Synthesis, characterisation and antimalarial activity of quinoline-pyrimidine hybrids

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ABSTRACT

The world suffers under the immense threat of malaria with about 1 million people dying and a further 500 million people getting infected and debilitated by the disease each year. It has a negative effect on the economic growth in developing countries that already battles with political unrest, civil wars, famine and the effect of diseases like tuberculosis and HIV/AIDS.

Resistance against the first line drugs such as the quinolines and the antifolate combination drugs makes the fight against malaria increasingly difficult and has prompted studies into alternative chemotherapeutic treatments of the disease. An efficient strategy to develop an effective and cheaper antimalarial compound appears to be the re-design of existing drugs and the exploitation of known parasite-specific targets.

In our search for novel drugs with improved antimalarial properties compared to the existing ones, we applied an emerging strategy in medicinal chemistry called hybridisation. This is the combination of two or more active ingredients into a single chemical entity to form a hybrid drug. The hybrid drug strategy has the potential advantage of restoring the effectiveness in antimalaria drugs such as the quinolines and the antifolate drugs. Artemisinin based and quinoline based hybrid drugs are demonstrative examples of the validity of such an approach.

Chloroquine used to be the first-choice drug in malaria treatment and prophylaxis ever since its discovery, but drug resistance has rendered it almost completely useless in treating *Plasmodium falciparum.* Today, it is still widely used in treating *Plasmodium vivax* malaria in resistance free areas. The historical success of the aminoquinoline antimalarial drugs supported our decision to include the quinoline pharmacophore in our study.

Pyrimethamine has been the most widely used antimalarial antifolate drug. It is used in malaria prophylaxis in combination with sulphonamides. Point mutations in the parasite's *dhfr* domain of the *dhfr* gene are severely compromising its antimalarial effectiveness.

The pharmacophores of chloroquine and pyrimethamine are a quinoline and a pyrimidine moiety, respectively. Through hybridisation of these two pharmacophores we hoped to bring about molecules with potent antimalarial properties and, thus restoring their antimalarial usefulness.

In this study we aimed to synthesise a series of quinoline-pyrimidine hybrids, determine their physicochemical properties and evaluate their antimalarial activity in comparison to that of chloroquine and pyrimethamine.

We successfully synthesised ten quinoline-pyrimidine hybrids by connecting a quinoline and a pyrimidine moiety *via* different linkers. The structures of the prepared hybrids were confirmed by nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS).

The experimental aqueous solubility of the compounds was determined to be higher at pH 5.5 than at pH 7.4 although no structure-physicochemical property could be drawn from this investigation.

The quinoline-pyrimidine hybrids were screened *in vitro* alongside chloroquine and pyrimethamine against the chloroquine-sensitive D10 strain of *Plasmodium falciparum.* The ether-linked hybrids tended to be more potent than the amine-linked ones. Compound **21,** exhibited the best antimalarial activity (IC_{50} = 0.08 µM) of all, and possessed activity similar to that of pyrimethamine (IC_{50} = 0.11 µM). None of the compounds proved to be as effective as chloroquine (IC_{50} = 0.03 µM).

Keywords: 4-aminoquinoline, pyrimethamine, hybrid drugs, malaria, drug resistance.

OPSOMMING

Die wêreld staan gebuk onder die enorme druk van malaria met ongeveer 1 miljoen sterftes en om en by 500 miljoen mense wat jaarliks deur die siekte geïnfekteer en verswak word. Dit het ’n negatiewe invloed op die ekonomiese groei in ontwikkelende lande veral waar dié lande alreeds lam gelê word deur politieke onrus, burgeroorloë, hongersnood en ander siektes soos tuberkulose en MIV/VIGS.

Weerstandigheid teen die eerste-linie geneesmiddels soos die kinoliene en die anti-folaat kombinasiegeneesmiddels, maak die geveg teen malaria al hoe moeiliker en het navorsing, om alternatiewe chemoterapeutiese geneesmiddels vir die behandeling van malaria te ontwikkel, genoodsaak. ’n Effektiewe strategie in die ontwikkeling van effektiewe, goedkoop anti-malariamiddels is die herformulering van reeds bestaande geneesmiddels asook om te werk op bekende parasiet-spesifieke teikens.

In ons poging om nuwe geneesmiddels te sintetiseer met anti-malaria eienskappe, het ons ’n relatief nuwe tegniek in medisinale chemie gevolg, genaamd hibridisasie. Dit is die kombinering van twee of meer aktiewe bestanddele as een chemiese entiteit om ’n hibriedgeneesmiddel te vorm. Hibridisasie het die potensiële voordeel dat dit die effektiwiteit van anti-malariamiddels soos die kinoliene en die anti-folaat geneesmiddels kan herstel. Artemisinien- en kinolien-gebaseerde hibriedgeneesmiddels is voorbeelde van die geloofwaardigheid van so ’n tegniek.

Sedert die ontdekking van chlorokien was dit die eerstekeuse geneesmiddel vir die behandeling en voorkomende behandeling van malaria maar het intussen omtrent alle effektiwiteit teen *Plasmodium falciparum* malaria verloor weens die ontwikkelelende weerstandigheid. Vandag word dit nog steeds wêreldwyd gebruik in die behandeling van *Plasmodium vivax* malaria waar daar nie weerstandigheid teenwoordig is nie. Die historiese sukses van die aminokinolien anti-malaria geneesmiddels het ons besluit, om die kinolien farmakofoor in ons studie te gebruik, ondersteun.

Pirimetamien is tot dusver die mees gebruikte anti-folaat geneesmiddel teen malaria. Dit word gebruik in die voorkomende behandeling van malaria in kombinasie met die sulfoonamiede. Punt mutasies in die parasiet se *dhfr*-eenheid van die *dhfr*-geen is egter besig om die effektiwiteit van hierdie klas geneesmiddels uit te wis.

Ons het die onderskeie farmakofore van chlorokien en pirimetamien as die 7-chlorkinolien- en die diaminopirimidien-eenhede geïdentifiseer. Deur middel van hibridisering van dié twee farmakofore, hoop ons om molekule te sintetiseer met sterk anti-malariële eienskappe en sodoende die handighed in van geneesmiddels soos die kinoliene en pirimetamien te herstel.

Die doel van die studie was om ’n reeks kinolien-pirimidienhibriede te sintetiseer, sekere fisies-chemiese eienskappe te bepaal en te toets vir enige merkwaardige anti-malaria aktiwiteit in vergelyking met dié van chlorokien.

Ons het tien kinoline-pirimidien hibriede suksesvol gesintetiseer deur ’n kinolien- en ’n pirimidienmolekuul met mekaar te verbind deur middel van veskillende verbindingsmolekule. Die strukture van die bereide hibriede is bevestig met kernmagnetieseresonansie-spektroskopie (KMR) en massaspektrometrie (MS).

Die eksperimentele wateroplosbaarheid van al die verbindings is hoër by ’n pH van 5.5 as by ’n pH van 7.4, maar geen struktuur-fisies-chemiese verwantskappe kon afgelei word na aanleiding van ons studie nie.

Die kinolien-pirimidien hibriede is *in vitro* getoets teen die chlorkiensensitiewe stam van *Plasmodium falciparum.* Die kinolien-pirimidieneters se anti-malaria aktiweit het beter vertoon as dié van die kinolien-pirimidienamiene. Verbinding **21**, met ’n feniel verbindingsmolekuul, het die beste anti-malaria-aktiwiteit getoon (IC_{50} = 0.08 µM) van almal en is vergelykbaar met dié van pirimetamien (IC_{50} = 0.11 µM). Geen van die verbindings het egter enigsins beter aktiwiteit as chlorokien getoon nie (IC_{50} = 0.03 µM).

Sleutelwoorde: 4-aminokinolien, pirimetamien, gehibridiseerde geneesmiddels, malaria, geneesmiddel weestandigheid.

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CHAPTER 1

INTRODUCTION AND PROBLEM STATEMENT

1.1 Introduction

Malaria is one of the world's most debilitating diseases and is caused by microscopic, apicomplexan parasites of various species of the *Plasmodium* genus. Half of the global population is under threat of infection with the malaria parasite, 1 million people die and a further 500 million get infected each year (WHO, 2009). The deadly impact of malaria is predominantly felt in Sub-Saharan Africa where approximately 750 000 children die each year of *Plasmodium falciparum* infections (Snow *et al*., 1999; Breman, 2001). This has monumental developmental and economic repercussions on a continent that already battles with political unrest, civil wars, famine and the effect of diseases like tuberculosis and HIV/AIDS (Adams *et al*., 2004).

Another worrying fact is that the occurring resistance to the first line drugs like the quinolines (chloroquine, amodiaquine and mefloquine) and the antifolate combination drugs (sulfadoxine and pyrimethamine) is making the fight against malaria increasingly difficult (Biagini *et al*., 2003). The first reported cases of resistance against the highly effective antimalaria drug, artemisinin, has occurred in South-East Asia (the Cambodia-Thailand border) and is a further cause of concern (WHO, 2009). The development of multi-drug resistance has prompted studies into alternative chemotherapeutic treatments for the disease.

Although several projects that focus on the search for a new class of compounds with novel modes of action are in place, only as little as 1-2% of these drugs will make it into clinical development due to certain specifications it has to abide to (Biagini *et al*., 2005). According to the Medicines for Malaria Venture (MMV), new antimalarial drugs must provide: efficacy against drug-resistant strains *of Plasmodium falciparum*, potential for intermittent treatments (for infants and pregnant women), safety in children younger than 6 months, and in pregnancy, efficacy against *Plasmodium vivax* (including radical cure), efficacy against severe malaria as well as transmission-blocking abilities (MMV, 2011). If a project leads to a new drug that fails to be more advantageous and have lesser toxicity than the existing drugs, such a project should be terminated. A more efficient strategy in the quest to develop an effective and cheaper antimalarial drug appears to be the re-design of existing drugs and the research on known parasite-specific targets (Biagini *et al*., 2005).

Chloroquine has been the antimalarial flag-ship drug since its discovery. Despite the fact that drug resistance made it virtually useless in treating *Plasmodium falciparum* malaria, it continues to be widely used in treating *Plasmodium vivax* malaria in resistance free areas (WHO, 2010b). Keeping the historical success of the aminoquinoline antimalarial drugs in mind, further research on these drugs seems viable. O'Neill (1998) justified future research on aminoquinoline antimalarial drugs by considering their proven effectiveness in the treatment and prophylaxis of malaria, the ease with which they can be synthesised whilst being inexpensive to produce and their relative non-toxicity.

Another antimalaria drug that has been contributing greatly to the treatment and prophylaxis of malaria is the antifolate drug, pyrimethamine. It has been the most widely used antimalarial antifolate drug but point mutations in the parasite's *dhfr* domain of the *dhfr* gene are wiping out its efficacy (Nzila, 2006).

Therefore, it is necessary to search for new derivatives of chloroquine and pyrimethamine with pronounced antimalarial activity.

1.2 Aim and objectives of study

In accordance to the above, the primary aim of this study was to synthesise a series of quinoline-pyrimidine hybrids, determine certain physicochemical properties, and to evaluate their antimalarial activity in comparison to that of chloroquine and pyrimethamine, and their physical combination.

The process of achieving this aim involved the following steps:

- Synthesis and characterisation of quinoline-pyrimidine hybrids, conjugated with different linkers and confirm their structures.
- Determination of the aqueous solubility and the partition coefficient of the hybrids at physiological pH 7.4 and parasitic food vacuole pH 5.5, and of any relationship of these properties with their structures.
- Evaluation of the *in vitro* antimalarial activity of the hybrids.

CHAPTER 2

EPIDEMIOLOGY OF MALARIA

2.1 Introduction

Man has known about malaria and its malicious effect for quite a while. Evidence of a disease causing malaria like symptoms has been found in early Chinese (NeiChing, The Canon of Medicine in 2700 BC), Indian (Sushruta in 500 BC) and Roman scripts. At first, malaria was attributed to toxic air rising from swamplands, but after Charles Laveran microscopically identified parasites in blood smears of patients suffering from malaria in 1880, a connection was made between the disease and a protozoan cause. In 1897, Ronald Ross provided the evidence that linked malaria transmittance to a mosquito vector; the female *Anopheles* mosquito (CDC, 2010).

Five species of malaria are identified as zoonotic parasites: *Plasmodium falciparum, P. vivax, P. malariae, P. ovale and P. knowlesi.* Of these, *P. falciparum* is known to cause the most severe cases of malaria and death. The parasites of *P vivax* and *ovale* species have the ability to become dormant inside their human host and, at a later date, cause a relapse of malaria. *Plasmodium knowlesi,* was at first thought to infect only non-human primates, but has emerged as a zoonotic malaria parasite (Cox-Singh *et al*., 2008).

2.2 Distribution of Malaria

Malaria is distributed worldwide, flourishing in the hot and humid conditions of tropical Africa, Asia, and South and Central America. The five malaria causing *Plasmodium* species have an overlapping geographical distribution throughout the world, but *P. falciparum* and *P. vivax* cause most of the infections. *Plasmodium falciparum* is the most common species in sub-Saharan Africa while *P. vivax* is the predominant species in India and South-America. *Plasmodium ovale* is mostly found in western Africa, while *P. malariae* is distributed in much the same way as *P. falciparum*, but to a lesser extent. Thus far, *P. knowlesi* cases have been localized to Southeast Asia, especially to Malaysia (Guerra *et al*., 2006, Cox-Singh *et al*., 2008).

2.2.1 Malaria in South-Africa

South-Africa is predominantly free of malaria except for the north-eastern parts of KwaZulu-Natal and the lower altitude areas of Limpopo and Mpumalanga, where it borders Swaziland, Zimbabwe and Mozambique (Figure 1) (NDOH, 2010). Preventative measures taken against the *Anopheles* vectors and the *Plasmodium* parasites in these regions have kept the malaria risk comparatively low. Outbreaks of malaria are highest during the rainy season that lasts from September to May (NDOH, 2003).

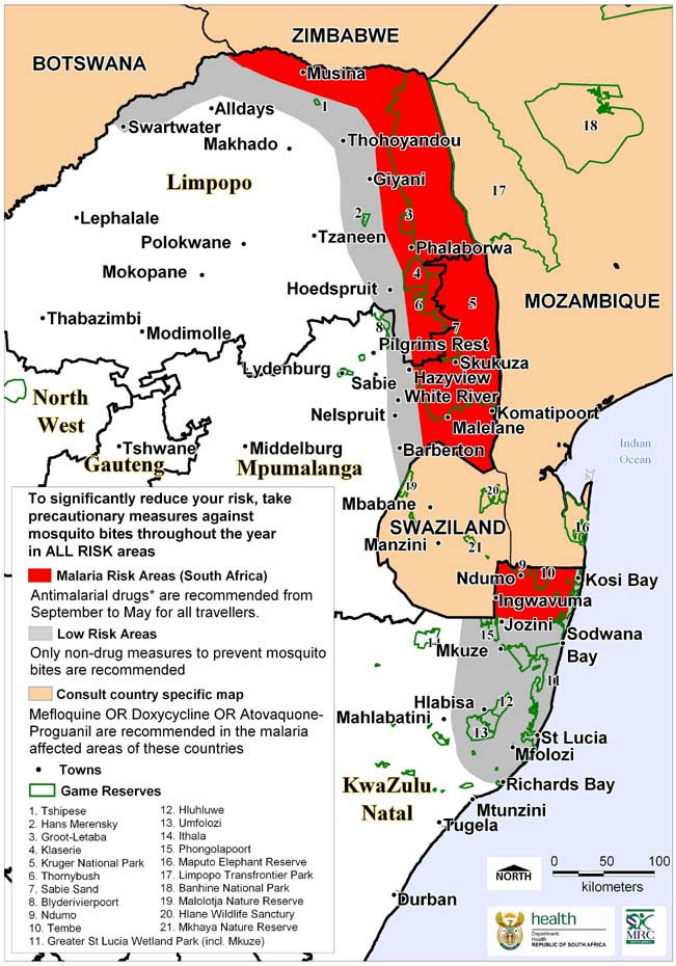


Figure 1 Map of malaria endemic areas in South Africa (NDOH, 2010)

2.2.2 Malaria worldwide

Malaria used to be spread throughout the world, but major campaigns to eradicate the disease was set in place during the post World War II era (Lewison & Srivastava, 2008). Several countries such as Australia, the Netherlands and Singapore, has been documented by WHO to have achieved complete malaria eradication between 1961 and 2010 (WHO, 2010b).

However, malaria is still causing serious health threats in countries in sub-Saharan-Africa, Latin America and Asia (Figure 2). *P. falciparum* is the predominant malaria species in sub-Saharan Africa and the cause of the immense mortality rate. During 2000 the death toll amongst children living in sub-Saharan Africa was estimated at 803 260 (Rowe *et al*., 2006). *P. vivax* is the most prevalent parasite worldwide due to its lower sensitivity to cool temperatures but it seldom causes the death of infected patients (Gething *et al*., 2011).

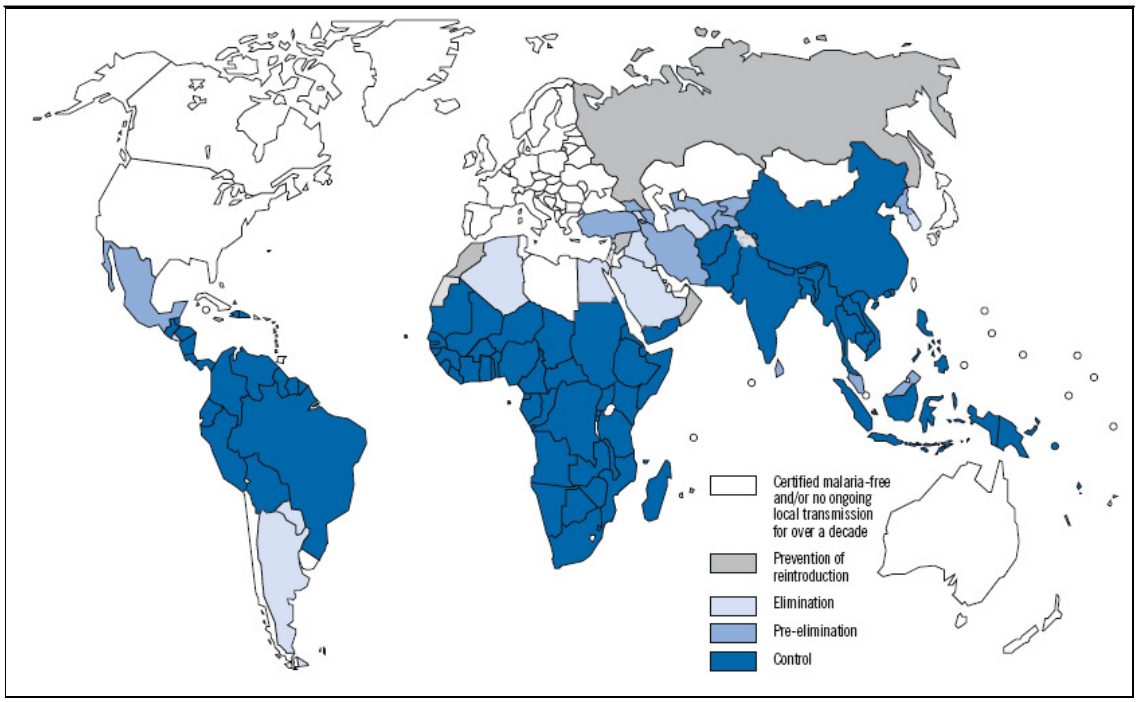


Figure 2 Malaria-free countries and malaria-endemic countries in phases of control, pre- elimination, elimination and prevention of reintroduction, end 2007 (WHO, 2008)

2.2.3 Factors influencing malaria distribution

The survival of the malaria parasite in a certain environment is dependent on the interactions between the parasite, host and vector. For effective malaria transmission to be accomplished, there have to be an abundance of *Anopheles* mosquitoes with a long enough lifespan to support sporogony and enough available hosts. Factors influencing the mosquito population are temperature, altitude, rainfall and the availability of breeding places (Breman, 2001).

Genetic and physiologic properties of the human host play a part in the global distribution of malaria. People living in endemic areas can develop immunity to malaria, which protects them against severe illness and death, although the immunity is only effective while the person is continuously exposed to the parasitic pathogens in that region. This type of

immunity is called premonition and is lost once a person gets isolated from those malarial antigens by leaving the endemic area (Langhorne *et al*. 2008).

Genetic diseases and polymorphisms have been linked to a decrease in malaria infections. The absence of *P. vivax* infections in western Africa is due to the fact that most of the populations do not have a specific receptor, called the Duffy blood group antigen, on the surface of their erythrocytes. Interaction between this receptor and the Duffy binding protein on the surface of merozoites are necessary for invasion of the erythrocytes. This gives a Duffy negative person complete protection against *P. vivax* (Arévalo-Herrera *et al*., 2005).

Some inherited erythrocyte disorders can provide protection against malaria. In cases such as ovalcytosis, a mutation in the erythrocytic membrane causes it to become rigid and inaccessible to merozoite invasion. Sickle cell anaemia and glucose-6-phosphate dehydrogenase deficiency are presumed to cause an inability to handle the extra oxidative stress placed upon the erythrocytes, because of the parasitic metabolism. Consequently, the erythrocytes are destroyed before the parasite can complete schizogony (Ayi *et al*. 2004, Williams, 2006).

2.3 The life cycle of malaria

To understand the pathology of malaria, one has to look at the life cycle of the *Plasmodium* parasite (Figure 3).

A human gets infected with malaria when a *Plasmodium-infected* female *Anopheles* mosquito takes a blood meal and inoculates sporozoites into the skin of the human host. From here the sporozoites enter the bloodstream through capillary endothelial cells. What follows are three asexual reproductive stages *via* the process of schizogony and a sexual reproductive phase:

- Liver stage or exo-erythrocytic schizogony (in human host)
- Erythrocytic stage or erythrocytic schizogony (in human host)
- Sexual stage or gametogenesis (in *Anopheline* vector)
- Sporogony (in *Anopheline* vector)

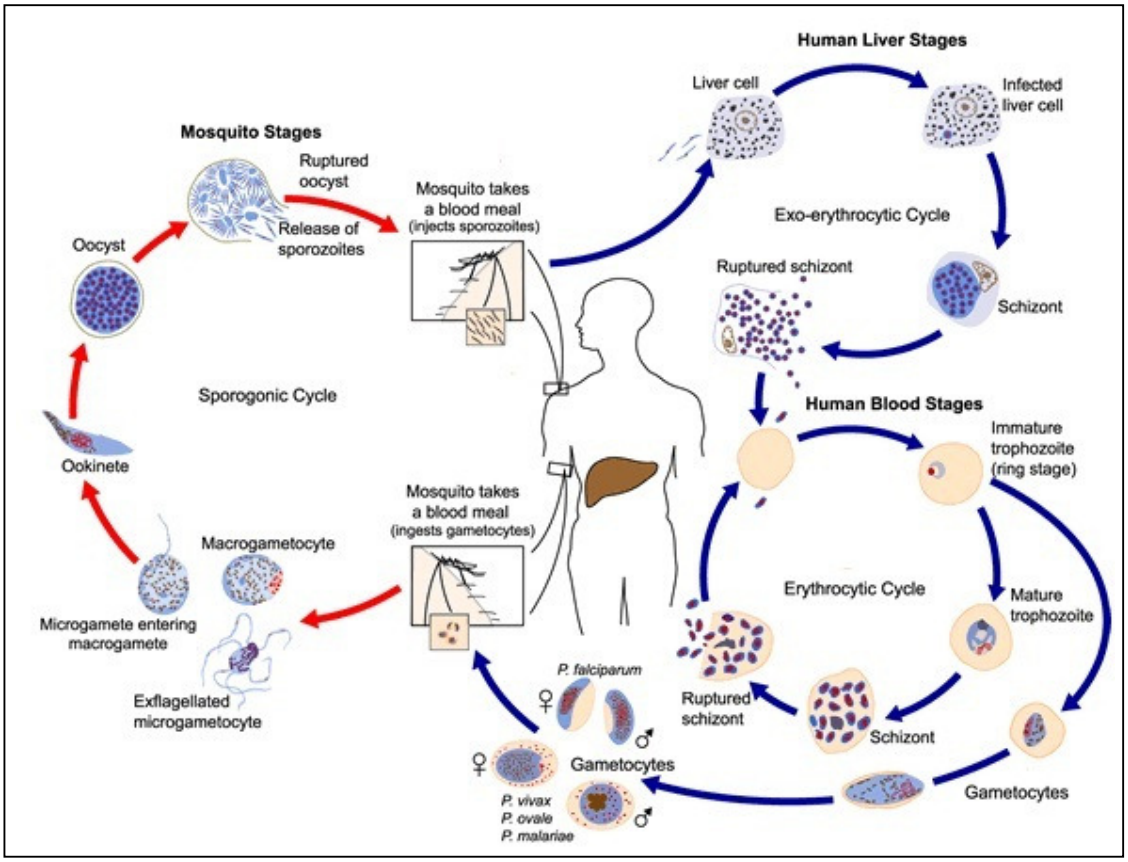


Figure 3 Life cycle of *Plasmodium falciparum*.

2.3.1 Liver Stage

Within an hour of inoculation the sporozoites infect hepatic cells where the process of pre-erythrocytic schizogony starts. After multiple rounds of division, the infected hepatocytes rupture and release thousands of merozoites, into the host's bloodstream where it invades the erythrocytes (Vaughan *et al.*, 2008). *Plasmodium vivax* and *P. ovale* differ from *P. falciparum* in that their sporozoites can enter a dormant phase known as the hypnozoite. Relapse malaria occurs when these hypnozoites reactivate weeks or even years later, undergo schizogony and start to invade the erythrocytes (Wiser, 2008).

2.3.2 Erythrocytic Stage

The merozoites actively invade the erythrocytes in a manner characteristic to apicomplexan parasites as follows. Firstly, the merozoites orientate themselves in such a way that their apical end is next to the host cell after which a tight junction is formed between the parasite and the erythrocyte. Next, the secretory organelles expel their contents into the host cell culminating in a parasitophorous vacuole which later mediates the rupture and the release of parasites from the infected erythrocyte (Baum *et al.*, 2008). The specificity with which the merozoites bind to erythrocytes is attributed to binding proteins on the membranes of the

erythrocytes. This protein specific interaction is a possible focus point for the development of malaria vaccine (Chitnis & Blackman, 2000).

Once inside the erythrocyte, the parasites develop through the ring, trophozoite and schizont stages. It ingests the host cell's haemoglobin and breaks it down to amino acids and the by product, hemozoin or malaria pigment. After several rounds of schizogony the host's erythrocytes rupture and release between sixteen and thirty two merozoites *per* infected erythrocyte together with antigens and waste products into the circulatory system (Miller *et al.*, 2002). The free merozoites are able to invade other erythrocytes to start another round of schizogony.

2.3.3 Sexual stage

After a number of asexual life-cycles, some of the merozoites differentiate into micro- or macrogametocytes, commencing the sexual reproductive stage. Ingestion of these gametocytes by the female *Anopheles* mosquito induces gametogenesis: the maturation of the gametocytes into micro- and macrogametes. The highly mobile microgamete fertilizes the macrogamete by fusing together to produce a zygote (Cowman & Crabb, 2006).

2.3.4 Sporogony

Within a day the zygote develops into an ookinete which is a motile, invasive phase that penetrates the midgut of the mosquito to reach the extracellular space. Here, the ookinete develops into an oocyst. An asexual process called sporogony produces thousands of sporozoites which are released upon maturation and the resulting rupture of the oocyst. The sporozoites migrate to the mosquito's salivary glands where it traverses the glands to settle in the lumen. The sporozoites will be released into a human host when the mosquito takes its next blood meal. This restarts the life-cycle (Wiser, 2008).

