by column chromatography over silica gel (96.5% DCM, 3.5% Et_3N). (Yield: 126.1 mg, 99%). R_f: 0.64 (96.5% DCM, 3.5% Et_3N)

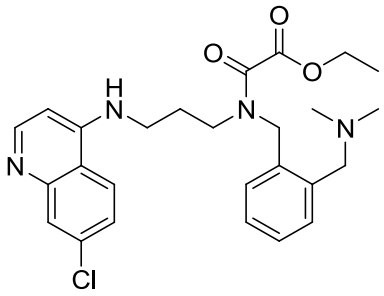
^{1}H NMR (400 MHz, DMSO-d_6, 130 °C) δ ppm 1.20 (m, 3 H, H7'), 2.13 (br. s., 6 H, H1'), 3.52 (m, 6 H, H9', H8', H2'), 4.21 (q, *J*=7.16 Hz, 2 H, H6'), 4.71 (s, 2 H, H3'), 6.44 (d, *J*=5.47 Hz, 1 H, H3), 7.25 (m, 4 H, Ar), 7.41 (d, *J*=8.98 Hz, 1 H, H7), 7.82 (d, *J*=1.95 Hz, 1 H, H9), 8.17 (d, *J*=9.37 Hz, 1 H, H6), 8.39 (d, *J*=5.47 Hz, 1 H, H2)

^{13}C NMR (151 MHz, DMSO-d_6, 130 °C) δ ppm 12.7 (C7'), 40.80 (C1'), 43.8 (C3'), 45.7 (C4'), 47.8 (C5'), 60.4 (C6'), 61.0 (C2'), 98.1 (C3), 116.9 (C5), 123.1 (C7), 123.7 (Ar), 126.4 (C6), 126.7 (Ar), 127.7 (Ar), 129.7 (Ar), 133.3 (C9), 134.4 (Ar), 135.3 (C8), 136.3 (Ar), 147.6 (C10), 150.0 (C2), 150.3 (C4), 162.4 (C4'), 163.4 (C5')

IR ATR 2926br w (NH), 1732 w (EtO-C=O), 1601 s, 1574 s (7-chloroquinoline), 1454 s (aromatic C=C)

HRMS (EI) *m/z* [M^+]+1 469.2018 [M^++1 $C_{25}H_{30}ClN_4O_3$ requires 469.2006]

Ethyl-2-((3-(7-chloroquinolin-4-ylamino)propyl)(2-((dimethylamino)methyl)benzyl) amino)-2-oxoacetate [F3]



150 mg (0.39 mmol) of **B3** was dissolved in dry DCM (3 mL) and Et_3N (2 mL), followed by the dropwise addition of ethyl 2-chloro-2-oxoacetate (65 µL, 0.59 mmol, 1.5 equivalents) at 0 °C for 2 hours under nitrogen. The product was purified by column chromatography over silica gel (96.5% DCM, 3.5% Et_3N). (Yield: 165.8 mg, 88%). R_f: 0.70 (90% DCM, 5% Et_3N, 5% MeOH)

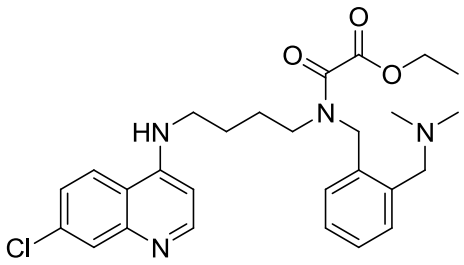
^{1}H NMR (400 MHz, DMSO-d_6, 130 °C) δ ppm 1.20 (br. s., 3 H, H7'), 1.95 (m, 2 H, H9'), 2.07 - 2.16 (m, 6 H, H1'), 3.29 (br. s., 4 H, H10', H8'), 3.36 (br. s., 2 H, H2'), 4.18 - 4.26 (q, 2 H, H6'), 4.74 (br. s., 2 H, H3'), 6.41 (d, *J*=5.27 Hz, 1 H, H3), 7.23 (br. s., 4 H, Ar), 7.38 (dd, *J*=8.98, 2.15 Hz, 1 H, H7), 7.80 (d, *J*=2.15 Hz, 1 H, H9), 8.14 (d, *J*=9.18 Hz, 1 H, H6), 8.41 (d, *J*=5.27 Hz, 1 H, H2)

^{13}C NMR (151 MHz, DMSO-d_6, 130 °C) δ ppm 12.7 (C7') 25.6 (C9'), 42.1 (C1'), 44.0 (C3'), 44.9 (C8'), 47.2 (C10'), 60.6 (C6'), 61.0 (C2'), 98.2 (C3), 117.2 (C5), 123.0 (C7), 123.4 (Ar), 126.4 (C6), 126.6 (Ar), 126.9 (Ar), 129.6 (Ar), 132.9 (C9), 134.5 (Ar), 135.3 (C8), 136.4 (Ar), 148.7 (C10), 149.5 (C2), 151.1 (C4), 161.6 (C4'), 162.4 (C5')

