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PTA coupled Ruthenium-aminochloroquinoline complexes: Antimalarials of the Future



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INTRODUCTION

Since the beginning of agriculture 10 000 years ago, malaria has been affecting mankind. This is because the humid conditions of the fields make the perfect environment for the *Anopheles* mosquito, which transmits the *Plasmodium* malaria parasite. This sickness can be very deadly, therefore there has always been an interest in finding a cure against it.

Quinine, the first antimalarial, was isolated from *Chinchona*, a peruvian tree, in 1632; but the malaria parasites developed a resistance against this medicine, obligating the scientific community to find new treatment possibilities. In 1934, chloroquine was developed as a quinine replacement, but, like its predecessor, the *Plasmodium* parasites again developed resistance and this problem of resistance persists with other antimalarials as well.

A new approach to overcome resistance is to couple antimalarial drugs or fragments of such drugs, with organometallic compounds to form metal complexes with antimalarial properties. Based on this principle, in 1994 Biot synthesized ferroquine, a chloroquine derivative coupled with a ferrocene molecule that not only overcomes parasitic resistance but also retains the antimalarial efficiency of the chloroquine, opening a door to infinite choices of creating new metalorganic antimalarial compounds.

The aim of this project is to develop and enhance an organometallic antimalarial: A *p*-cymene ruthenium-chloroquine compound to which a phosphine (PTA) molecule can be coordinated in order to make the complex water soluble, and possibly increasing its antimalarial activity. This project is the continuation of my bachelor work, in which the ruthenium chloroquine and halogenide coordinated ligand complexes were developed. The objective of this study was to synthesize ruthenium complexes of this ligand, to introduce 1,3,5-triaza-7-phosphaadamantane (PTA) as an additional ligand and (if possible) compare the antimalarial properties of the PTA derivatives to the corresponding parent complexes. As the present study is a direct continuation of my B.Sc. research, my B.Sc. thesis will be cited extensively [1].

MALARIA

Malaria is a protozoan sickness caused by five species of the Plasmodium parasite:

- *Plasmodium falciparum*
- *Plasmodium vivax*
- *Plasmodium knowlesi*
- *Plasmodium malariae*
- *Plasmodium ovale*

The *P. falciparum* parasite is the most dangerous species because it can enter the brain, thus causing coma, and death. Symptoms of malaria include fevers, chills, vomiting, sweats, hepatic and renal failure.

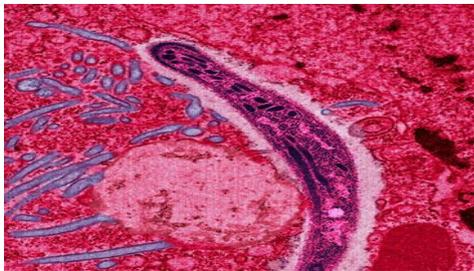


Fig 1. (left) A Plasmodium parasite [2] (right) an Anopheles mosquito [3]

Plasmodium is transmitted via *Anopheles* mosquitoes. The mosquito is a very good vector for spreading the sickness; acting as a hypodermic needle, biting the host, contaminating it with the parasite. [1]

As Fig. 2 shows, the mosquito inserts the parasite into the host, the parasite sporozoites travel through the capillary vessels to the liver, where they enter the hepatic cells to incubate (hepatic schizonts). Two weeks later, the parasite ruptures the cell, spreading its merozoites to the blood system, finding the red blood cells, entering into and feeding on them. To satisfy its biological requirements, the parasite digests and degrades large quantities of hemoglobin.

At this stage of the sickness the symptoms appear, especially the fevers and the chills. After this, the parasite stays in the blood, prepared to leave the host, waiting for a bite by another mosquito, which will serve as a carrier to reach another host. [1]

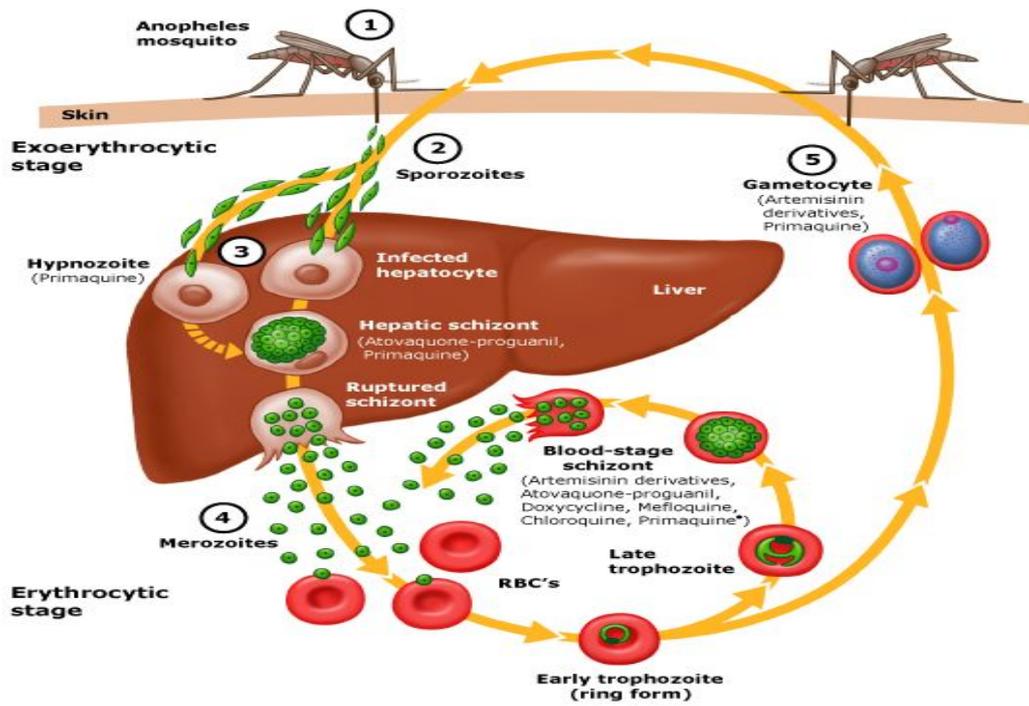


Fig. 2 The malaria life cycle [3]

It is estimated that more than 3.3 billion of persons are at risk of acquiring the parasite. The *Anopheles* mosquito lives in tropical humid regions:

