J. Chem. Soc. Nigeria, Vol. 47, No. 3, pp 573 – 592 [2022]

BIOORGANOMETALLIC FERROQUINE AND RELATED COMPOUNDS AS ANTIMALARIAL CHEMOTHERAPEUTIC AGENTS: A SHORT REVIEW

T. A. Abubakar[1]*, U. B. Eke[2] and A. Salisu[1]

[1]Chemistry Department, Sule Lamido University, Kafin Hausa, P.M.B. 048, Kafin Hausa
[2]Chemistry Department, University of Ilorin, P.M.B.1515, Ilorin, Nigeria
Corresponding author: aabu_tenimu@slu.edu.ng, +2348036331983

ABSTRACT

Drug resistant *Plasmodium falciparum* is a major threat to global health. Eradicating the parasite in endemic regions, especially sub-Saharan Africa is daunting. Milestones and a general target of 2030 have been set for this. However, funding and disruptions due to Ebola virus disease epidemic and COVID-19 pandemic are some of the cog in the wheel of the malaria eradication program. The recent WHO approval of the first malaria vaccine is a hope raiser and is expected to merely catalyze the eradication effort. Chemoprevention is a key malaria eradication strategy. Ferroquine, an organometallic chloroquine-ferrocene conjugate with effective antimalarial properties is capable of overcoming resistant strains and restoring chloroquine antimalarial properties. It has a novel mechanism of action. Like chloroquine, it forms complex with Fe(III)PPIX, strongly inhibit ß-hematin formation and drug accumulation in the acidic digestive vacuole. ferroquine is more lipophilic at cytosolic pH and cannot be pumped out of digestive vacuole thereby evading the chloroquine resistance mechanism. Clinical trials showed the drug candidate to be safe and tolerable. The ferroquine molecule can be conveniently synthesized via a reductive amination reaction involving a condensation of 7-chloroquinolin-4-amine and amino ferrocenyl aldehyde in a single stage procedure. It is well characterized. Studies revealed that covalently bonded chloroquine-ferrocene is responsible for the molecule's efficacy against *P. falciparum*. The pure chloroquine or ferrocene moieties are less active. Structural adjustments in amino group side chain, changes in position of the ferrocenyl organometallic group, substituting the Fe with other bioactive metals (Ru, Rh, Os) and substituting chloroquine for other organic molecules with antimalarial properties, all resulted in analogues with potent antimalarial properties similar to or better than chloroquine but not as effective as ferroquine.

Keywords: Ferroquine, Metalo-chloroquine, bioorganometallics, Metallocene, Chloroquine-Ferrocene

INTRODUCTION

The World Health Organization (WHO) Global Technical Strategy (GTS) for malaria, the Rollback Malaria Advocacy Plan (RMAP) and the Action and Investment to defeat malaria (AIM) in conjunction with the Sustainable Development Goal (SDG) has set year 2030 as the target date for the eradication of malaria [1]. With about 229 million cases and 409,000 malaria deaths estimated to have occurred in 2018 and 2019 [2], the target look daunting especially as the 2019 figure was about 13 million cases higher than the 216 million for the year 2016 [3]. About 94% of the 2019 cases were in the WHO African region where 194 M total malaria cases were reported with Nigeria being the worst hit in the continent with 27% and 23% of the African cases of malaria and malarial death respectively [2]. In this region *P falciparum* is

ISSN 0795 - 22066

responsible for 99.7 of the cases in 2019 with a burden of 93 and 94% malaria cases and death respectively of the global 228 million cases and 405,000 deaths estimated for the year 2018 [4].

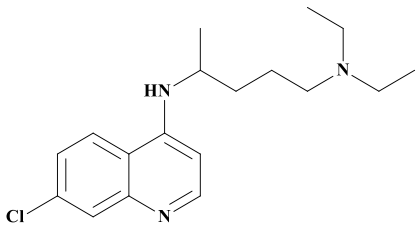
The picture of this scenario is gloomy especially as evidence at the moment is pointing to impeded progress. This is compounded by challenges in funding and the COVID-19 disruptions [5]. With children less than five years estimated to account for 67% of death. The recent success of the only vaccine under trial is heartwarming [6]. An efficient vaccine may just be the impetus to reducing this continuing threat facing sub-Saharan Africa from *P. falciparum*. A vaccine can in the long run solve the problem of antimalarial drug resistance and infant mortality arising from malaria in the region. But at the moment, the malaria vaccine is at best contemplated as a complementary effort to strategies set at eliminating the falciparum parasite [5] The good news is the RTS,S vaccine developed by GSK and her collaborator which recently passed the pilot programme in Ghana, Kenya and Malawi and active against *P falciparum* is that four doses administered on children can prevent them against malaria [6]. This is expected to put an end to the annually estimated death of over 250,000 African children.

Greater effort is required to be stimulated for actions against *P. falciparum* pathogen, especially in adults. Malaria is life threatening and devastating, in complex malaria zones, for instance in 2017, Borno state, North East Nigeria, where as a result of insecurity due to insurgency by Boko Haram terrorists, humanitarian and basic health workers could not reach most part of the state, by August 2016 child death rates of up to 8.4 deaths per 10,000 children per day was observed due to high level transmission of *Plasmodium falciparum*, representing the largest contributor to morbidity. Other such complex cases at that time include areas in Sudan, Yemen, and Bavarian Republic of Venezuela. Curiously, of the $572 million total research and development spending for malaria in 2016, the three main financiers, accounting for about 70%, were the US Government National Institute of Health, the Bill and Melinda Gates Foundation and pharmaceutical and biotechnology companies, all from “non-malaria endangered zones” [3]. There is yet no improvement in this regard as governments of countries of endemic regions still contribute only 30% of the 2.7 billion funding for control and elimination in 2018. Strains of the *P falciparum* implicated in malaria deaths in most part of Africa such as the Borno case show resistance to common antimalarial drugs such as chloroquine, mefloquine, etcetera which is largely responsible for the prevalence of this disease. One way to sustaining the fight against malaria is the development of resistance overcoming drugs. Research is ongoing in this direction, which is based on the principles of bioorganometallic chemistry. It seeks to introduce organometallic moieties into well-known antimalarial drugs with the hope of enhancing activity and eliminating resistance [7].

ISSN 0795 - 22066

CHLOROQUINE AND THE MALARIA PARASITE

Chloroquine (CQ) **1** was first synthesized in 1934 by Hans Andersag. CQ was for a long time the most effective and well tolerated antimalarial, it is safe and low cost [8]. Chloroquine is used in both prevention and in treatment of malaria which is caused by mosquito bite. The mosquito bite injects the body with malaria parasites which can live in body tissues such as red blood cells or the liver. Different medications may be required to treat parasites inside the red blood cells and those in the liver or other body tissues [9].



1

Even though the exact mechanism of action of CQ is not clear, it is generally accepted that CQ exerts it toxicity by interfering with the conversion of free heme to hemzoin [10,11]. Even the exact mechanism by which CQ inhibits hemzoin formation is not fully understood but because CQ can bind heme, suggests sequestration and possibly prevent heme from being incorporated into the inert crystalline polymer, hemzoin (HZ). The accumulation of metabolic waste (heme) due to the digestion of hemoglobin results in the killing of malaria parasite. Hemoglobin degradation and detoxification of heme occur in the digestive vacuole (DV); a lysosomal isolated acidic compartment where hemoglobin is digested by the parasite. Chloroquine builds up in the DV. It accumulates up to several thousand folds. This selective accumulation of Chloroquine in the DV is reported to be favoured by the protonation and ion trapping of the CQ due to the low local acid pH of the DV, the weak basic properties of the drug, the active uptake of CQ by a parasite transporter(s), and the existence of a specific receptor in the DV which can suitably bind CQ molecules [8,10,12].