IR ATR 2939 w, 2815 w (NH), 1737 m (EtO-C=O), 1644 s (N-C=O), 1609 m, 1577 vs (7-chloroquinoline), 1450 m (aromatic C=C)

HRMS (EI) *m/z* [M^+]+1 483.2178 [M^++1 $C_{26}H_{32}ClN_4O_3$ requires 483.2163]

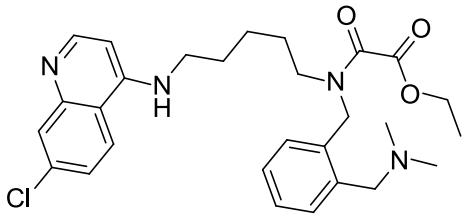
Ethyl2-((4-(7-chloroquinolin-4-ylamino)butyl)(2-((dimethylamino)methyl)benzyl) amino)-2-oxoacetate [F4]



253.6 mg (0.64 mmol) of **B4** was dissolved in dry DCM (3 mL) and Et_3N (2 mL), followed by the drop wise addition of ethyl 2-chloro-2-oxoacetate (107 µL, 0.96 mmol, 1.5 equivalents) at 0 °C for 4 hours under nitrogen. The product was purified by column chromatography over silica gel (95% DCM, 5% Et_3N). (Yield: 186 mg, 59%).R_f: 0.34 (95% DCM, 5% Et_3N)

^{1}H NMR (400 MHz, DMSO-d_6, 130 °C) δ ppm 1.21 (br.s, 3 H), 1.67 (br. s., 4 H), 2.10 - 2.21 (m, 6 H), 3.31 (d, *J*=7.42 Hz, 4 H), 3.41 (br. s., 2 H), 4.26 (br. s., 2 H), 4.72 (br. s., 2 H), 6.46 (d, *J*=5.27 Hz, 1 H), 7.15 - 7.29 (m, 4 H), 7.40 (dd, *J*=9.08, 2.25 Hz, 1 H), 7.81 (d, *J*=2.15 Hz, 1 H), 8.23 (d, *J*=8.98 Hz, 1 H), 8.41 (d, *J*=5.47 Hz, 1 H)
^{13}C NMR (151 MHz, DMSO-d_6, 130 °C) δ ppm 12.9 (C7'), 23.9 (C9'), 24.9 (C10'), 41.7 (C1'), 44.0 (C3'), 45.7 (C8'), 46.4 (C11'), 60.6 (C6'), 60.9 (C2'), 98.1 (C3), 117.1 (C5), 123.1 (C7), 123.3 (Ar), 126.3 (C6), 126.6 (Ar), 126.8 (Ar), 129.6 (Ar), 133.0 (C9), 134.6 (Ar), 135.4 (C8), 136.5 (Ar), 148.5 (C10), 149.8 (C2), 150.9 (C4), 161.5 (C4'), 162.4 (C5')
IR ATR 3271br w, 2928 m (NH), 1736 m (EtO-C=O), 1651 s (N-C=O), 1602 s, 1578 s (7-chloroquinoline), 145 m (aromatic C=C)
HRMS (EI) *m/z* [M^+]+1 497.2340 [M^++1, C27H34ClN4O3 requires 497.2319]

Ethyl 2-((5-(7-chloroquinolin-4-ylamino)pentyl)(2-((dimethylamino)methyl)benzyl) amino)-2-oxoacetate [F5]



164 mg (0.4 mmol) of **B5** was dissolved in dry DCM (3 mL) and Et_3N (2 mL), followed by the dropwise addition of ethyl 2-chloro-2-oxoacetate (67 µL, 0.6 mmol, 1.5 equivalents) at 0 °C for 4 hours under nitrogen. The product was purified by column chromatography over silica gel (99.5% DCM, 0.5% Et_3N). (Yield: 150 mg, 74%) R_f: 0.46 (95% DCM, 5% Et_3N)

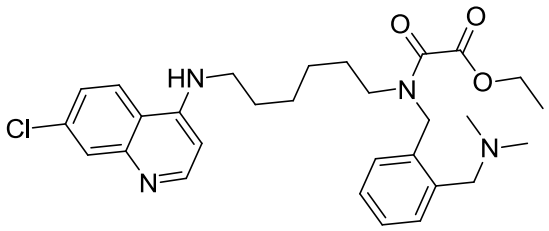
^{1}H NMR (400 MHz, DMSO-d_6, 130 °C) δ ppm 1.18 - 1.32 (m, 3 H, H7'), 1.37 (br. s., 2 H, H10'), 1.55 - 1.63 (m, 2 H, H9'), 1.67 (br. s., 2 H, H11'), 2.15 (br. s., 6 H, H1), 2.88 (br. s., 2 H, H8') 3.26 (br. s., 4 H, H12'), 3.40 (br. s., 2 H, H2'), 4.28 (br. s., 2 H, H6'), 4.72 (br. s., 2 H, H3'), 6.43 (d, *J*=4.88 Hz, 1 H, H3), 7.25 (br. s., 4 H, Ar), 7.37 (d, *J*=8.98 Hz, 1 H, H7), 7.78 (br. s., 1 H, H9), 8.20 (d, *J*=8.98 Hz, 1 H, H6), 8.39 (d, *J*=5.08 Hz, 1 H, H2)