2.3.5 Pathology of malaria

The clinical manifestations of malaria are solely due to activities taking place during the erythrocytic stage (Malaguarnera & Musumeci, 2002). A person infected by any of the malaria species will initially experience flu-like symptoms like headache, slight fever, muscle pain and nausea. What follows are the febrile attacks, known as paroxysms, characteristic of a malaria infection (Table 1).

Table 2. Stages of malaria paroxysms (Wiser, 2008)

COLD STAGE	HOT STAGE	SWEATING STAGE
• Experiencing intense cold • Vigorous shivering • Lasts 15-60 minutes	• Intense heat • Dry burning skin • Throbbing headache • Lasts 2-6 hours	• Profuse sweating • Declining temperature • Exhaustion and fatigue leading to sleep • Lasts 2-4 hours

The periodicity of the paroxysms is due to the synchronized development of the schizonts. All the malarial parasites within a host are approximately at the same developmental stage (i.e., merozoite, trophozoite, schizont) resulting in erythrocytic schizogony to happen in a synchronous manner (24 hours for *P. knowlesi,* 48 hours for *P. falciparum*, *P. vivax* and *P. ovale* and 72 hours for *P. malaria*). This leads to the simultaneous rupture of the infected erythrocytes, the release of merozoites into the host's circulatory system and the subsequent malarial paroxysms (Wiser, 2008).

The release of pro-inflammatory cytokines, like tumour necrosis factor-alpha (TNF-a), are stimulated as a response to the "dumping" of parasitic waste products and antigens into the host's bloodstream (Malaguarnera & Musumeci, 2002) and has been linked to the development of the febrile attacks (Karunaweera *et al*., 1992).

P. falciparum is capable of the most lethal attacks and if left untreated, culminates into severe malaria. Infections by *P. vivax, ovale* and *malariae* are rarely lethal, but are a cause of great morbidity.

2.3.5.1 Pathology of *Plasmodium falciparum*

Three properties of the infection by *P. falciparum* parasites that make it more lethal than the other malaria infections are:

- the high level of parasitemia
- their ability to invade all types of erythrocytes
- induction of structural changes to infected erythrocytes

Plasmodium falciparum produces ten to hundred times more parasites than other *Plasmodium* species which leads to the destruction of higher quantity of erythrocytes and the release of antigens into the host's circulatory system; thus a more severe malaria attack (Wiser, 2008).

Plasmodium falciparum parasites are able to infest all types of erythrocytes, in comparison to *P. vivax* which prefers reticulocytes (Miller *et al*., 2002). This non-selective invasion of erythrocytes is further supported by an *in vitro* study done by Chotivanich *et al*. (2000) that suggested that *falciparum* parasites in patients with severe malaria is more virulent than parasites from patients with uncomplicated *falciparum* malaria.

The surface of infected erythrocytes is changed during *P. falciparum* infections. An example is the enhanced permeability of the erythrocytic membrane during the trophic phase which enables the in- and outflux of a wide variety of low molecular weight solutes that helps to satisfy the increased feeding and waste removal needs of the parasites (Kirk *et al*., 1993).

Another modification is the formation of "knoblike" protrusions that is associated with the adherence of infected erythrocytes to endothelial cells. Cytoadherence is mediated by interactions between protein ligands on the surface of the infected erythrocytes e.g. *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP1) and various receptors on the vascular endothelial cells. The affected erythrocytes sequestrate in the capillaries and post-capillary venules of the host in organs such as the brain, lung, gut, heart and placenta resulting in severe complications. This protects the parasite from being destroyed in the spleen (Craig & Scherf, 2001). Some of the *P. falciparum* infected erythrocytes bind to uninfected erythrocytes to form a rosette like clump; a phenomenon called "rosetting". These clumps can block micro-vascular flow and contribute to severe malaria (Mercereau-Puijalon *et al*., 2008)

The three most common syndromes associated with severe *falciparum* malaria, and most often correlated with death are: cerebral infection, severe anaemia and metabolic acidosis. Other complications of severe malaria are: renal failure, circulatory collapse (shock), hypoglycaemia, impaired consciousness, repeated generalised convulsions, prostration or weakness, abnormal bleeding or coagulation, haemoglobinuria, jaundice and hyperpyrexia (WHO, 2000).

2.4 Control of Malaria

The closely integrated symbiosis of the parasite, the human host and the mosquito vector provide two strategies that can be used in the fight against the disease:

- Vector control
- Disease control

2.4.1 Vector Control

Vector control is aimed at killing the malaria infected female *Anopheles* mosquitoes (or their larvae) or minimizing the contact between human hosts and the mosquitoes. To this end, the World Health Organisation prescribes the use of insecticide treated nets and the indoor residual spraying (IRS) of targeted households that are at high risk (WHO, 2009). Complimentary to these measures, environment-based interventions such as the drainage of breeding sites or the managing of stream water flow to kill the mosquito's larvae (Konradsen *et al*, 2004). However, resistance to the pyrethroid insecticides used in treating the nets is a cause of concern. In 2000, South Africa had a dramatic increase in malaria incidence (64 000 cases and 423 deaths) and it was linked to the appearance of the *Anopheles funestus* mosquito, a species showing metabolic resistance to the pyrethroids. The controversial toxin DDT had to be re-introduced in the IRS strategy, and it took immediate effect with the death toll falling to 67 in 2005 (Tren & Bate, 2004).

2.4.2 Disease controlling

Control of the disease is achieved by chemotherapeutic treatment of people with malaria or the prophylactic treatment of people living in or visiting malaria endemic areas. To exercise the most effective antimalarial therapy, factors such as the parasite species, severity of the disease as well as the age, and immune status of the patient need to be considered. The WHO and the South-African National Department of Health published guidelines on the standard treatment with malarial chemotherapy (NDOH, 2010; WHO, 2010b).

The ways in which antimalarial drugs exhibit their activity on the parasite can be categorised as follows:

- *Tissue schizontocides which a*ct upon the liver stage and prevent the invasion of the parasites into the erythrocytes by eliminating developing tissue schizonts or latent hypnozoites and thus preventing relapse.
- *Blood schizontocides which a*ct upon the blood stage of the parasite, eliminating it in the erythrocytes. Acute infections are treated with fast-acting blood schizontocides while slow-acting blood schizontocides are used for prophylaxis.
- *Gametocytocides* are antimalarial agents that prevent infection in mosquitoes by eliminating sexual forms of the parasite in hepatic circulation.
- *Sporontocides:* These antimalarial agents render gametocytes non-infective in the mosquito (Sweetman, 2002; Goldsmith, 1998).

2.4.3 Antimalarial drugs

The classification of antimalaria drugs is based on their structures as follows:

2.4.3.1 Quinoline based

2.4.3.1.1 History of chloroquine

One of the first treatments of malaria was the powdered bark from the cinchona tree (Bruce-Chwatt, 1988). In 1820, one of the active compounds found in the bark, quinine, was extracted by two French pharmacists, P.J. Pelletier and J.B. Caventou (Kumar *et al*., 2009). In the years that followed, quinine became the preferred malaria treatment throughout the world.

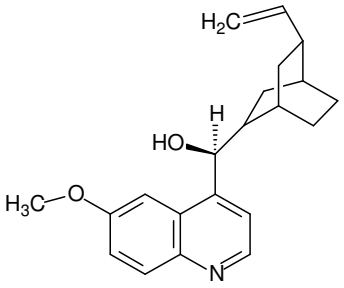


Figure 4 Structure of quinine

With World War I, began the search for synthetic antimalarial drugs. At that time, malaria was still prevalent in many European countries including Germany (Hamoudi & Sachs, 1999). As a result of the War, Germany got isolated from all sources of quinine. By incorporating the work of Guttman and Ehrlich (1891), Schulemann and his German colleagues modified the structure of methylene blue (Vennerstorm *et al*, 1995). They introduced basic side chains (aminoalkylamino side chains) to the structure of methylene blue and one of the resulting compounds showed remarkable antimalarial potential (Wainwright; 2008). Combination of this side chain with a quinoline nucleus led to the development of pamaquine, an 8-aminoquinoline. It was the first synthetic quinoline compound that exhibited antimalaria activity in human *P. falciparum* malaria and the only one with antimalarial activity against the gametocyte phase of the parasite, at that time (WHO; 1955). Later research conducted in the United States of America saw the development of primaquine from pamaquine in 1945; the structural difference being the tertiary amino group of pamaquine, replaced with a primary amine in primaquine (WHO, 1955).

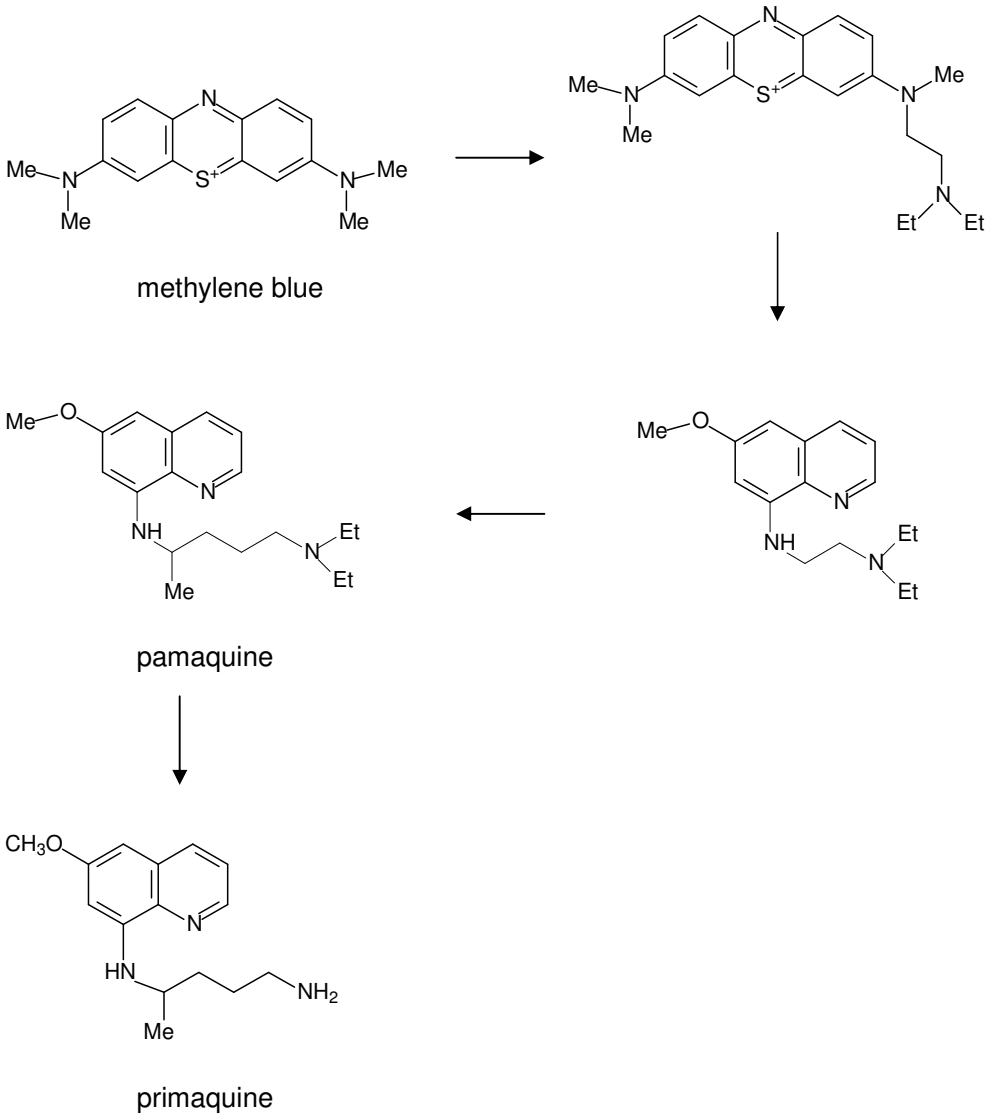
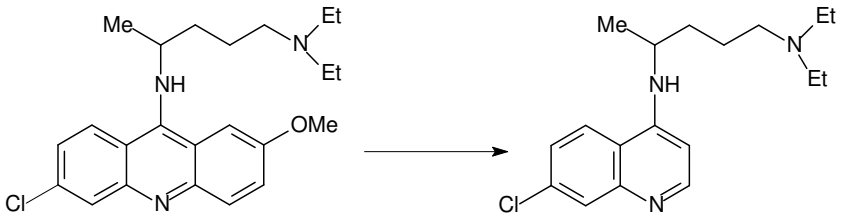


Figure 5 Structures of methylene blue, pamaquine and primaquine

The toxicity of pamaquine and its lack of schizontocidal activity led to further research and scientists at Bayer in German tried to attach the pamaquine side chain to various heterocyclic nuclei. After testing approximately 12 000 compounds, an acridine compound called quinacrine was developed in 1931 and subsequently marketed as Mepacrine (WHO, 1955). Replacement of the acridine moiety of quinacrine with a quinoline nucleus, while keeping the amino side chain, lead to the development of chloroquine (Greenwood, 1995).



quinacrine chloroquine

Figure 6 Structures of quinacrine and chloroquine

2.4.3.1.2 Quinoline derivatives

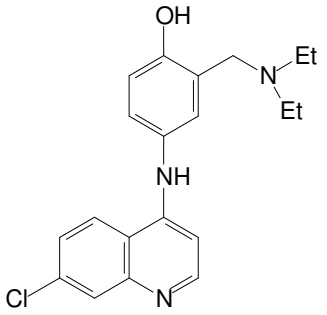
This class of anti malarial drugs is structurally related to quinine and contains a quinoline ring moiety and various aminoalkyl side chains. Quinolines are blood schizontocides and only have activity against the erythrocytic stage of the malaria parasite, except primaquine that is highly effective against the gametocytes of all the *Plasmodium* species as well as the hypnozoites of *P. vivax* and *P. ovale*, and is used to treat and prevent relapse malaria.

Quinine and quinidine are stereoisomers and alkaloids derived from the bark of the cinchona tree. Both quinine and quinidine can be used to treat severe malaria *via* parenteral admission, but quinidine has cardio specific toxicity (Ashley *et al*, 2006).

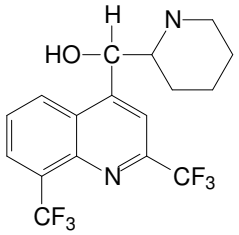
The most famous antimalarial drug in this group is the 4-aminoquinoline, chloroquine. Since its development in 1946, it was extensively used in chemotherapeutic treatment and chemoprophylaxis of malaria. This caused widespread resistance and chloroquine is currently virtually useless in treating *Plasmodium falciparum* malaria. It is, however, still used in treating malaria caused by the other *Plasmodium* species but an increasing number of cases of chloroquine resistance in *P. vivax* are being reported (Baird, 2004).

Some of the adverse effects of chloroquine are pruritus, rash, headache, gastrointestinal disturbance and rarely hair loss and convulsions. Acute overdose is life threatening due to fatal cardiac arrhythmias (WHO, 2010a).

Amodiaquine, a 4-anilinoquinoline, is structurally related to chloroquine, but differs in the side chain where the presence of an aryl ring gives it efficacy against some chloroquine resistant strains of *P. falciparum*. Amodiaquine has adverse effects similar to chloroquine, but has an increased risk of agranulocytosis and hepatoxicity. The World Health Organisation no longer recommends its use malaria prophylaxis (WHO, 2010a).



amodiaquine



mefloquine

Figure 7 Structures of amodiaquine and mefloquine

Mefloquine is a quinoline methanol drug with a long elimination half-life and is therefore an excellent prophylactic drug amongst non-immune travellers because of the once-a-week dosage (CDC, 2010; Shapiro & Goldberg, 2006). Due to neuropsychiatric side effects such as seizures, acute psychosis and nightmares, it is contraindicated in patients with epilepsy, a history of neuropsychiatric disease and patients recovering from cerebral malaria. A link between mefloquine use during pregnancy and stillbirths has been reported, but not yet confirmed (Winstanley *et al*., 2004)

Primaquine, an 8-aminoquinoline, is a tissue schizontocide with intrahepatic activity and the ability to kill hypnozoites of *P. vivax* and *P. ovale* and thus prevent malaria relapse. In combination with a blood schizontocide, a radical malaria cure can be achieved. Primaquine is contra-indicated in patients with G6PD deficiency and may cause haemolytic anaemia. The mechanism of action has not been configured yet (Vale *et al*., 2009).

2.4.3.1.3 Mechanism of action of the quinoline derivatives

The precise mechanism of action of the quinolines is not known, but the fact that the antimalarial activity is exerted exclusively during the erythrocytic stage (except for primaquine), while the parasites are feeding on the host cell's haemoglobin, has strongly suggested that it interferes with the way in which haemoglobin is degraded in the parasitic food vacuole (O'Neil *et al*, 1998).

One of the waste products of the parasite's haemoglobin diet is ferriprotoporphyrin IX (FP); a toxic iron containing molecule. The parasite detoxifies this molecule *via* a crystallization reaction that converts FP into non-toxic hemozoin or malaria pigment (Ridley, 1996).

The most accepted hypothesis is that chloroquine forms a complex with FP that inhibits the formation of hemozoin and results in an accumulation of toxic molecules in the parasite that eventually kills it (Dorn *et al*., 1998).

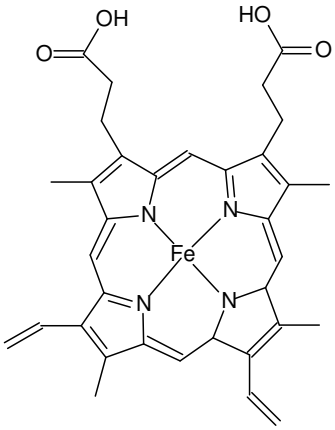


Figure 8 Structure of Ferriprotoporphyrin IX

Chloroquine becomes trapped and accumulates inside the parasitic food vacuole because of its ability to be protonated in an acidic environment. Chloroquine is a diprotic weak base with pKa values at 8.1 and 10.2. Whilst chloroquine can traverse the membranes of infected erythrocytes and move from a physiologic pH of 7.4, down the pH gradient into the parasitic food vacuole in its unprotonated and monoprotonated configurations, it becomes membrane impermeable once it is diprotonated in the acidic compartment of the parasite at pH 5.5 (Fig 11) (O'Neill *et al*, 2006).

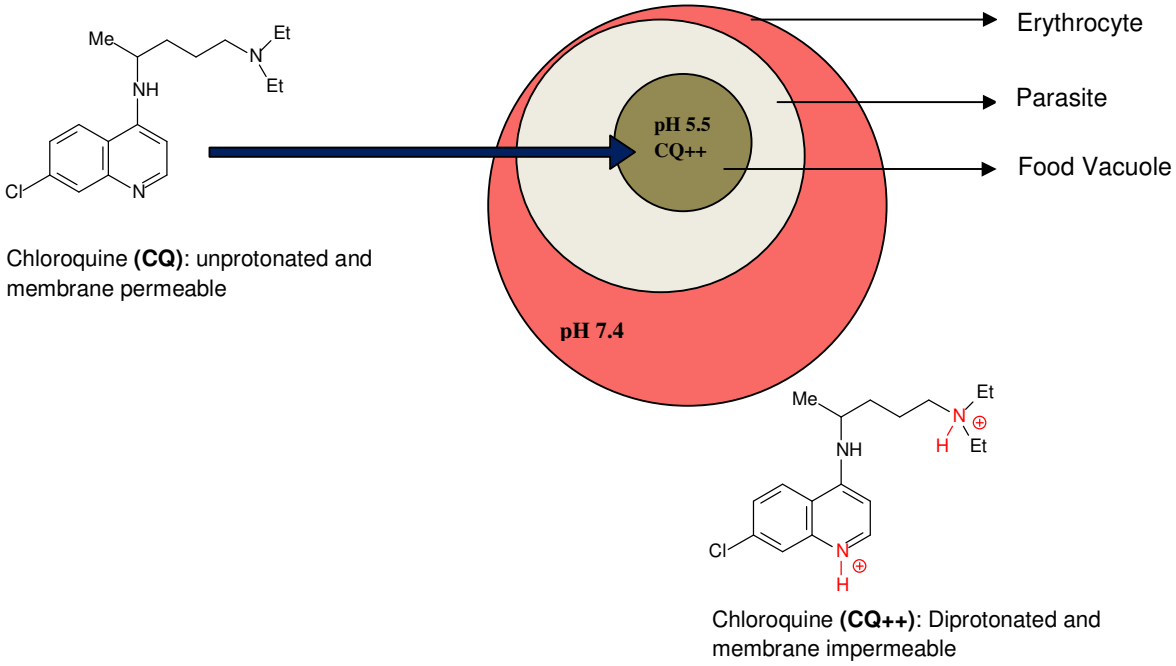
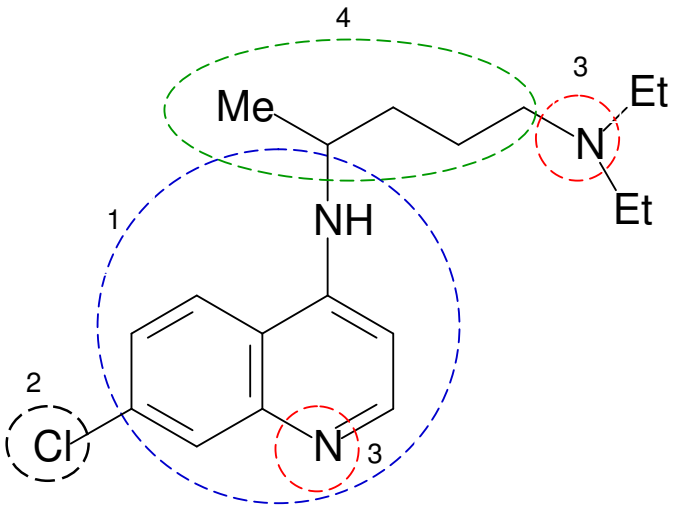


Figure 9 Accumulation of CQ in the parasitic food vacuole (O'Neill *et al*., 2006).

2.4.3.1.4 Structure activity of the chloroquinoline derivatives

The 7-chloro group on the quinoline nucleus seems to be essential for antimalarial activity since its replacement with other electron donating or electron withdrawing groups resulted in a loss of activity (Kaur *et al.*, 2010).

The aminoalkyl side chain attached to 4-aminoquinolines has been researched extensively by experimenting different chain lengths and moiety sizes in the side chain. De *et al.* (1996) found that diaminoalkane side chains shorter than four carbons and longer than seven carbons had activity against chloroquine sensitive and chloroquine resistant strains of *P. falciparum* while Ridley *et al.* (1996) synthesized a library of 4-aminoquinolines with an inter-nitrogen distance of two to three carbons that exhibited activity against chloroquine resistant strains of *P. falciparum.*



1. **Interaction with hematin**: The 4-aminoquinoline serves as the haem binding template.
2. **β-hematin inhibition**: 7-chloro group is required for correct charge distribution and high affinity binding to haem
3. **Accumulation in the food vacuole**: weak basicity afforded by quinoline and terminal tertiary amine assist in vacuolar accumulation through pH trapping
4. **Alkyl side chain**: For optimal efficacy, the carbon chain length has been determined at four carbons. Shorter chains render more active molecules but these are prone to acute toxicity and metabolic drawbacks. Furthermore, together with the terminal nitrogen, it influences the physicochemical properties of the molecule.

Figure 10 Proposed structure activity relationships for chloroquine (Biot *et al, 2005;* O'Neil *et al.*, 2006)

2.4.3.1.5 Mechanism of drug resistance against the quinoline derivatives

The ability of resistant strains of *P. falciparum* to prevent the accumulation of chloroquine in their digestive food vacuoles helped with the formation of hypotheses on how quinoline resistance is acquired (Bray *et al*., 1998).

The most supported hypothesis is the efflux of chloroquine out of the digestive food vacuole *via* a transporter protein. Mutations in the *pfcrt* gene produced strains of *P. falciparum* with a transporter protein in the membrane of their food vacuole called the *Plasmodium falciparum* chloroquine resistance transporter (PfQRT). It is believed to prevent quinoline accumulation and thus the formation of chloroquine-FP bindings (Carlton *et al*., 2001).

2.4.3.2 Hydroxynaphthaquinone derivatives

Exploitation of a specific oxidation site in the mitochondria, coenzyme Q, of blood stage parasites was the strategy followed that lead to the development of atovaquone. Atovaquone is effective in chloroquine resistant *P. falciparum,* but due to rapid development of drug resistance against it, it is now only used in combination therapy with proguanil as the drug Malanil® (Looareesuwan *et al*., 1999).

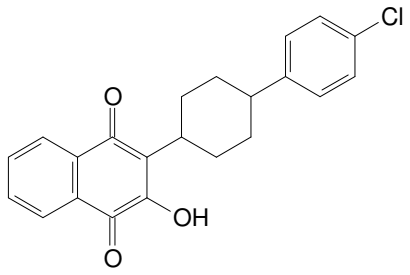
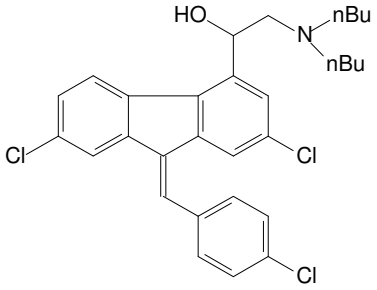


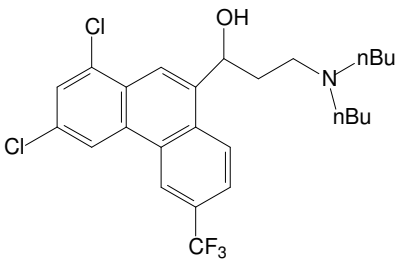
Figure 11 Structure of atovaquone

2.4.3.3 Aryl-amino-alcohol derivatives

Lumefantrine and halofantrine are classified as aryl-amino-alcohol antimalarial drugs. Other drugs in this class are quinine and mefloquine. Lumefantrine is used in combination with the artemisinin derivative, artemether as Coartem® (Ezzet et al., 2000). A comparative study done by Van Agtmael concluded that the lumefantrine-artemether combination had a superior parasite clearance time and tolerability (does not cause QT prolongation) than halofantrine monotherapy (Van Agtmael et al., 1999)



lumefantrine

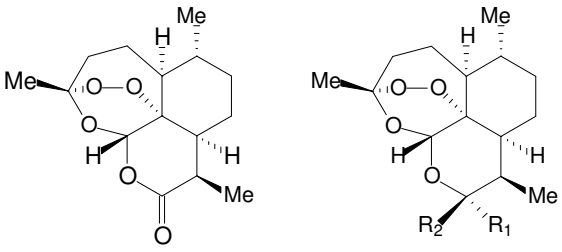


halofantrine

Figure 12 Structures of lumefantrine and halofantrine

2.4.3.4 Sesquiterpene lactones

The traditional Chinese fever treatment for at least the past two millennia has been *Artemisia annua* or the sweet wormwood plant (Ashley *et al*, 2006). The active ingredient of this plant, artemisinin or qinghaosu is a sesquiterpene lactone that is extracted from the leaves. It has an unusual 1,2,4-trioxane moiety in its chemical structure that is believed to be the focus point of the antimalarial activity. Artemisinin has a limited therapeutic use due to its low solubility in oil and water necessitating its reduction to dihydroartemisinin from which a series of more potent analogues e.g. artemether, artether and sodium artesunate was synthesized (Biagini *et al*, 2003).