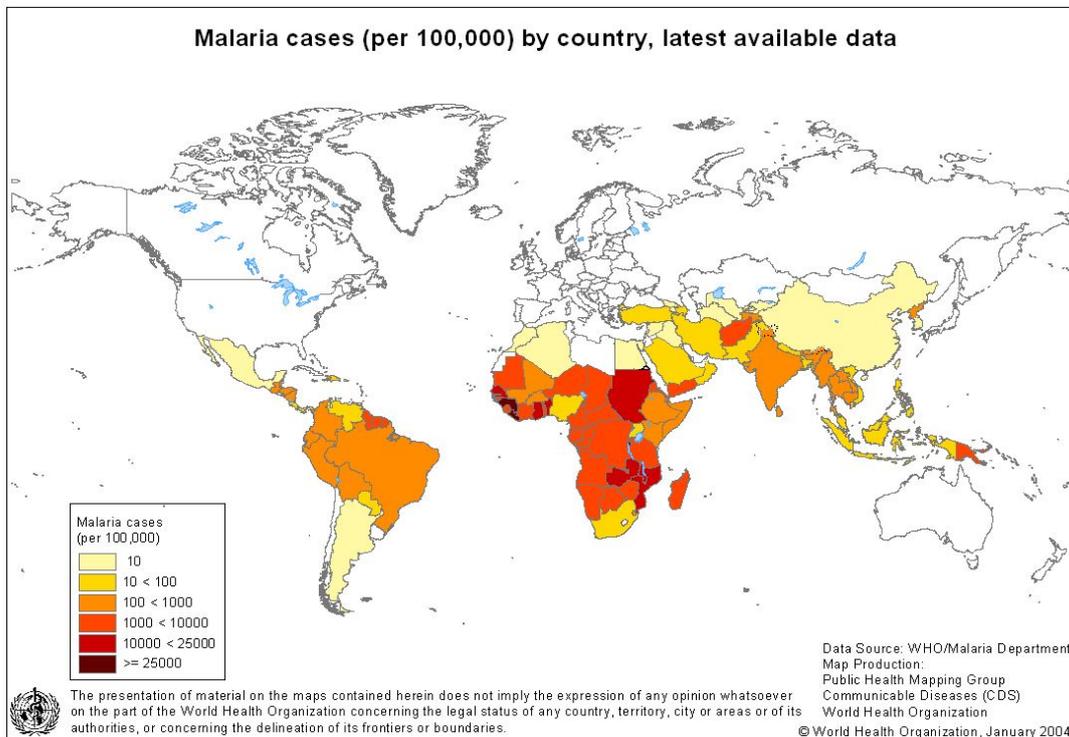


Fig 3. Malaria cases in the world [4]

In 2010, more than 655 000 deaths were registered, 91% of these being in the African region, and approximately 85% of these deaths were children not older than 5

years[5]. The World Health Organization has malaria in their priority list because of the many people affected by this disease.

ANTIMALARIALS: THE PAST

Malaria has been known for 10 000 years; it began in old Egypt when agriculture was born. Because of water necessity for food harvesting, the perfect humid environment was made for the mosquito to live. The main food source of the mosquito is human blood, therefore while feeding, it spreads the parasite everywhere. At the beginning and for a long time it was believed that the cause of the sickness was the putrid air conditions, hence its name “mal-aria” which means “bad air”. There has always been a strong interest in finding a cure against malaria, the first, quinine, being discovered by a French countess in Peru [1]:

Quinine: One of the components of the bark of the Chinchona tree, this compound was long used as an antimalarial by the indigenous population long before its “discovery” in 1632. In 1820, quinine was isolated by two French chemists and it is also a component of tonic water. The compound is still an important antimalarial, resistance was first observed in 1910.

Chloroquine: While looking for a quinine replacement, a German scientist developed this compound in 1934. With chloroquine, a new class of antimalarials was born: **the 4-aminoquinolines**. It was studied in depth by American researchers, especially during World War II [1]. Dismissed because of its toxicity, it was later discovered that if taken in small quantities, chloroquine is very effective against malaria, making it the principal antimalarial remedy recommended by WHO (After WWII). But in 1957 parasite resistance was observed, thus increasing the studies for a new alternative in form of coordination with transition metals, which not only increases its effectivity but overcomes the resistance of the parasite against the medicament.

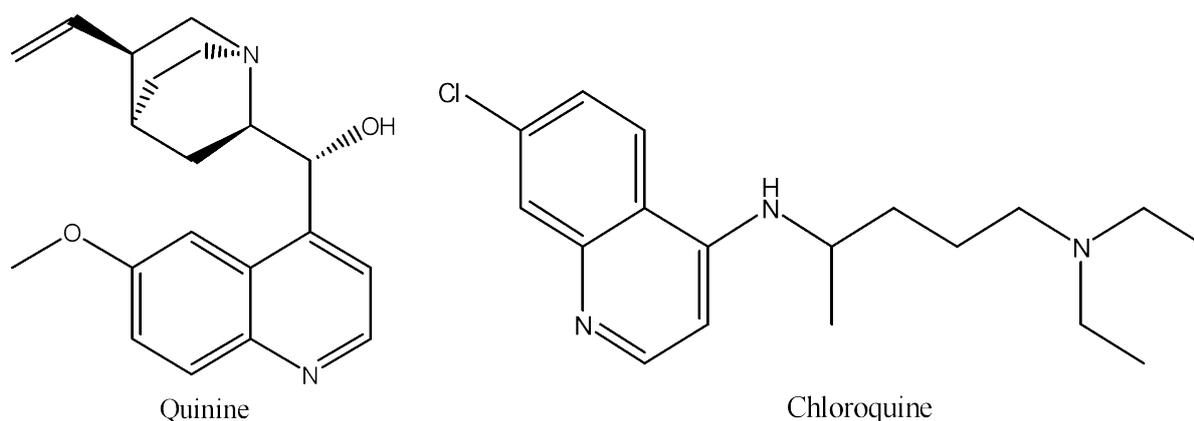


Fig. 5 Chemical structures of Quinine and Chloroquine

Mefloquine: This anti *P. falciparum* medicine, which was created in 1974 by the US Army Medical Research and Development command, WHO and Hoffmann-La Roche, its use was discontinued because parasite resistance was shown in 1985 in Asia, shortly after the drug became available for public use [6].

Artemisinin: Found in the *Artemisa annua* tree, this compound was isolated by Chinese researchers who studied its efficacy against *P. Falciparum* in mice in 1970. Human studies began in 1979 and the results were published in 1979 in the Chinese Medical Journal [1]. In 2015, Youyou Tou won the Nobel Prize in Medicine because of her discovery of Artemisinin. Artemisinin and other artemether group medicaments are the main antimalarials in South-East Asia. [6]

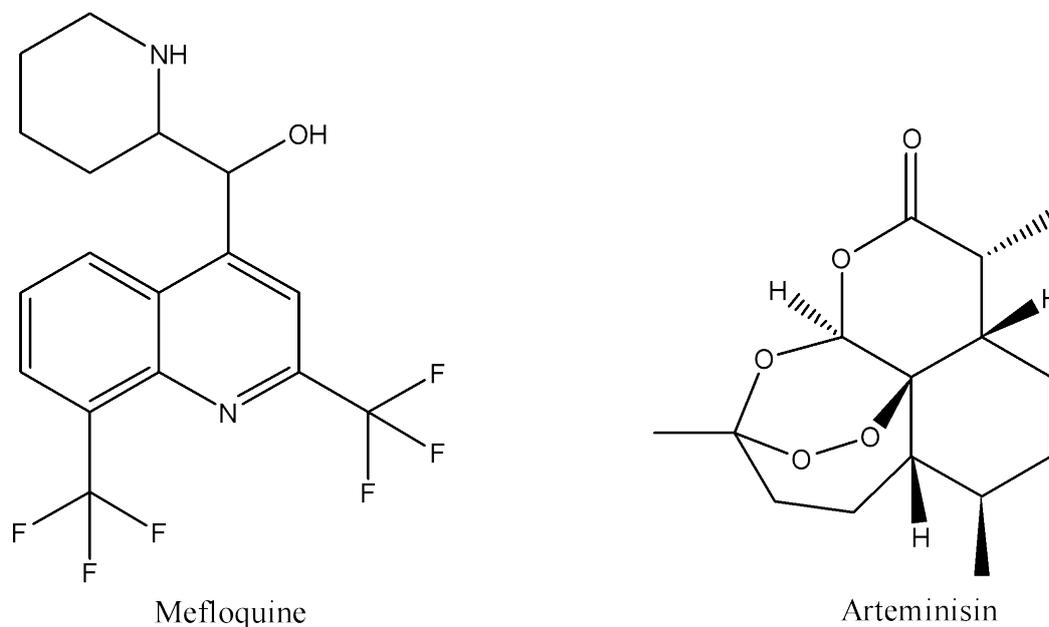


Fig. 6 Chemical structures of Mefloquine and Artemisinin

ANTIMALARIALS: THE PRESENT

Artemisinin-based Combination Therapy (ACT)

ACT is an artemisinin based treatment that combines this drug with other antimalarial drugs, increasing its efficacy against the malady. Artemisinin derivatives are active against all of the parasite's blood stages, resulting in shorter fever clearance times compared to other antimalarials [1]. But in 2009 it was observed that humans receiving ACT treatment required longer time to get rid of the parasites [6].