^{13}C NMR (151 MHz, DMSO-d_6, 130 °C) δ ppm 12.9 (C7'), 23.2 (C10'), 25.8 (C9'), 27.0 (C1'), 41.9 (C3'), 44.0 (C8'), 46.5 (C12'), 60.6 (C6'), 60.8 (C2'), 98.1 (C3), 117.1 (C5), 123.1 (C7), 123.2 (Ar), 126.2 (C6), 126.5 (Ar), 126.8 (Ar), 126.8 (Ar), 129.6 (C9), 132.8 (Ar), 134.6 (C8), 136.5 (Ar), 148.6 (C10), 149.7 (C2), 150.9 (C4), 161.5 (C4'), 162.4 (C5')

IR ATR 2938br w, 2858 w (NH), 1737 m (EtO-C=O), 1651 s (N-C=O), 1609 w, 1577 vs (7-chloroquinoline), 1450 m (aromatic C=C)

HRMS (EI) *m/z* [M^+]+1 511.2493 [M^++1, $C_{28}H_{36}ClN_4O_3$ requires 511.2476]

Ethyl 2-((6-(7-chloroquinolin-4-ylamino)hexyl)(2-((dimethylamino)methyl)benzyl) amino)-2-oxoacetate [F6]



152 mg (0.36 mmol) of **B6** was dissolved in dry DCM (3 mL) and Et_3N (2 mL), followed by the drop wise addition of ethyl 2-chloro-2-oxoacetate (59 µl, 0.535 mmol, 1.5 equivalents) at 0 °C for 6 hours under nitrogen. The product was purified by column chromatography over silica gel (99.5% DCM, 0.5% Et_3N). (Yield: 146 mg, 73%). R_f: 0.58 (95% DCM, 5% Et_3N)

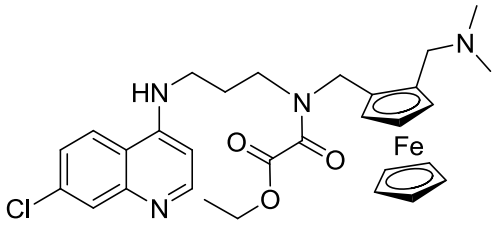
^{1}H NMR (400 MHz, DMSO-d_6, 130 °C) δ ppm 1.22 (br. s., 3 H, H7'), 1.29 (br. s., 2 H, H10'), 1.39 (br. s., 2 H, H11'), 1.55 (m, 2 H, H9'), 1.68 (m, 2 H, H12'), 2.16 (s, 6 H, H1'), 3.27 (br. s., 4 H, H12', H8'), 3.41 (s, 2 H, H2'), 4.29 (br. s., 2 H, H6'), 4.72 (br. s., 2 H, H3'), 6.44 (d, *J*=5.27 Hz, 1 H, H3), 7.16 - 7.31 (m, 4 H, Ar), 7.37 (dd, *J*=8.89, 2.25 Hz, 1 H, H7), 7.78 (d, *J*=1.95 Hz, 1 H, H9), 8.21 (d, *J*=8.98 Hz, 1 H, H6), 8.40 (d, *J*=5.47 Hz, 1 H, H2)

^{13}C NMR (151 MHz, DMSO-d_6, 130 °C) δ ppm 12.9 (C7'), 25.3 (C10'), 25.5 (C11'), 27.3 (C9'), 42.0 (C12'), 43.1 (C1'), 44.0 (C3'), 46.5 (C8'), 47.0 (C13'), 60.6 (C6'), 60.8 (C2'), 98.1 (C3), 117.1 (C5), 123.1 (C7), 123.2 (Ar), 126.3 (C6), 126.5 (Ar), 126.8 (Ar), 129.6 (Ar), 132.8 (C9), 134.6 (Ar), 135.4 (C8), 136.4 (Ar), 148.6 (C10), 149.8 (C2), 151.0 (C4), 161.5 (C4'), 162.4 (C5')

IR ATR 2935br w, 2857 w (NH), 1737 m (EtO-C=O), 1651 s (N-C=O), 1609 w, 1577 vs (7-chloroquinoline), 1450 m (aromatic C=C)

HRMS (EI) *m/z* [M^+]+1 525.2610 [M^++1, $C_{29}H_{38}ClN_4O_3$ requires 525.2632]