Inside the DV, CQ binds to ferriprotoporphyrin X **(**FPIX) and prevents the polymerization of FPIX into hemzoin (HZ). Both FPIX and CQ complex and free FPIX are reported to be toxic to the malaria parasite. Most of the FPIX generated during hemoglobin digestion exits the DV where it is degraded by glutathione (GSH). Therefore, inhibition of HZ formation by CQ is not sufficient to explain drug action [12,13]. However the seemingly illusive exact drug mechanism of action is not the major concern, the real problem confronting malaria chemotherapy is the ability of the pathogen to mutate and become drug resistant [10]. Extensive use of CQ leads to development of CQ-resistant parasite, *P. falciparum* strains. Some twenty years after CQ introduction, *P. falciparum* CQ resistance was first noticed at the Thari-Cambodia border in the late 1950s and has spread to major malaria-endemic countries [8]. Mutations in target gene, increased production of target, decrease in drug accumulation and drug inactivation are identified as some of the ways parasites have evolved to overcome drug toxicity. Most often, drug resistance involves changes in the genetic makeup of the drug target so that the drug

ISSN 0795 - 22066

no longer binds or inhibits the target cell wall. It could also be due to expression of higher levels of target cell through increased transcription translation, gene replication and similar events.

CQ resistance is therefore a function of the reduced amount of CQ that accumulates in the DV [10]. Research has questioned this decreased accumulation and it is thought to be related to changes in drug efflux implicating the ATP-dependent transporter [12]. Tackling this epidemic at the moment largely involves the organic modification of CQ and combination therapy [14-16]. More recently the tactics is changing to inorganic derivatives since organic drug molecules are known to eventually succumb to drug resistance [17-20]. This new designs involve the incorporation of organometallic moiety into CQ molecule or other organic drugs to improve action and overcome resistance.

Impact of Covid-19 Pandemic on Malaria and Chloroquine

At the onset of the Covid-19 pandemic, predictions were made of how the impact of the pandemic will affect malaria, and malaria deaths expected to double in 12 months [21]. Africa, a major malaria endemic zone was therefore, forecasted as an epicentre of Covid-19 due to her fragile medical facilities and poor economy [22]. There were far-reaching alerts on government and policy makers of malaria endemic nations on the need for adequate preparations for the anticipated covid-19 spread which was expected to strain the medical facilities and healthcare professionals of this region. Some of such calls were made based on the past experience of Ebola disease outbreak where higher than expected malaria deaths were recorded during the period of the outbreak. The surge in morbidity and mortality due to malaria during the EVD outbreak was estimated to be due to cut in supply of drugs, insecticide treated net (ITN), other medicals and shortage of medics which put stress on antimalarial programs [23,24].

In accordance to expectations, available data indicate that though the lockdown and the scare of covid-19 pandemic might have affected the malaria case in the region. There are reports on the delay in ITN distribution [24]. Interestingly, some of the malaria endemic regions of the world recorded the least cases of infection and deaths arising from covid-19. The twist about malaria endemic regions and Covid-19 is the emerging realisation that the region's reversal of the expected fate is indeed connected to the previous burden of malaria [24]. What is responsible for the low cases in these regions? Scientific hypothesis, among other things points to previous exposure to antimalarial drugs [21,25] conferring immunity against SARS-COV-2. Covid-19 prevalence, burden and fatality rates are lower in malaria endemic countries whose populations are exposed to malaria but higher in malaria free countries. These populations that are previously exposed to malaria antigen are thought to have conferred on them antimalarial immunity [25].

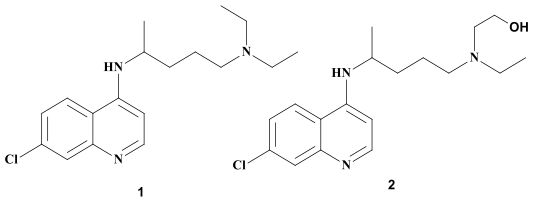
Further scientific evidences such as seroprevalence testing for populations with insignificant cases of

ISSN 0795 - 22066

Covid-19 were required to shed more light on these claims. Such studies provided insight into why Black Africans were mostly affect in northern Italy, African-American were severely affected in USA. But home in Africa, Africans were not highly affected. Was it a case of defensive immunity against the virus in Africa due to previous administration of antimalarial drugs? Since there are regions of the world with similar climate and weather as Africa where the virus still spread widely [26]. Recent findings by Bamigboye *et al*., suggests Africa's youthful population, apart from exposure to previous infections like malaria, human development/healthcare quality and the effect of UV-light, heat and humidity as responsible for Africa's seeming immunity to the COVID-19 pandemic [27].

Chloroquine and hydroxychloroquine were widely reported to be effective as inhibitors of the SARS-COV-2 [28-33], the drugs are capable of both prevention of the entry of the virus into cell and blocking its transportation within cell [28]. They do these by the terminal glycosylation of the SARS-COV-2 receptor ACE2 and blockade of sialic acid receptors [34]. Certain Coronaviruses activate a mitogen-activated protein kinase (MAPK) pathway which triggers their downstream targets which aid assembly and spread of virus. CQ has been reported as an inhibitor of the MAPK thereby mitigating the spread of virus through the channel [35]. But endogenously generated CO is a known activator of MAPK [36,37]. Therefore cigarette smoking may have serious adverse effects on SARS-COV-2 disease especially for patients with underlying airway diseases.

Administration of CQ/HCQ **2** also reported to cause Fe starvation in host by inhibiting the transferrin receptor 1 (TFR1) complex. The process of Fe release from the iron storage enzyme transferrin (TF) is also inhibited by CQ. Non-investigated report suggests that iron starvation is suspected to affect the life cycle of SARS-COV-2. There are reported evidences of inhibitory effect of iron starvation in several human viruses [35]. Inhibition of/ transferrin receptor 1 (TFR1) complex and the process of Fe release from the Fe storage enzyme FT are some of the ways CQ/HCQ treatment causes iron starvation



While there abound scientific evidence that pre-exposure to CQ/HCQ or their outright administration can serve as a form of prophylaxis against covid-19. The action of feroquine (FQ) in this regard has not been documented. FQ belong to the 4-aminoquinolines sub class of the quinolines derivatives including CQ **1**, HCQ **2** and amodiaquine. It is the only metal containing member of the entire quinoline family [38].