RESISTANCE TO ANTIMALARIALS: HEMOZOIN

Hemozoin is a crystalline brown pigment that is a product of the hemoglobin catabolism in the parasite's digestive vacuole [7]:

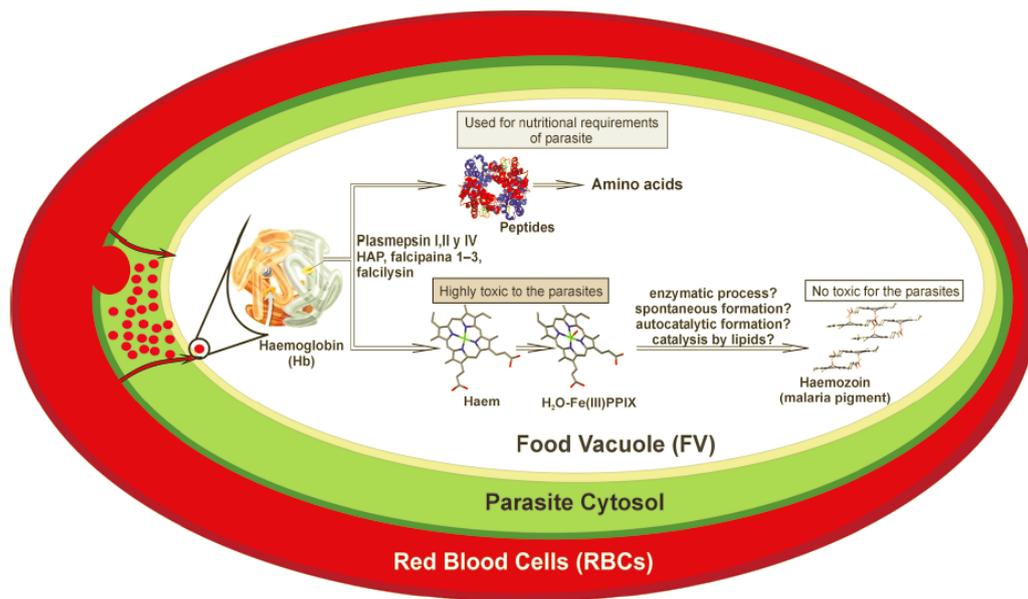


Fig. 7 Hemozoin production [8]

Being the main source of energy and nutrients for the parasite, up to 80% of the host's hemoglobin is digested by *Plasmodium*. This procedure also kills the blood cells and spreads the parasite further in the host system. The digestion of hemoglobin, generates free heme b, a very toxic compound for the parasite. Because of the parasite's inability to excrete the free heme, and it not possessing a heme oxygenase to recover the iron and detoxifying the heme, the parasite aggregates the heme into an insoluble crystal in a biocrystallization process that produces hemozoin, which, transformed into a crystal can be expelled from the body of the parasite. [9]

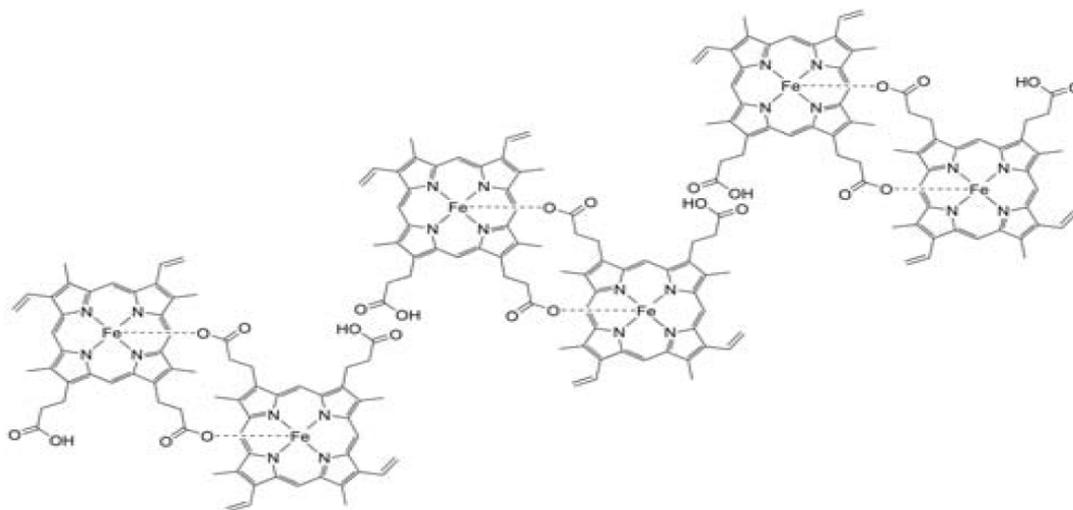


Fig 8. Crystalline form of hemozoin [9]

ANTIMALARIALS: THE FUTURE

The main objective of the new antimalarials is to stop the hemozoin formation. Because this process is not completely understood, a synthetic form of hemozoin has

been created: β -Hematin. This compound is chemically identical to hemozoin and consists of dimers of Fe(III)(protoporphyrin IX) (ferric heme) connected through reciprocal iron propionate bonds (*cf.* Fig. 8). It has been found that the β -hematin formation is an enzyme-independent water/lipid interface process. With these advances, the hemozoin biomineralisation process is more understood [10].

Studies have shown that the principal interaction between 4-aminoquinolines and β -hematin indicates the formation of a π - π complex through coplanar interaction between the quinoline aromatic ring and the porphyrin system [10]. Antimalarials based on 4-aminochloroquinolines act by accumulating in the digestive vacuole, where they form complexes with hemin to inhibit its conversion to hemozoin. Because of the acidic environment of the digestive vacuole, protonation of the quinoline N-bond and the side chain terminal nitrogen occurs, and it prevents the resultant positively charged molecule from leaving the food vacuole of the parasite [11]. This drug accumulation is known as pH trapping, and so the hemozoin formation does not take place. Fig. 9 illustrates the critical structural elements of 4-aminoquinoline/chloroquine molecules that are important for this process:

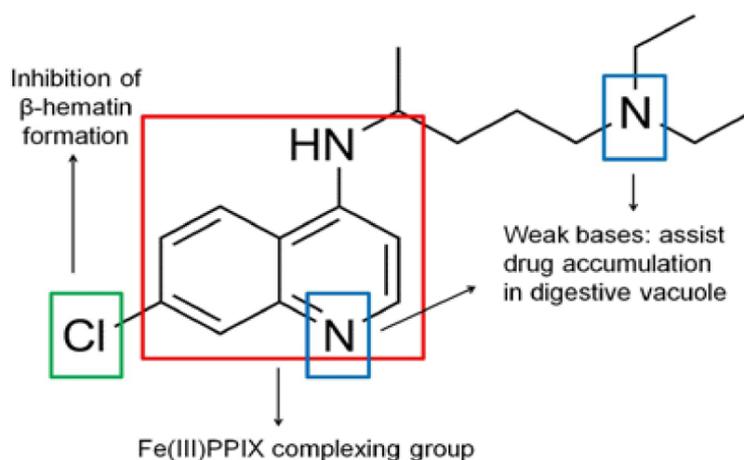


Fig. 9 Structure and explanation of chloroquine, taken from L. Glans (2012) [12]

Chloroquine parasitic resistance still persist, this being the main problem of the antimalarials. This situation forces the scientific community to find new ways to solve this problem. Ferroquine (Fig. 10) a potent organometallic antimalarial agent was created by Biot in 1994. Biot coupled ferrocene, an organometallic iron based compound, with a quinoline to create it. Having passed phase IIb clinical trials, ferroquine opens more doors for the investigation of a new class of antimalarials: **The chloroquine transition metal complexes.**