Ethyl2-((4-(7-chloroquinolin-4-ylamino)propyl)(2-((dimethylamino)methyl) ferrocenyl)amino)-2-oxoacetate [E2]



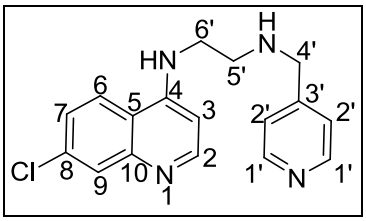
95 mg (0.19 mmol) of **A3** was dissolved in dry DCM (3 mL) and Et_3N (2 mL), followed by the drop wise addition of ethyl 2-chloro-2-oxoacetate (33 µl, 0.29 mmol, 1.5 equivalents) at 0 °C for 5 hours under nitrogen. The product was purified by column chromatography over silica gel (96% DCM, 4% Et_3N). (Yield: 62 mg, 55%).R_f: R_f: 0.47 (96% DCM, 4% Et_3N)

^{1}H NMR (400 MHz, DMSO-d_6, 130 °C) δ ppm 1.16 (br. s., 3 H, H7'), 1.92 (d, *J*=9.76 Hz, 2 H, H9'), 2.10 (s, 6 H, H1'), 2.99 (br. s., 4 H, H8', H10'), 3.24 (br. s., 2 H, H6'), 3.43 (br. s., 2 H, H2'), 3.53 (d, *J*=12.89 Hz, 1 H, H3'a), 4.08 (s, 5 H, Cp), 4.18 (br. s., 1 H, Cp'), 4.24 (br. s., 1 H, Cp'), 4.42 (br. s., 3 H, Cp', H3'b), 6.40 (d, *J*=5.47 Hz, 1 H, H3), 7.37 (dd, *J*=8.98, 1.95 Hz, 1 H, H7), 7.77 (d, *J*=2.34 Hz, 1 H, H9), 8.15 (d, *J*=8.98 Hz, 1 H, H6), 8.41 (d, *J*=5.08 Hz, 1 H, H2)

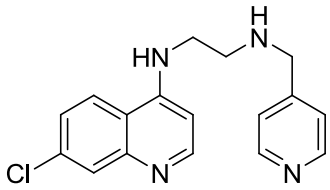
^{13}C NMR (151 MHz, DMSO-d_6, 130 °C) δ ppm 12.9 (C7'), 26.7 (C9'), 43.6 (C1'), 43.9 (C3'), 44.9 (C8'), 56.2 (C10'), 60.7 (C6'), 65.7 (C2'), 68.4 (Cp'), 68.9 (Cp), 69.6 (Cp'), 69.8 (Cp'), 83.5 (Cp'), 98.2(C3), 117.2 (C5), 122.9 (C7), 123.2 (C6), 127.0 (C9), 132.7 (C8), 148.8 (C10), 149.4 (C2), 151.0 (C4), 160.7 (C4'), 162.4 (C5')

IR ATR 2939br w, 2815 (NH), 1733 m (EtO-C=O), 1644 s (N-C=O), 1610 w, 1577 vs (7-chloroquinoline), 1136 m, 1105 m (ferrocene)

HRMS (EI) *m/z* [M^+]+1 591.1837 [M^++1 $C_{30}H_{36}ClFeN_4O_3$ requires 591.1825]



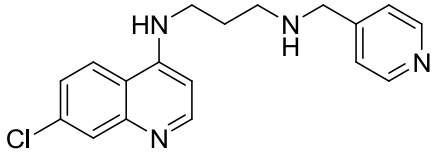
N^1-(7-chloroquinolin-4-yl)-N^2-(pyridin-4-ylmethyl)ethan-1,2-diamine [G2]



100 mg (0.451 mmol) of **1** and 114 mg 4-(bromomethyl)pyridine hydrobromide (0.451 mmol) was dissolved in acetonitrile (3 mL) after which (0.19 mL, 3 equivalents) of Et_3N was added. The reaction was stirred overnight. The product was purified by column chromatography over silica gel (86% EtOAc, 7% Et_3N, 7% MeOH). (Yield: 64 mg, 45.5%). R_f: 0.36 (90% DCM, 5% Et_3N, 5% MeOH)