ORGANOMETALLIC HYBRIDS IN ANTIMALARIAL CHEMOTHERAPY

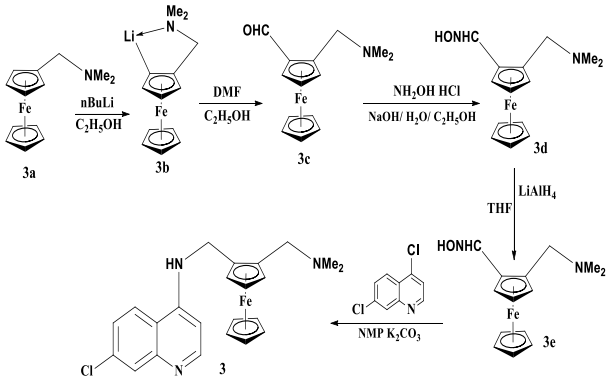
Modification of chloroquine side chain leading to improvement in activity against both sensitive and

ISSN 0795 - 22066

resistant strains of *P. falciparum* is well reported [14,39,40]. However such improvement may not be enough for combating the current scourge of the malaria epidemic. Since the problem with the treatment of malaria is largely associated with the issue of resistance, developing a drug molecule capable of overcoming resistance is likely the way out. Organometallic fragments attached to organic drug molecules are believed to improve activity and capable of reversing drug resistance [20,41]. Current applications include antitumour and antibacterial hybrids similar to the ferrocene-tamoxifen hybrid [42,43]. This has been reviewed elsewhere [44,45]. The ferroquine-chloroquine moiety 7-chloro-4-[[[2-[(N,N-dimethylamino)-methylferrocenyl]methyl] amino] quinolone [46] which has been named ferroquine **3** is the pioneering work reported on organometallic antimalarial hybrid [46]. Ferroquine was found t9o be active against chloroquine-resistant strain of P. falciparum *in vitro* among other synthesized analogues derived from substitution of the dimethylamino side chain with various tertiary amino groups

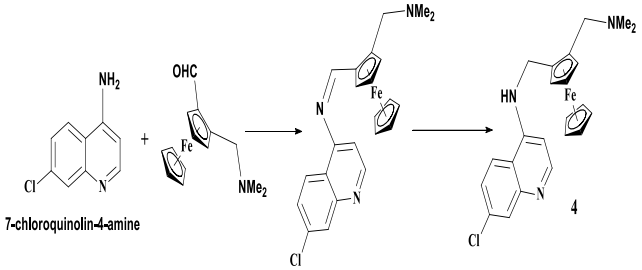
Synthesis of Ferroquine

The preparation of ferroquine was reported by Biot and coworkers [47-50] starting with the metallation of [(dimethylamino) methyl] ferrocene **3a** in the presence of n-butyllithium to yield **3b**. This is followed by the coupling of the product with N,N-dimethyl formamide under nitrogen atmosphere at room temperature to yield the aldehyde **3c** which is converted to the corresponding oxime **3d** with hydroxylamine. The oxime intermediate is then reduced with $LiAlH_4$ to give the amine derivative **3e**. The amine moiety is eventually condensed with 4,7 dichloroquinoline giving rise to ferroquine.



Scheme 1: Route to the preparation of ferroquine by Biot and coworkers.

In a related procedure, Ferey et al., invented a simplified and more covenient route to the bioorganometallic molecule making use of the aldehyde specie **3c** as starting material thereby avoiding the use of explosive/expensive reagents such as $LiAlH_4$, hydroxylamine and generation of unstable oxime intermediate. The method couples the aldehyde amino ferrocene **3c** with 7-chloroquinolin-4-amine to give ferroquine [51].



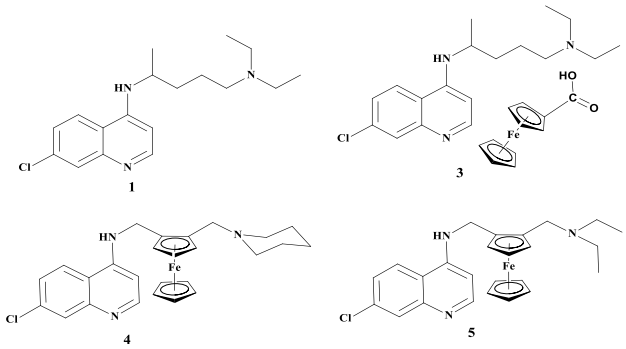
Scheme 2: Route to one stage synthesis of ferroquine by Ferey *et al.*

The reductive amination reaction involves several steps in a single stage producing ferroquine in good yield. Ferroquine can be in the form of free base or salt-like.

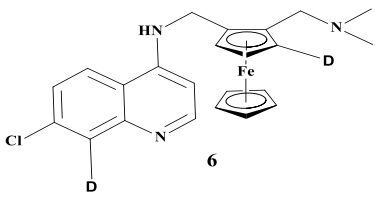
ISSN 0795 - 22066

WAR AGAINST *P. FALCIPARUM: IN VITRO* AND *IN VIVO* ANTIMALARIAL ACTION OF CHLOROQUINE-FERROCENE ORGANOMETALLIC HYBRIDS

About the biggest challenge associated with choloroquine administration in the treatment of P. falciparum at the moment is largely that of resistance. Therefore overcoming drug resistance strains is critical to resolving the issues surrounding the high rate of mortality and morbidity due to malaria. Because FQ exhibit comparable activity with CQ against FG3 strain and a far higher one against highly CQ-resistant strains; FCM6, FCM17 and FG1. FQ might just be the antimalarial wonder candidate to overcome P. falciparum. The iron metal in the compound is believed to greatly impact the activity of FQ. Plasmodium is known to have fervor for iron; therefore the addition of iron to the chloroquine molecule might enhance the removal of the chloroquine resistance of plasmodium parasites. This is chiefly the basis for tethering of the iron-containing ferrocenyl moiety to chloroquine to give rise to a new organometallic compound capable of restoring plasmodium drug resistance. Detailed *in vitro* antimalarial activities of the ferrocenyl-chloroquine and related analogues against *P falciparum* is documented [48]



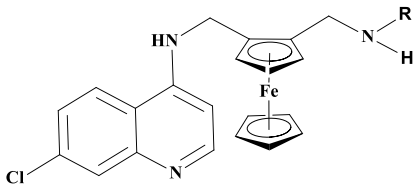
Futher investigation of the activities of the ferrocenyl-chloroquine compounds against chloroquine-susceptible strain SGE2 and the highly chloroquine-resistant strains; FCM6 & FCM17 revealed a trend consistent with previous report [46]; the compounds were more effective than chloroquine against the chloroquine resistant parasites. A tartaric acid form of the compound found to be more effective than chloroquine and more effective than a ferrocene tartaric acid of chloroquine reveals that the compound is more effective when chloroquine is covalently bonded to ferrocene and associated with tartaric acid for solubility. Investigation shows that ferrocene must be covalently bonded to chloroquine for efficacy. The ferrocene molecule has no antimalarial property but only enhances chloroquine activity when attached to the drug molecule. The analogue completely restored chloroquine's efficacy against chloroquine-resistant parasites. A double deuterium-labelled analogue of ferroquine **6** have been synthesized with the hope of using it in the examination of the kinetics of its accumulation and release by parasites for elucidation of the metabolites which will help in the understanding of its interaction with malaria pigment and general drug pharmacokinetics [52].



But in order to have a grasp of the understanding of the mechanism of action of ferroquine, knowledge

ISSN 0795 - 22066

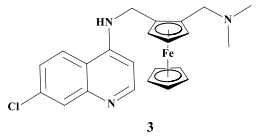
of the chemistry of its metabolism is required. Chloroquine metabolism is reported to take place in the liver by oxidation process via the cytochrome P-450 enzyme system [53]. A side chain de-ethylation initially produced monodesethylchloroquine (DECQ) and finally didesethylchloroquine (di-DECQ) is the main metabolization pathway. Ferroquine was thus expected to exhibit similar metabolic character, that is, produce the corresponding dimethyl metabolites. This led to the synthesis of a series of compounds **7a-d** bearing various secondary amino groups in order to examine the effect of substitution on the antimalarial activity.



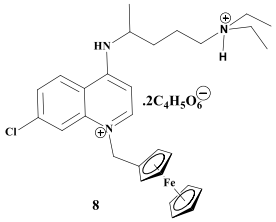
7a, R = Me
7b, R = Et
7c, R = t-Bu
7d , R = H

The nature of the compounds differs only in the nature of the amino group attached to the terminal N atom. The result showed that similar to the chloroquine's major metabolites; didesethylferrochloroquine **7d** is less active than monodesethylferrochloroquine **7a.** The didesethylferrochloroquine was twice less potent against chloroquine-resistant strain Dd2 and potent against chloroquine-sensitive strain HB3 as ferroquine. The didesethylferrochloroquine displayed a lower potency against chloroquine-resistant strain of *P. falciparum* compared to ferroquine but remain more active than chloroquine.