CHLOROQUINE TRANSITION METAL COMPLEXES

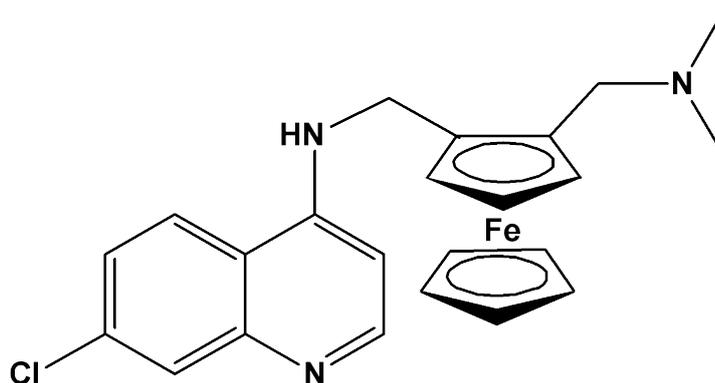


Fig. 10 Structure of ferroquine.

The antimalarial principle of chloroquinoline is its ability to prevent hemozoin formation in the food vacuole of the parasite. Hemozoin is very toxic, killing the parasite and brings the sickness to an end. The big problem of all antimalarials is the resistance that the parasite builds over time upon being exposed to the antimalarial drug.

The success of metal-based drugs like cisplatin (anticancer), awakened the interest of the scientific community for inorganic medicinal chemistry and the search for complexes of other transition metals with interesting biological properties [14]. The transformation of antimalarial products of known activity by addition of a transition metal into its molecular structure is very important in biological systems, as the binding capabilities and reactivities of transition metals may alter the properties of the drugs [15].

The method of complexing metals with antimalarials has been most widely explored with chloroquine, which is a cost-effective antimalarial drug with a good safety profile, making it the perfect compound for metal complexes. It was observed that when complexed with a metal, the antimalarial activity of chloroquine is not only restored but in addition parasitic resistance is overcome. The lower parasitic resistance of this chloroquinoline complex is attributed to the high lipophilicity of metal complexes and the structural modification of chloroquinoline [16].

To complex the chloroquinoline a transition metal an arene ring in the structure is often used, because the ring structure stabilizes the metal center and helps to increase the lipophilicity of CQ, which is believed to be of importance for transmembrane transport (into the cell/parasite). Based on this principle, ferrocene, an iron cyclopentadiene organometallic compound, was incorporated into the chloroquinoline framework to create ferroquinoline (Fig. 10), the first metalorganic drug that consist of a covalently coupled ferrocenyl group by a 4-aminoquinoline and a basic alkylamine [16]. It is important to mention that ferrocene did not have the expected results when coordinated with other antimalarials (mefloquine, quinine, artemisinin) this was because of their instability in acidic conditions [16].

Racemic mixtures of ferroquinoline does not affect its antimalarial properties, studies between ferroquinoline enantiomers have not shown any significant difference in their effectivity.

Both ferroquinoline and chloroquinoline accumulate in the digestive vacuole, thus preventing hemozoin formation. Both are weak bases with the ability of accumulation in the digestive vacuole, but there are also significant differences between both compounds: One of them is the lower pKa of ferroquinoline, and ferroquinoline also produces big quantities of toxic hydroxyl radicals in the parasitic vacuole. Another reason for the high antimalarial activity of ferroquinoline is probably its preferred localization at the lipid-water interface. It is assumed that ferroquinoline might prevent the hemozoin modification by maintaining the hemozoin in the aqueous environment. [16]

There are studies that include the complexation between chloroquinoline and organometallic compounds based on metals like ruthenium, iridium and rhodium, that show the same properties as ferroquinoline, thus increasing the possibility of creating of new antimalarials. This area of research is still under development, but it has a very big potential.

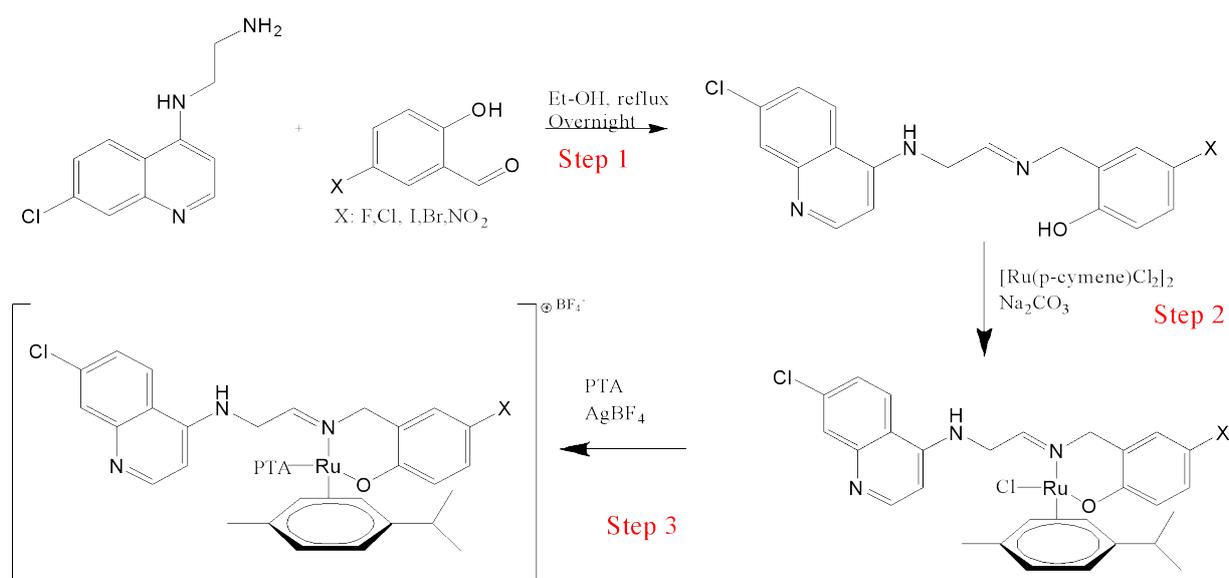
MY WORK

The goal of this study is to continue the work of my previous project [1]: To produce several variations of metal 4- aminochloroquine complexes, continuing the studies carried out before by Lotta Glans [12] and Erik Ekengaard [13].

The path to follow is the same as outlined in [1]:

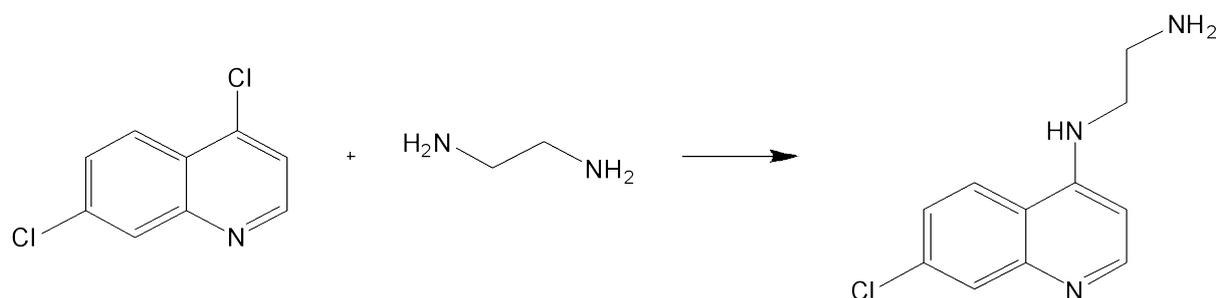
- Synthesis of the chloroquine root ligand compound N'(7-chloroquine-4-yl-ethane-1,2-diamine, which was achieved in the previous project [1].
- Formation of ligands through the reaction of the root ligand starting material with halogen-containing salicylaldehydes. In the previous project two ligands were successfully made, in this project more ligands were to be produced.
- Each ligand should be coordinated to a Ru-cymene fragment, and the resultant complex should subsequently be derivatized with the phosphine PTA (1,3,5-triaza-7-phosphaadamantane) in order to increase aqueous solubility of the complex.