^{1}H NMR (400 MHz, CHLOROFORM-*d*) *δ* ppm 3.09 (t, *J*=5.66 Hz, 2 H, H5'), 3.43 (t, *J*=5.76 Hz, 2 H, H6'), 3.90 (s, 2 H, H4'), 6.40 (d, *J*=5.66 Hz, 1 H, H3), 7.30 (d, *J*=5.47 Hz, 2 H, H2'), 7.39 (dd, *J*=8.98, 1.95 Hz, 1 H, H7), 7.81 (d, *J*=8.98 Hz, 1 H, H9), 7.98 (d, *J*=1.95 Hz, 1 H, H6), 8.49 (d, *J*=5.47 Hz, 1 H, H2), 8.57 (d, *J*=5.66 Hz, 2 H, H1'),
^{13}C NMR (75 MHz, CHLOROFORM-*d*) *δ* ppm 42.1 (C5'), 47.1 (C6'), 52.1 (C4'), 99.1 (C3), 117.2 (C5), 121.3 (C7), 122.9 (C2'), 125.4 (C6), 128.4 (C9), 135.0 (C8), 148.7 (C3'), 148.8 (C10), 149.9 (C1'), 149.9 (C2), 151.7 (C4)
IR ATR 1601 m, 1579 vs (7-chloroquinoline), 1368 m (pyridine)

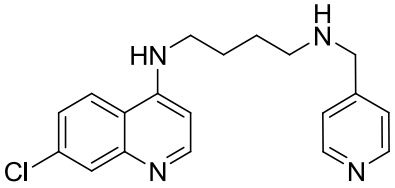
N^1-(7-chloroquinolin-4-yl)-N^3-(pyridin-4-ylmethyl)propan-1,3-diamine [G3]



100 mg (0.42 mmol) of **2** and 107 mg 4-(bromomethyl)pyridine hydrobromide (0.42 mmol) was dissolved in 50/50 mixture of DCM and Et_3N. The reaction was stirred overnight. The product was purified by column chromatography over silica gel (86% EtOAc, 7% Et_3N, 7% MeOH). (Yield: 30.2 mg, 22%). R_f: 0.52 (86% DCM, 7% MeOH, 7% Et_3N)

^{1}H NMR (400 MHz, CHLOROFORM-*d*) *δ* ppm 1.95 (m, 2 H, H6), 2.92 (m, , 2 H, H5'), 3.39 (t, *J*=5.96 Hz, 2 H, H7'), 3.84 (s, 2 H, H4'), 6.28 (d, *J*=5.66 Hz, 1 H, H3), 7.10 (dd, *J*=8.89, 1.66 Hz, 1 H, H7), 7.25 (d, *J*=5.47 Hz, 2 H, H2'), 7.53 (d, *J*=8.98 Hz, 1 H, H9), 7.88 (d, *J*=1.37 Hz, 1 H, H6), 8.40 (d, *J*=5.47 Hz, 1 H, H2), 8.56 (d, *J*=5.47 Hz, 2 H, H1')
^{13}C NMR (101 MHz, CHLOROFORM-*d*) *δ* ppm 27.4 (C6'), 43.4 (C5'), 48.8 (C7'), 52.9 (C4'), 98.2 (C3), 117.1 (C5), 122.0 (C7), 123.0 (C3'), 124.9 (C6), 127.5 (C9), 135.0 (C8), 147.9 (C3'), 148.3 (C10), 150.0 (C1'), 150.6 (C2), 150.9 (C4)
IR ATR 1606 m, 1578 vs (7-chloroquinoline), 1366 m (pyridine)

***N*^{1}-(7-chloroquinolin-4-yl)-*N*^{4}-(pyridin-4-ylmethyl)butan-1,4-diamine [G4]**

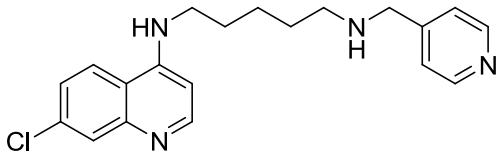


100 mg (0.40 mmol) of **3** and 101 mg 4-(bromomethyl)pyridine hydrobromide (0.40 mmol) was dissolved in 50/50 mixture of acetonitrile and Et_3N (3 mL). The reaction was stirred overnight. The product was purified by column chromatography over silica gel (90% DCM, 5% Et_3N, 5% MeOH). (Yield: 48.8 mg, 35.8%).R_f: 0.4 (90% DCM, 5% MeOH, 5% Et_3N)

^{1}H NMR (400 MHz, CHLOROFORM-*d*) *δ* ppm 1.73 (m, 2 H, H6'), 1.90 (m, 2 H, H7'), 2.75 (t, *J*=6.74 Hz, 2 H, H6'), 3.36 (t, *J*=6.74 Hz, 2 H, H8'), 3.86 (s, 2 H, H4'), 6.39 (d, *J*=5.47 Hz, 1 H, H3), 7.25 (d, *J*=2.15 Hz, 1 H, H7), 7.28 (m, 2 H, H2'), 7.73 (d, *J*=8.98 Hz, 1 H, H9), 7.95 (d, *J*=2.15 Hz, 1 H, H6), 8.47 (d, *J*=5.47 Hz, 1 H, H2), 8.57 (m, 2 H, H1')