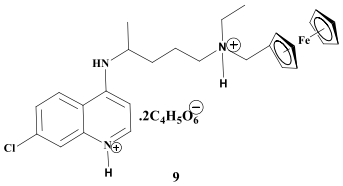
Sequel to the discovery that the attachment of the ferrocene moiety to chloroquine improves efficacy of the drug, similar approach has been adopted in the ferrocene-tamoxifen conjugate where the position of the ferrocene moiety was varied in the ferrocifen/hydroxyferrocifen framework already reviewed elsewhere [45], the new organometallic anticancer compound is in pre-clinical trial, to assess the structure-activity relationship [49], the ferrocene moiety's position in ferroquine was varied similarly to the ferrocene-tamoxifen hybrid to assess the effect on biological properties. They are shown in **8** and **9**



3



8



9

Attachment of ferrocenyl methyl to the pyridine N of chloroquine **8**, resulted in lower antimalarial activity of the compound compared to chloroquine. But substituting an ethyl group with a ferrocenyl methyl group in the chloroquine structure led to increased activity for the organometallic analogue. The new compound reversed the chloroquine resistance. All analogues in the series displayed activity against all chloroquine-sensitive and -resistant strains of *P falciparum* tested except FCR3 strains[49]. However in a separate effort to confirm the *in vitro* and *in vivo* activity of ferroquine [54], *in vitro* assay revealed that ferroquine exhibited varying levels of activity on chloroquine-susceptible *P falciparum* strains compared to

ISSN 0795 - 22066

chloroquine, ferroquine was about twice as active on HB3 strain and six to ten times on the resistant strains FCR3 and Dd2. *In vivo* studies on mice infected with drug-sensitive strain of *P. berghei* showed that the administration of chloroquine and ferroquine exhibited quite similar ED_{50} values just as the ED_{90} of ferroquine was lower but not significantly different from that of chloroquine. Curative effect on mice infected with vinckei vinckei strains was reported to indicate that ferroquine curative treatment was five times more potent than chloroquine against the chloroquine-susceptible form and twenty-two times more potent against the chloroquine-resistant lineage. Preliminary toxicity investigation of the drugs using mouse lymphoma cells revealed ferroquine was more toxic than chloroquine, however, administration of 8.4 mg kg^{-1} day^{-1} of ferroquine for four days observed for up to 14 days after the last administration of ferroquine did not record any death [54].

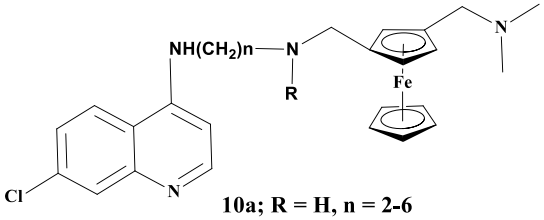
Because the *P. falciparum* strains remain the dominant causative agent of malaria in Africa and in furtherance to the investigation of ferroquine efficacy against the pathogen *in vitro* studies of some 55 Senegalese isolates of *P. falciparum* was conducted and the outcome compared to nine administered antimalarial drugs [55]. The ferroquine was discovered to be slightly more potent against chloroquine-susceptible isolates than against chloroquine-resistant parasites and 35-fold more potent than chloroquine against chloroquine-resistant parasites. The organometallic candidate drug was also more active than quinine, mefloquine, amodiaquine, cycloguamil and pyrimethanine. However, halofantrine, which has failed clinical trial, artesunate and atovaquone were more active than ferroquine.

Feroquine and all ferrocene-chloroquine analogues which are reported to exhibit better antimalarial properties than chloroquine have all been tested as a mixture of their enantiomers. It is the racemic mixtures that were found to be active against the multidrug-resistant *P. falciparum* strains. Since some biological systems can discriminate between a pair of enantiomers as different substances, different enantiomer may in many cases induce different responses in these systems. As have been reported for certain racemic pairs of ferrocifen and its analogues the desired activity may reside in only one enantiomer. It may not be impossible that the other pair could be inactive, competitive or completely toxic. In view of the foregoing the pure enantiomers of ferroquine was isolated and the antimalarial activity assessed and compared to that of the racemic mixture [56]. *In vitro* investigation of the antimalarial activity of the compounds against the chloroquine sensitive strain HB3 and chloroquine resistant strain Dd2 showed that the ferroquine enantiomers displayed equal levels of activity, which is twice as effective as chloroquine or their racemic mixture. The *in vivo* curative effect on mice showed ferroquine racemate cured 100 percent of the animals infected with either chloroquine susceptible or resistant *P. vinckel vinckel* strains. A minor difference in the potency of the enantiomers was observed *in vivo* with (+)FQ displaying a better curative effect than the (-)FQ

ISSN 0795 - 22066

pair. The toxicities of the ferroquine species were similar. But in all cases they were reported to show good security index. Furtherance to this and previous findings that the ferroquine molecule owe it activity over chloroquine to the covalent attachment of ferrocene to chloroquine, the influence of the ferrocenyl moiety on the *in vitro* biological activity of metallocenic based antimalarials on *P. falciparum* strains have been reported [46]. In investigating the role of ferrocenyl moiety in ferroquine, a series of ruthenocene analogues including quinolone and pyridine complexes to help differentiate between effects consistent with structure activity relationship previously reported for 4-aminoquinolines and those associated ferrocene moiety (discussed below). From the comparison of the results of the antiplasmodial studies of ferrocene and ruthenocene analogues of 4-aminoquinolines as well as antimalarial- ferrocene analogues of 4-aminoquinoline, pyridine and quinolines. There was no significant difference between the ferrocene and ruthenocene analogues which suggests that the difference in chemical behavior of these moieties is insignificant. The lipophilicity and size of the moieties may therefore be important since lipophilicity may aid passage through membranes and lead to a greater affinity for hematin.

The bioorganometallic approach to new drug discovery in the area of antimalarial agents have extended it search beyond the discrete ferroquine molecule. Ferrocene conjugation to other administered drugs or introduction of biologically active compounds into the ferroquine structure has also been explored. Amine, urea [57] quinoline [50] ferroquine analogues as well as the mefloquine and quinine ferocenic analogues [58] have been reported. The dual drug model of ferroquine with thiosemicarbazone [59] and the *in vitro* investigation of the antimalarial property of ruthenoquine, where the Fe in ferroquine is substituted with Ru [60] is documented. The effect of the introduction of another metal centre in a ferroquine-like structure engendering a heterobimetallic species have also been reported [61] and the results are inspiring. The amine and urea analogues of ferroquine **10a-b** with varying length of the methylene spacer between the two nitrogen atoms in the side chain were found to be more active than chloroquine against chloroquine-sensitive D10 and resistant K1 strains [57].