Dihydroartemisinin	R_1 = H, R_2 = OH
Artemether	R_1 = H, R_2 = OMe
Arteether	R_1 = H, R_2 = OEt
Sodium artesunate	R_1 = H, R_2 = $OCO(CH_2)_2CO_2Na$

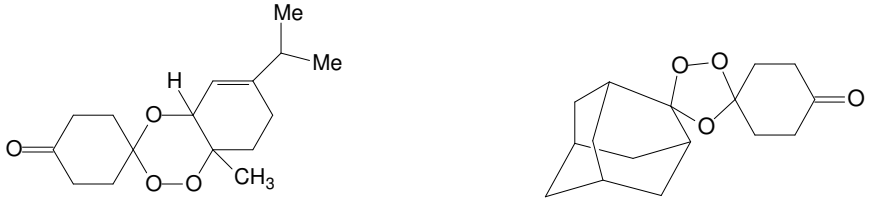
Figure 13 Artemisinin and artemisinin derivatives

Artemisinin is a very potent blood schizontocide that kills the asexual stages of all *Plasmodium* species. It has the ability to clear the parasites 10 - 100 fold more effectively than other antimalarial drugs. The artemisinins, alongside primaquine, is also the only antimalarial drugs to kill the gametocytes of *P. falciparum* (WHO, 2010a).

The artemisinin derivatives are generally well tolerated with side effects including mild gastrointestinal disturbances, dizziness, tinnitus and neutropenia and, are thought to be relatively safe during pregnancy according to studies done by McGready *et al*. (2001). Due to their very short plasma half-lives, these drugs are used in combination therapy with other antimalarials to prevent the development of resistance. Therefore *a*rtemisinin mono-therapy should be avoided to prolong the development of such resistance (WHO, 2010a). According to WHO, artemisinin-based combination therapies (ACTs) are the best treatment for uncomplicated *falciparum* malaria. Although resistance to the artemisinins is slow to develop, cases of treatment failure due to resistance have been reported at the Thailand-Cambodia border (WHO, 2009).

The suggested mechanism by which artemisinin exerts its intraerythrocytic effect on the malaria parasites, derives from its unusual chemical structure. The mechanism includes the cleavage of the endoperoxide-bridge by monomeric haem leading to the formation of carbon-centred free radicals which in turn alkylate haem and other parasitic biomolecules. The process causes cell damage which result in parasite death (Robert & Meunier, 1998).

Artemisinin raw material is obtained in minor quantity from the leaves of the mother plant, *Artemisia annua*, which triggered the search for the design of cheaper, synthetic molecules containing the endoperoxide, pharmacophore of artemisinin. Such molecules have been synthesised (trioxane and trioxolane) and are considered as potent new antimalarial drugs (Vennerstorm *et al*., 2004).



trioxane trioxolane

Figure 14 Structures of trioxane, trioxolane

2.4.3.5 Antifolates

At first, the use of the antifolate drugs was in treating tumour diseases, such as leukaemia, (Farber et al., 1947) after which their role expanded to the treatment of other rapid dividing cells, such as bacteria and parasites. Their mechanism of action lies in their ability to disrupt metabolic pathway responsible for the production of folic acid, an essential co-factor in the synthesis of nucleic acids (Fig 15). Antimalarial antifolates can be divided into two classes depending on their point of action on the folic acid pathway.

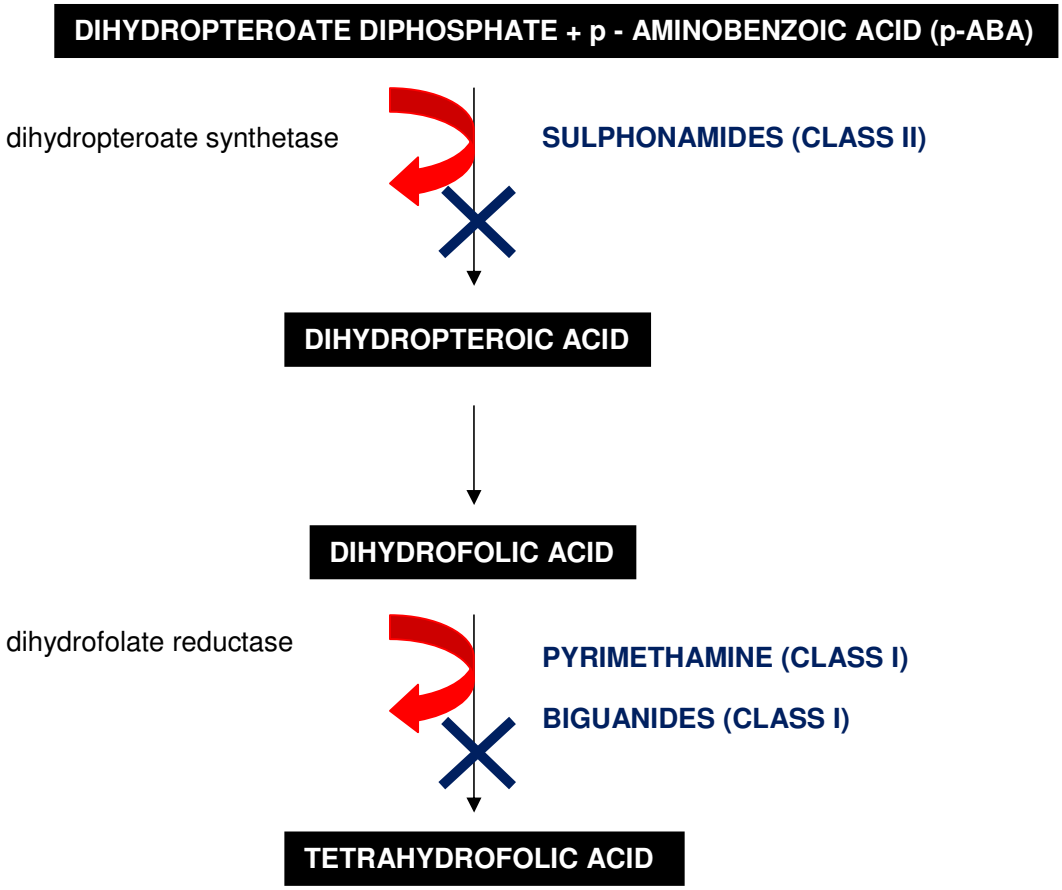
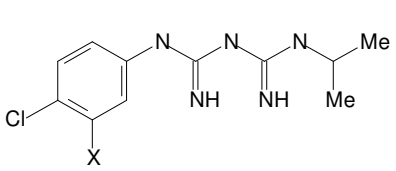


Figure 15 The folic acid pathway

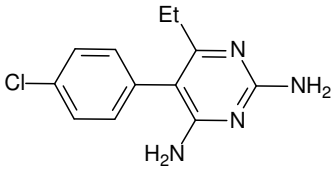
2.4.3.5.1 Class I antifolates

Proguanil, chlorproguanil and pyrimethamine are antifolate drugs that act by inhibition of dihydrofolic acid reductase (DHFR), the enzyme responsible for the reduction of dihydrofolic acid to tetrahydrofolic acid. The result is failure of nucleotide division during schizont formation in the liver- and erythrocytic stage (Shapiro & Goldberg, 2006).



proguanil, X = H,

prochlorguanil, X = Cl



pyrimethamine

Figure 16 Structures of the biguanides, proguanil and prochlorguanil, and pyrimethamine

2.4.3.5.2 Class II antifolates

Dapsone and sulphadoxine are structural analogs of para-aminobenzoic acid (PABA) and competitively inhibits the enzyme, dihydropteroate synthase (DHPS), from catalysing the condensation of dihydropteridine pyrophosphate and PABA to form dihydropteroate. In combination with a DHFR e.g. pyrimethamine (Fansidar®), the folic acid pathway are disrupted at two different points and a synergistic action is achieved (Shapiro & Goldberg, 2006). However, the effectiveness of the antifolates is compromised by drug resistance due to point mutations that cause amino acid substitution in the *dhfr* and *dhps* genotypes of the parasite (Nzila, 2006).

dapsone sulphadoxine

Figure 17 Structures of the sulphonamides, dapsone and sulphadoxine

2.5 Hybrid drugs

The simultaneous treatment of multiple drug targets (polypharmacology) is a tactic used by clinicians to achieve an optimal patient outcome. “Drug cocktails”, where two or more single outcome tablets are used or multicomponent drugs, with two or more active ingredients co-formulated as one tablet, are the currently available methods of polypharmacology. A more recent approach towards polypharmacology is the binding of two or more active ingredients together as a single chemical entity and thus forming a hybrid of the two drugs (Morphy & Rankovic, 2005)

A hybrid molecule is defined as a “chemical entity with two or more structural domains, having different biological functions and dual activity” and thus describing a single molecule that acts as two distinct pharmacophores (Meunier, 2007).

Hybrid molecules can be classified as (Morphy and Rankovic, 2005):

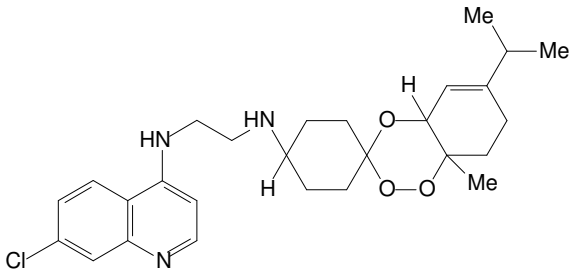
- *Conjugates*: An entity of two pharmacophores separated by a stable linker group that is not found in any of the individuals.
- *Cleavage conjugates*: A compound with linkers that are designed to be metabolised, releasing the individual entities to interact independently.
- *Fused hybrids:* The distance between the different entities is reduced by a small linker in such a manner that the framework of the different pharmacophores are touching.

- *Merged hybrids*: The two pharmacophores are bound together at a commonality in the structures rendering a smaller, simpler molecule.

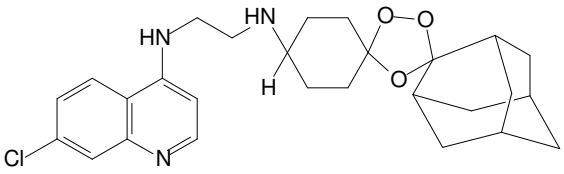
Recently Muregi and Ishih (2010) reviewed potential antimalarial hybrid molecules that have been synthesised over the last decade. Artemisinin and quinoline-based hybrids have been the predominantly researched entities (Lombard *et al*., 2010; 2011).

2.5.1 Artemisinin-based hybrids

Trioxaquines and trioxolaquines are the combined pharmacophores of the artemisinin derived endoperoxide analogs, 1,2,4-trioxane and 1,2,4-trioxalane, and an aminoquinoline moiety. These molecules have a dual method of action by incorporating the haem alkylating properties of the peroxide moiety and the anti-hemozoin properties of the quinoline moiety. The quinoline moiety should contribute to the accumulation of the molecule in the parasite food vacuole. The trioxaquines had a greater antimalarial effect on sensitive and resistant strains than the individual moieties, indicating a synergistic effect of the hybrids (Araújo *et al*., 2009). Other artemisinin-based hybrids are trifluoromethylartemisinin and mefloquine (Grellepois *et al*., 2005), and artemisinin and quinine (Walsh *et al*., 2007).



trioxaquine



trioxolaquine

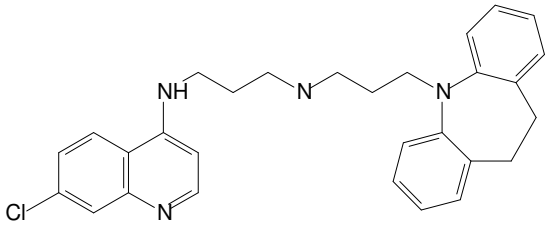
Figure 18 Structures of trioxaquine and trioxolaquine

2.5.2 Quinoline-based hybrids

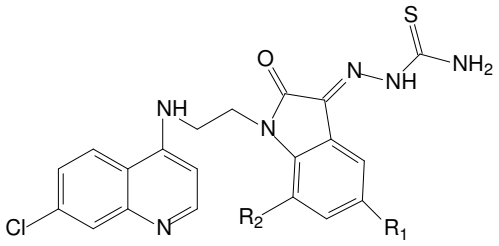
One of the strategies behind quinoline-based hybrids is reversing the resistance attributed to the PfCRT that export the quinoline derivatives out of the parasitic food vacuole. Drugs like imipramine and verapamil has shown the ability to undo this efflux-driven resistance (Van Schalkwyk & Egan, 2006). Burgess synthesised a ‘reversed chloroquine’ molecule consisting

of a 7-chloroquinoline moiety and an imipramine moiety with IC_{50} values lower than that of chloroquine against both chloroquine-sensitive and resistant strains of *P. falciparum*. The remarkable antimalarial activity of this hybrid molecule makes it a viable approach to restore the quinolines as a first line antimalarial drug (Burgess *et al*., 2006).

A potent quinoline-based hybrid with dual activity against the malaria parasite was synthesised by Chiyanzu. A 4-aminoquinoline was hybridised with thiosemicarbazone derivatives of isatin to produce potent *P. falciparum* growth inhibitors. The quinoline moiety again provided the molecule with the ability to accumulate inside the parasite food vacuole and the inhibition of hemozoin formation while the thiosemicarbazone moiety could inhibit cysteine proteases of *P. falciparum* (Chiyanzu *et al*., 2005).



aminoquinoline-imipramine hybrid



R_1 = H, Me, Cl

R_2 = H

aminoquinoline based isatin derivatives

Figure 19 Structures of an aminoquinoline-imipramine hybrid and aminoquinoline based isatin derivatives

Other aminoquinoline hybrids that are under investigation are double prodrugs of the 8-aminoquinoline, primaquine (Vangapandu *et al*., 2003) and ferrocene-quinoline derivatives (N'Da et al., 2010; 2011)

CHAPTER 3

ARTICLE FOR SUBMISSION

Chapter 3 contains the manuscript of an article to be submitted to the Journal of Pharmacy and Pharmacology. The article contains the background, aims, all the experimental details and results of this study, including the physicochemical properties and *in vitro* biological results of quinoline-pyrimidine hybrid drugs. The article is prepared according to the Guide for Authors that can be found on the website of this journal (http://www.onlinelibrary.wiley.com/journal/10.1111/(ISSN)2042-7158/homepage/ForAuthors.html), except that for easy reading figures, schemes and tables are inserted at their logical places as they would appear in the printed version.

Synthesis, characterisation and antimalarial activity of quinoline-pyrimidine hybrids

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Abstract

Objectives The aim of this study was to synthesise a series of quinoline-pyrimidine hybrids, determine their values for selected physicochemical properties and evaluate their *in vitro* antimalarial activity.

Methods The hybrids were brought about in a two-step process by coupling a quinoline and a pyrimidine moiety *via* various linkers. Their structures were confirmed by NMR and MS spectroscopy. The aqueous solubility and log D values where determined in phosphate buffered saline at physiological pH 7.4 and parasitic food vacuole pH 5.5. The hybrids were screened *in vitro* alongside chloroquine and pyrimethamine against the chloroquine sensitive D10 strain of *P. falciparum*.

Key findings The aqueous solubility of all the compound were greater at pH 5.5 than at pH 7.4 but no structure-physicochemical property could be drawn from this investigation. The IC_{50} values revealed all of the hybrids to possess antimalarial activity against the D10 strain. None of the compounds showed better activity than chloroquine. However, hybrid **21**, featuring a piperazine linker showed the best antimalarial activity of all, exhibiting similar activity than pyrimethamine.

Conclusions Hybridisation of a quinoline and a pyrimidine moiety renders compounds with moderate to good antimalarial activity, though none with more potency than that of chloroquine. Nevertheless it did lead to one hybrid with similar antimalarial potency to that of pyrimethamine and is thus worthwhile investigating a broader series of quinoline-pyrimidine hybrids.

Keywords: 4-aminoquinoline, pyrimethamine, hybrid drugs, malaria, drug resistance.

1. Introduction

Malaria treatment is a growing therapeutic challenge due to the rapid appearance of multi-drug resistant *Plasmodium* parasites [1]. Chloroquine and the combination drug, pyrimethamine/sulfadoxine, used to be the first line drugs in malaria treatment and prophylaxis but is now virtually useless against *Plasmodium falciparum* parasites [2].

Quinoline-based antimalarial drugs such as chloroquine are structurally derived from quinine, a compound extracted from the bark of the cinchona tree [3]. The proposed mechanism of action of these drugs is the formation of a toxic complex between the quinoline and ferriprotoporphyrin IX (FP), a waste product of haemoglobin digestion, inside the food vacuole of malaria parasites [4].

Resistance to the 4-aminoquinolines is thought to be due to mutations in the *pfcrt* gene of *P. falciparum.* The resultant mutated parasite strains have a transporter protein in the

membrane of their food vacuole called the *Plasmodium falciparum* chloroquine resistance transporter (PfQRT). This transporter protein causes the efflux of quinoline out of the food vacuole and hereby prevents the formation of quinoline-FP bindings [5].

Historically, the quinoline antimalarial drugs proved to be very effective in treating malaria while having the advantages of being easy to synthesise and relatively non-toxic [6]. In attempts to restore the antimalarial efficacy of these drugs, much research has been done on structural changes in the quinoline moiety [7] as well as the aminoalkyl side chain [8, 9]

The simultaneous treatment of multiple drug targets (polypharmacology) is a tactic used by clinicians to achieve an optimal patient outcome. “Drug cocktails”, where two or more single outcome tablets are used or multicomponent drugs, with two or more active ingredients co-formulated as one tablet, are the currently available methods of polypharmacology. A recent strategy in pharmaceutical chemistry is the synthesis of compounds that contain two or more pharmacophores in a single entity [10]. Burgess *et al* [11] synthesised hybrid molecules consisting of a chloroquinoline moiety and an imipramine moiety with IC_{50} values lower than that of chloroquine against both chloroquine-sensitive and resistant strains of *P. falciparum*. The remarkable antimalarial activity of this hybrid molecule makes hybridisation a viable approach in the attempt to restore the quinolines as a first line antimalarial drug.

The antifolates are drugs that exhibit their antimalarial activity by disrupting the folic acid pathway of the parasites. Of these drugs, pyrimethamine has been the most widely used, but point mutations in the parasite’s *dhfr* domain of the *dhfr* gene are wiping out its antimalarial effectiveness [12]. The antifolates and quinolines target independent areas in the *Plasmodium* physiology; that is the folate-pathway and the parasitic food vacuole respectively [13]. A molecule containing these two moieties is anticipated to exhibit both antimalarial properties of the quinolines as well as pyrimethamine as explained by Meunier [14].

In the search for new, potent antimalarial drugs we synthesised hybrid entities by combining a quinoline moiety with a pyrimidine moiety, the respective moieties of the chloroquine and pyrimethamine.

The aim of this study was to synthesise quinoline-pyrimidine hybrids, determine their physicochemical properties such as (S_w) and distribution coefficient (log D) and evaluate their antimalarial activity compared to that of chloroquine and pyrimethamine.

2. Materials and methods

2.1. Materials

4,7-dichloroquinoline and 2,6-diamino-4-chloroquinoline were purchased from Hangzhou Dayangchem Co., Ltd (China). 2-aminoethan-1-ol, 2-aminopropan-1-ol, 3-aminopropan-1-ol, 2-(2-aminoethoxy)ethan-1-ol, 2-aminobutan-1-ol, 1,2-diaminoethane, 1,3-diaminopropane, 1,4-diaminobutane, piperazine and 1,4-diaminobenzene were purchased from Sigma-Aldrich, Ltd. HPLC grade acetonitrile was obtained from Labchem South Africa. All the reagents and chemicals were of analytical grade.

2.2. General procedures

Thin-layer chromatography (TLC) was performed using silica gel plates ($60F_{254}$ Merck). Preparative flash column chromatography was carried out on silica gel (230-240 mesh, G60 Merck) and silica gel 60 (70-230 mesh ASTM, Fluka).

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 600 spectrometer (at a frequency of 600.17 and 150.913 MHz, respectively) in deuterated chloroform ($CDCl_3$), methanol (MeOD) or dimethylsulfoxide (DMSO-d_6). Chemical shifts are reported in parts per million (δ ppm) using tetramethylsilane (TMS) as internal standard. The splitting pattern abbreviations are as follows: s (singlet), d (doublet), dd (doublet of doublets), dt (doublet of triplets), t (triplet), tt (triplet of triplet), q (quartet), dq (doublet of quartets), p (pentaplet), h (hexaplet) and m (multiplet). Mass spectra were obtained on a Waters Synapt G2 spectrometer equipped with electrospray ionization sources. The melting points were measured on a Stuart Melting Point SMP 10 and given in degrees Celsius (^{o}C)

2.3. High performance liquid chromatography (HPLC)

The HPLC system consisted of an Agilent 1100 series auto sampler, Agilent 1100 series variable wave detector (VWD) and Agilent 1100 series isocratic pump. A Zorbax Eclipse XDB C18, 5 µm (150 x 4.60 mm) column was used and the Agilent Chemstation rev A08.03 for LC systems software package for data analysis.

The compounds were quantified using a gradient method (A = 0.2 % triethylamine in H_2O, pH 7.0, B = acetonitrile) at a flow rate of 1 ml/min with 20 µl standard sample injections. The gradient consisted of 25 % of solvent B (ACN) until 1 min, then increased linearly to 95 % of B after 10 min, and held for 15 min, where after the instrument was re-equilibrated to the starting conditions for 5 min. A calibration plot of peak area versus drug concentration for each compound showed excellent linearity ($0.993 < r^2 \leq 1$) over the concentration range (0–2000 µg/ml) employed for the assays. The absorption maximum for compounds (**12** – **21**) as well as for pyrimethamine was at 210 nm; this wavelength was consequently used for the

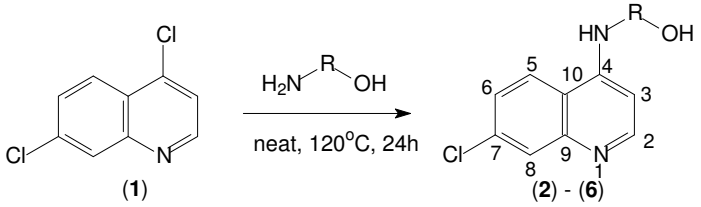
HPLC detection. New mobile phase was prepared for each sample batch that was analysed by HPLC. The peak retention times (t_R) were 3.64 min for (**12**), 3.67 min for (**13**), 3.73 min for (**14**), 3.41 min for (**15**), 3.99 min for (**16**), 3.50 min for (**17**), 3.59 min for (**18**), 3.72 min for (**19**), 3.97 min for (**20**) and 3.75 min for (**21**).

3. Experimental procedures

The synthesis of quinoline–pyrimidine hybrids followed a two step process. In the first step either hydroxyl- or amine-functionalised quinolines are synthesised by nucleophilic substitution of either amino alcohols or diamines to 4,7-dichloroquinoline. In the second, a given intermediate is treated with 2,6-diamino-4-chloropyrimidine in the presence of sodium hydride, leading to the corresponding hybrid.

3.1. Synthesis of hydroxyl-functionalised quinolines

The hydroxyl-functionalised quinolines (**2** - **6**) were synthesised by using, with slight modifications, the general method reported by N'Da *et al.* [15] and is described as follows: a mixture of 4,7-dichloroquinoline (4,951g; 25mmol) and an aminoalcohol (250 mmol; 10.0 equiv.) were refluxed and stirred at 120°C for 24 hours. The progress of the reaction was monitored by thin layer chromatography (TLC). After completion, the reaction mixture was poured into distilled water and the precipitate was filtered. The residue was recrystallised in ethyl acetate (EtOAc) to yield the pure intermediate product. (See Scheme 1). The NMR and MS data of all compounds are reported.



Compounds	R
(**2**)	$(CH_2)_2$
(**3**)	CH_2-$CH(CH_3)$
(**4**)	$(CH_2)_3$
(**5**)	$(CH_2)_2$-O-$(CH_2)_2$
(**6**)	$CH(CH_2$-$CH_3)$-CH_2

Scheme 1: Synthesis of hydroxyl-functionalised quinolines (**2** – **6**)

*3.1.1 2-[(7-Chloroquinolin-4-yl) amino] ethan-1-ol (**2**) (See Scheme 1)*

Compound (**2**) was obtained in 94% (5.3 g) yield as an off-white solid. Mp: 220-221 °C. ^{1}H NMR (600 MHz, DMSO): δ (ppm) 8.37 (d, *J* = 5.3 Hz, 1H, H-2), 8.25 (d, *J* = 2.0 Hz, 1H, H-8), 7.77 (d, *J* = 9.0 Hz, 1H, H-5), 7.39 (dd, *J* = 2.2, 8.6 Hz, 1H, H-6), 6.48 (d, *J* = 5.3 Hz, 1H, H-3), 4.87 (s, 1H, H-11), 3.65 (t, *J* = 4.9 Hz, 2H, H-13), 3.47 (td, *J* = 5.2, 6.01 Hz, 2H, H-12). ^{13}C NMR (151 MHz, DMSO): δ (ppm) 151.89 (C-2), 150.25 (C-4), 133.38 (C-7), 127.49 (C-8), 124.07 (C-5), 124.02 (C-6), 98.07 (C-3), 58.7 (C-13), 45.13 (C-12).

3.1.2 1-[(7-Chloroquinolin-4-yl) amino] propan-2-ol (**3**) *(See Scheme 1)*

Compound (**3**) was obtained in 89% (5.3 g) yield as an off-white solid. Mp: 172-175 °C. ^{1}H NMR (600 MHz, MeOD): δ (ppm) 8.37 (d, *J* = 5.3 Hz, 1H, H-2), 8.13 (d, *J* = 2.5.0 Hz, 1H, H-8), 7.81 (d, *J* = 8.9 Hz, 1H, H-5), 7.44 (dd, *J* = 2.0, 8.7 Hz, 1H, H-6), 6.61 (d, *J* = 5.3 Hz, 1H, H-3), 4.88 (s, 1H, H-11), 3.89 – 3.59 (m, 2H, H-14), 3.36 (m, 1H, H-13), 1.30 (d, *J* = 5.4 Hz, 3H, H-12). ^{13}C NMR (151 MHz, MeOD): δ (ppm) 151.53 (C-2), 150.94 (C-4), 135.01 (C-7), 126.16 (C-8), 124.68 (C-5), 124.25 (C-6), 98.49 (C-3), 65.14 (C-14), 49.92 (C-13), 19.82 (C-12).

3.1.3 3-[(7-Chloroquinolin-4-yl) amino] propan-1-ol (**4**) *(See Scheme 1)*

Compound (**4**) was obtained in 96% (5.7 g) yield as an off-white solid. Mp: 142-145 °C. ^{1}H NMR (600 MHz, MeOD): δ (ppm) 8.37 (d, *J* = 5.3 Hz, 1H, H-2), 8.02 (d, *J* = 2.0 Hz, 1H, H-8),

7.76 (d, *J* = 9.0 Hz, 1H, H-5), 7.36 (dd, *J* = 2.1, 8.2 Hz, 1H, H-6), 6.51 (d, *J* = 5.3 Hz, 1H, H-3), 4.88 (s, 1H, H-11), 3.89 – 3.59 (t, *J* = 4.9 Hz, 2H, H-14), 3.45 (td, *J* = 5.1, 6.8 Hz, 2H, H-13), 2.17 – 1.93 (m, 2H, H-12). ^{13}C NMR (151 MHz, MeOD): δ (ppm) 152.73 (C-2), 152.47 (C-4), 136.27 (C-7), 127.70 (C-8), 125.95 (C-5), 124.10 (C-6), 99.61 (C-3), 60.89 (C-14), 41.37 (C-12), 32.19 (C-13).