^{13}C NMR (101 MHz, CHLOROFORM-*d*) *δ* ppm 26.3 (C6'), 27.6 (C7'), 43.2 (C5'), 48.7 (C8'), 52.6 (C4'), 98.8 (C3), 116.9 (C5), 121.5 (C7), 122.9 (C3'), 125.4 (C6), 127.7 (C9), 135.3 (C8), 147.9 (C3'), 148.8 (C10'), 149.9 (C1'), 150.4 (C2), 150.8 (C4)
IR ATR 1604 m, 1579 vs (7-chloroquinoline), 1368 m (pyridine)

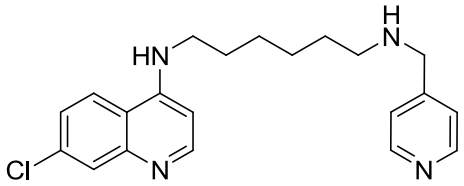
***N*^{1}-(7-chloroquinolin-4-yl)-*N*^{5}-(pyridin-4-ylmethyl)pentan-1,5-diamine [G5]**



100 mg (0.38 mmol) of **4** and 95 mg 4-(bromomethyl)pyridine hydrobromide (0.38) was dissolved in 50/50 mixture of acetonitrile and Et_3N (3 mL). The reaction was stirred overnight. The product was purified by column chromatography over silica gel (90% DCM, 5% Et_3N, 5% MeOH). (Yield: 31 mg, 23%) R_f: 0.4 (90% DCM, 5% MeOH, 5% Et_3N)

^{1}H NMR (300 MHz, CHLOROFORM-*d*) *δ* ppm 1.58 (m, 4 H, H6', H7'), 1.79 (m, 2 H, H8'), 2.67 (t, *J*=1.00 Hz, 2 H, H5'), 3.33 (m, 2 H, H9'), 3.82 (s, 2 H, H4'), 6.40 (d, *J*=5.43 Hz, 1 H, H3), 7.26 (m, 2 H, H2'), 7.35 (dd, *J*=8.95, 2.20 Hz, 1 H, H7), 7.68 (d, *J*=8.95 Hz, 1 H, H9), 7.96 (d, *J*=2.20 Hz, 1 H, H6), 8.52 (d, *J*=5.43 Hz, 1 H, H2), 8.55 (m, 2 H, H1')
^{13}C NMR (75 MHz, CHLOROFORM-*d*) *δ* ppm 24.8 (C7'), 28.7 (C6'), 29.8 (C8'), 43.1 (C5'), 49.2 (C9'), 52.8 (C4'), 99.0 (C3),117.0 (C5),120.9 (C7),122.9 (C3'),125.3 (C6),128.6 (C9),135.0 (C8), 148.8 (C3'), 149.4 (C10), 149.8 (C1'), 149.8 (C2), 151.7 (C4)
IR ATR 1604 m, 1579 vs (7-chloroquinoline), 1368 m (pyridine)
HRMS (EI) *m/z* [M^+]+1 355.1688 [M^++1, $C_{20}H_{24}N_4Cl$ requires 355.1689]

N^1-(7-chloroquinolin-4-yl)-N^6-(pyridin-4-ylmethyl)hexan-1,6-diamine [G6]



120 mg (0.43 mmol) of **5** and 109 mg 4-(bromomethyl)pyridine hydrobromide (0.43) was dissolved in 50/50 mixture of acetonitrile and Et_3N (5 mL). The reaction was stirred overnight. The product was purified by column chromatography over silica gel (90% DCM, 5% Et_3N, 5% MeOH). (Yield: 33 mg, 53%) R_f: 0.44 (90% DCM, 5% MeOH, 5% Et_3N)

^{1}H NMR (300 MHz, CHLOROFORM-*d*) *δ* ppm 1.50 (m, 6 H, H6', H7', H8'), 1.76 (m, 2 H, H9'), 2.63 (t, *J*=6.97 Hz, 2 H, H5'), 3.30 (t, *J*=1.00 Hz, 2 H, H10'), 3.81 (s, 2 H, H4'), 6.40 (d, *J*=5.28 Hz, 1 H, H3), 7.26 (m, 2 H, H2'), 7.35 (dd, *J*=8.95, 2.20 Hz, 1 H, H7), 7.67 (d, *J*=8.95 Hz, 1 H, H9), 7.95 (d, *J*=2.20 Hz, 1 H, H6), 8.53 (m, 3 H, H1', H2)
^{13}C NMR (75 MHz, CHLOROFORM-*d*) *δ* ppm 27.0 (C7'), 27.1 (C8'), 28.8 (C6'), 30.0 (C9'), 43.2 (C5'), 49.3 (C10'), 52.8 (C4'), 99.0 (C3), 117.1 (C5), 120.9 (C7), 123.0 (C3'), 125.3 (C6), 128.7 (C9), 134.9 (C8), 149.0 (C3'), 149.5 (C10), 149.8 (C1'), 151.9 (C2)
IR ATR 1606 m, 1576 vs (7-chloroquinoline), 1373 m (pyridine)