Scheme 1 illustrates the different steps:



Scheme 1. Chemical syntheses to be carried out within this project

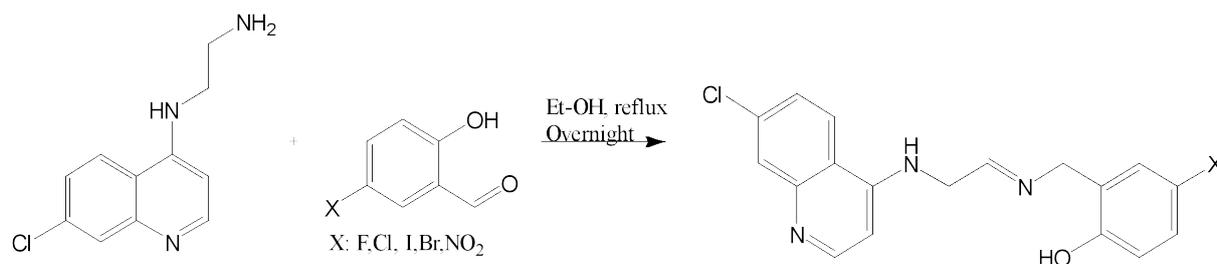
RESULTS AND DISCUSSION:



Scheme 2. Reaction 1

Reaction 1 was developed in the previous project, therefore the production of the rootligand was easily made following the previously described procedure [1].

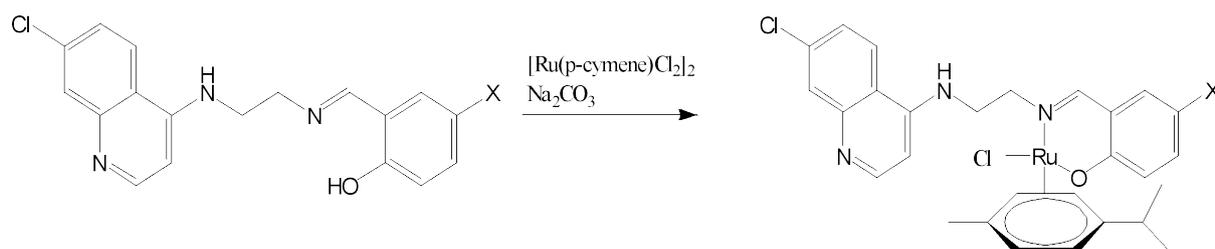
With this product, the desired ligands were made effortlessly following the procedure described by Erik Ekengard [13] (Scheme 3).



Scheme 3. Ligand reactions (reaction 2-6)

This led to the synthesis of the halide and nitro salicylchloroquinoline ligands, which were found to be very hygroscopic [1]. In the previous project there were unsuccessful attempts at synthesis of the corresponding ruthenium *p*-cymene complexes. There were many problems, e.g. temperature sensibility of the reaction or the possibility of making one- or two-pot reactions for the phosphine addition. One of the goals of the continuation of this project was to produce the chloroquinone-based complexes.

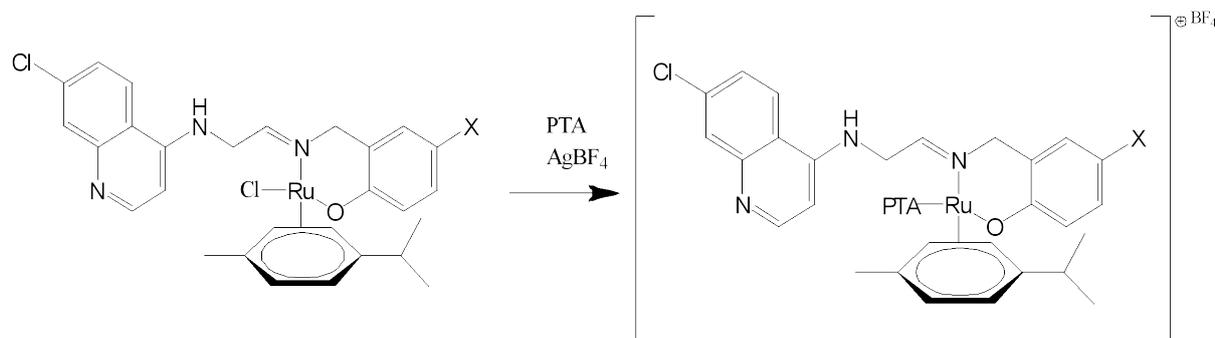
It was decided on a two-pot synthesis, the first step being the coordination of the ruthenium-*p*-cymene salt to the ligand (Scheme 4).



Scheme 4. Complex reaction

A number of modifications of the synthetic procedure were made. Firstly, the base for the deprotonation (triethylamine) was changed to potassium carbonate, as the solvent methanol was chosen instead of the original dichloromethane. The reaction was carried out at a temperature reaction of -60°C , thus solving the temperature sensibility problem of the product, and the reaction time was at least 7-12 hours. It is important to note that, as mentioned in [1], the addition of ruthenium-*p*-cymene chloride dissolved in dichloromethane was made dropwise and after a deprotonation of at least 2 hours. As suggested in [1], the raw product was filtrated via celite, the solvent was evaporated in *vacuo* and the product cleaned again by sonication, using diethylether as a solvent. This synthesis was reproduced with all ligands, and all complexes are not soluble in water.

Water solubility is a very important aspect of any potential antimalarial because it may facilitate drug transport and thus increase its efficacy. To make the complexes water soluble, the water soluble phosphine PTA (1,3,5-triaza-7-phosphaadamantane) was coordinated to the complex, replacing the chloride ligand (Scheme 5):



Scheme 5. PTA coordination to the Ruthenium complex

This reaction was possible because of the lability of one chloride ligand, thus making rapid aquation possible. A silver salt (AgBF_4) was added to precipitate AgCl and then PTA was coordinated to the complex. Methanol was chosen as a solvent, because the better solubility of the starting complex as well as the PTA derivative in this solvent. The synthesis was made under nitrogen-atmosphere and carried out at room temperature. After a reaction time of aprox. 10 hours, the solvent was removed in *vacuo* and the product was cleaned by sonication using diethylether as solvent. Two PTA complexes were made: F and Br.

The PTA complexes were soluble in water, in contrast to the parent Ru-complexes; this is shown in Fig.11. However, satisfactory ^1H NMR spectra could not be obtained. Even though the ^1H NMR spectra showed the presence of a (coordinated) PTA molecule, impurities were detected. The same problem was encountered when the ^{31}P NMR spectrum was acquired. However, a much cleaner ^{31}P NMR spectrum could be obtained when D_2O was used as solvent instead of deuteriochloroform, while the ^1H NMR spectrum still showed the presence of impurities.