3.1.4 2-{2-[(7-Chloroquinolin-4-yl) amino] ethoxy} ethanol-1-ol (**5**) *(See Scheme 1)*

Compound (**5**) was obtained as an off-white powder in yield of 88% (5.85 g). Mp: 130-132 ℃. ^{1}H NMR (600 MHz, MeOD): δ (ppm) 8.35 (d, *J* = 5.2 Hz, 1H, H-2), 8.09 (d, *J* = 1.9 Hz, 1H, H-8), 7.77 (d, *J* = 8.3 Hz, 1H, H-5), 7.45 (dd, *J* = 2.2, 8.9 Hz, 1H, H-6), 6.79 (d, *J* = 6.3 Hz, 1H, H-3), 4.93 (s, 1H, H-11), 3.77 (t, *J* = 28.3 Hz, 2H, H-16), 3.72-3.70 (t, *J* = 27.6 Hz, 4H, H-13 & H-15), 3.60 (t, *J* = 24.2 Hz, 2H, H-12). ^{13}C NMR (151 MHz, MeOD): δ (ppm) 152.72 (C-2), 152.43 (C-4), 136.34 (C-7), 127.58 (C-8), 126.02 (C-5), 124.37 (C-6), 99.80 (C-3), 70.25 (C-15), 69.82 (C-13), 62.21 (C-16), 43.95 (C-12).

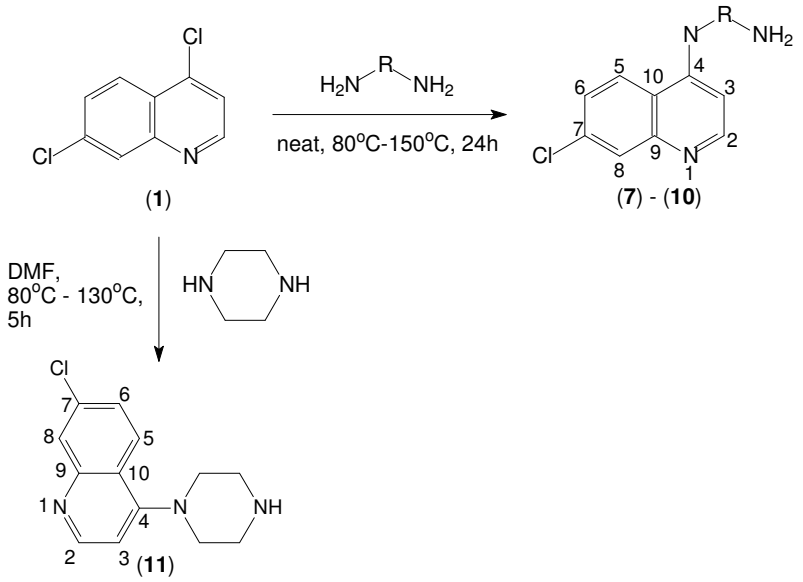
3.1.5 2-[(7-Chloroquinolin-4-yl) amino] butan-1-ol (**6**) *(See Scheme 1)*

Compound (**6**) was obtained in 86% (5.4 g) yield as an off-white solid. Mp: 201-203 ℃. ^{1}H NMR (600 MHz, DMSO): δ (ppm) 8.36 (d, *J* = 5.2 Hz, 1H, H-2), 8.10 (d, *J* = 2.5 Hz, 1H, H-8), 7.76 (d, *J* = 8.9 Hz, 1H, H-5), 7.46 (dd, *J* = 2.5, 8.9 Hz, 1H, H-6), 6.79 (d, *J* = 5.3 Hz, 1H, H-3), 4.81 (s, 1H, H-11), 3.89 – 3.59 (m, H, H-14), 3.36 (m, 2H, H-15), 1.82 – 1.45 (m, 2H, H-13), 0.90 (t, *J* = 7.4 Hz, 3H, H-12). ^{13}C NMR (151 MHz, DMSO): δ (ppm) 151.79 (C-2), 150.29 (C-4), 133.36 (C-7), 127.37 (C-8), 124.41 (C-5), 123.77 (C-6), 98.89 (C-3), 62.44 (C-15), 55.98 (C-14), 23.54 (C-13), 10.66 (C-12).

3.2. Synthesis of amine-functionalised quinolines

The amine-functionalised quinolines (**7** - **9**) was synthesised by means of the process reported on by N'Da *et al.* [16], with slight modifications, and is described as follows: a mixture of 4,7-dichloroquinoline (4,951g, 25 mmol) and a diamine (250 mmol; 10.0 equiv) was heated at 80 ℃ for one hour and then at 135 ℃ for five hours while stirring vigorously. For amine-functionalised quinolines (**10** & **11**), a mixture of the quinoline and the diamine was dissolved in anhydrous dimethylformamide (DMF) after which the method was used as described for compounds (**7** - **9**). The process was monitored by thin layer chromatography. At the end of the reaction, the reaction mixture was cooled to room temperature while stirring. The reaction mixture was basified with 1M NaOH (50ml) and washed with distilled water (150ml) where after the product was extracted with hot EtOAc (3x 150ml). The organic phase was dried over $MgSO_4$ for an hour and the solvent removed under reduced pressure. The resulting residue was redissolved in boiling ethyl acetate, and allowed to recrystallise at 0-5℃ to afford the amine-functionalised intermediate (see Scheme 1). The NMR data of all

compounds are reported.



Compounds	R
(**7**)	$(CH_2)_2$
(**8**)	$(CH_2)_3$
(**9**)	$(CH_2)_4$
(**10**)	p-C_6H_4

Scheme 2: Synthesis of amine-functionalised quinolines (**7** – **11**)

3.2.1 N-(2-aminoethyl)-7-chloroquinolin-4-amine (**7**) *(See Scheme 2)*

Compound (**7**) was obtained as an off-white powder. ^{1}H NMR (600 MHz, CD_3OD): δ (ppm) 8.35 (d, *J* = 5.3 Hz, 1H, H-2), 8.08 (d, *J* = 23.8 Hz, 1H, H-8), 7.74 (d, *J* = 37.8 Hz, 1H, H-5), 7.39 (dd, *J* = 5.2, 9.6 Hz, 1H, H-6), 6.55 (d, *J* = 5.2 Hz, 1H, H-3), 3.45 (t, *J* = 6.1 Hz, 2H, H-13), 2.99 (t, *J* = 6.1 Hz, 2H, H-12). ^{13}C NMR (151 MHz MHz, CD_3OD): δ (ppm) 152.83 (C-2), 149.68 (C-4), 136.34 (C-7), 127.62 (C-8), 124.34 (C-5), 118.82 (C-6), 98.71 (C-3), 46.37 (C-13), 40.88 (C-12).

3.2.2 N-(3-aminopropyl)-7-chloroquinolin-4-amine (**8**) *(See Scheme 2)*

Compound (**8**) was obtained as a feintly off-yellow powder. ^{1}H NMR (600 MHz, CD_3OD): δ (ppm) 8.34 (d, *J* = 2.2 Hz, 1H, H-2), 8.10 (d, *J* = 39.7 Hz, 1H, H-8), 7.76 (d, *J* = 7.5 Hz, 1H, H-5), 7.38 (dd, *J* = 2.8, 8.6 Hz, 1H, H-6), 6.51 (d, *J* = 2.2 Hz, 1H, H-3), 4.93 (s, 1H, H-11), 3.38

(t, *J* = 35.3 Hz, 2H, H-14), 2.82 (t, *J* = 6.5 Hz, 2H, H-12), 1.85 (m, 2H, H-13). ^{13}C NMR (151 MHz, CD_3OD): δ (ppm) 152.68 (C-2), 149.67 (C-4), 136.29 (C-7), 127.61 (C-8), 125.96 (C-5), 124.28 (C-6), 99.65 (C-3), 41.73 (C-14), 40.28 (C-12), 32.03 (C-13).

3.2.3 N-(4-aminobutyl)-7-chloroquinolin-4-amine (**9**) *(See Scheme 2)*

Compound (**9**) was obtained as an off-white powder. ^{1}H NMR (600 MHz, DMSO): δ (ppm) 8.37 (d, *J* = 5.3 Hz, 1H, H-2), 8.26 (d, *J* = 9.0 Hz, 1H, H-8), 7.76 (d, *J* = 1.5 Hz, 1H, H-5), 7.44 (dd, *J* = 2.8, 9.7 Hz, 1H, H-6), 6.43 (d, *J* = 5.3 Hz, 1H, H-3), 3.23 (t, *J* = 25.72 Hz, 2H, H-15), 2.63-2.52 (m, 4H, H-12 & H-13), 1.77-157 (m, 2H, H-14). ^{13}C NMR (151 MHz, DMSO): δ (ppm) 151.92 (C-2), 150.09 (C-4), 133.30 (C-7), 127.47 (C-8), 124.13 (C-5), 123.93 (C-6), 98.59 (C-3), 42.40 (C-15), 41.45 (C-12), 30.90 (C-14), 25.34 (C-13).

3.2.4 1-N-(7-chloroquinolinyl-yl) benzene-1,4-diamine (**10**) *(See Scheme 2)*

Compound (**10**) was obtained as a dark-brown powder. ^{1}H NMR (600 MHz, DMSO): δ (ppm) 8.79 (d, *J* = 5.9 Hz, 1H, H-2), 8.39 (d, *J* = 9.0 Hz, 1H, H-8), 8.31 (d, *J* = 5.2 Hz, 1H, H-5), 7.81 (dd, *J* = 1.5, 8.2 Hz, 1H, H-6), 7.48 (d, *J* = 8.8 Hz, 1H, H-3), 6.98 (d, *J* = 8.0 Hz, 2H, H-13 & H-17), 6.64 (d, *J* = 5.2 Hz, 2H, H-14 & H-16), 5.13 (s, 2H, H-18). ^{13}C NMR (151 MHz, DMSO): δ (ppm) 151.80 (Ar, *C*-NH_2), 150.05 (C-2), 149.46.10 (C-4), 133.60 (NH-Ar, NH-*C*), 127.53 (C-7), 126.37 (C-8), 124.33 (C-5), 124.22 (C-6), 117.58 (C-14 & C-16), 114.54 (C-14 & C-17), 100.24 (C-3).

3.2.5 7-Chloro-4-(piperazin-1-yl) quinoline (**11**) *(See Scheme 2)*

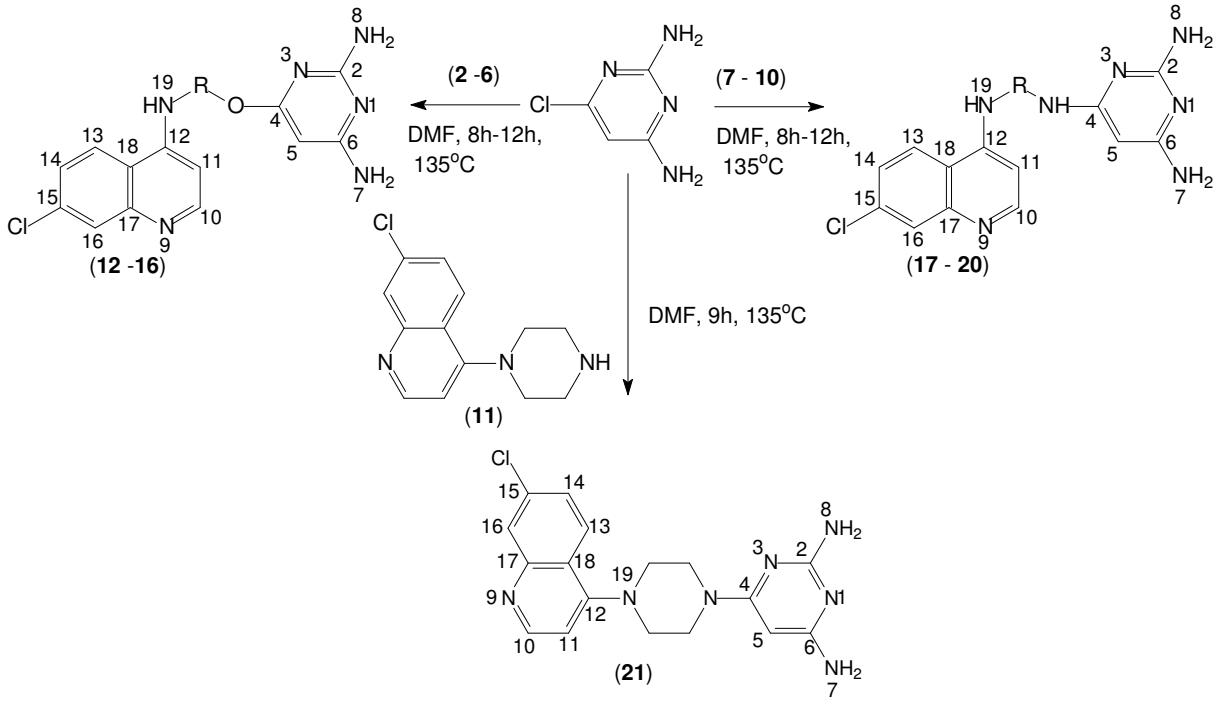
Compound (**11**) was obtained as an off-white powder. ^{1}H NMR (600 MHz, DMSO): δ (ppm) 8.67 (d, *J* = 5.0 Hz, 1H, H-2), 7.79 (d, *J* = 5.4 Hz, 1H, H-8), 7.52 (d, *J* = 9.0 Hz, 1H, H-5), 7.45 (dd, *J* = 1.9, 9.0 Hz, 1H, H-6), 6.94 (d, *J* = 5.0 Hz, 1H, H-3), 3.06 (t, *J* = 8.3 Hz, 4H, H-13 & H-15), 2.94 (t, *J* = 4.0Hz, 4H, H-12 & H-16). ^{13}C NMR (151 MHz, DMSO): δ (ppm) 156.88 (C-2), 152.18 (C-4), 133.46 (C-7), 128.03 (C-8), 126.10 (C-5), 125.58 (C-6), 109.22 (C-3), 53.21 (C-13 & C-15), 45.51 (C-14 & C-16).

3.3. The synthesis of quinoline-pyrimidine hybrids

3.3.1 General procedure for the synthesis of pyrimidine-ethers and -amines of quinoline

The second step in the process was the etherification of given hydroxyl-functionalised quinolines to pyrimidine and the amidation of given amine-functionalised quinolines to pyrimidine as depicted in Scheme 3: A mixture of a given intermediate (**2** - **11**) (10 mmol) and 90% sodium hydride (NaH, 10 mmol) in a 1:1 ratio was stirred in DMF at room temperature for 1 hr. While continuously stirring, the mixture was heated to 135 ℃ and 2,6-diamino-4-chloro-pyrimidine (50 mmol; 5 equiv.) was added proportionately over 30 minutes. The process was monitored by thin layer chromatography. After completion, the mixture was

dissolved in MeOH and purified by flash chromatography on silica gel eluting with MeOH and DCM (ratios MeOH:DCM = 4:1) as mobile phase. Products emerged as off white, brown and yellow powders. ^{1}H and ^{13}C NMR chemical shifts, melting points as well as ES+MS data of compounds **(12** – **21)** are reported.



Compounds	R
(**12**)	$(CH_2)_2$
(**13**)	CH_2-$CH(CH_3)$
(**14**)	$(CH_2)_3$
(**15**)	$(CH_2)_2$-O-$(CH_2)_2$
(**16**)	$CH(CH_2$-$CH_3)$-CH_2
(**17**)	$(CH_2)_2$
(**18**)	$(CH_2)_3$
(**19**)	$(CH_2)_4$
(**20**)	p-C_6H_4

Scheme 3: Synthesis of quinoline-pyrimidine hybrids (**12** – **21**).

3.3.1.1 6-{2-[(7-chloroquinolin-4-yl)amino]ethoxy}pyrimidine-2,4-diamine (**12**)

Compound (**12**) was purified by flash silica gel column chromatography eluting with methanol (MeOH) and dichloromethane (DCM): MeOH:DCM (4:1) to give light yellow powder: 1.67 g (51.1%) yield. Melting point: 203-203.5 ℃ $C_{15}H_{15}ON_6Cl$. ^1H NMR (600 MHz, MeOD) δ 9.22 (d, *J* = 5.4 Hz, 1H, H-10,), 9.05 (d, *J* = 9.0 Hz, 1H, H-13), 8.60 (d, *J* = 2.0 Hz, 1H, H-16),

8.33 (t, *J* = 5.1 Hz, 1H, H-19), 8.24 (dd, *J* = 9.0, 2.0 Hz, 1H, H-14), 7.39 (d, *J* = 5.5 Hz, 1H, H-11), 6.86 (s, 2H, H-7), 6.72 (s, 2H, H-8), 5.87 (s, 1H, H-5), 5.17 (t, *J* = 5.6 Hz, 2H, H-21), 4.39 (q, *J* = 5.4 Hz, 2H, H-20). ^{13}C NMR (151 MHz, MeOD) δ 179.50 (C-4), 175.63 (C-6), 172.50 (C-2), 161.61 (C-10), 159.72 (C-12), 158.56 (C-17), 143.11 (C-15), 137.02 (C-16), 133.83 (C-14), 133.67 (C-13), 126.97 (C-18), 108.46 (C-11), 85.95 (C-5), 71.95 (C-21), 51.61 (C-20). MS ES+ 331.1 ($M+H^+$).

3.3.1.2 6-{2-[(7-chloroquinolin-4-yl)amino]propoxy}pyrimidine-2,4-diamine (**13**)

Derivative (**13**) was purified by flash silica column chromatography eluting with MeOH:DCM (4:1) to produce an off-white powder: 1.59 g (45.8%) yield. Melting point: 220-221 °C. $C_{16}H_{17}ON_6Cl$. ^{1}H NMR (600 MHz, DMSO) δ 8.43 (d, *J* = 5.4 Hz, 1H, H-10), 8.21 (d, *J* = 9.0 Hz, 1H, H-13), 7.77 (d, *J* = 1.9 Hz, 1H, H-16), 7.55 (t, *J* = 5.3 Hz, 1H, H-19), 7.40 (d, *J* = 10.9 Hz, 1H, H-14), 6.64 (d, *J* = 5.4 Hz, 1H, H-11), 6.02 (s, 2H, H-7), 5.90 (s, 2H, H-8), 5.31 (h, *J* = 6.1 Hz, 1H), 5.01 (s, 1H, H-5), 3.49 (dt, *J* = 13.0, 6.2 Hz, 1H, H-22a), 3.37 (d, *J* = 5.4 Hz, 1H, H-22b), 1.28 (d, *J* = 6.2 Hz, 3H, H-20). ^{13}C NMR (151 MHz, DMSO) δ 169.60 (C-4), 166.05 (C-6), 162.88 (C-2), 152.06 (C-10), 150.03 (C-12), 149.06 (C-17), 133.37 (C-15), 127.50 (C016), 124.09 (C-14), 124.01 (C-13), 117.31 (C-18), 99.01 (C-11), 76.86 (C-5), 67.90 (C-22), 47.52 (C-21), 18.31 (C-20). MS ES+ 345.1 ($M+H^+$).

3.3.1.3 *6-{3-[(7-chloroquinolin-4-yl)amino]propoxy}pyrimidine-2,4-diamine* (**14**)

Derivative (**14**) was purified by flash silica column chromatography eluting with MeOH:DCM (4:1) to produce a brown powder: 1.57 g (45.5%) yield. Melting point: 228-230 °C. $C_{16}H_{17}ON_6Cl$. ^{1}H NMR (600 MHz, DMSO) δ 8.37 (d, *J* = 5.4 Hz, 1H, H-10), 8.25 (d, *J* = 9.0 Hz, 1H, H-13), 7.78 (d, *J* = 2.4 Hz, 1H, H-16), 7.44 (dd, *J* = 9.0, 2.2 Hz, 1H, H-14), 7.34 (t, *J* = 5.2 Hz, 1H, H-19), 6.47 (d, *J* = 5.5 Hz, 1H, H-11), 6.03 (s, 2H, H-7), 5.85 (s, 2H, H-8), 5.07 (s, 1H, H-5), 4.19 (t, *J* = 6.2 Hz, 2H, H-22), 3.36 (q, *J* = 6.7 Hz, 2H, H-20), 2.01 (p, 2H, H-21). ^{13}C NMR (151 MHz, DMSO) δ 170.12 (C-4), 166.06 (C-6), 163.02 (C-2), 151.97 (C-10), 150.16 (C-12), 149.04 (C-17), 133.49 (C-15), 127.48 (C-16), 124.17 (C-14), 124.13 (C-13), 117.50 (C-18), 98.73 (C-11), 76.20 (C-5), 62.62 (C-22), 39.44 (C-20), 27.62 (C-21). MS ES+ 345.1 ($M+H^+$).

3.3.1.4 6-2-{2-[(7-chloroquinolin-4-yl)amino]ethoxy}ethoxy)pyrimidine-2,4-diamine (**15**)

Derivative (**15**) was purified by flash silica column chromatography eluting with MeOH:DCM (4:1) to produce an off-white powder: 1.98 g (52.8%) yield. Melting point: 109-110 °C. $C_{17}H_{19}O_2N_6Cl$. ^{1}H NMR (600 MHz, DMSO) δ 8.38 (d, *J* = 5.3 Hz, 1H, H-10), 8.24 (d, *J* = 9.0 Hz, 1H, H-13), 7.78 (s, 1H, H-16), 7.44 (d, *J* = 8.9 Hz, 1H, H-14), 7.33 (t, *J* = 5.1 Hz, 1H, H-19), 6.51 (d, *J* = 5.4 Hz, 1H, H-11), 6.01 (s, 2H, H-7), 5.87 (s, 2H, H-8), 5.03 (s, 1H, H-5), 4.22 (t, 2H, H-24), 3.69 (t, *J* = 5.7 Hz, 4H, H-21 & H-23), 3.46 (q, *J* = 5.4 Hz, 2H, H-20).

^{13}C NMR (151 MHz, DMSO) δ 169.85 (C-4), 166.02 (C-6), 162.90 (C-2), 151.95 (C-10), 150.05 (C-12), 149.09 (C-17), 133.41 (C-15), 127.53 (C-16), 124.14 (C-14), 124.03 (C-13), 117.43 (C-18), 98.79 (C-11), 76.19 (C-5), 68.95 (C-21), 68.12 (C-23), 63.85 (C-24), 42.29 (C-20). MS ES+ 375.1 ($M+H^+$).

3.3.1.5 6-{2-[(7-chloroquinolin-4-yl)amino]butoxy}pyrimidine-2,4-diamine (**16**)

Derivative (**16**) was purified by flash silica column chromatography eluting with MeOH:DCM (4:1) to produce a cream-white powder: 2.15 g (59.9%) yield. Melting point: 221-223 °C. $C_{17}H_{19}ON_6Cl$. ^{1}H NMR (600 MHz, DMSO) δ 8.37 (d, *J* = 5.5 Hz, 1H, H-10), 8.33 (d, *J* = 9.0 Hz, 1H, H-13), 7.77 (d, *J* = 2.2 Hz, 1H, H-16), 7.42 (dd, *J* = 9.1, 2.2 Hz, 1H, H-14), 7.02 (d, *J* = 8.0 Hz, 1H, H-19), 6.60 (d, *J* = 5.8 Hz, 1H, H-11), 6.02 (s, 2H, H-7), 5.89 (s, 2H, H-8), 5.00 (s, 1H, H-5), 4.31 (dd, *J* = 10.9, 6.8 Hz, 1H, H-23a), 4.13 (dd, *J* = 10.9, 5.3 Hz, 1H, H-23b), 3.89 (h, *J* = 5.9 Hz, 1H, H-22), 1.73 (tt, *J* = 13.0, 7.4 Hz, 1H, H-21a), 1.69 – 1.60 (m, 1H, H-21b), 0.92 (t, *J* = 7.4 Hz, 3H, H-20). ^{13}C NMR (151 MHz, DMSO) δ 170.10 (C-4), 166.14 (C-6), 163.02 (C-2), 152.11 (C-10), 150.38 (C-12), 149.23 (C-17), 133.70 (C-15), 127.48 (C-16), 124.41 (C-14), 124.21 (C-13), 117.54 (C-18), 99.27 (C-11), 76.46 (C-5), 66.12 (C-23), 53.29 (C-22), 23.94 (C-21), 10.55 (C-20). MS ES+ 359.1 ($M+H^+$).

3.3.1.6 4-N-{2-[(7-chloroquinolin-4-yl)amino]ethyl}pyrimidine-2,4,6-triamine (**17**)

Derivative (**17**) was purified by flash silica column chromatography eluting with MeOH:DCM (4:1) and recrystallised in EtOAc to produce light yellow crystals: 1.83 g (55.4%) yield. Melting point: 229-230 °C. $C_{15}H_{16}N_7Cl$. ^{1}H NMR (600 MHz, DMSO) δ 8.40 (d, *J* = 5.4 Hz, 1H, H-10), 8.18 (d, *J* = 9.0 Hz, 1H, H-13), 7.77 (d, *J* = 2.2 Hz, 1H, H-16), 7.59 (s, 1H, H-19), 7.39 (dd, *J* = 9.0, 2.1 Hz, 1H, H-14), 6.52 (d, *J* = 5.5 Hz, 1H, H-11), 6.45 (s, 1H, H-22), 5.61 (d, *J* = 17.7 Hz, 4H, H-7 & H-8), 4.87 (s, 1H, H-5), 3.46 (m, 2H, H-21), 3.33 (q, *J* = 5.6 Hz, 2H, H-20). ^{13}C NMR (151 MHz, DMSO) δ 164.26 (C-4), 163.99 (C-6), 162.92 (C-2), 152.09 (C-10), 150.10 (C-12), 148.98 (C-17), 133.36 (C-15), 127.43 (C-16), 124.14 (C-13), 124.05 (C-14), 117.31 (C-18), 98.61 (C-11), 74.36 (C-5), 43.77 (C-21), 38.47 (C-22). MS ES+ 330.1 ($M+H^+$)

3.3.1.7 4-N-{3-[(7-chloroquinolin-4-yl)amino]propyl}pyrimidine-2,4,6-triamine (**18**)

Derivative (**18**) was purified by flash silica column chromatography eluting with MeOH:DCM (4:1) to produce a light yellow powder: 1.24 g (36.0%) yield. Melting point: 196-197 °C. $C_{16}H_{18}N_7Cl$. ^{1}H NMR (600 MHz, DMSO) δ 8.37 (d, *J* = 5.4 Hz, 1H, H-10), 8.25 (d, *J* = 9.1 Hz, 1H, H-13), 7.77 (d, *J* = 2.2 Hz, 1H, H-16), 7.43 (dd, *J* = 9.0, 2.2 Hz, 1H, H-14), 7.30 (t, *J* = 5.2 Hz, 1H, H-19), 6.46 (d, *J* = 5.4 Hz, 1H, H-11), 6.14 (s, 1H, H-23), 5.54 (s, 2H, H-7), 5.35 (s, 2H, H-8), 4.84 (s, 1H, H-5), 3.29 (q, *J* = 6.6 Hz, 2H, H-20), 3.18 (m, 2H, H-22), 1.85 (p, *J* = 6.9 Hz, 2H). ^{13}C NMR (151 MHz, DMSO) δ 164.34 (C-4), 163.97 (C-6), 162.92 (C-2),

151.96 (C-10), 150.09 (C-12), 149.08 (C-17), 133.39 (C-15), 127.49 (C-16), 124.10 (C-13), 124.06 (C-14), 117.49 (C-18), 98.72 (C-11), 40.31 (C-20), 38.22 (C-22), 27.84 (C-21). MS ES+ 344.1 ($M+H^+$).