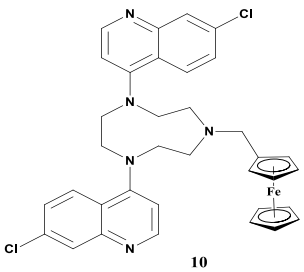


10a; R = H, n = 2-6
10b; R = CONHBn, n = 2-6

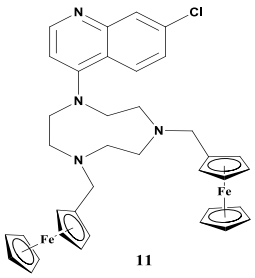
Correlation of length of side chain and ease of oxidation of the ferrocenyl moiety and antimalarial activity showed that the length of the methylene spacer was a major determinant of antimalarial activity. Because bisquinolines have been reported to be active on *P. falciparum*[62-64] a ferrocene derivatized triazacyclononane which will generate a bisquinoline is expected to exhibit potent antimalarial activity [50], Two ferrocenyl bisquinoline derivatives **10**, **11** screened against *P. falciparum* strains showed that the bisquinoline

ISSN 0795 - 22066

analogues were found to be more efficient on the chloroquine resistant strain than chloroquine but less active on the chloroquine-sensitive strain tested compared to ferroquine.

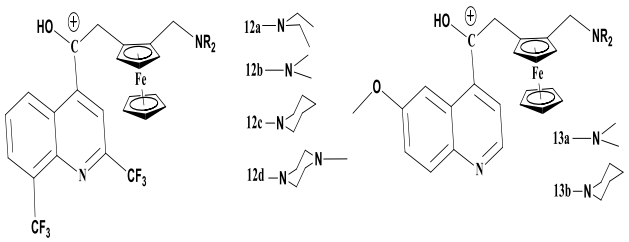


10



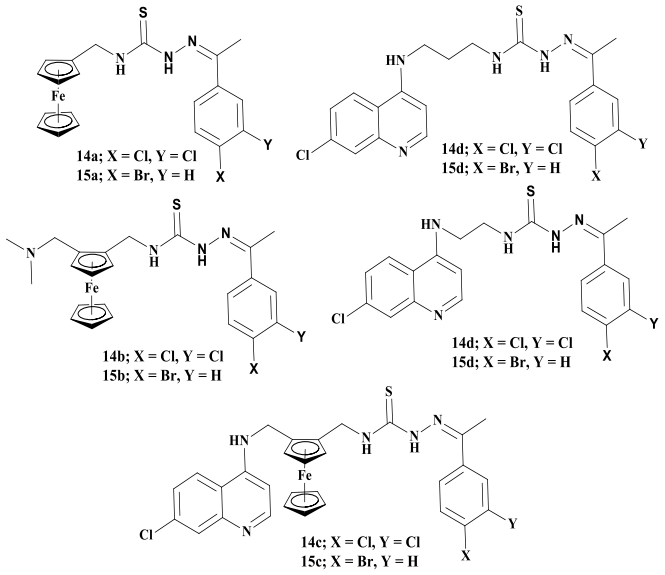
11

Bisferrocene analogues of the derivatized quinolines were much less active on both strains examined. With the successful replacement of carbon chain of chloroquine with a ferrocenyl group which afforded the organometallic compound; ferroquine, which have demonstrated activity against chloroquine resistant parasites. Biot and coworkers employed similar strategy with other antimalarial drug molecules, they tethered the ferrocene moiety to mefloquine and quinine to evaluate the antimalarial activity [58]. *In vitro* susceptibility screening of the organometallics against P. falciparum strains including chloroquine and mefloquine resistant strains showed that the compounds exhibited lower antimalarial activity compared to mefloquine and quinine. Therefore the ferrocenyl mefloquine and quinine derivatives are not as active antimalarial agents compared to the chloroquine analogues like ferroquine.

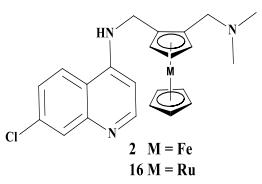


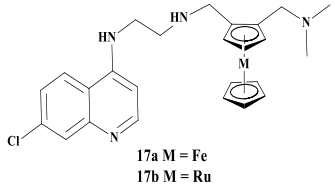
The discovery of thiosemiacbazones (TSC) as new lead that kill species of protozoan parasites via inhibition of cysteine protease has stimulated special interest in this class of compound. A series of TSC conjugate of ferroquine were evaluated against four different *P. falciparum* strains from different geographical and antimalarial resistance background of Africa, Indochina and South America [59]. The organometallic compounds were also examined against the parasitic cysteine protease falcipan-2 to determine their potential targets in the malaria parasite. All organometallic ferroquine-thiosemicarbazone conjugates and pure organic chloroquine-thiosemicarbazones series evaluated were much more active than the ferrocenyl-thiosemicarbazone compounds. Data suggests that the basic amino group may improve potency, probably by aiding the drug transport to the active food vacuole of the parasite. The chimeras of thiosemicarbazone-ferroquine were the most active of the series of analogues examined against the different strains of *P. falciparum*. The purely organic derivatives showed comparable potency. However, introduction of the ferrocene moiety did not increase antimalarial activity. Ferroquine displayed better inhibitory character towards falcipan-2 compared to the corresponding flexible alkyl analogue.

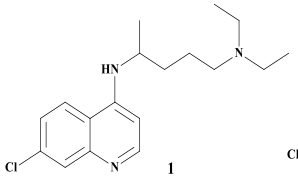
ISSN 0795 - 22066

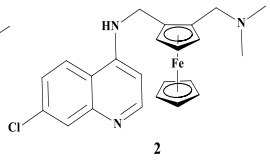


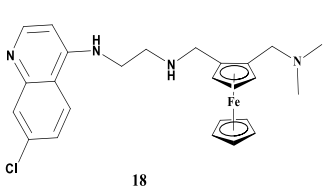
Ferrocene and ruthenocene have similar chemistry but differ in reactivity; hence replacing ferrocene with ruthenocene in ferroquine might be expected to exhibit interesting properties similar to ferroquine. Ruthenocene derivatives of chloroquine where the ferrrrocene group in ferroquine is substituted with ruthenocene **16** and another where the ruthenocene is at a different position **17b** on the side chain with a two carbon methylene spacer [60]. Antimalarial assay of the ruthenocene derivatives against chloroquine-sensitive D10 and the resistant strain K1 of P. *falciparum* revealed that the ruruthenocene-chloroquine compounds have high antimalarial activities which are comparable to the ferroquine analogues. However the ferroquine analogues are more potent [61]. In the quest to whether an additional metal centre in ferroquine and its analogues will have effect on antimalaria activity, some ferroquine analogues were coordinated to new metal centres. Examination of the activity of the new complexes against both chloroquine sensitive and resistant strains of four ligands and their heterometallic complexes was reported to indicate that all the ferrocene derived ligand-like species (**1**, **2**, **18**, **19**) exhibit higher activity than chloroquine and except for the chloroquine complexes of Au and Rh, all other complexes examined are less effective than the corresponding ligand.

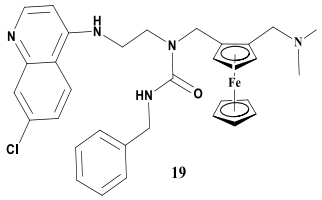










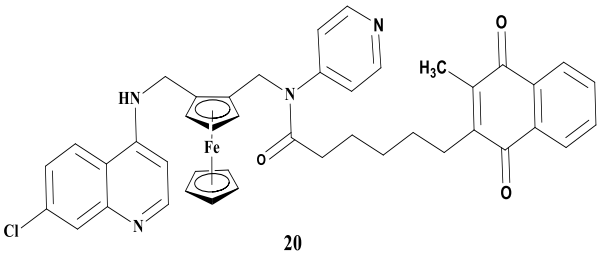


The ferrocenyl ligands (**2**, **18**, **19**) are also more effective than the gold or rhodium complexes (structures not shown) of chloroquine. Therefore introduction of the second metal upon coordination makes the oxidation of the ferrocenyl moiety difficult and thus appear antagonistic to the properties of the complexes.