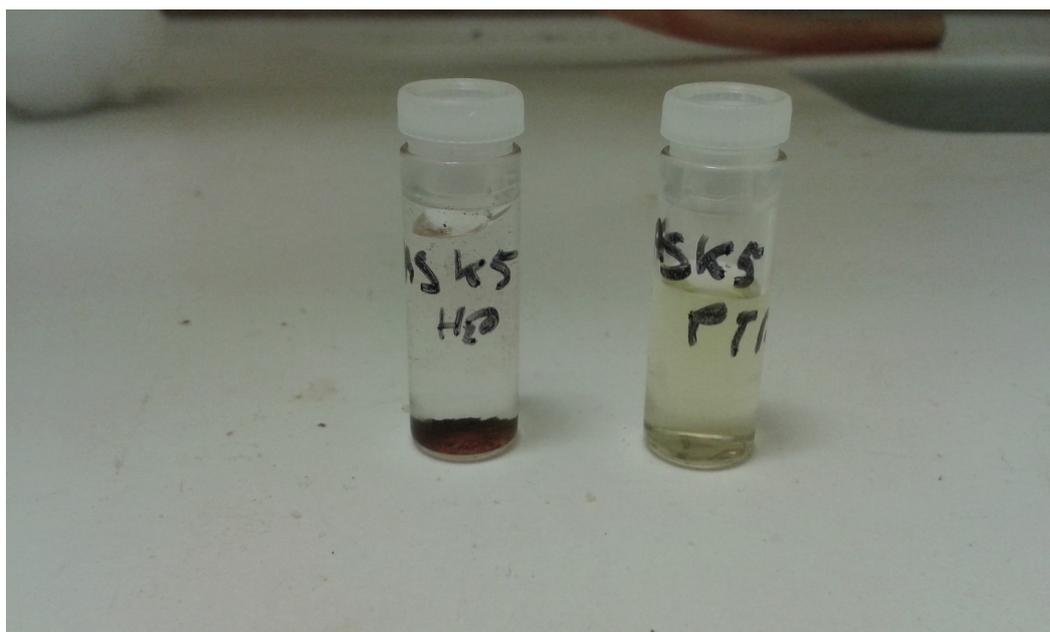


Fig 11. Parent ruthenium complex (left) and PTA ruthenium complex dissolved in water (Br ligand)

In order to purify products, various recrystallization attempts were unsuccessfully made on both parent and PTA complexes. The PTA-complexes could be somewhat purified by dissolving them in water and subsequent filtration, whereupon black specks of impurities were removed. After this purification the ^1H NMR spectrum was clearer but impurities could still be detected. On the other hand, the ^{31}P NMR spectra showed a distinctive at -30 ppm that did not belong to free PTA (150 ppm). The significant shift is in accordance with coordination of to the ruthenium complexes.

These results indicate that water-soluble PTA derivatives of the Ru cymene complexes

can be made, and this chemistry should be pursued further and also investigated with other substituted quinoline-salicylaldehyde ligands (not only the haloderivates). Furthermore, PTA derivatives of other metal complexes (Ir, Rh) with the quinoline ligands may be synthesized and their antimalarial properties investigated. Fig. 12 shows the ^{31}P NMR spectra of PTA and the two PTA-complexes.

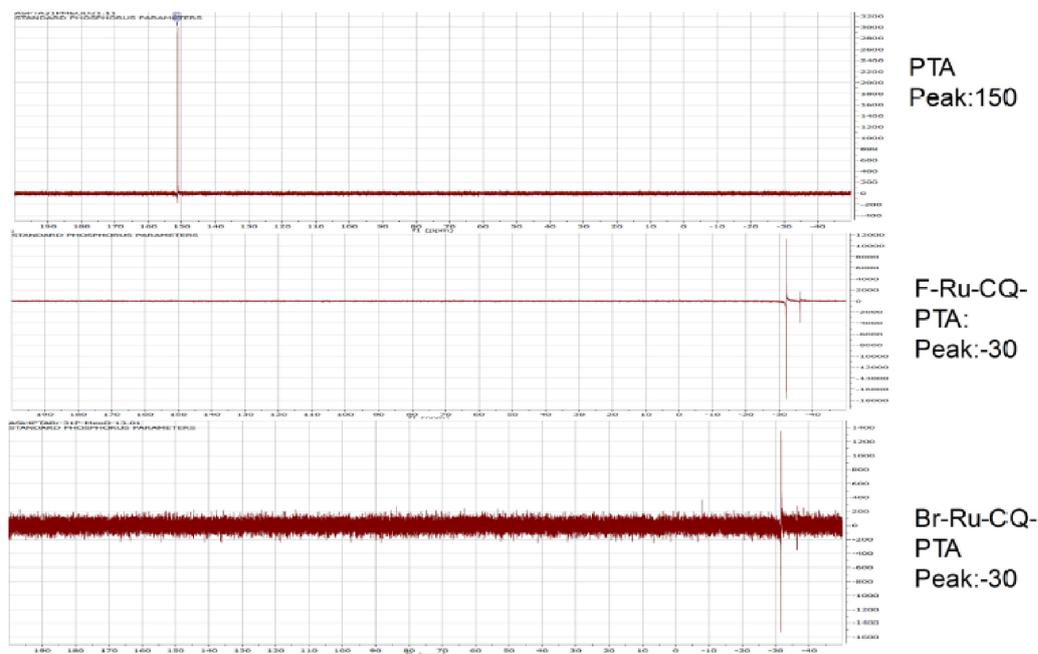


Fig 12. Phosphorus NMR spectra of PTA, F-Ru-CQ-PTA complex and Best regards-Ru-CQ-PTA complex.

EXPERIMENTAL PROCEDURES:

All reagents used were purchased from Sigma Aldrich unless otherwise stated. NMR spectra were recorded using a Varian Inova 500 MHz spectrometer. Reaction conditions are stated in the synthetic procedures (below).

N'-(7-chloroquinolin-4-yl)ethane-1,2-diamine (1): 4,7-dichloroquinoline (0.200 g, 10.07 mmol) and ethylenediamine (0.648 g, 0.101 mmol, 10 eq) in 30 mL of ethanol, were refluxed at 93°C under N_2 atmosphere and left to stir for 22 hours. Half of the solvent was *removed in vacuo*, and the resulting solution was poured into icewater. The resulting white precipitate was filtered and left to dry. The product was a pearly white powder (1.731 g, 7.8 mmol, 77.5%). [1]
 $^1\text{H NMR}$ (CDCl_3): 8.54(d, 1H), 7.96(d, 1H), 7.74(d, 1H), 7.38(dd, 1H), 6.42(d, 1H), 3.34(q, 2H), 3.13(t, 2H).

N-(2-((5-Fluoro-2-hydroxyphenyl)methylimino)ethyl)-7-chloroquinolin-4-amine (2): 5-Fluoro-2-hydroxybenzaldehyde (0.140 g, 1.0 mmol) and N'-(7-chloroquinolin-4-yl)ethane-1,2-diamine (0.222 g, 1.0 mmol) were refluxed (80°C) in 25 mL ethanol overnight. The solvent was *removed in vacuo* and the product was

dried under vacuum. The product was obtained as a hygroscopic yellow powder (0.3165 g 0.921 mmol, 92%). [13]

¹H NMR (500 MHz, CDCl₃): 12.72(s, 1H), 8.55 (d, 1H), 8.34 (s, 1H), 7.99 (d, 1H), 7.65 (d, 1H), 7.37 (dd, 1H), 7.06 (m, 1H), 6.94 (t, 1H), 6.92 (t, 1H), 6.51 (d, 1H), 5.40 (m, 1H), 3.99 (dd, 2H), 3.76 (dd, 2H)

N-(2-((5-Chloro-2-hydroxyphenyl)methylimino)ethyl)-7-chloroquinolin-4-amine

(3): 5-Chloro-2-hydroxybenzaldehyde (0.314 g, 2.0 mmol) and N'-(7-chloroquinolin-4-yl)ethane-1,2-diamine (0.444 g, 2.0 mmol) were refluxed (80°C) in 50 mL ethanol overnight. The solvent was *removed in vacuo* and the product was dried under vacuum. The product was obtained as a hygroscopic yellow powder (0.5044 g, 1.4 mmol, 70%). [13]