3.3.1.8 4-N-{4-[(7-chloroquinolin-4-yl)amino]butyl}pyrimidine-2,4,6-triamine (**19**)

Derivative (**19**) was purified by flash silica column chromatography eluting with MeOH:DCM (4:1) to produce an off-white powder: 877 mg (24.5%) yield. Melting point: 159-160 °C. $C_{17}H_{20}N_7Cl$. ^1H NMR (600 MHz, DMSO) δ 8.37 (d, *J* = 5.3 Hz, 1H, H-10), 8.25 (d, *J* = 9.0 Hz, 1H, H-13), 7.77 (s, 1H, H-16), 7.43 (dd, *J* = 10.9, 5.1 Hz, 1H, H-14), 7.30 (t, *J* = 4.9 Hz, 1H, H-19), 6.46 (t, *J* = 5.5 Hz, 1H, H-11), 6.05 (s, 1H, H-24), 5.54 (d, *J* = 12.3 Hz, 2H, H-7), 5.34 (d, *J* = 9.7 Hz, 2H, H-8), 4.83 (d, *J* = 14.1 Hz, 1H, H-5), 3.27 (dq, *J* = 12.6, 6.4 Hz, 2H), 3.21 – 3.10 (m, 2H), 1.67 (p, *J* = 7.3 Hz, 2H), 1.57 (p, *J* = 6.9 Hz, 2H). ^{13}C NMR (151 MHz, DMSO) δ 164.31 (C-4), 163.95 (C-6), 162.90 (C-2), 151.97 (C-10), 150.09 (C-12), 149.11 (C-17), 133.37 (C-15), 127.49 (C-16), 124.12 (C-13), 123.99 (C-14), 117.46 (C-18), 98.70 (C-11), 73.90 (C-5), 42.24 (C-20), 40.04 (C-23), 26.89 (C-22), 25.41 (C-21). MS ES+ 358.1 ($M+H^+$).

3.3.1.9 *4-N-{4-[(7-chloroquinolin-4-yl)amino]phenyl}pyrimidine-2,4,6-triamine* (**20**)

Derivative (**20**) was purified by flash silica column chromatography eluting with MeOH:DCM (4:1) to produce a dark yellow powder: 476mg (12.5%) yield. Melting point: 222-223 °C. $C_{19}H_{16}N_7Cl$. ^1H NMR (600 MHz, DMSO) δ 8.98 (s, 1H, H-19), 8.58 (s, 1H, H-26), 8.42 (d, *J* = 9.0 Hz, 1H, H-10), 8.38 (d, *J* = 5.4 Hz, 1H, H-13), 7.85 (d, *J* = 2.2 Hz, 1H, H-16), 7.71 – 7.61 (m, 2H, H-21 & H-25), 7.52 (dd, *J* = 8.9, 2.3 Hz, 1H, H-14), 7.23 – 7.14 (m, 2H, H-22 & H-24), 6.68 (d, *J* = 5.4 Hz, 1H, H-11), 5.81 (s, 2H, H-7), 5.64 (s, 2H, H-8), 5.21 (s, 1H, H-5). ^{13}C NMR (151 MHz, DMSO) δ 164.66 (C-4), 162.93 (C-6), 161.47 (C-2), 151.92 (C-10), 149.55 (C-12), 149.09 (C-17), 138.88 (C-20), 133.78 (C-23), 132.26 (C-15), 127.60 (C-16), 124.63 (C-13), 124.46 (C-22 & C-24), 124.37 (C-14) , 120.01 (C-21 & C-25), 117.91 (C-18), 100.80 (C-11), 76.37 (C-5) MS ES+ 378.1 ($M+H^+$).

3.3.1.10 *6-[4-(7-chloroquinolin-4-yl)piperazine-1-yl]pyrimidine-2,4-diamine* (**21**)

Derivative (**21**) was purified by flash silica column chromatography eluting with MeOH:DCM (4:1) and recrystallised in EtOAc to give yellow crystals: 1.92 g (53.9%) yield. Melting point: 154-155 °C. $C_{17}H_{18}N_7Cl$. ^1H NMR (600 MHz, DMSO) δ 8.71 (d, *J* = 5.0 Hz, 1H, H-10), 8.10 (d, *J* = 9.0 Hz, 1H, H-13), 7.99 (d, *J* = 2.2 Hz, 1H, H-16), 7.56 (dd, *J* = 9.0, 2.2 Hz, 1H, H-14), 7.03 (d, *J* = 5.1 Hz, 1H, H-11), 5.77 (s, 2H, H-7), 5.54 (s, 2H, H-8), 5.12 (s, 1H, H-5), 3.19 (m, 4H, C-21 & C-23), 3.16 (d, *J* = 3.6 Hz, 4H, C-20 & C-24). ^{13}C NMR (151 MHz, DMSO) δ 165.32 (C-4), 163.76 (C-6), 162.76 (C-2), 156.28 (C-10), 152.23 (C-12), 149.65 (C-

17), 133.62 (C-15), 128.06 (C-16), 126.14 (C-13), 125.86 (C-14), 121.41 (C-18), 109.57 (C-11), 74.21 (C-5), 51.56 (C-20 & C-24), 43.64 (C-21 & C-23). MS ES+ 356.1($M+H^+$).

3.4. Physicochemical properties

3.4.1. Solubility

The aqueous solubility values (S_w) of compounds (**12** – **21**) and pyrimethamine were obtained by preparing saturated solutions in phosphate buffers at pH 7.4 and pH 5.5 (Table 1). The slurries were stirred with magnetic bars in a water bath at 37 ℃ for 24 h. An excess of solute was present at all times to provide saturated solutions. After 24 h, the solutions were filtered and analyzed directly by HPLC to determine the concentration of solute dissolved in the solvent. The experiment was performed in triplicate [17].

3.4.2. Experimental log D

Equal volumes of *n*-octanol and phosphate buffer solution of pH 7.4 were saturated with each other under vigorous stirring for at least 24 h. An excess amount of compound was mixed with 0.75 ml of pre-saturated *n*-octanol; the solution was then stoppered and agitated for 10 min in 1.50 ml graduated tubes (0.5 ml division). Subsequently 0.75 ml of pre-saturated buffer was transferred to the tubes containing the before mentioned solutions.

The tubes were stoppered and agitated for 45 min then centrifuged at 4 000 rpm (1503 G) for 30 min. The *n*-octanol and aqueous phases were allowed to separate at room temperature for 5 min, where after their volume ratio (v/v; *n*-octanol:buffer) was determined. The volume ratio was found in all cases to be 1.

Of the n-octanol phase, 250 µl were diluted with methanol to a factor of four, filtered and analysed by HPLC. The phosphate buffer phase was filtered and measured directly by HPLC. From this data the concentrations of the derivative in both phases were determined. The log D values (log (octanol: pH 7.4 buffer partition coefficient)) were calculated as logarithmic ratios of the concentrations in the *n*-octanol phase to the concentrations in the buffer. The experiment was performed in triplicate and the results expressed as means are listed in Table 1.

3.5. In vitro *biological studies*

The derivatives were tested in triplicate against the D10 strain of *Plasmodium falciparum*. Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method reported by Trager and Jensen [18]. Quantitative assessment of antiplasmodial activity *in vitro* was determined *via* the parasite lactate dehydrogenase assay using a modified method described by Makler [19].

The test samples were prepared as a 2 mg/mL stock solution in 10% dimethyl sulfoxide (DMSO) and sonicated to enhance solubility. Stock solutions were stored at -20℃. Further

dilutions were prepared on the day of the experiment. Chloroquine was used as the reference drugs in all experiments. A full-dose response was performed for all compounds to determine the concentration inhibiting 50% of parasite growth (IC_{50}).

For a range starting from 100 ng/mL the following was applied: The starting concentration of 100 ng/mL was serially diluted, 2-fold in complete medium to give ten dilutions with the lowest concentration being 0.2 ng/mL. Thus the concentrations considered were 100 ng/mL, 50 ng/mL, 25 ng/mL, 12.5 ng/mL, 6.25 ng/mL, 3.125 ng/mL, 1.56 ng/mL, 0.78 ng/mL, 0.39 ng/mL and 0.195 ng/mL. The same dilution technique was used for all samples. The samples are tested in triplicate. The solvents to which the parasites were exposed to had no measurable effect on the parasite viability. The IC_{50}-values were obtained using a non-linear dose-response curve fitting analysis via Graph Pad Prism v.4.0 software, and the values on molar basis in Table 2 were obtained by dividing those on mass basis by the molecular weight of each compound.

4. Results and Discussion

4.1. Chemistry

The quinoline-pyrimidine hybrids were prepared by covalent binding of 4,7-dichloroquinoline to an appropriate amino alcohol or diamine linker to form five hydroxyl-functionalised and five amine-functionalised intermediate molecules (**2** – **11**). 2,6-diamino-4-chloropyrimidine was then respectively bound to each of the intermediates to form hybridised pyrimidine-ethers and -amines of quinoline (**12** – **21**). The yield of the purified products ranged from 12.5 - 59.9 %. Purification was done by flash column chromatography.

The chemical structures of compounds (**12** – **21**) were confirmed by NMR and MS ES+ data. In the ^{1}H NMR spectrum, the quinoline moiety was identified by four distinctive doublet signals (H-10, H-13, H-16 and H-11) and one doublet of doublet (H-14) signal in the upper end of the spectrum (the 9.22 and 6.46 ppm region) that accounts for the aromatic hydrogens. The diaminopyrimidine moiety was identified by three characteristic singlets for the amino hydrogens (H-7 and H-8) and the solitary aromatic hydrogen (H-5) in the 6.86-4.84 ppm region. In the ^{13}C NMR spectrum, the quinoline moiety was identified by the signals in the region of 152.09, 150.10, 148.98, 133.36, 127.43, 124.14, 124.05, 117.31 and 98.61 ppm which corresponds to the aromatic carbons: C-10, C-12, C-17, C-15, C-16, C-13, C-14, C-18 and C-11. The presence of the pyrimidine moiety was determined by three signals in the region of 164.26, 163.99 and 162.92 ppm that correspond to the aromatic carbons, C-4, C-6 and C-2 and a distinctive signal in the 74.36 ppm region corresponding to aromatic carbon, C-5.

The MS ES+ data for the compounds confirmed the presence of molecular ions at 331.1

($M+H^+$) for (**12**), 345.1 ($M+H^+$) for (**13**), 345.1 ($M+H^+$) for (**14**), 375.1 ($M+H^+$) for (**15**), 359.1 ($M+H^+$) for (**16**), 330.1 ($M+H^+$) for (**17**), 344.1 ($M+H^+$) for (**18**), 358.1 ($M+H^+$) for (**19**), 378.1 ($M+H^+$) for (**20**) and 356.1 ($M+H^+$) for (**21**) which corresponds to the molecular formulae $C_{15}H_{15}ON_6Cl$, M_w = 330.78 (**12**), $C_{16}H_{17}ON_6Cl$, M_w = 344.80 (**13**), $C_{16}H_{17}ON_6Cl$, M_w = 344.80 (**14**), $C_{17}H_{19}O_2N_6Cl$, M_w = 374.83 (**15**), $C_{17}H_{19}ON_6Cl$, M_w = 358.83 (**16**), $C_{15}H_{16}N_7Cl$, M_w = 329.79 (**17**), $C_{16}H_{18}N_7Cl$, M_w = 343.82 (**18**), $C_{17}H_{20}N_7Cl$, M_w = 357.85 (**19**), $C_{19}H_{16}N_7Cl$, M_w = 377.84 (**20**) and $C_{17}H_{18}N_7Cl$, M_w = 355.83 (**21**).

4.2. Aqueous solubility (S_w) and experimental log D (Table 1)

Aqueous solubility (S_w) and lipophilicity influence a drug molecule's membrane permeability which in turn, determines the distribution of the molecule in the body. In order for the drug molecule to reach the systemic circulation, it must possess some lipophilic properties to permeate biological membranes as well as enough hydrophilic properties to permit an efficient drug delivery [20].

The experimentally determined distribution coefficients (log D), a pH dependant version of the partition coefficient, are the logarithmic ratios of octanol solubility to water (a PBS buffer) solubility at a given pH value and serve as an indication of an investigated compound's *in vivo* behaviour. The octanol mimics the biological membrane while the PBS buffer represents the cytosol and the digestive vacuole of the parasite, at pH 7.4 and 5.5, respectively. The results are listed in Table 1 for compounds (**12 – 21**) and pyrimethamine. From this data, the lipid solubility was deduced using the equation: $\log S_{oc} = \log D + \log S_w$.

Chloroquine and pyrimethamine are basic molecules that are protonated at pH levels lower than the physiological pH of 7.4 (chloroquine pKa 8.38 and pKa 10.18; [21] and pyrimethamine pKa 7.36; [22]. This is in accordance with the ability of the quinolines and pyrimethamine to permeate biological membranes at a physiologic pH (unionised form) and accumulate in high concentrations in the acidic environment of the malaria parasite's food vacuole (ionised form) [23]. Thus, the synthesised hybrids should possess, high hydrophilicity in the acidic conditions of pH 5.5 (protonated) and high lipophilicity at the physiological pH 7.4 (unprotonated).

And, the results of this study support these facts. Indeed, each hybrid exhibited a greater aqueous solubility at pH 5.5 than at pH 7.4 and inversely, a higher lipophilicity at pH 7.4 than at 5.5 (Table 1). With the exception of hybrid **13**, no hybrid was more hydrophilic or lipophilic than pyrimethamine, irrespective of the media. Hybrid **20**, featuring a phenyl linker, was the least hydrophilic of all and this is presumably due to its inability to protonate in either medium; the linker being notoriously lipophilic. Compounds **13** (S_w = 1445.23 mM) and **19** (S_w = 937.73 mM) were the most hydrophilic hybrids at pH 5.5 and pH 7.4, respectively. On

contrary, compound **15** (S_{oc} = 598.42 mM) was the most lipophilic in the acidic medium while hybrid **16** (S_{oc} = 5682.83 mM) displayed the highest lipophilicity in the neutral conditions. Compound **15** features two ethylene oxide units in the linker. This may explain its good solubility in both acidic and neutral media as a result of H-bond formation between water molecules and the intra chain oxygen atoms of ethylene oxide [24]

Moreover, for a given linker, the amine-linked hybrids tend to be more water soluble than their ether-linked counterpart, presumably as a result of additional protonation at pH 5.5 apart from the hydrogen bonding. Indeed, the amine-linked hybrids possess a diprotic nature in the acidic medium. While both the amine and the ether hybrids can be protonated at the quinoline ring nitrogen heteroatom (pKa 8.1) at pH 5.5, the amine hybrids undergo an additional protonation at their terminal nitrogen [25]. When one considers, ethyl linker, hybrid **17** is more water soluble than **12** (523.07 *versus* 303.29 mM). This finding was not observed at pH 7.4.

No structure-physicochemical properties could be drawn from this study.

Table 1 Aqueous solubility (S_w), partition coefficients (Log D) and lipid solubility (S_{oc}) of quinoline-pyrimidine hybrids (**12** – **21**) and pyrimethamine

Compound	pH 5.5 S_W (mM)[a]	Std	pH 5.5 log D [a]	Std	pH 5.5 S_{OC} (mM) [b]	pH 7.4 S_W (mM)[a]	Std	pH 7.4 log D [a]	Std	pH 7.4 S_{OC} (mM) [b]
(**12**)	303.29	0.001	-0.303	0.040	150.96	234.51	0.014	0.895	0.038	1841.46
(**13**)	1445.23	0.091	-1.341	0.192	65.91	254.93	0.017	0.924	0.031	2140.04
(**14**)	304.89	0.021	-0.082	0.004	252.43	117.11	0.004	1.01	0.053	1198.38
(**15**)	1166.83	0.072	-0.290	0.03	598.42	843.06	0.01	-0.279	0.039	443.46
(**16**)	40.96	0.006	1.073	0.102	484.81	19.12	0.003	2.473	0.052	5681.83
(**17**)	523.07	0.030	-0.070	0.004	445.20	118.91	0.008	0.780	0.066	716.50
(**18**)	883.63	0.075	-0.735	0.022	162.66	616.02	0.006	0.110	0.044	793.59
(**19**)	1035.27	0.010	-0.737	0.075	189.69	937.73	0.057	0.492	0.010	2911.24
(**20**)	8.13	0.000	[c]	-	-	[c]	-	[c]	-	-
(**21**)	659.07	0.022	-0.992	0.066	67.13	7.03	0.001	[c]	-	-
PYR	1163.82	0.031	0.758	0.020	6666.31	149.72	0.001	1.933	0.204	12831.57

Aqueous solubility (S_w), standard deviation (STD), partition coefficient (Log D), Lipid solubility (S_{OC}), Pyrimethamine (PYR). [a] Determined experimentally, each value represents the mean and STD of 3 measurements. [b] Calculated from log SOC = log D + log SW. [c] Compound not water soluble enough to determine value.

4.3 In vitro *antimalarial activity (Table 2)*

The antimalarial activity of the quinoline-pyrimidine hybrids were screened *in vitro* alongside chloroquine and pyrimethamine against the chloroquine sensitive D10 strain, a clone of human *Plasmodium falciparum* malaria. The results are reported in Table 2.

As can be seen, all synthesised hybrids were active against the D10 strain. The IC_{50} values were found in the 0.08 – 0.89 µM range. None of them displayed better potency than chloroquine (IC_{50} = 0.03 µM). Hybrid **21** (IC_{50} = 0.08 µM) which contains a piperazine linker showed antiplasmodial activity comparable to that of pyrimethamine (IC_{50} = 0.11 µM). This activity may be attributed to the fact that hybrid **21** accumulates to a higher concentration in the digestive vacuole of the parasite given that the piperazine-linker is a tertiary amine and thus gets protonated to a more easily in this acidic medium. This hybrid seems to be the most active of all.

Overall, the ether-linked hybrids tend to be generally more potent than the amine-linked ones. This result is somewhat surprising. Indeed, one would expect the amine-linked hybrids to accumulate to higher concentrations in the parasitic digestive vacuole and therefore be more active because of their diprotic nature at pH 5.5. Hybrids **12** (IC_{50} = 0.23 µM), **13** (IC_{50} = 0.24 µM), **14** (IC_{50} = 0.22 µM), **16** (IC_{50} = 0.16 µM) and **20** (IC_{50} = 0.22 µM) exhibited similar antimalarial activity in the 0.20 µM order. The fact that none of the hybrids possessed better antimalarial activity than chloroquine, the flagship drug against malaria, suggests that the addition of the pyrimidine ring antagonises the activity of the quinoline moiety. However, the findings of this study can only rigorously be confirmed upon investigation of broader series of hybrids.

Table 2. Antimalarial activity of quinoline-pyrimidine hybrids (**12** – **21**), chloroquine and pyrimethamine.

Compound	MW	IC_{50} (ng/ml) [a]	IC_{50} (µM) [a]
(**12**)	330.78	77.1	0.23
(**13**)	344.80	83.9	0.24
(**14**)	344.80	75.5	0.22
(**15**)	374.83	331.9	0.89
(**16**)	358.83	56.2	0.16
(**17**)	329.79	103.6	0.31
(**18**)	343.82	287.6	0.84
(**19**)	357.85	206.6	0.58
(**20**)	377.84	83.1	0.22
(**21**)	355.83	27.9	0.08
PYR	248.72	26.7	0.11
CQ	319.87	10.9	0.03

Concentration inhibiting 50% of parasite growth (IC_{50}); standard deviation (STD);
[a] determined experimentally, data represents the mean and STD of 3 measurements;

5. Conclusion

We have successfully synthesised a series of quinoline-pyrimidine hybrids (**12** – **21**). The structures of the synthesised compounds were validated by means of NMR and MS spectroscopy. The physicochemical properties namely aqueous solubility (S_w) and distribution coefficient (log D) were determined experimentally. Overall, the hybrids were found more water soluble at pH 5.5 than at pH 7.4, and inversely displayed higher lipophilicity at pH 7.4 than at 5.5. However, no structure-physicochemical property could be drawn from this investigation. The antiplasmodial activity screening showed all the hybrids to be active against the chloroquine-sensitive D10 strain of *Plasmodium falciparum*. None of the synthesised compounds showed better activity than chloroquine. However, hybrid **21** (IC_{50} = 0.08 µM) which features a piperazine linker showed antimalarial potency similar to that of pyrimethamine (IC_{50} = 0.11 µM), and therefore is worthwhile being further investigated against the resistant strains of *Plasmodium falciparum*.

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CHAPTER 4

SUMMARY AND FINAL CONCLUSIONS

This section strives to unify the outcomes of this study and draws overall conclusions from all data, to point out relevance and specific advances made by this study, to point out remaining scientific questions and to propose appropriate prospective studies.

The burden of malaria presses heavily on the developing world especially in those countries that already battles with political unrest, civil wars, famine and the effect of diseases like tuberculosis and HIV/AIDS (Adams et al., 2004).

Resistance against the first line drugs is making the fight against malaria increasingly difficult for it necessitates the continuous revision and assessment of treatment policies (Bosman & Olumese, 2004). This has prompted studies into alternative chemotherapeutic treatments for the disease. An efficient strategy to develop effective and cheaper antimalarial compound appears to be the re-design of existing drugs and the work on known parasite-specific targets (Biagini *et al.*, 2005).

Hybridisation is an emerging strategy in medicinal chemistry and involves the binding of two or more active ingredients together as a single chemical entity (Morphy & Rankovic, 2005). Artemisinin- (Araújo *et al.*, 2009) and quinoline based (Burgess *et al.*, 2006) hybrid drugs with proven antimalarial activity have been successfully synthesised.

In this study we aimed to synthesise a series of quinoline-pyrimidine hybrids, tested it for any significant antimalarial activity and compared it to the antimalarial activity of chloroquine and pyrimethamine.

The process of achieving this aim involved the following steps:

- Synthesis and characterisation of quinoline-pyrimidine hybrids, conjugated with different linkers and confirm their structures.
- Determination of the aqueous solubility and the partition coefficient of the hybrids at physiological pH 7.4 and parasitic food vacuole pH 5.5, and of any relationship of these properties with their structures.
- Evaluation of the *in vitro* antimalarial activity of the hybrids.

The quinoline-pyrimidine hybrids were successfully synthesised via synthetic organic methods and their structures were verified by NMR and MS spectroscopy.

The results of this study indicated that each hybrid exhibited a greater aqueous solubility at pH 5.5 than at pH 7.4 and inversely, a higher lipophilicity at pH 7.4 than at 5.5. With the exception of hybrid **13**, no hybrid was more hydrophilic or lipophilic than pyrimethamine, irrespective of the media. Hybrid **20**, featuring a phenyl linker, was the least hydrophilic of all and this is presumably due to its inability to protonate in either medium; the linker being notoriously lipophilic. Compounds **13** (S_w = 1445.23 mM) and **19** (S_w = 937.73 mM) were the most hydrophilic hybrids at pH 5.5 and pH 7.4, respectively. On contrary, compound **15** (S_{oc} = 598.42 mM) was the most lipophilic in the acidic medium while hybrid **16** (S_{oc} = 5682.83 mM) displayed the highest lipophilicity in the neutral conditions. Compound **15** features two ethylene oxide units in the linker. This may explain its good solubility in both acidic and neutral media as a result of H-bond formation between water molecules and the intra chain oxygen atoms of ethylene oxide (Solomon *et al*, 2007)

Moreover, for a given linker, the amine-linked hybrids tend to be more water soluble than their ether-linked counterpart, presumably as a result of additional protonation at pH 5.5 apart from the hydrogen bonding. Indeed, the amine-linked hybrids possess a diprotic nature in the acidic medium. While both the amine and the ether hybrids can be protonated at the quinoline ring nitrogen heteroatom (pKa 8.1) at pH 5.5, the amine hybrids undergo an additional protonation at their terminal nitrogen (O'Neill *et al*., 2006). When one considers, ethyl linker, hybrid **17** is more water soluble than **12** (523.07 *versus* 303.29 mM). This finding was not observed at pH 7.4. No structure-physicochemical properties could be drawn from this study.

All synthesised hybrids were active against the chloroquine-sensitive D10 strain. The IC_{50} values were found in the 0.08 – 0.89 µM range. None of them displayed better potency than chloroquine (IC_{50} = 0.03 µM). Hybrid **21** (IC_{50} = 0.08 µM) which contains a piperazine linker showed antiplasmodial activity comparable to that of pyrimethamine (IC_{50} = 0.11 µM). This activity may be attributed to the fact that hybrid **21** accumulates to a higher concentration in the digestive vacuole of the parasite given that the piperazine-linker is a tertiary amine and thus gets protonated more easily in the acidic medium. This hybrid seems to be the most active of all.

Overall, the ether-linked hybrids tend to be generally more potent than the amine-linked ones. This result is somewhat surprising. Indeed, one would expect the amine-linked hybrids to accumulate to higher concentrations in the parasitic digestive vacuole and therefore be more active because of their diprotic nature at pH 5.5. Hybrids **12** (IC_{50} = 0.23 µM), **13** (IC_{50} =

0.24 µM), **14** (IC_{50} = 0.22 µM), **16** (IC_{50} = 0.16 µM) and **20** (IC_{50} = 0.22 µM) exhibited similar antimalarial activity in the 0.20 µM order. The fact that none of the hybrids possessed better antimalarial activity than chloroquine, the flagship drug against malaria, suggests that the addition of the pyrimidine ring antagonises the activity of the quinoline moiety. However, the findings of this study can only rigorously be confirmed upon investigation of broader series of hybrids.

This study showed the hybrids were found more water soluble at pH 5.5 than at pH 7.4., however, no structure-physicochemical property could be drawn. The biological results indicate that all the hybrids have activity against the chloroquine-sensitive D10 strain of *Plasmodium falciparum*. None synthesised compound showed better activity than chloroquine, but hybrid **21** showed antimalarial potency similar to that of pyrimethamine.