5.3. Detergent mediated β-Haematin Formation Assay

The β-Haematin inhibition assay was developed at Vanderbilt University using a specific detergent called NP-40 to create the lipid-water interface at which haemozoin formation occurs naturally.[7] The assay we followed is the method described by *Carter et al*. and was modified for manual liquid delivery using a multichannel pipette. Three stock solutions of the test compounds were made, a 10 mM (Stock 1), 2 mM (Stock 2) and 0.4 mM (Stock 3) respectively by dissolving each sample in DMSO. 200 µL of the 10mM solution was mixed with 800 µL DMSO to obtain the 2 mM solution, and 200 µL of that solution mixed with 800 µL DMSO to obtain the 0.4 mM solution. In the 96-well plate, drugs were delivered in triplicate with concentrations raging from 500 µM to 0 µM with a final DMSO volume of 10 µL in each well. The bottom table indicated the different volumes of each stock solution added, and the drug concentrations in each well.

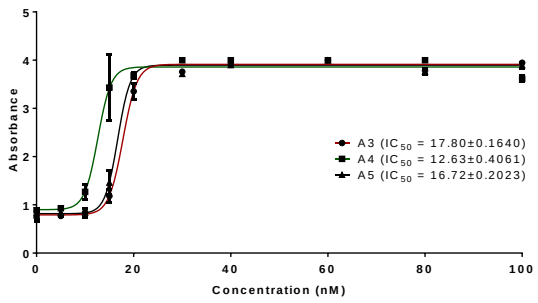
Well number	1	2	3	4	5	6	7	8	9	10	11	12
Final concentration of drug (µM)	0	5	10	15	20	30	40	60	80	100	200	500
Volume (µL) of drug solution	0	2.5	5	7.5	10	3	4	6	8	10	4	10
Volume (µL) of solvent (DMSO)	10	7.5	5	2.5	0	7	6	4	2	0	6	0

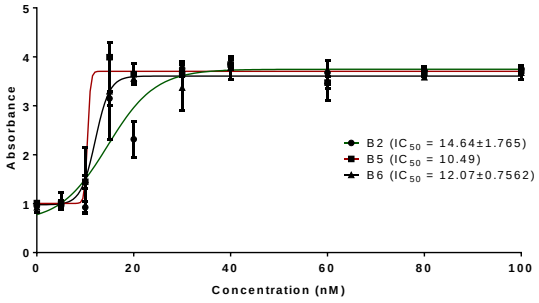
Well numbers 1 - 5 contained stock solution 3, well numbers 6 - 10 contained stock solution 2 and well number 11 - 12 contained stock solution 1. After the specific amount of stock solution has been added to a well and the corresponding volume of DMSO, distilled H_2O (70 µL) was added to each well, followed by NP-40 (20 µL, 30.55 µM). Afterwards, a 25mM haemin stock solution in DMSO was prepared followed by sonication for complete dissolution. Haemin solution (177.76 µL) was added to a 2M

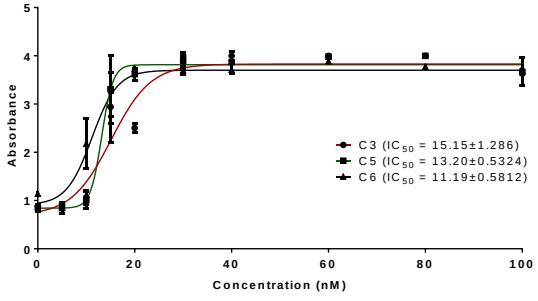
acetate buffer (20 mL, pH – 4,8) and vortexed to make the suspension as homogenous as possible. 100 µL of this solution was added to each well. The plate was then covered and incubated at 37 °C for 5 – 6 hours. After the incubation time has elapsed, analysis of the plate was carried out using the pyridine-ferrichrome method.[8] A pyridine solution (32 µL, 50% pyridine, 20% acetone, 20% water and 10% 2M HEPES) was added to each well. Acetone (60 µL) was added to each well to assist with mixing. The plate was read at 405 nm on a SpectraMax 340 PC384 plate reader and the data was processed using Graphpad Prism v6.01. Each compound's absorbance vs. concentration (nM) data was plotted, and then using a Sigmoidal-dose response curve (with variable slope) function to determine each compound's IC_{50} value.

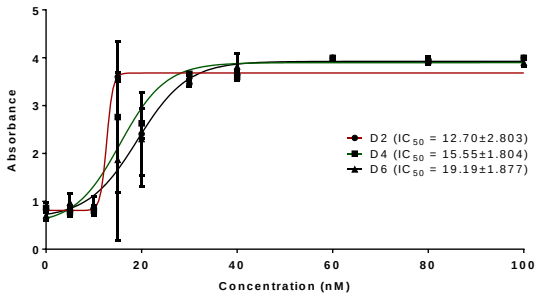
The following graphs plot the change in absorbance values with increase in drug concentration for all the compounds tested with this assay.