Elevation of glutathione (GSH) content in *P. falciparum* is established to lead to increase resistance to chloroquine while its depletion in resistance strains restore sensitivity to the drug [65]. GSH is a powerful antioxidant that prevents damage to important cellular components; it is known to protect the malaria parasite from oxidative damage. Because GSH is found almost exclusively in its

ISSN 0795 - 22066

reduced form, the high intracellular GSH levels depend on the efficient reduction of glutathione disulfide (GSSG) by glutathione disulfide reductase (GR). Thus GR inhibitors were developed to fight malaria [66-69] and to reverse chloroquine resistance [70]. Drug combination is well known to be beneficial in the treatment of malaria, but simple drug combination can cause problem due to different pharmacokinetics. Hence the binding of active molecule via a covalent linker seems to be a plausible alternative. Binding of both active entities allows for increased bioavailability of the final dual molecule and to merge active molecules with independent modes of action to prevent the emergence of resistance. Based on this dual prodrug strategy, ferroquine moiety has been used as template for new antimalarial ferrocenic dual molecules [65] by linking a ferroquine derivative to a GR inhibitor or GSH depletor. Result of the antimalarial activity of the ferroquine analogues and the dual drug derivatives tested against chloroquine-susceptible (NF54) and chloroquine resistant (K1) strains of *P. falciparum* show that the activity of the ferroquine analogues are similar to ferroquine. The dual molecules based on ferroquine attached to GR inhibitor were reported to be highly active against the malaria parasites. They were more active than the inhibitor, but their activity is however lower than the parent ferroquine analogue. Those of the dual molecule derived from GSH depletor including the depletors displayed potent antimalarial activity. Their reactivity was found to be midway those of chloroquine and ferroquine. Expectedly glutathione reductase inhibition studies revealed no inhibition was observed for ferroquine, similarly, the dual molecules showed no inhibition of the *P. falciparum* GR except for a slight inhibition observed for one of the GR inhibitor-ferroquine analogue **20**.



20

FERROQUINE: MECHANISM OF ACTION

Detailed investigations of mode of action and pharmacokinetics of ferroquine have been reported. From previous studies of 4-aminoquinoline analogues of chloroquine it has been suggested that the 4-aminoquinoline, the 7-chloro group and the basic tertiary amino group in the side chain and the quinoline N are essential for strong complex formation with Fe(III)PPIX, inhibition of β-hematin formation and drug accumulation in the acidic parasite food vacuole respectively. Investigation of physicochemical parameters such as association with Fe(III)PPIX, strength of inhibition of β-hematin formation, pka and lipophilicity can be useful in exploring the likely mechanism of ferroquine antimalarial action The interaction of ferroquine with monomeric hematin performed at pH 7.5, and monitoring of soret band of hematin at 402 nm as a function of ferroquine concentration gave the association constant log k = 4.95 ± 0.05 lower than the chloroquine's log k = 5.52 ± 0.05 but still in the same range. Subjecting hematin to conditions used in preparation of β-hematin and

ISSN 0795 - 22066

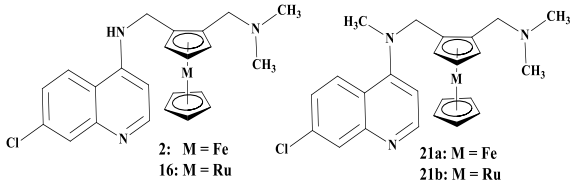
quantification of the inhibition of β-hematin formation by deprotonated ferroquine indicated β-hematin was strongly inhibited by ferroquine, making it a more potent inhibitor than chloroquine.

Theoretical molecular modelling suggested similar mode of interaction of ferroquine or chloroquine with hematin. The apparent partition coefficient (logD) of chloroquine and ferroquine at vacuolar and cytosolic pHs (5.2 and 7.4 respectively) determined to evaluate drug accumulation in the food vacuole revealed considerable larger lipophilicity at cytosolic pH for ferroquine compared to chloroquine (more than 100-fold). Only a slight difference in lipophilicity was noticed at vacuolar pH (~ 3-fold). Comparison of their pKa values shows that in the absence of special transport mechanism, ferroquine is a weaker base and would be expected to accumulate in the food vacuole less than chloroquine [71]. In the report of the random clinical phase I trials of ferroquine [72], the pKa of ferroquine in African volunteers was assessed, the safety, tolerability, maximum tolerated dose and pharmacokinetic of the drug candidate was found to be active against both chloroquine susceptible and resistance *P. falciparum*. Ferroquine in combination with appropriate partner drug constitute one of the most important new antimalarial compounds, a potential candidate for the development of a single dose antimalarial treatment. Since the treatment of chloroquine-resistant parasites accumulate compound suspected to be GSH in the digestive vacuole, understanding the detail mechanism of action of ferroquine and any antimalarial drug for that matter, require an investigation of the oxidative conditions inside *P. falciparum* and the effect of *P. falciparum* erythrocyte reinvasion [73], Ferroquine was found to play key role in merozoites reinvasion, a clear advantage of ferroquine over the organic parent drug chloroquine. This is an indication that the organometallic drug offer hope in overcoming the disadvantages of chloroquine. From chloroquine to ferroquine or any of their derivatives, the result of test on susceptible and resistance strains are rarely the same, even though the mechanism of action of both chloroquine and ferroquine is linked to the heme detoxification pathway in the digestive vacuole of *P. falciparum*. It is rational to suggest different molecular modes of action in susceptible and resistance parasites [74]. This hypothesis was put to test by Dubar and coworkers. Experiments performed to study the subcellular distribution of ferroquine and chloroquine within infected red blood cells (iRBCs) on W2 strains exposed for 30 minutes to 40 nM ferroquine or chloroquine led to the conclusion that chloroquine-resistance parasites accumulate ferroquine but effluxes chloroquine out of the digestive vacuole. Transporter proteins are reported to be unable to transport ferroquine out of DV, hence allowing ferroquine to evade the resistance mechanism [74].

The discovery of sulphur content in the digestive vacuole requires investigation even though the presence of a sulphur-containing protein may not be excluded. The rational hypothesis was that the subcellular accumulation of sulphur was due to a higher concentration of glutathione in infected red blood cells (iRBCs) treated with chloroquine. Two

ISSN 0795 - 22066

pathways were thus envisaged; (i) Ferroquine in the presence of H_2O_2 generates OH^- radicals capable of causing damage to the membrane of the proteins of the parasite digestive vacuole (ii) ferroquine causes the death of malaria parasite by disrupting hemozoin formation resulting in the accumulation of toxic heme [74]. With the earlier implication of the redox behaviour of the ferrocene moiety and intramolecular hydrogen bonding in the lateral side chain of ferroquine on antimalarial activity, the specific role of the metal species and intramolecular hydrogen bonding on activity and resistance by investigating whether substituting Fe and Ru will prevent the redox reaction and if the presence of a methyl group on the 4-amino group in methyl ferroquine and methyl ruthenoquine will prevent the formation of intramolecular hydrogen bonding and the overall effect on activity and resistance. The authors considered the properties relevant to antimalarial activity for four compounds ferroquine, ruthenoquine, methylferroquine **21a** and methylruthenoquine **21b** [75].



The results of crystal structure analysis, density functional theory (DFT) calculations, and solution NMR experiments indicated the presence of intramolecular hydrogen bonding between the unprotonated terminal tertiary amino group and the 4-amino group of the quinolone in the crystal structure, and between N11 and N24 which lead to suggesting a folded conformation for the molecules. The presence of the methyl group on the molecules was also found to impact on the conformation of the molecule. Such folded conformers were expected to be more lipophilic than open conformations and thus enhances drug transport through membranes.