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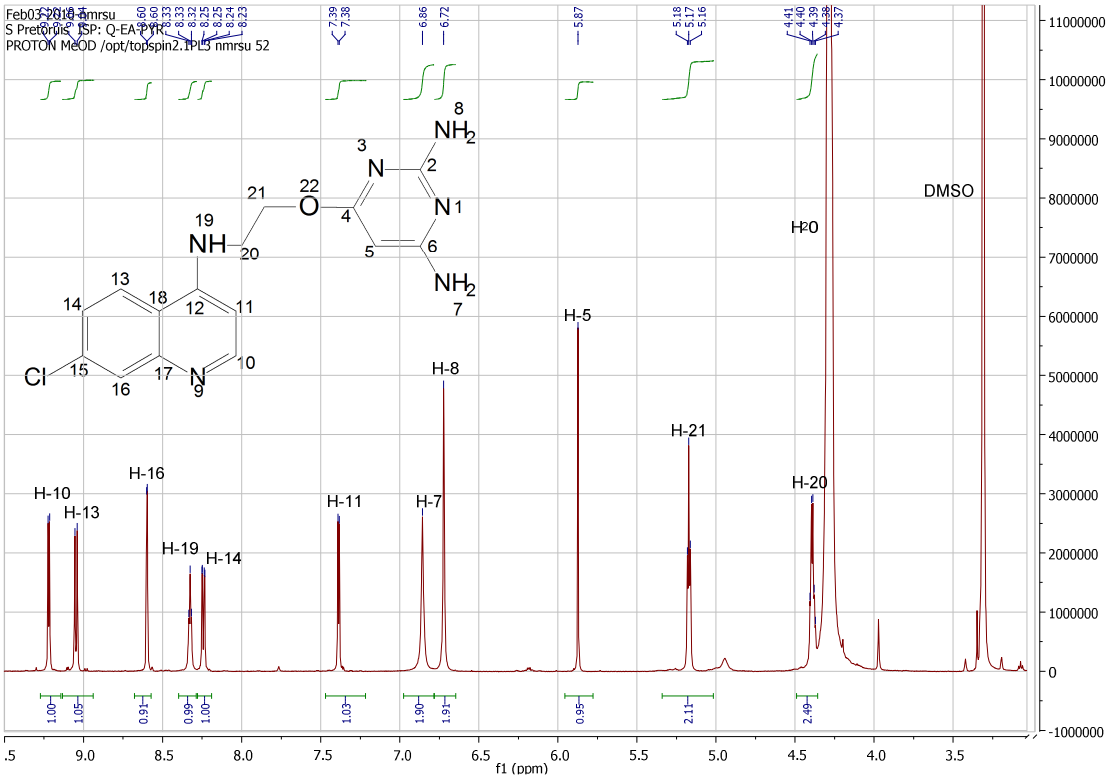
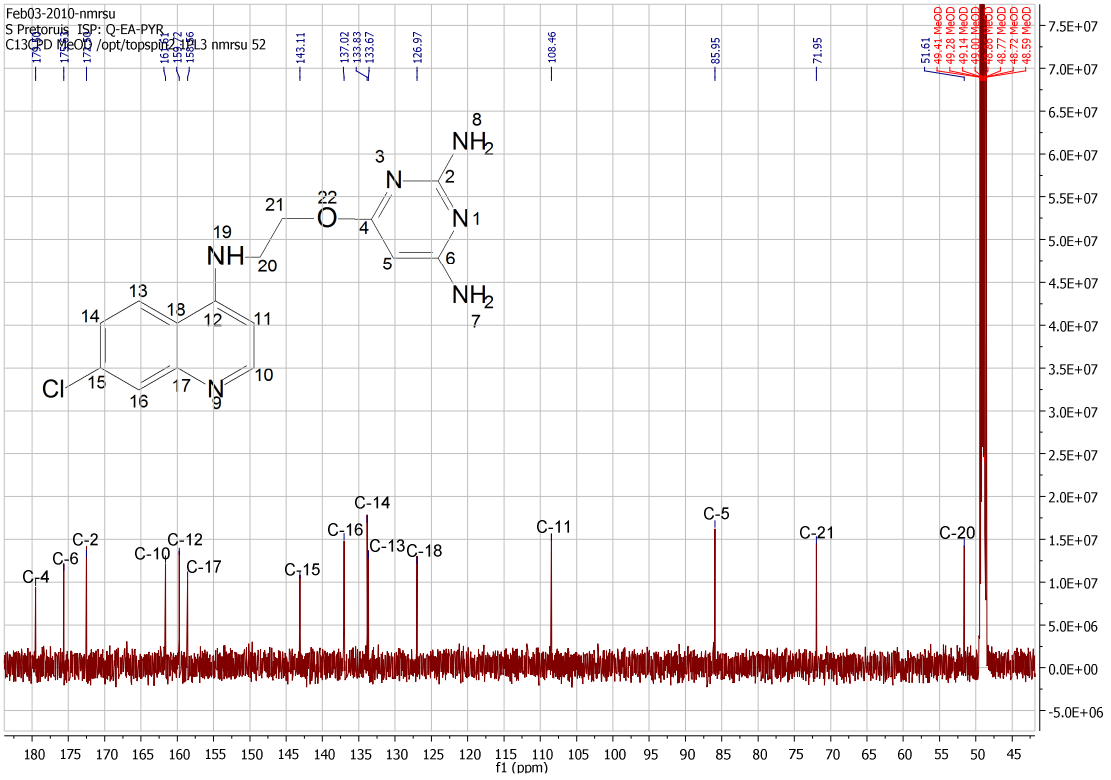
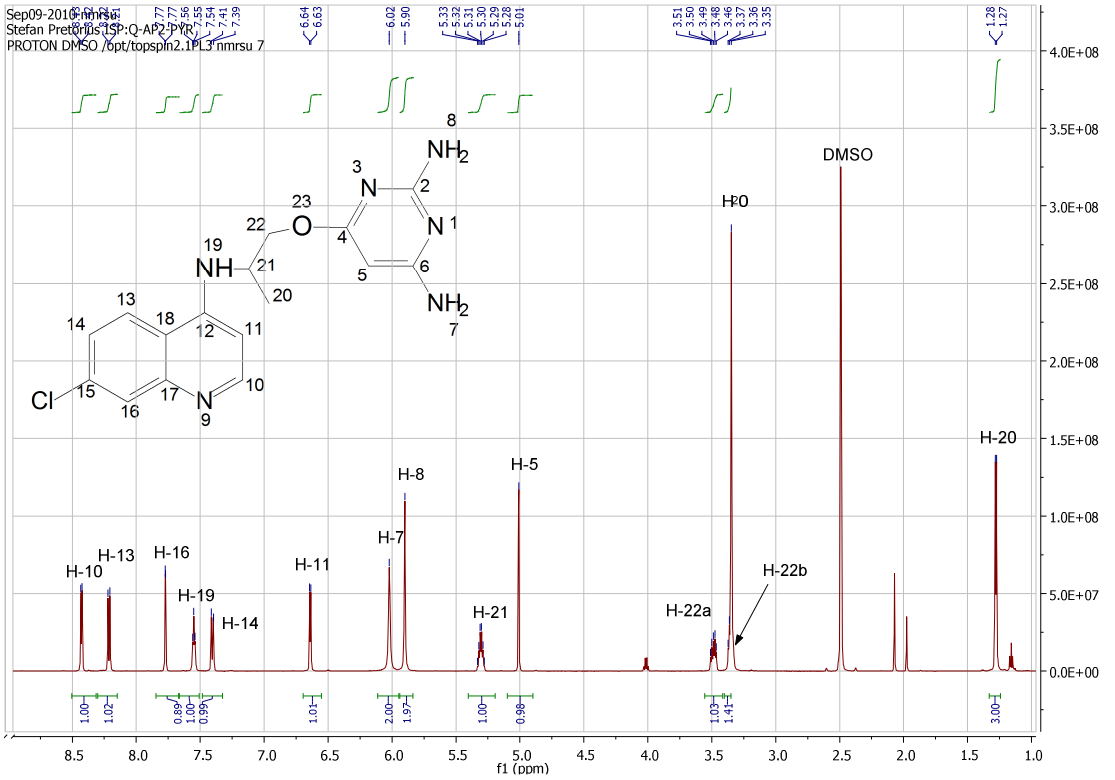
Spectrum 1: ^{1}H NMR of (12)



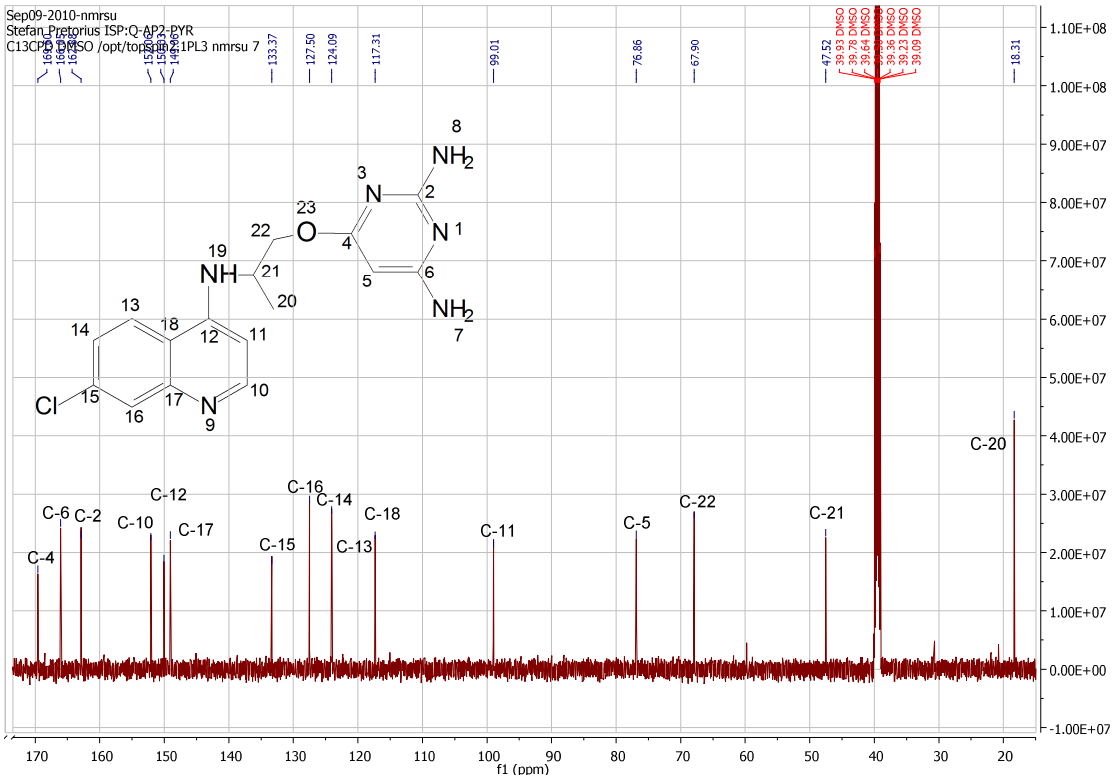
Spectrum 2: ^{13}C NMR of (12)



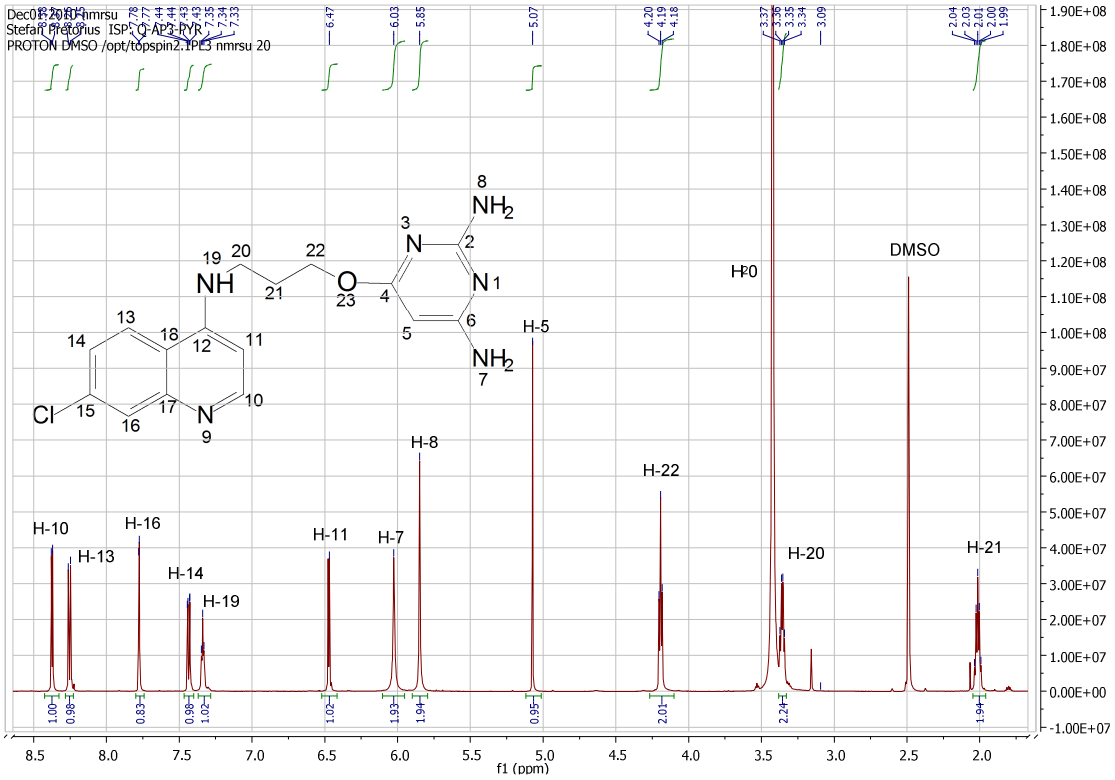
Spectrum 3: ^{1}H NMR of (13)



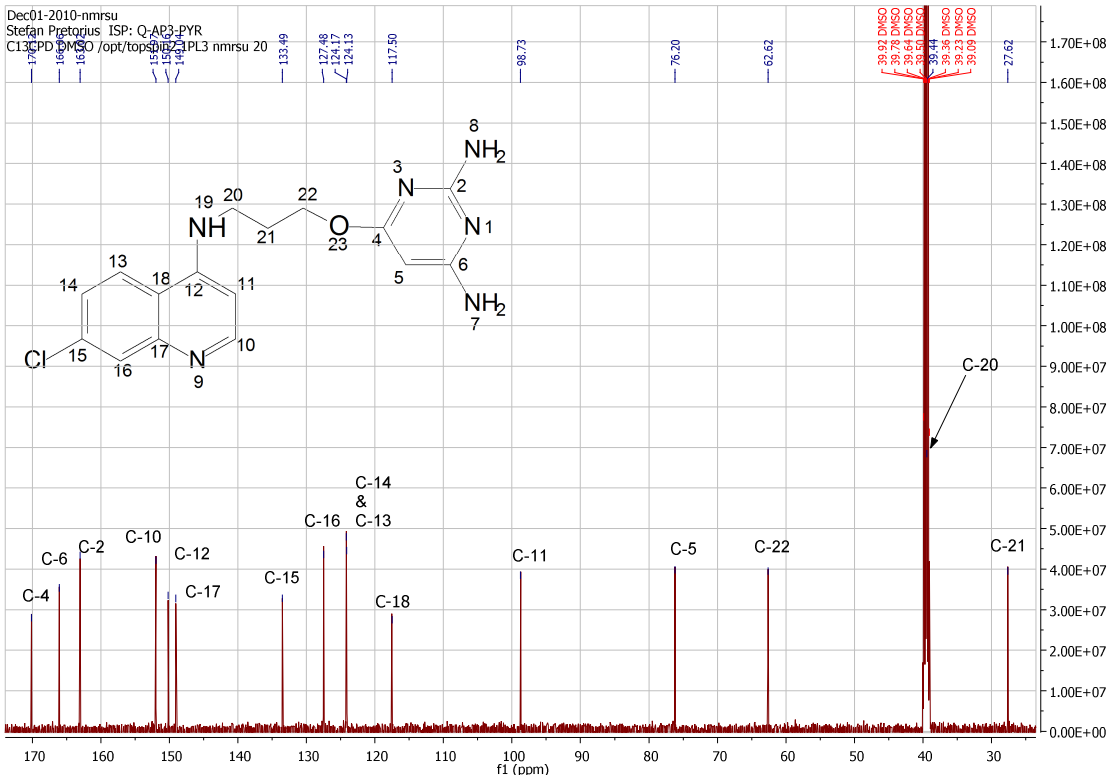
Spectrum 4: ^{13}C NMR of (13)



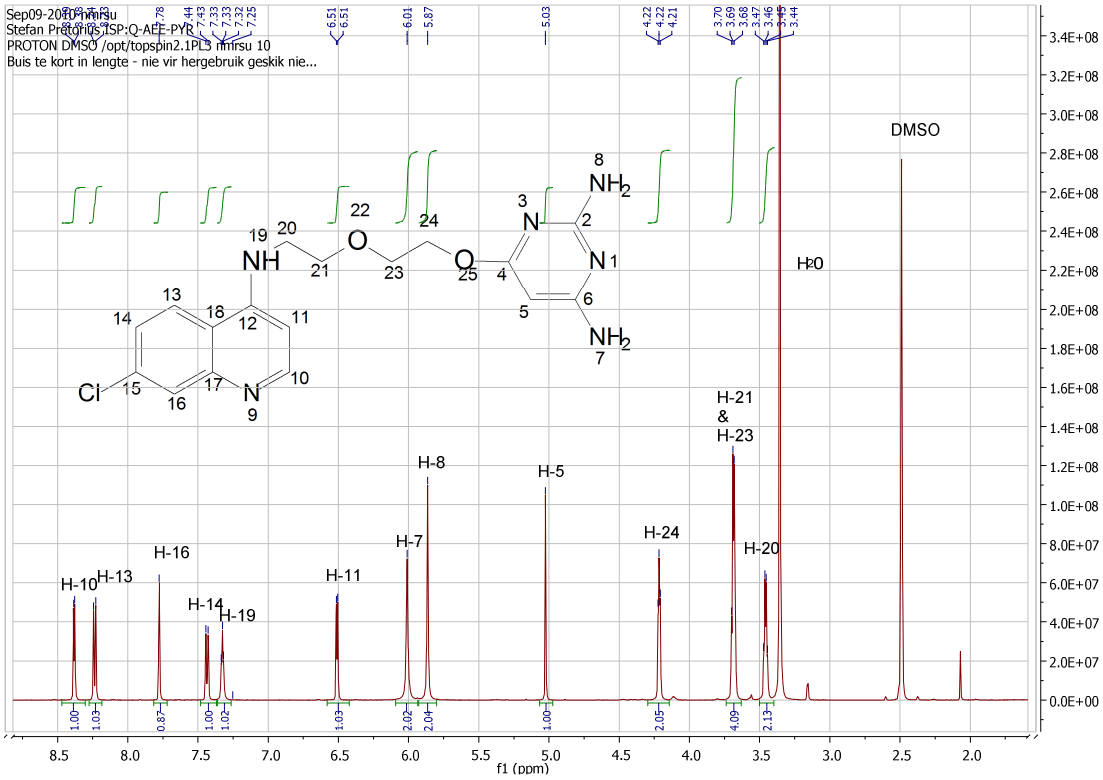
Spectrum 5: ^{1}H NMR of (14)



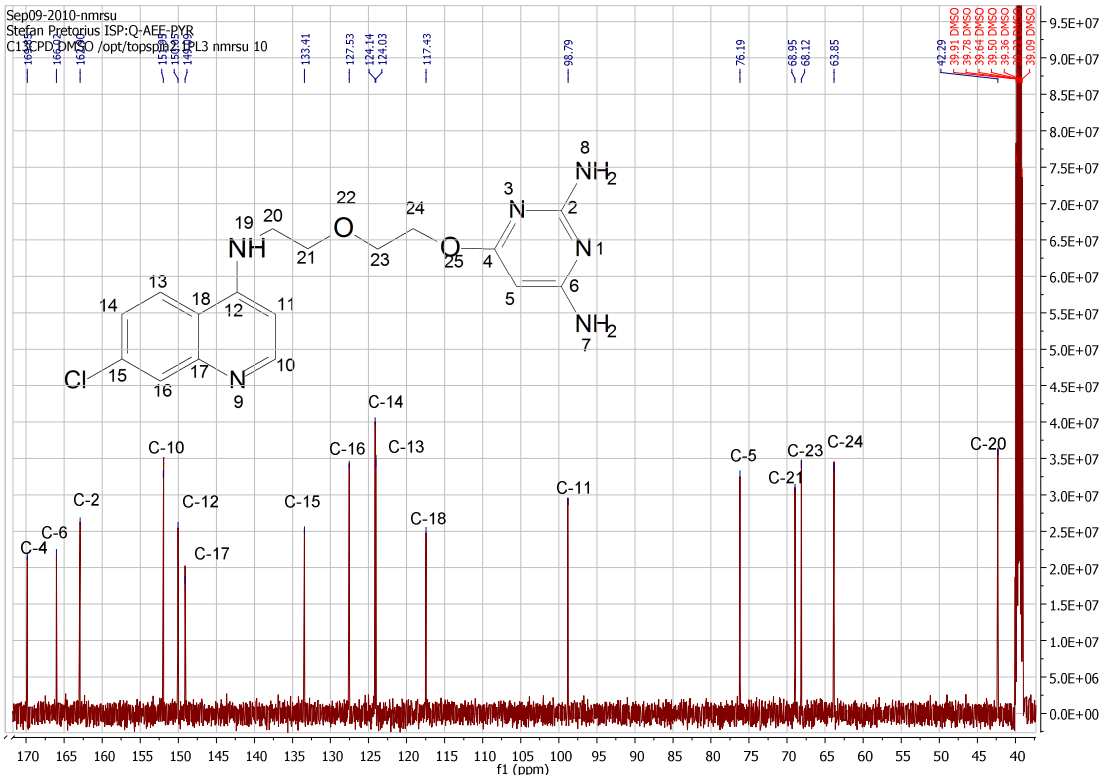
Spectrum 6: ^{13}C NMR of (14)



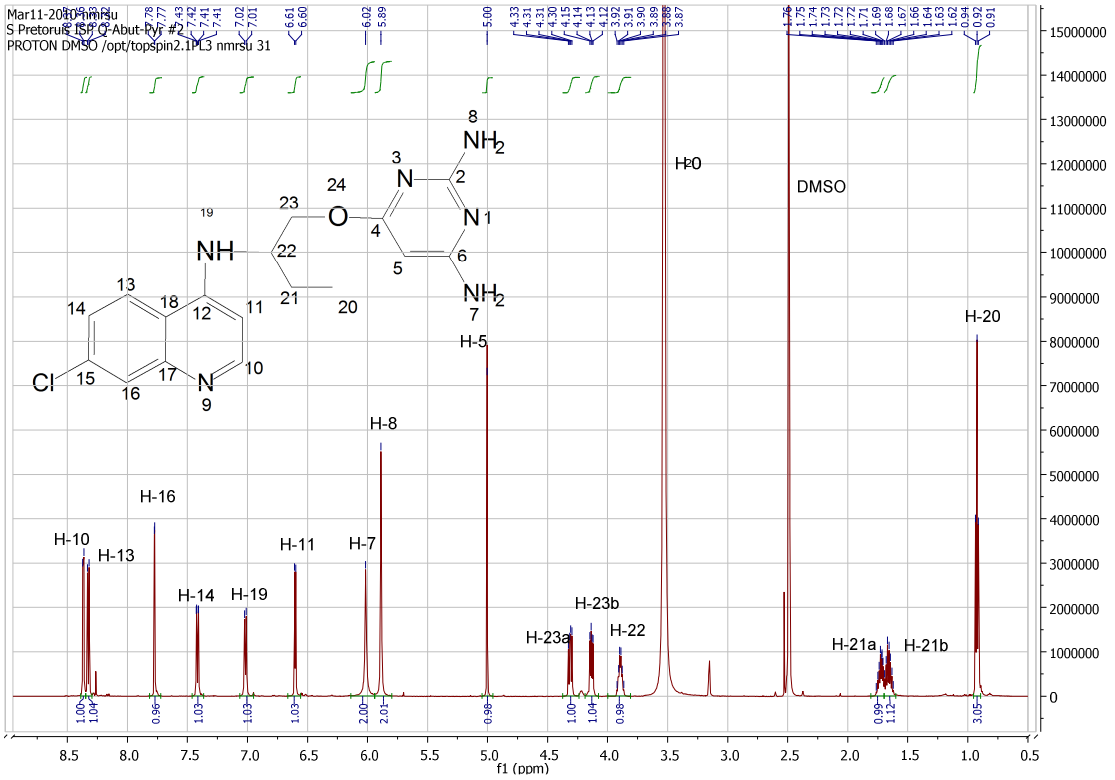
Spectrum 7: ^{1}H NMR of (15)



Spectrum 8: ^{13}C NMR of (15)



Spectrum 9: ^{1}H NMR of (16)



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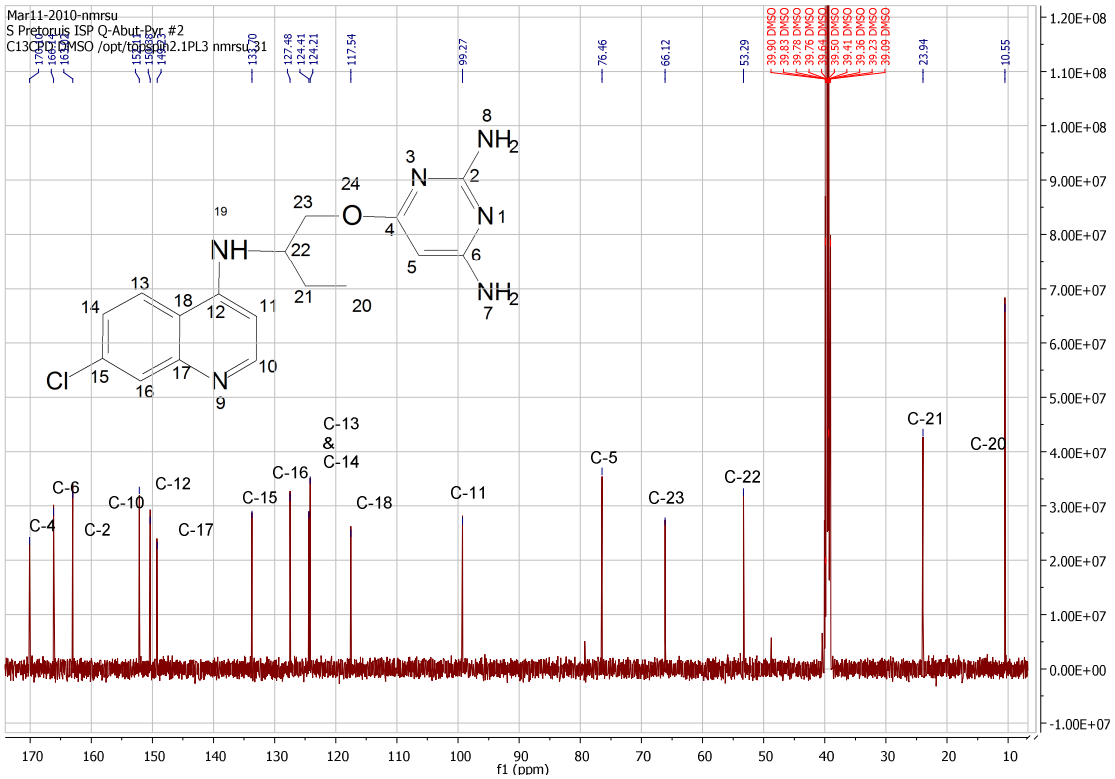

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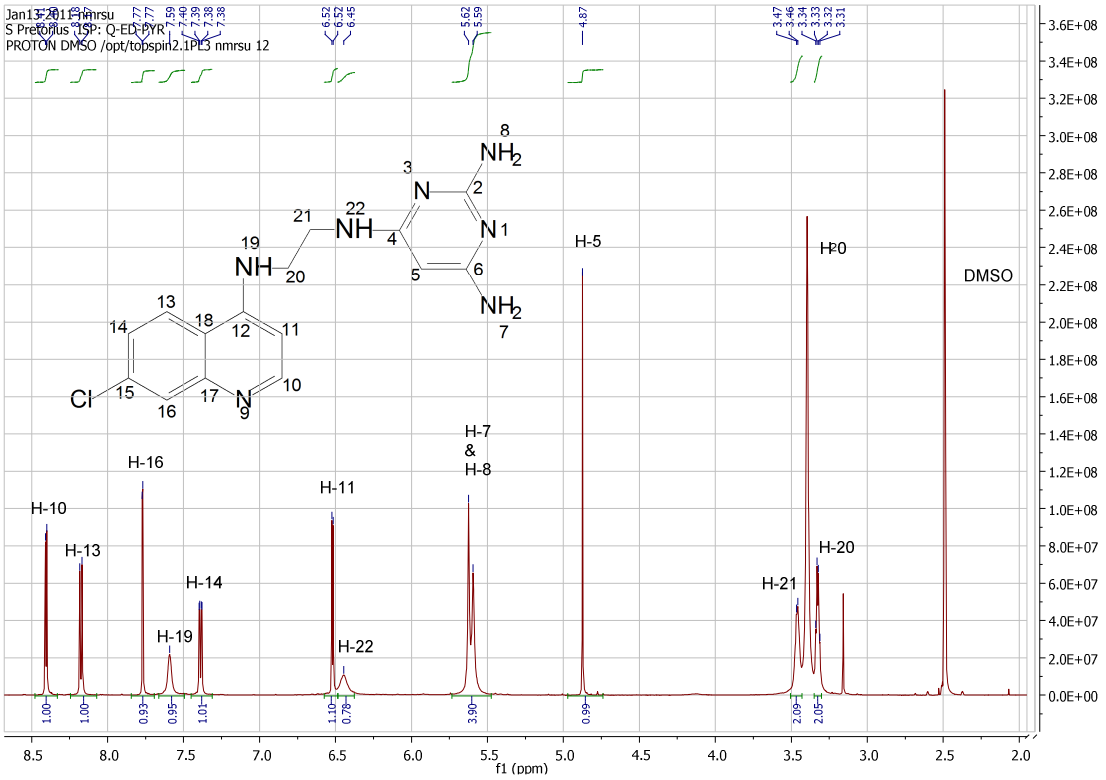