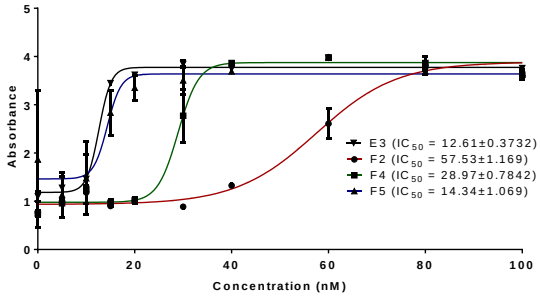
All the compounds appearing in each graph belongs to a specific series, and each compound's corresponding IC_{50} value is included.

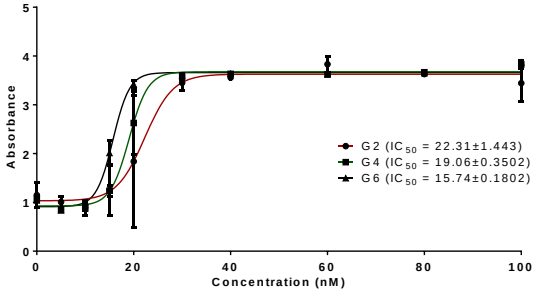












5.4. Whole cell testing

The test samples were tested in triplicate on one or two separate occasions against chloroquine sensitive (CQS) strain of *Plasmodium falciparum* (NF54). Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen (1976).[9] Quantitative assessment of antiplasmodial activity *in vitro* was determined via the parasite lactate dehydrogenase assay using a modified method described by Makler (1993).

The test samples were prepared to a 20 mg/mL stock solution in 100% 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 (CQ) was used as the reference drug in all experiments. A full dose-response 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 100 µg/mL, which was then serially diluted 2-fold in complete medium to give 10 concentrations; with the lowest concentration being 0.2 µg/mL. The same dilution technique was used for all samples. Quinoline based compounds were tested at a starting concentration of 1000ng/mL. Compound AH6 was tested at a starting concentration of 1000 ng/mL. Compounds 32, 33 and 40 were tested at 100 ng/mL. CQ was tested at a starting concentration of 1000 ng/mL. The highest concentration of solvent to which the parasites were exposed to had no measurable

effect on the parasite viability (data not shown). The IC_{50}-values were obtained using a non-linear dose-response curve fitting analysis via Graph Pad Prism v.4.0 software.

5.5. Crystal Structure

A single crystal was covered in a small amount of paratone oil and mounted on a glass fibre. X-ray intensity data was collected at 100 K on a Bruker SMART APEX CCD with 1.75 kW graphite monochromated Mo radiation. The detector to crystal distance was 60 mm. Data were collected by omega scans. The data were scaled and reduced using the *APEXII* software unit. Unit cell dimensions were refined on all the data and the space group was assigned on the basis of systematic absences and intensity statistics. The structure was solved and refined using *SHELX97*.[10] Hydrogen atoms are placed in calculated positions and included in the model during later stages of the refinement. The program *X-SEED*[11] an interface to *SHELX*, was used during the structure solution and refinements.

Table 1. Crystal data and structure refinement

N^1-(2-(7-chloroquinolin-4-ylamino)ethyl)-N^2-(2-((dimethylamino)methyl)benzyl)oxalamide (**D2**)

Empirical formula	$C_{23}H_{26}ClN_5O_2$	
Formula weight	439.94	
Temperature (K)	101(2)	
Wavelength (Å)	0.71073	
Crystal system	triclinic	
Space group	*P*-1	
Unit cell dimensions (Å, °)	*a* = 6.895(2)	α = 89.021(4)
	b = 11.606(4)	β = 88.388(4)
	c = 13.905(4)	γ = 84.940(4)
Volume (Å)	1107.9(6)	
Z	2	
Calculated density (g cm^{-3})	1.319	
Absorption coefficient (mm^{-1})	0.202	
F_{000}	464	
Crystal size (mm^3)	0.41 x 0.40 x 0.11	
θ range for data collection (°)	1.47 to 27.61	
Miller index ranges	$-8 \le h \le 8$, $-14 \le k \le 15$, $-\le l \le 18$	
Reflections collected	12291	
Independent reflections	4987 [R_{int} = 0.0482]	
Completeness to θ_{max} (%)	97.4	
Max. and min. transmission	0.9781 and 0.9216	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4987 / 0 / 282	
Goodness-of-fit on F^2	1.028	
Final *R* indices $[I > 2\sigma(I)]$	*R*1 = 0.0533, *wR*2 = 0.1378	
R indices (all data)	*R*1 = 0.0770, *wR*2 = 0.1521	
Largest diff. peak and hole (e $Å^{-3}$)	0.508 and -0.575	

5.6. References

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