CONCLUSION

The impact of malaria on the health system and economy of endemic countries is devastating. A successful malaria vaccine will be a great lifeline in these societies. The number of children lost annually to this monster will be mitigated and thus a better outlook. This will eventually save the future from the billions of dollars in research fund spending on the development of antimalarial resistant drugs. As Africa and other concerned regions await this future, we also need to address the population that is currently hosting the deadly parasite and those that will contract it before a full vaccination program. The fate of this group will depend largely on an efficient drug candidate capable of dealing with both susceptible and drug resistant strains of *P. falciparum*. The promising path at the moment is organometallic/antimalarial drug hybrids dealt with here and elsewhere. They offer great potential in reversing resistance and effectively overcoming the malaria parasite. Ferroquin is in clinical trials [76-78] and several others are promising candidates, ferroquin seem the most promising of this class of compounds.

The synthesis, full characterization and biological evaluation of these molecules revealed that the metal containing compounds exhibit mechanisms

ISSN 0795 - 22066

similar to the wholly organic molecules. The iron metal and ferrocenyl moieties are reported to contribute to enhancing structure-activity relationship thereby increasing activity and overcoming resistance. A malaria vaccine and a successful antimalarial drug that can significantly reverse drug resistance is surely the way out of the age-long burden of *P. falciparum* malaria in Africa. Such antimalarial drug will help in removing the biggest disease burden in sub-Saharan Africa. And bioorganometallic chemistry would have made its greatest impact in medicine and positioned along with its exploits in areas such as transport of small molecules in the activation of molecular enzymes and biological pathways [79,80], the search for better estrogen receptor modulators [81], novel antibiotics [82] and etcetera, bioorganometallic chemistry will be making the world a healthier space.

REFERENCES

[1] WHO, *Global technical strategy for malaria 2016–2030*. Geneva, 2015.

[2] "World malaria report 2020," 2020.

[3] "World malaria report 2017," Luxembourg, 2017.

[4] "World malaria report 2019."

[5] "Global technical strategy for malaria: 2016–2030 2021 Update," 2021.

[6] "WHO recommends ground breaking malaria vaccine for children at risk." [Online]. Available: www.who.int/news/item/06-10-2021. [Accessed: 19-Nov-2009].

[7] G. Jaouen and S. Michèle, Eds., *Bioorganometallic Chemistry*. Weinheim, Germany: Wiley-VCH, 2015.

[8] R. Thomé, S. Costa, P. Lopes, F. Trindade, M. Costa, and L. Verinaud, "Chloroquine : Modes of action of an undervalued drug," *Immunol. Lett.*, vol. 153, no. 1–2, pp. 50–57.

[9] "Chloroquine phosphate," *Drugs and Medication*. [Online]. Available: https://www.webmedicom/drug/2/drug.8633/chloroquine-oral/details.

[10] "Mechanism of drug action and resistance," *Focus on Antimalarials*. [Online]. Available: https://www.tulane.edu/wiser/protozoology/notes/drugs.html.

[11] A. Martin *et al.*, "Potential role of Plasmodium falciparum exported protein 1 in the chloroquine mode of action," *IJP Drugs Drug Resist.*, vol. 8, no. 1, pp. 31–35, 1998.

[12] H. Ginsburg and M. Krugliak, "Chloroquine - some open questions on its antimalarial mode of action and resistance," *Drug Resist. Updat.*, vol. 2, pp. 180–187, 1999.

[13] D. G. Russelltt and D. E. Goldberg, "On the molecular mechanism of chloroquine ' s antimalarial action," vol. 93, no. October, pp. 11865–11870, 1996.

[14] S.-J. Yeo, D.-X. Liu, H. S. Kim, and H. Park, "Anti-malarial effect of novel chloroquine derivatives as agents for the treatment of malaria," *Malar. J.*, vol. 16, no. 1, p. 80, 2017.

[15] L. Kremer, J. Gut, and P. J. Rosenthal, "7-Chloroquinoline – isatin Conjugates : Antimalarial , Antitubercular , and Cytotoxic Evaluation," pp. 622–629, 2014.

[16] L. Kremer, "4-Aminoquinoline- b -Lactam Conjugates : Synthesis , Antimalarial , and Antitubercular Evaluation," pp. 191–197, 2014.

[17] J. Davies and D. Davies, "Origins and Evolution of Antibiotic Resistance," vol. 74, no. 3, pp. 417–433, 2010.

ISSN 0795 - 22066

[18] G. Annunziato, "Strategies to Overcome Antimicrobial Resistance (AMR) Making Use of Non-Essential Target Inhibitors : A Review," *Int. J. o f Mol. Sci.*, vol. 20, no. 5844, 2019.

[19] R. K. Sodhi and S. Paul, "Metal Complexes in Medicine : An Overview and Update from Drug Design Perspective," vol. 14, no. 1, pp. 001–008, 2019.

[20] A. Valente, A. Podolski-reni, I. Poetsch, N. Filipovi, I. Turel, and P. Heffeter, "Metal- and metalloid-based compounds to target and reverse cancer multidrug resistance," *Drug Resist. Updat.*, vol. 58, no. August, pp. 1–33, 2021.

[21] P. E. Napoli, "Global Spread of Coronavirus Disease 2019 and Malaria : An Epidemiological Paradox in the Early Stage of A Pandemic," *Clin. Med. (Northfield. Il).*, vol. 9, no. 1138, pp. 1–6, 2020.

[22] E. Sherrard-smith *et al.*, "The potential public health consequences of COVID-19 on malaria in Africa," *Nat. Med.*, vol. 26, no. September, 2020.

[23] S. Africa, W. Africa, S. Leone, and G. Fund, "What impact is Covid-19 having on global malaria efforts ? How are the global malaria community and the RBM Partnership to End Malaria responding to Covid-19 ? Protect those most vulnerable – pregnant women and children under five in malaria affected countries – with life-saving malaria tools and prioritize safe delivery of these," 2016.

[24] G. Chowell and K. Mizumoto, "Preparedness is essential for malaria-endemic regions during the COVID-19 pandemic," *Lancet*, vol. 395, pp. 1094–1096, 2020.

[25] M. Tripathi, "Comparative analyses revealed reduced spread of COVID-19 in malaria endemic countries," 2020.

[26] E. C. Aurora Parodi, "Coronavirus disease 2019 (COVID 19) and Malaria Have anti glycoprotein antibodies a role?," *Med. Hypotheses*, vol. 110036, no. January, 2020.

[27] E. L. Bamgboye, Omiye Jesutofunmi A., O. J. Afolaranmi, and R. D. Mogamat, "COVID-19 Pandemic: Is Africa Different?," *J. Natl. Med. Assoc.*, vol. 13, no. 3, pp. 324–335, 2020.

[28] J. Liu *et al.*, "Hydroxychloroquine , a less toxic derivative of chloroquine , is effective in inhibiting SARS-CoV-2 infection in vitro," *Cell Discov.*, vol. 6, no. 16, pp. 6–9, 2020.

[29] P. Colson, J. Rolain, J. Lagier, P. Brouqui, and D. Raoult, "Chloroquine and hydroxychloroquine as available weapons to fight COVID-19," *Int. J. Antimicrob. Agents*, no. xxxx, p. 105932, 2020.

[30] R. Chang, "Repositioning chloroquine as an ideal antiviral prophylaxis against COVID-19 -Time is now Original Manuscript Title : Repositioning chloroquine as an ideal antiviral prophylaxis against COVID-19 – Time is now Institute of East-West Medicine , New York , U," *Drug Discov. Today*, no. March, 2020.