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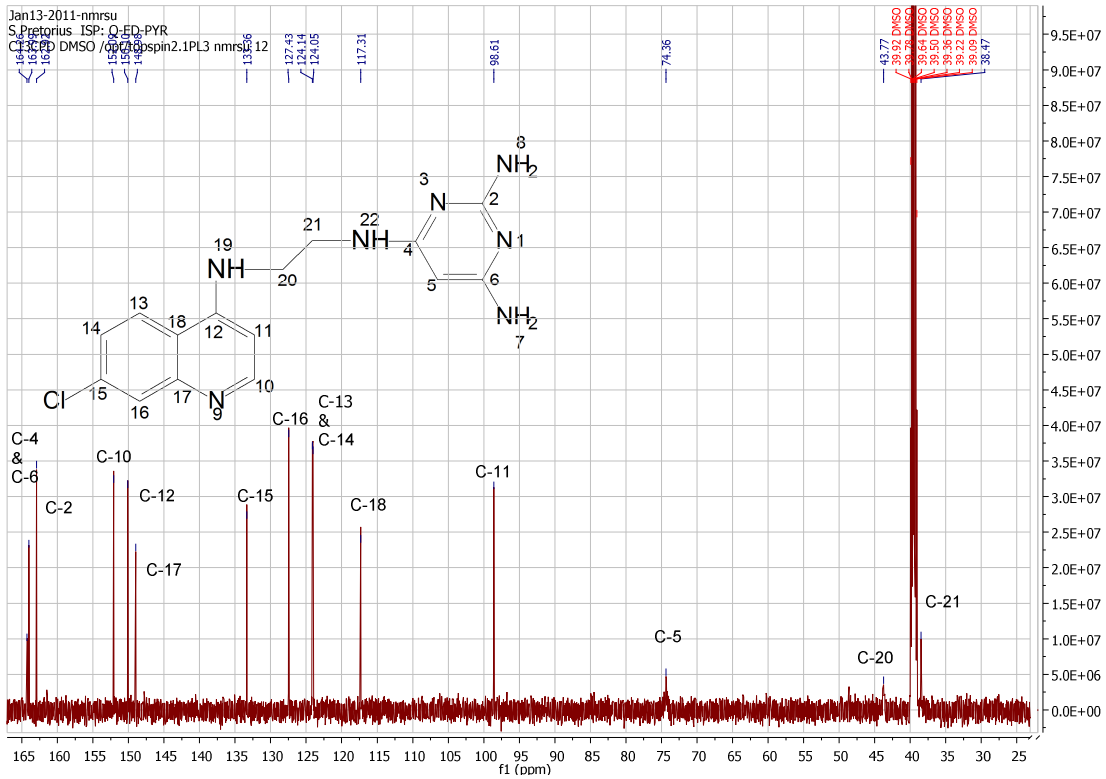
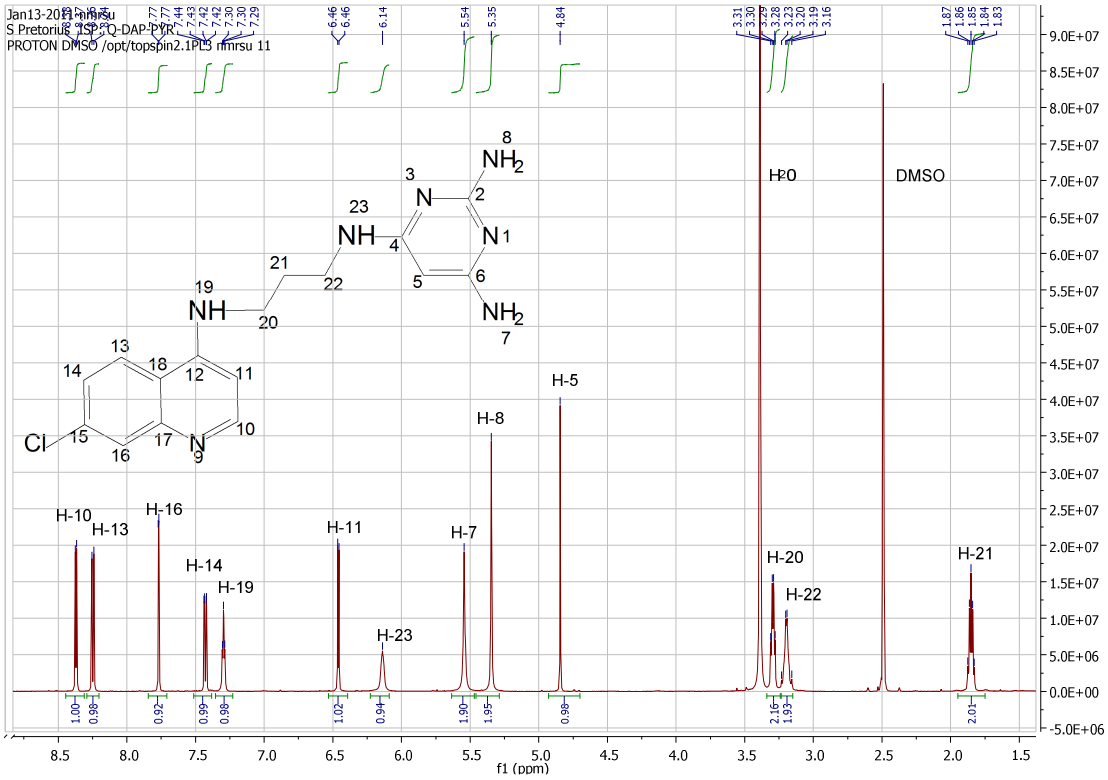


Spectrum 13: ^{1}H NMR of (18)



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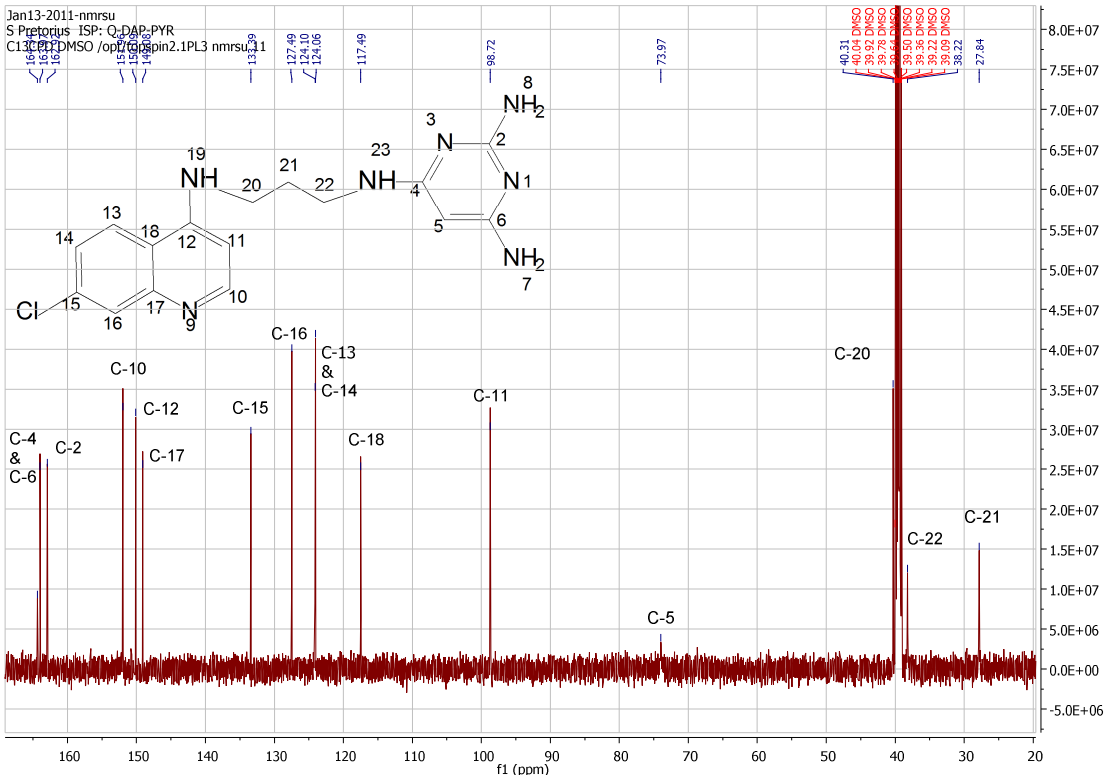
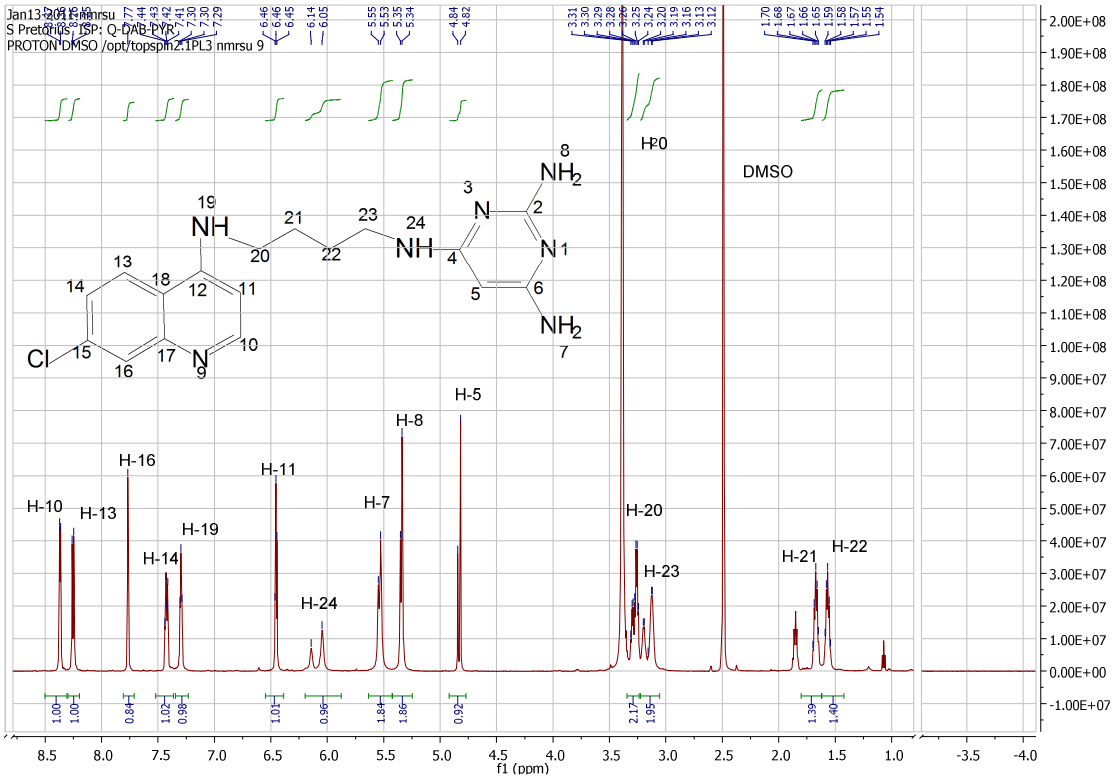
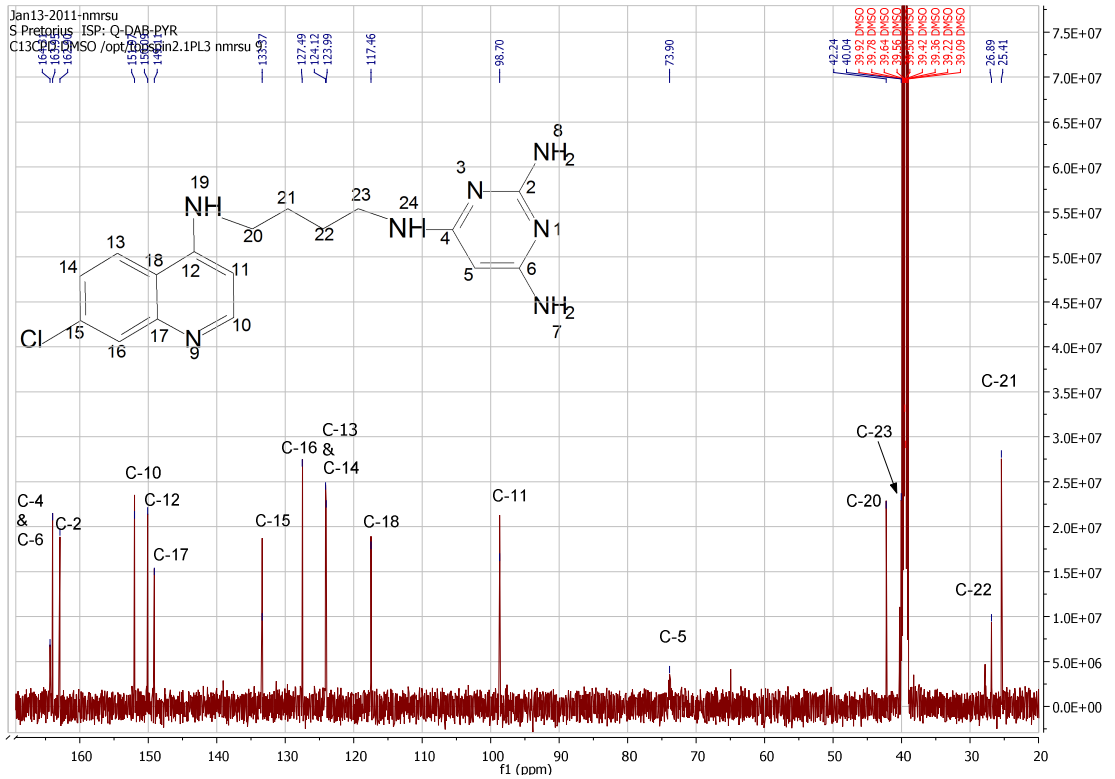


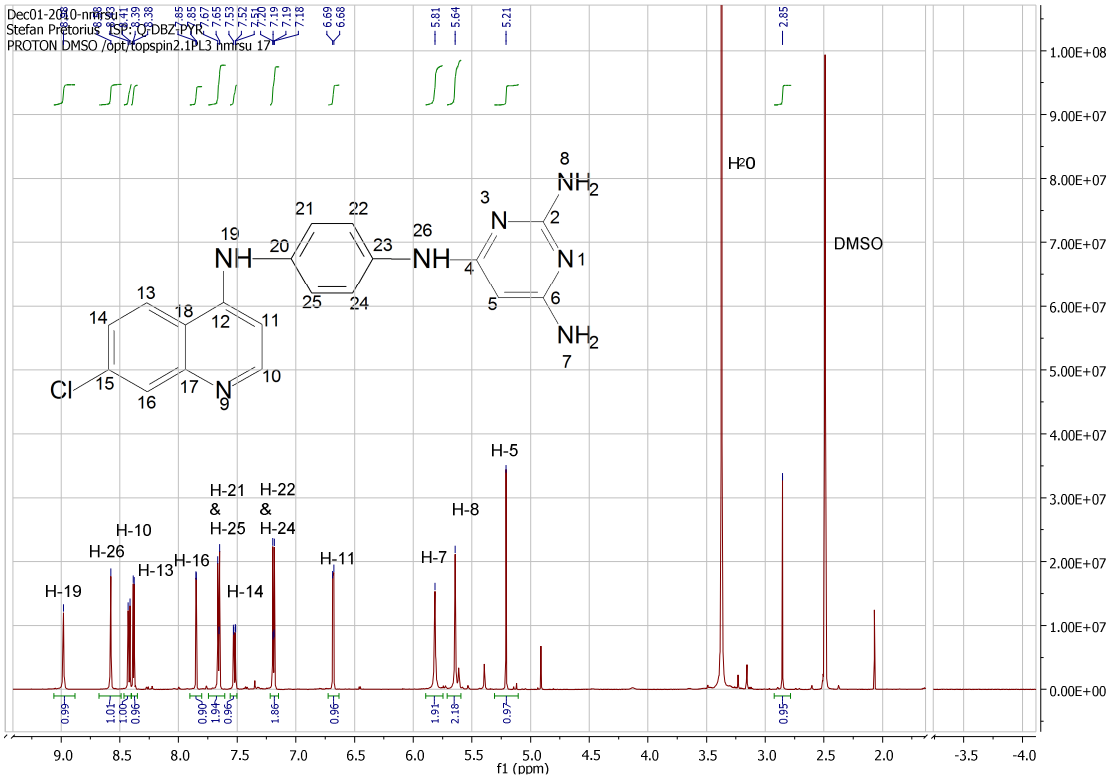
Spectrum 15: ^{1}H NMR of (19)



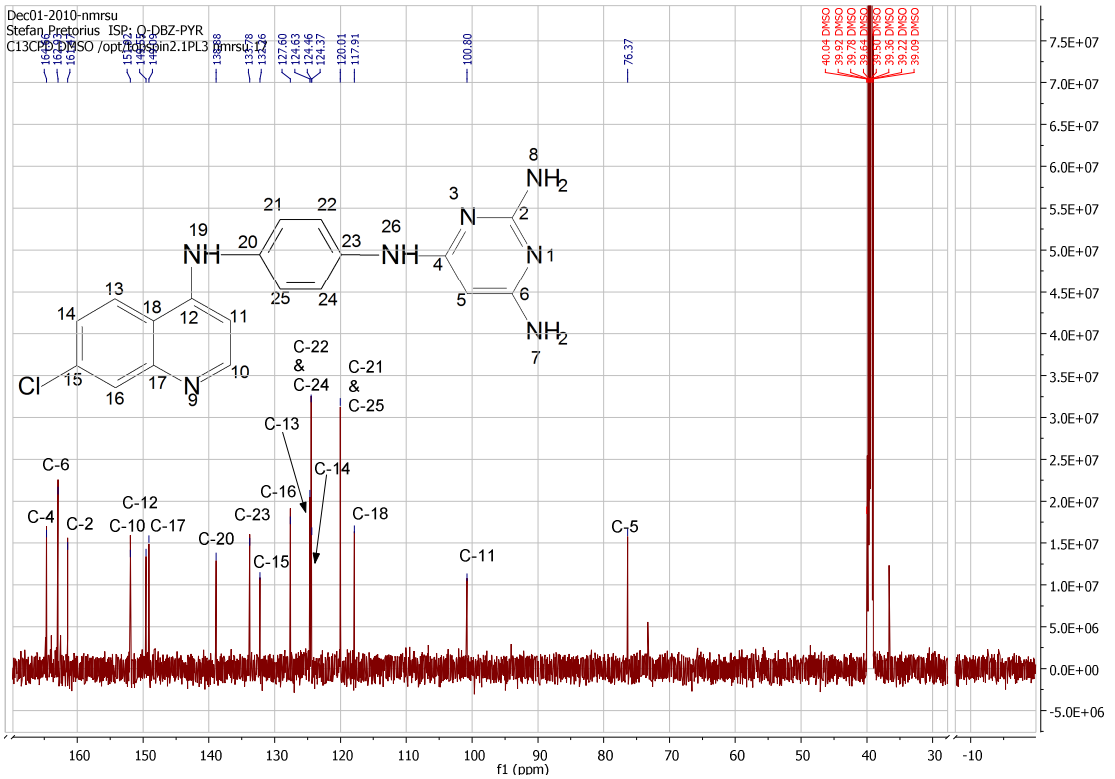
Spectrum 16: ^{13}C NMR of (19)



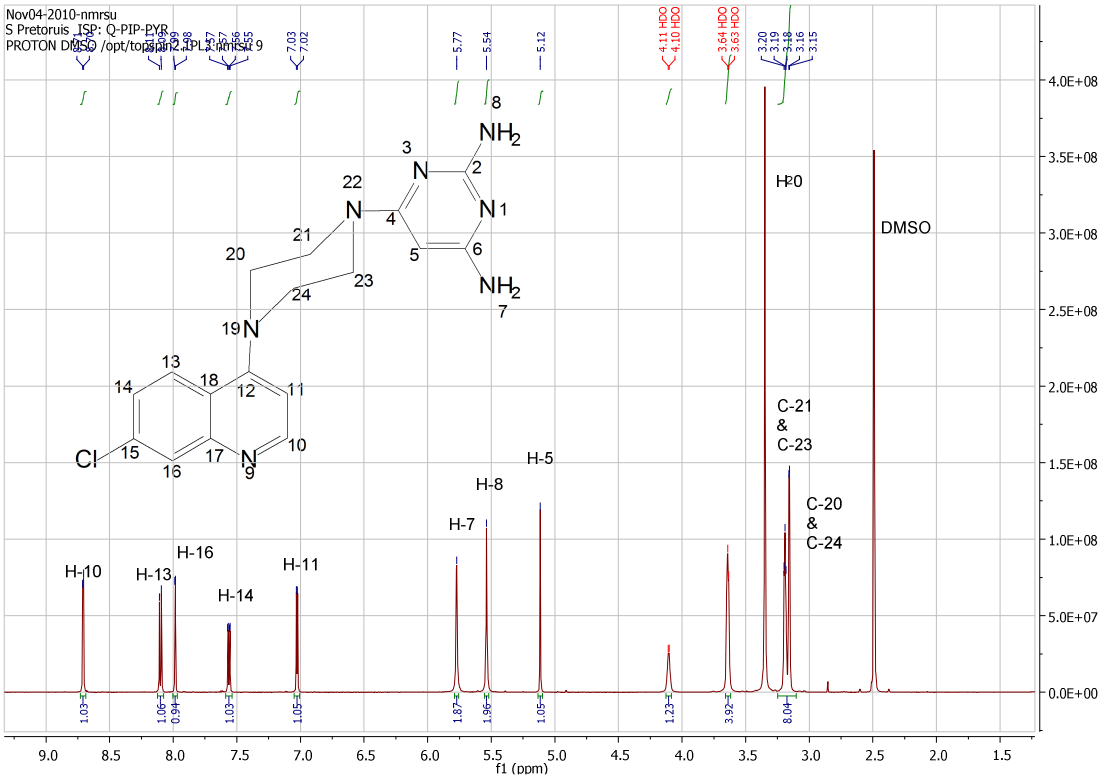
Spectrum 17: ^{1}H NMR of (20)



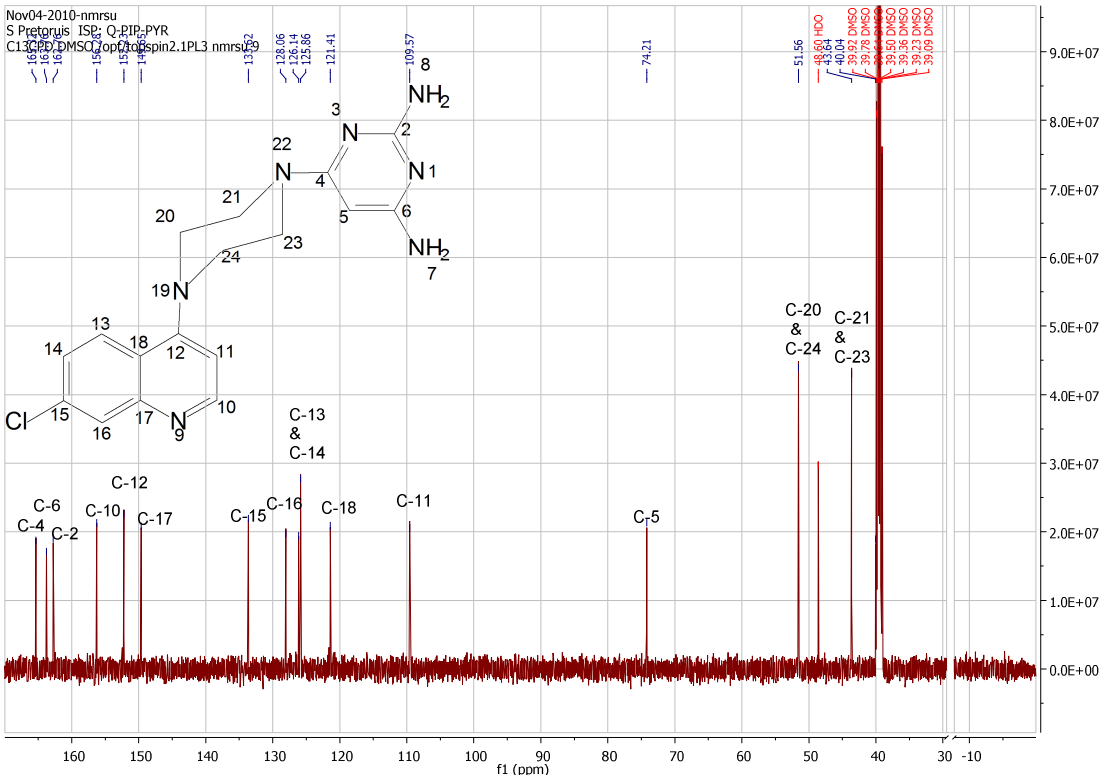
Spectrum 18: ^{13}C NMR of (20)