¹H NMR (CDCl₃) 12.94 (s, 1H), 8.54 (d, 1H), 8.32 (s, 1H), 7.98(d, 1H), 7.69 (d, 1H), 7.36 (dd, 1H), 7.29 (d, 1H), 7.19 (d, 1H), 6.92 (d), 6.51 (d, 1H), 3.99 (t), 3.77 (dd, 2H)

N-(2-((5-Iodo-2-hydroxyphenyl)methylimino)ethyl)-7-chloroquinolin-4-amine (4):

5-Iodo-2-hydroxybenzaldehyde (0.248 g, 1.0 mmol) and N'-(7-chloroquinolin-4-yl)ethane-1,2-diamine (0.222 g, 1.0 mmol) were refluxed(80°C) in 50 mL ethanol overnight. The solvent was *removed in vacuo* and the product was dried under vacuum. The product was obtained as a hygroscopic white-yellow powder (0.2715 g, 0.61 mmol, 60%). [13]

¹H NMR (DMSO-d₆) 13.49 (s, 1H), 8.49 (d, 1H), 8.39 (s, 1H), 7.78(d, 1H), 7.73 (d, 1H), 7.44 (dd, 1H), 6.70 (d, 1H), 6.60 (d, 1H), 3.88 (t, 1H), 3.62 (dd, 1H)

N-(2-((5-Bromo-2-hydroxyphenyl)methylimino)ethyl)-7-chloroquinolin-4-amine

(5): 5-Bromo-2-hydroxybenzaldehyde (0.418 g, 2.0 mmol) and N'-(7-chloroquinolin-4-yl)ethane-1,2-diamine (0.444 g, 2.0 mmol) were refluxed(80°C) in 50 mL ethanol overnight. The solvent was *removed in vacuo* and the product was dried under vacuum. The product was obtained as a hygroscopic yellow powder (0.272 g, 0.120 mmol, 60%). [13]

¹H NMR (DMSO-d₆) 13.49 (s, 1H), 8.51 (d, 1H), 8.40 (s, 1H), 8.23(d, 1H), 7.78 (d, 1H), 7.61 (s, 1H), 7.47 (d, 1H), 7.44(s, 1H), 6.88 (d, 1H), 6.61(d, 1H), 3.89(m,2H), 3.64 (m, 2H)

N-(2-((5-Nitro-2-hydroxyphenyl)methylimino)ethyl)-7-chloroquinolin-4-amine (6):

5-Iodo-2-hydroxybenzaldehyde (0.239 g, 1.43 mmol) and N'-(7-chloroquinolin-4-yl)ethane-1,2-diamine (0.315 g, 1.43 mmol) were refluxed(80°C) in 50 mL ethanol overnight. The solvent was *removed in vacuo* and the product was dried under vacuum. The product was obtained as a hygroscopic white-yellow powder (0.281 g, 0.76 mmol, 38%). [13]

¹H NMR (DMSO-d₆) 13.49 (s, 1H), 8.49 (d, 1H), 8.39 (s, 1H), 7.78(d, 1H), 7.73 (d, 1H), 7.44 (dd, 1H), 6.70 (d, 1H), 6.60 (d, 1H), 3.88 (t, 1H), 3.62 (dd, 1H)

(η^6 -*p*-cymene)N-(2-((5-Fluoro-2-hydroxyphenyl)methylimino)ethyl)-7-

chloroquinolin-4-amine)chlororuthenium(II) (7): To a solution of N-(2-((5-Fluoro-2-hydroxyphenyl)methylimino)ethyl)-7-chloroquinolin-4-amine (0.200 g, 0.58 mmol) dissolved in 20mL of ethanol, potassium carbonate (0.080g, 0.58mmol) was added and the mixture was left to stir for at least 2h. Then (*p*-cymene)ruthenium(II) chloride dimer (0.178 g, 0.129 mmol) dissolved in dichloromethane, was added dropwise to the solution and it was left to stir overnight at -60°C. The raw product was filtered through celite and solvent was evaporated *in vacuo*. After sonication (in

diethylether), the product was obtained as a dark red solid (0.212 g, 0.76 mmol, 60%).

¹H NMR (CDCl₃) 8.49 (s, 1H), 7.95 (s, 1H), 7.73 (d, 1H), 7.21(s, 1H), 7.19 (s, 1H), 6.94 (t, 1H), 6.83 (dd, 1H), 6.82 (br, 1H), 6.48 (s 1H), 5.52 (d, 1H), 5.40 (d, 1H), 4.98 (d,1H), 4.52 (m, 1H), 4.25 (m, 2H), 3.88 (m,1H), 2.80 (td, 1H), 2.30 (d, 3H), 1.25 (d, 3H), 1.14 (d, 3H)

η^6 -*p*-cymene)N-(2-((5-Bromo-2-hydroxyphenyl)methylimino)ethyl)-7-chloroquinolin-4-amine)chlororuthenium(II) (8): To a solution of N-(2-((5-Bromo-2-hydroxyphenyl) methylimino) ethyl)- 7-chloroquinolin-4-amine (0.200 g, 0.495 mmol) dissolved in 20 mL of ethanol, potassium carbonate (0.068 g, 0.50 mmol) was added and the mixture was left to stir for at least 2h. Then (*p*-cymene)ruthenium(II) chloride dimer (0.151 g, 0.2475 mmol) dissolved in dichloromethane, was added dropwise to the solution and it was left to stir overnight at -60°C. The raw product was filtered through celite and solvent was evaporated *in vacuo*, after sonication (in diethylether), the product was obtained as a orange solid (0.279 g , 0.41 mmol, 83%).

¹H NMR (CDCl₃) 8.50 (s, 1H), 7.97 (s, 1H), 7.74 (d, 1H), 7.20(s, 1H), 7.19 (s, 1H), 6.95 (t, 1H), 6.83 (dd, 1H), 6.81 (br, 1H), 6.50 (s 1H), 5.51 (d, 1H), 5.43 (d, 1H), 4.99 (d,1H), 4.54 (m, 1H), 4.25 (m, 2H), 3.87 (m,1H), 2.79 (td, 1H), 2.31 (d, 3H), 1.25 (d, 3H), 1.15 (d, 3H)

η^6 -*p*-cymene)N-(2-((5-Bromo-2-hydroxyphenyl)methylimino)ethyl)-7-chloroquinolin-4-amine)chlororuthenium(II)PTA(9): η^6 -*p*-cymene)N-(2-((5-Bromo-2-hydroxyphenyl)methylimino)ethyl)-7-chloroquinolin-4-amine)chlororuthenium(II) (0.100 g, 0.148 mmol) and 1,3,5-Triaza-7-phosphaadamantane(PTA) (0.023 g, 0.148 mmol) were dissolved in 20-30 mL of methanol and left to stir under nitrogen atmosphere, then (0.057 g, 0.029 mmol) of AgBF₄ dissolved in 0.5 mL of methanol was added dropwise and the solution was left to stir overnight. The raw product was filtered through celite and the solvent was *evaporated in vacuo*. For analysis the raw green-yellow product was dissolved in D₂O, dissolved and *reduced in vacuo*. The yield of this reaction was very low, but it was enough for ¹HNMR and ³¹PNMR analysis.