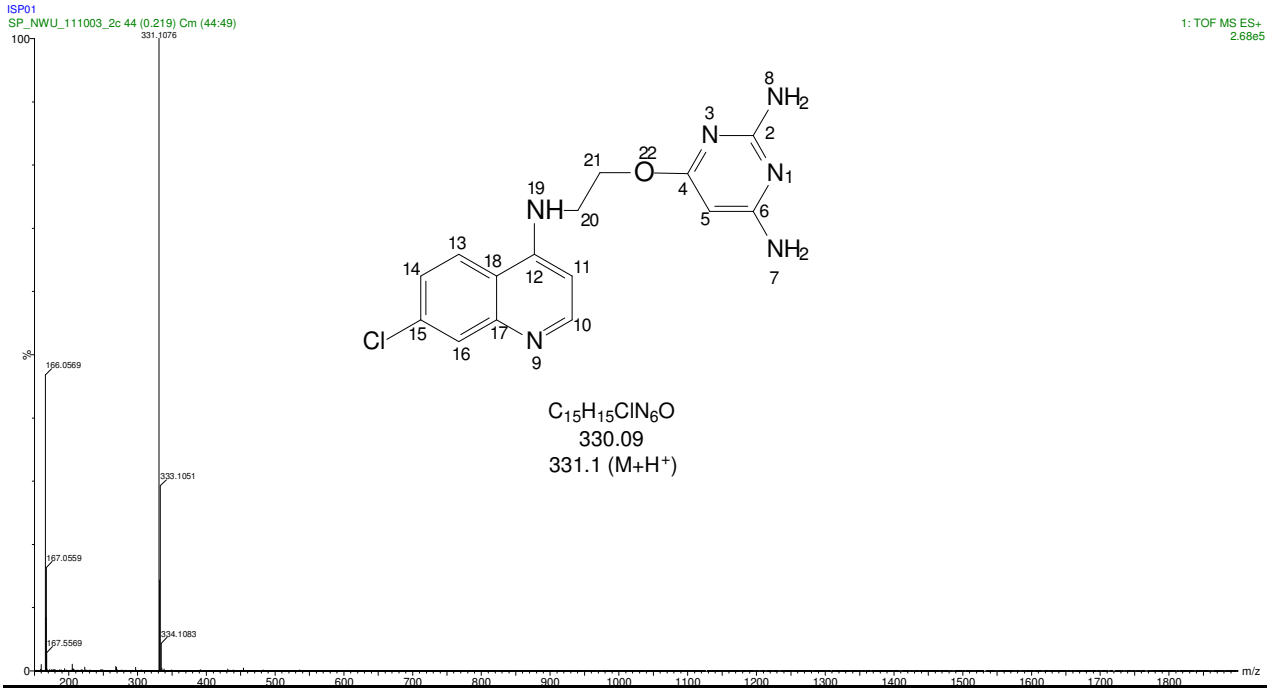
Spectrum 19: ^{1}H NMR of (21)



Spectrum 20: ^{13}C NMR of (21)

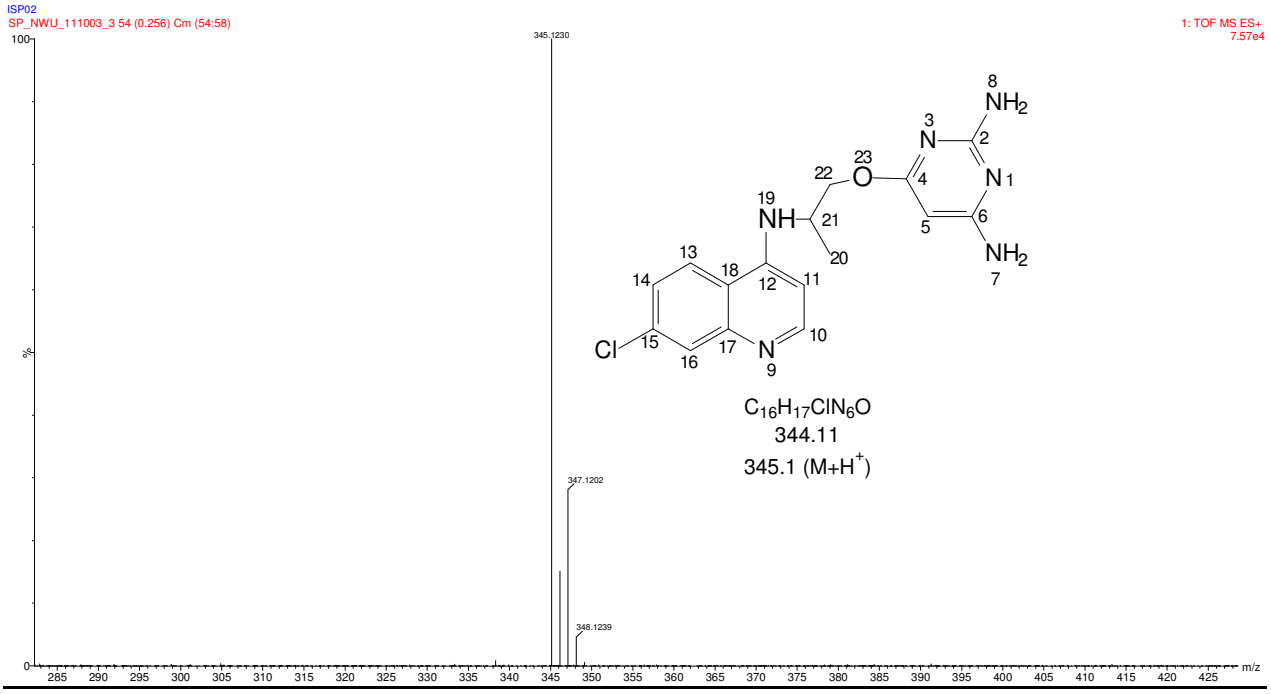


SPECTRUM 21: MS ES+ of (12)



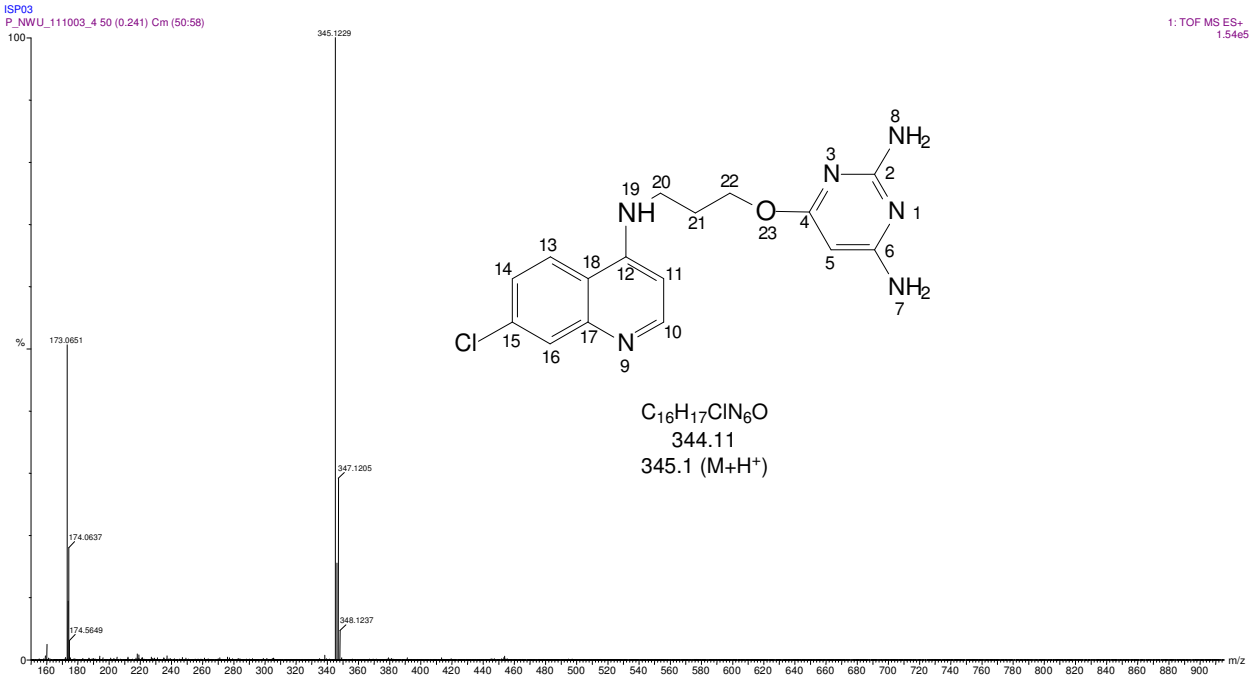
$C_{15}H_{15}ClN_6O$
330.09
331.1 ($M+H^+$)

SPECTRUM 22: MS ES+ of (13)



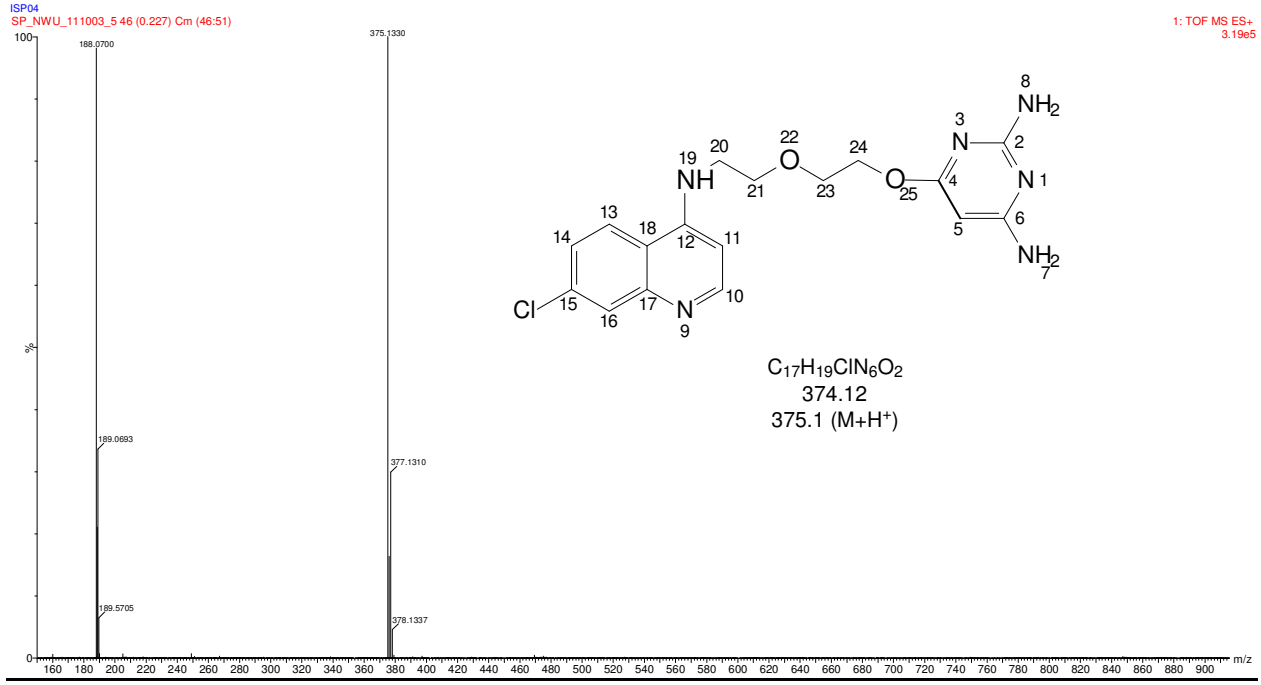
$C_{16}H_{17}ClN_6O$
344.11
345.1 ($M+H^+$)

SPECTRUM 23: MS ES+ of (14)



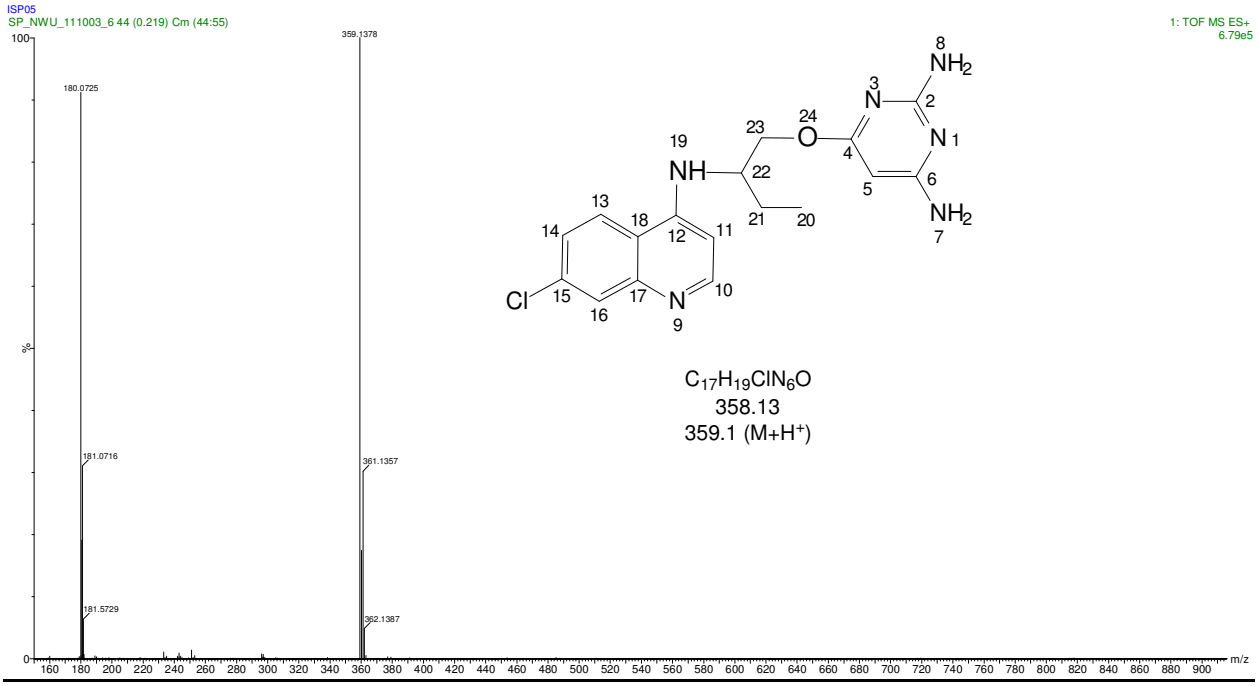
$C_{16}H_{17}ClN_6O$
344.11
345.1 ($M+H^+$)

SPECTRUM 24: MS ES+ of (15)



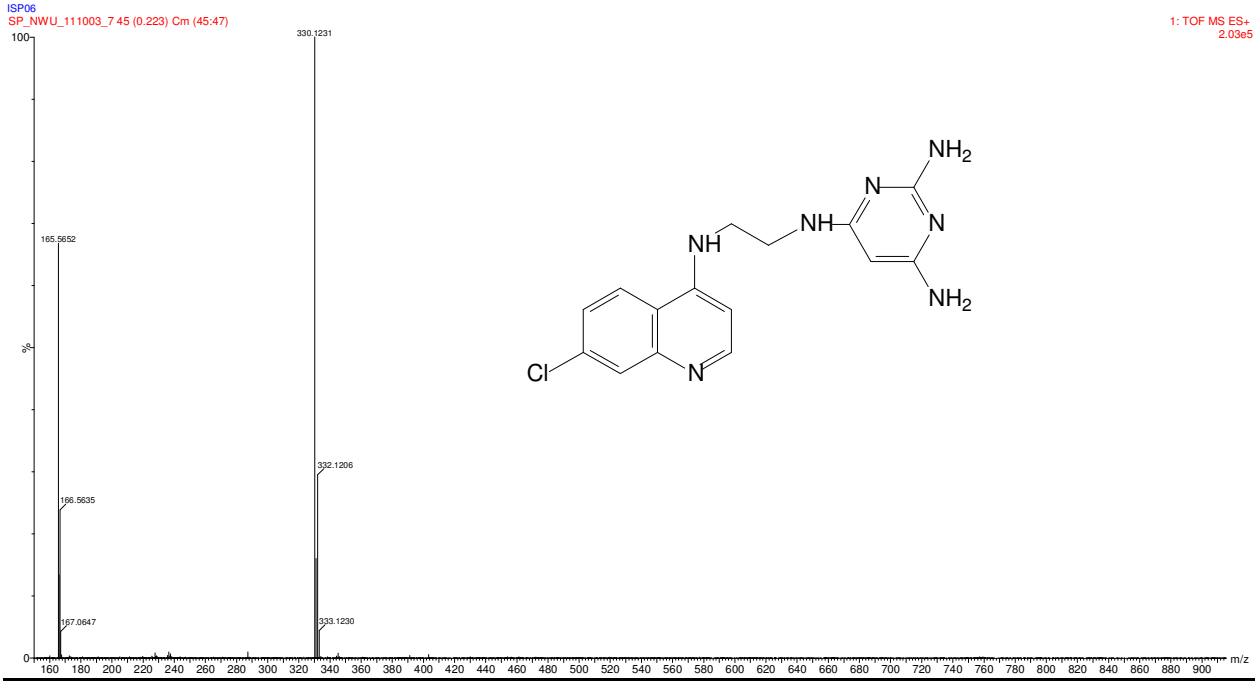
$C_{17}H_{19}ClN_6O_2$
374.12
375.1 ($M+H^+$)

SPECTRUM 25: MS ES+ of (16)

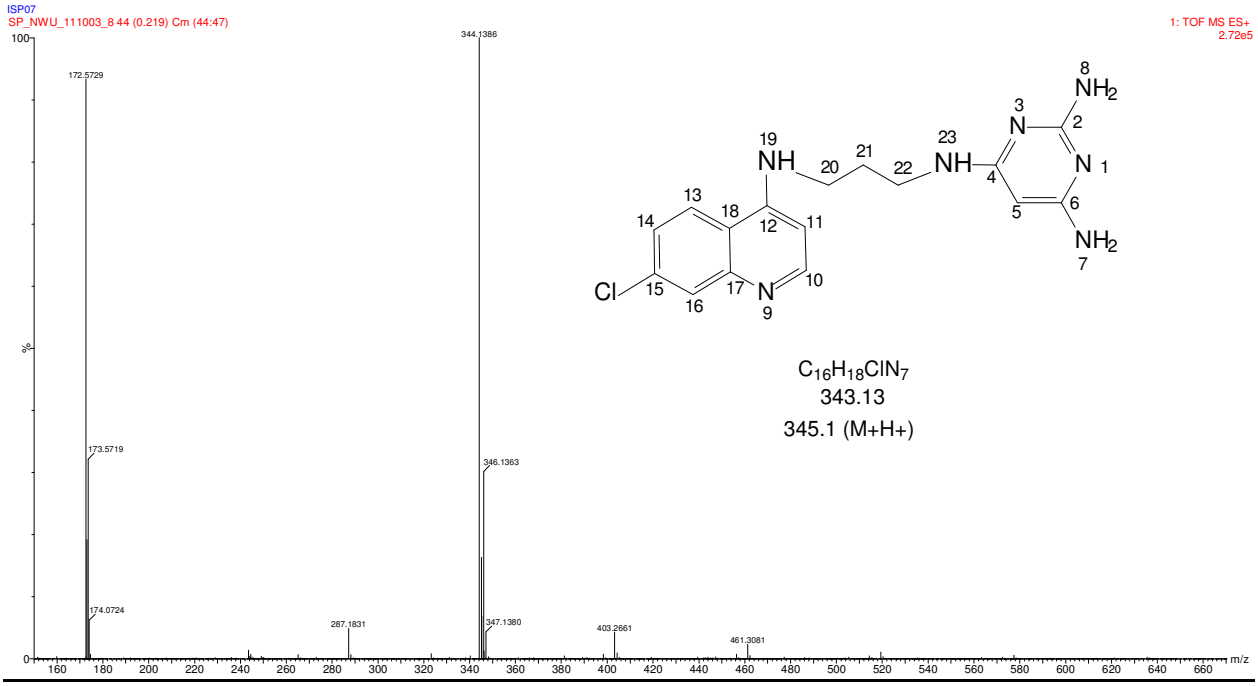


$C_{17}H_{19}ClN_6O$
358.13
359.1 ($M+H^+$)

SPECTRUM 26: MS ES+ of (17)

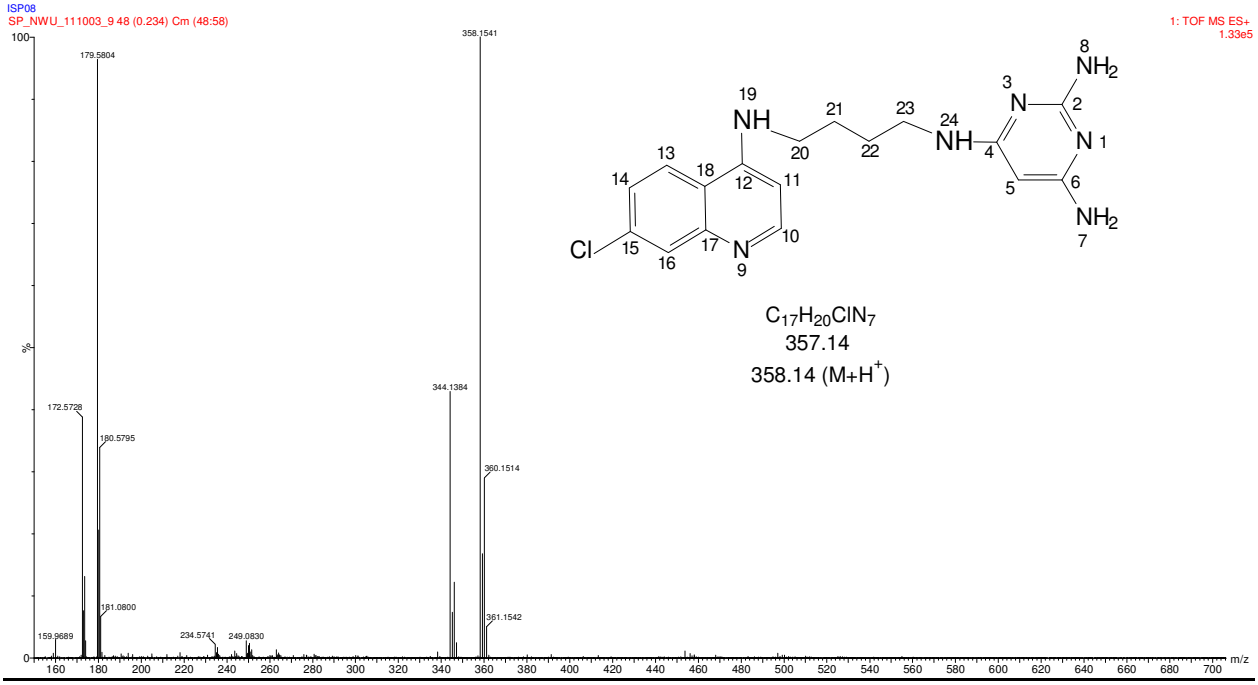


SPECTRUM 27: MS ES+ of (18)



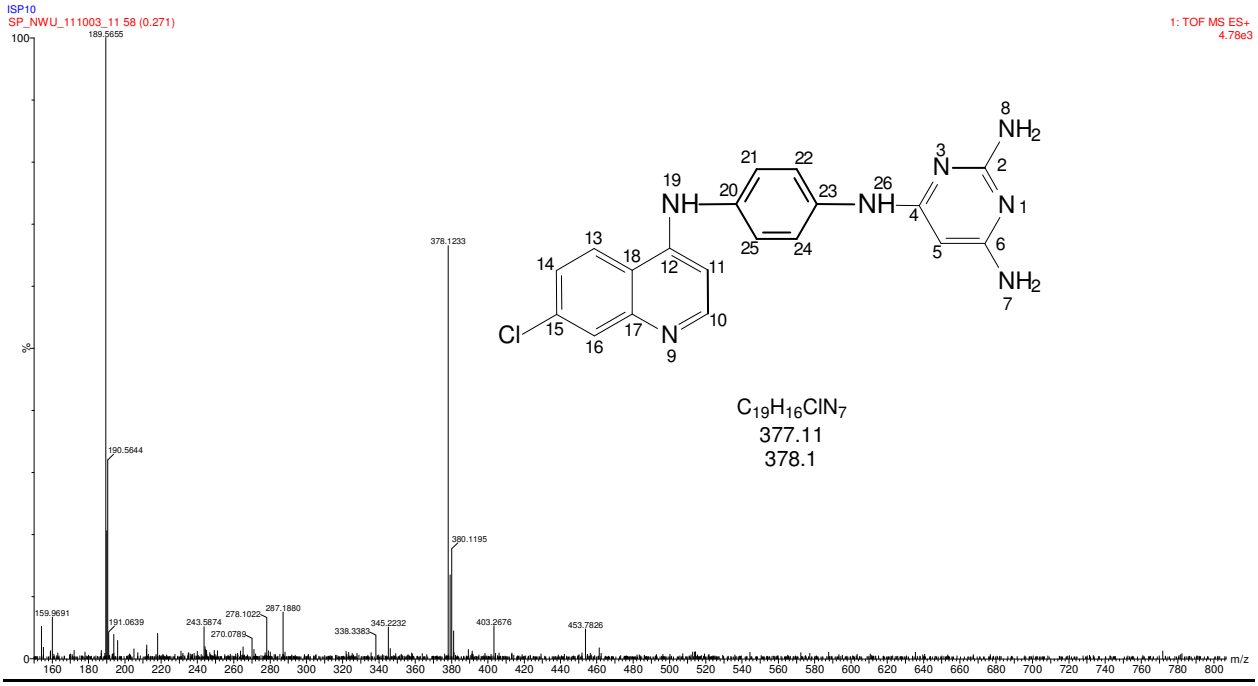
$C_{16}H_{18}ClN_7$
343.13
345.1 (M+H+)

SPECTRUM 28: MS ES+ of (19)



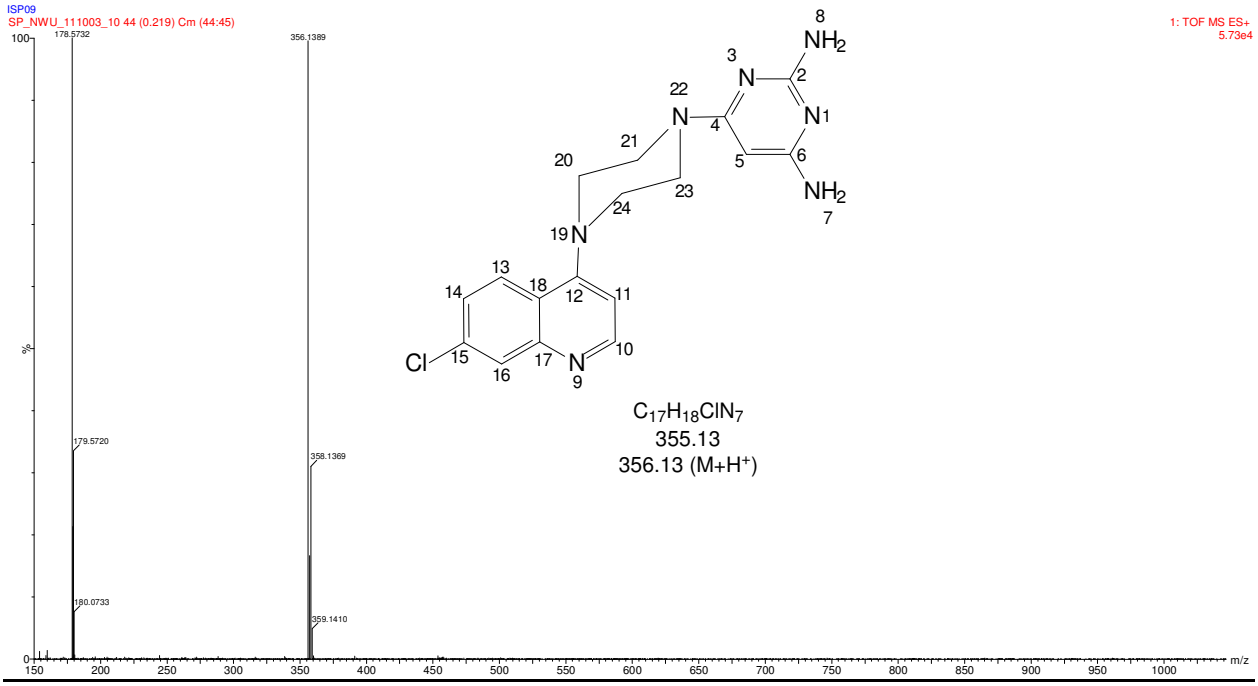
$C_{17}H_{20}ClN_7$
357.14
358.14 ($M+H^+$)

SPECTRUM 29: MS ES+ of (20)



$C_{19}H_{16}ClN_7$
377.11
378.1

SPECTRUM 30: MS ES+ of (21)



$C_{17}H_{18}ClN_7$
355.13
356.13 ($M+H^+$)