¹H NMR (D₂O) 8.43 (s, 1H), 8.08 (s, 1H), 7.93 (d, 1H), 7.58 (s, 1H), 7.13 (t, 1H), 2.94 (s,1H), 2.53 (dd, 1H). **³¹P NMR** (D₂O) -30

η^6 -*p*-cymene)N-(2-((5-Fluoro-2-hydroxyphenyl)methylimino)ethyl)-7-chloroquinolin-4-amine) chlororuthenium(II)PTA(10): η^6 -*p*-cymene)N-(2-((5-Fluoro-2-hydroxyphenyl)methylimino)ethyl)-7-chloroquinolin-4-amine) chlororuthenium (II) (0.062 g, 0.110 mmol) and 1,3,5-Triaza-7-phosphaadamantane (PTA) (0.016 g, 0.110 mmol) were dissolved in 20- 30 mL of methanol and left to stir under nitrogen atmosphere, then (0.038 g, 0.220 mmol) of AgBF₄ dissolved in 0.5 mL of methanol was added dropwise and the solution was left to stir overnight, the raw product was filtered through celite and solvent was evaporated *in vacuo*. For analysis the raw green product was dissolved in D₂O, and reduced *in vacuo*, yield of this reaction was very low, but it was enough for ¹HNMR and ³¹PNMR analysis.

¹H NMR (D₂O) 8.44 (s, 1H), 8.08 (s, 1H), 7.93 (d, 1H), 7.67 (s, 1H), 7.09 (t, 1H), 2.94 (s,1H), 2.53 (dd, 1H). **³¹P NMR** (D₂O) -30

SUMMARY AND CONCLUSIONS

- Malaria is a sickness that affects approx. 3 million people yearly.
- The main problem with the antimalarials drugs is the resistance towards the drugs that the malaria parasites develop rapidly.
- There have been advances in preventing hemozoin formation by coupling 4-aminochloroquine groups with metals to decrease the parasite resistance but maintaining its antimalarial effectivity.
- This is still work in progress and in the development phase.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Malaria is a disease that is transmitted by mosquitoes; it has been transmitted affecting humanity for more than 10.000 years, it affects between 700 000 and 2.7 million people per year and it can be deadly, therefore there is a large and increasing interest in developing a cure against it.

Quinine, the first antimalarial, was isolated from the *chinchona* tree in 1632. This compound was widely used in the form of tonic water, but afterwards, a decrease of its antimalarial effectivity was observed and in 1910 it was concluded that this situation was because the parasite that causes malaria had developed a resistance against the quinine.

This situation inspired the scientific community to find new options to fight the sickness. In 1934, chloroquine was developed by German scientists and was the most used antimalarial until the 1950's, when parasitic resistance again occurred. Other medicaments like mefloquine and artemisinin have been developed but the resistance problem still exists thus maintaining interest in the development of for new treatments.

The success of the anticancer medicine cisplatin and other metal-based drugs has increased interest in inorganic medicinal chemistry. Therefore scientists came to the idea of coupling a known antimalarial with a organometallic species thus creating organometallic antimalarial complexes that are not only effective but the parasite does not develop resistance against it. Studies of ferroquine, a chloroquinoline derivative coupled with a ferrocene molecule, showed fantastic results and it is in phase IIb of medical trials.

Chloroquine derivatives may be coupled with metals like ruthenium, iron, iridium and rhodium, and the first studies showed similar results (increased antimalarial activity without the resistance) to those from the ferroquine studies, indicating the great future and potential of these compounds. This chemistry is still on in the development phase.

This project is involved with the development of organometallic antimalarial compounds. Specifically ruthenium-chloroquine complexes coupled with a water-soluble phosphine molecule (PTA) were targeted in order to create antimalarial complexes with good aqueous solubility, which may be of importance to, their antimalarial effectivity.

POPULÄRVETENSKAPLIG SAMMANFATTNING (SVENSKA)

Malaria är en sjukdom som överförs av myggor; det har överförts påverka mänskligheten i mer än 10 000 år, det påverkar mellan 700 000 och 2,7 miljoner människor per år och det kan vara dödligt, därför finns det ett stort och ökat intresse för att utveckla en bot mot den.

Quinin, den första antimalarialen, isolerades från chinchona-trädet år 1632. Denna förening användes i stor utsträckning i form av tonisk vatten, men efteråt observerade man en minskning av dess antimalarial effektivitet och år 1910 drogs slutsatsen att denna situation berodde på att parasit som orsakar malaria hade utvecklat ett motstånd mot quinin.

Denna situation inspirerade det vetenskapliga samfundet att hitta nya alternativ för att bekämpa sjukdomen. År 1934 utvecklades klorokin av tyska forskare och var den mest använda antimalarialen till 1950-talet, när parasitmotståndet upprepades. Andra läkemedel som mefloquin och artemisinin har utvecklats men resistansproblemet finns fortfarande och därmed bibehåller intresse för utveckling av nya behandlingar.

Framgången av anticancermedicin cisplatin och andra metallbaserade läkemedel har ökat intresse för oorganisk medicinsk kemi. Därför kom forskare till idén om att koppla en känd antimalariell med en organometallisk art och därigenom skapa organometalliska antimalariella komplex som inte bara är effektiva, utan parasiten utvecklar inte motstånd mot den. Studier av ferroquin, ett kloroquinolinderivat kopplat till en ferrocenmolekyl, visade fantastiska resultat och ligger i fas IIb av medicinska prövningar.

Kloroquinderivatet kan kopplas med metaller som rutenium, järn, iridium och rodium, och de första studierna visade liknande resultat (ökad antimalariell aktivitet utan motstånd) mot de från ferroquinstudierna, vilket indikerar den stora framtiden och potentialen hos dessa föreningar. Denna kemi finns fortfarande i utvecklingsfasen.

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REFERENCES

[1] M.A. Silva Quintero, "Ruthenium-Aminochloroquinoline complexes: The antimalarials of the future", Bachelor Thesis, 2016, Lund University.

[2]<http://biology.plosjournals.org/perlserv/?request=get-toc&issn=1545-7885&volume=3&issue=6>, CC BY 2.5.

[3]http://scientistsagainstmalaria.net/sites/default/files/styles/full_post/public/Anopheles.jpg?itok=WpGi5yfc

[4] WHO/Malaria Department. Copyright: World Health Organisation, 2004

[5] WHO Malarial Report 2010. Copyright: World Health Organization, 2010

[6] <http://www.mmv.org/malaria-medicines/parasite-lifecycle>

[7] W. Castro, M. Navarro, "Complejos metálicos como potenciales antimaláricos con blanco de acción: Hemozoína", *Avan en Quím*, 7(2), 135-144 (2012)

[8] W.Castro, M.Navarro, C. Biot "Bioorganometallic Compounds with Antimalarial Targets: Inhibiting Hemozoin Formation", *Organometallics* 31, 5715-5727 (2012)

- [9] M. Olivier, K. Van de Dam, " Malarial Pigment Hemozoin and the Innate Inflammatory Response", *Front in Immu*, 2014, 5; 25
- [10] W. Castro, M. Navarro, "Complejos metálicos como potenciales antimaláricos con blanco de acción: Hemozoína", *Avan en Quím*, 7(2), 135-144 (2012)
- [11] L. Coronado, C. Nadovich, "Malarial Hemozoin: To target to tool", *Biochim Biophys Acta*. 2014 Jun; 1840(6): 2032–2041.
- [12] Source: L. Glans, "Organometallic complexes with antimalarial properties", Doctoral Thesis, 2012, Lund University
- [13] E. Ekengard, "Half-sandwich complexes with aminoquinoline ligands-synthesis, antiplasmodial and antimycobacterial activities", Doctoral Thesis, 2015, Lund University.
- [14] A. Martinez, C. S. Rajapakse, "Synthesis, Characterization and in vitro Antimalarial and Antitumor activity of New Ruthenium(II) Complexes of Chloroquine", *Inorg Chem*. 2009; 48(3): 1122-1131.
- [15] M. Navarro, W. Castro, "Metal-chloroquine derivates as possible anti-malarial drugs: evaluation of anti-malarial activity and mode of action", *Malar. J*; 2014, 13:471
- [16] W. Wani, E. Jameel, "Ferroquine and its derivates: New generation of antimalarial agents", *Eur. J. Med Chem*; 101 (2015) 534-551