[31] C. A. Devaux, J. Rolain, P. Colson, and D. Raoult, "New insights on the antiviral effects of chloroquine against coronavirus : what to expect for COVID-19 ?," *Int. J. Antimicrob. Agents*, vol. 55, no. December 2019, 2020.

[32] P. B. Nina and A. P. Dash, "Hydroxychloroquine as Prophylaxis or Treatment for COVID-19 : What Does the Evidence Say ?," *Indian J. Public Health*, vol. 64, no. 2, pp. 125–127, 2020.

[33] N. Principi and S. Esposito, "Chloroquine or hydroxychloroquine for prophylaxis of COVID-19," *Lancet Infect. Dis.*, vol. 20, no. 10, p. 1118, 2020.

[34] S. Satarker, T. Ahuja, M. Banerjee, V. B. E, S. Dogra, and T. Agarwal, "Hydroxychloroquine in COVID-19 : Potential Mechanism of Action Against SARS-CoV-2," vol. 19, no. M, 2020.

[35] E. Quiros, G. Biasiotto, P. Magro, and I. Zanella, "The possible mechanisms of action of 4-aminoquinolines (chloroquine /

ISSN 0795 - 22066

hydroxychloroquine) against Sars-Cov-2 infection (COVID-19): A role for iron homeostasis ?," *Pharmacol. Res.*, vol. 158, no. April, p. 104904, 2020.

[36] R. Motterlini *et al.*, "CORM-A1 : a new pharmacologically active carbon monoxide-releasing molecule," *FASEB J.*, vol. 19, pp. 284–286, 2005.

[37] A. Friebe, G. Schultz, and D. Koesling, "Sensitizing soluble guanylyl cyclase to become a highly CO-sensitive enzyme," *EMBO J.*, vol. 15, no. 24, pp. 6863–6868, 1996.

[38] J. Vitte, M. Michel, S. Mezouar, and A. B. Diallo, "Immune Modulation as a Therapeutic Option During the SARS-CoV-2 Outbreak : The Case for Antimalarial Aminoquinolines," vol. 11, no. August, pp. 1–15, 2020.

[39] V. R. Solomon *et al.*, "Synthesis and Antimalarial Activity of Novel Side Chain Modified Antimalarial Agents Derived from 4-Aminoquinoline," *Med. Chem.*, no. 4, pp. 446–456, 2008.

[40] D. P. Iwaniuk *et al.*, "Synthesis and antimalarial activity of new chloroquine analogues carrying a multifunctional linear side chain," *Bioorg. Med. Chem.*, vol. 17, no. 18, pp. 6560–6566, 2009.

[41] C. A. Vock *et al.*, "Development of Ruthenium Antitumor Drugs that Overcome Multidrug Resistance Mechanisms," *J. Med. Chem.*, vol. 27, no. 50, pp. 2166–2175, 2007.

[42] P. Biegan and M. Arruebo, "Brief survey on organometalated antibacterial drugs and metal-based materials with antibacterial activity," *RSC Chem. Biol.*, no. 2, pp. 368–386, 2021.

[43] M. Zaki, "Scope of organometallic compounds based on transition metal-arene systems as anticancer agents : starting from the classical paradigm to," *RSC Adv.*, vol. 9, pp. 3239–3278, 2019.

[44] U. B. Eke and T. A. Abubakar, "Bioorganometallic compounds in medicine : The search for new antibacterial agents," *World J. Biomed. Pharm. Sci.*, vol. 1, no. 1, pp. 22–27, 2015.

[45] T. A. Abubakar and U. B. Eke, "Organometallic-Tamoxifen Hybrids : A Chronology of the Search for New Selective Estrogen Receptor Modulator," *J. Chem. Soc. Niger.*, vol. 45, no. 2, pp. 234–252, 2020.

[46] C. Biot, G. Glorian, A. L. Maciejewski, and B. S. Jacques, "Synthesis and antimalaria activity in vitro and in vivo of a new ferrocene-chloroquine analogue," *J. Med. Chem.*, vol. 40, no. 97, pp. 3715–3718, 1997.

[47] C. Biot *et al.*, "Synthesis and antimalarial Activity in vitro and in vivo of a New Ferrocene-Chloroquine Analogue," *J. Med. Chem.*, vol. 40, pp. 3715–3718, 1997.

[48] O. Domarle *et al.*, "In vitro antimalarial activity of a new organometallic analog, ferrocene-chloroquine," *Antimicrob. Agents Chemother.*, vol. 42, no. 3, pp. 540–544, 1998.

[49] C. Biot *et al.*, "Novel metallocenic compounds as antimalarial agents. Study of the position of ferrocene in chloroquine," *J. Organomet. Chem.*, vol. 589, no. 1, pp. 59–65, 1999.

[50] C. Biot, J. Dessolin, I. Ricard, and D. Dive, "Easily synthesized antimalarial ferrocene triazacyclononane quinoline conjugates," *J. Organomet. Chem.*, vol. 689, no. 25 SPEC. ISS., pp. 4678–4682, 2004.

[51] V. Ferey, J. Mateos-Caro, R. Mondiere, P. Vayron, and S. Vigne, "United States Patent: Method for the synthesis of Ferroquine by Convergent Reductive Amination," US 8,497,375 B2, 2013.

[52] C. Biot, S. Caron, L. MacIejewski, and J. Brocard, "Synthesis and characterization of a double deuterium-labelled ferrochloroquine," *J. Label. Compd. Radiopharm.*, vol. XLI, pp. 911–918, 1998.

ISSN 0795 - 22066

[53] C. Biot *et al.*, "Synthesis and antimalarial activity in vitro of potential metabolites of ferrochloroquine and related compounds," *Bioorganic Med. Chem.*, vol. 7, no. 12, pp. 2843–2847, 1999.

[54] L. Delhaes *et al.*, "In vitro and in vivo antimalarial activity of ferrochloroquine, a ferrocenyl analogue of chloroquine against chloroquine-resistant malaria parasites," *Parasitol. Res.*, vol. 87, no. 3, pp. 239–244, 2001.

[55] B. Pradines *et al.*, "In vitro activities of ferrochloroquine against 55 Senegalese isolates of Plasmodium falciparum in comparison with those of standard antimalarial drugs," *Trop. Med. Int. Heal.*, vol. 7, no. 3, pp. 265–270, 2002.

[56] L. Delhaes *et al.*, "Synthesis of ferroquine enantiomers: First investigation of effects of metallocenic chirality upon antimalarial activity and cytotoxicity," *ChemBioChem*, vol. 3, no. 5, pp. 418–423, 2002.

[57] K. Chibale, J. R. Moss, M. Blackie, D. Van Schalkwyk, and P. J. Smith, "New amine and urea analogs of ferrochloroquine: Synthesis, antimalarial activity in vitro and electrochemical studies," *Tetrahedron Lett.*, vol. 41, no. 32, pp. 6231–6235, 2000.

[58] C. Biot *et al.*, "Synthetic ferrocenic mefloquine and quinine analogues as potential antimalarial agents," *Eur. J. Med. Chem.*, vol. 35, no. 7–8, pp. 707–714, 2000.

[59] J. Gut, C. Biot, B. Pradines, P. J. Rosenthal, and K. Chibale, "Design , synthesis , and antimalarial activity of structural chimeras of thiosemicarbazone and ferroquine analogues," vol. 17, pp. 6434–6438, 2007.

[60] P. Beagley, M. A. L. Blackie, K. Chibale, C. Clarkson, J. R. Moss, and P. J. Smith, "Synthesis and antimalarial activity in vitro of new ruthenocene-chloroquine analogues," *J. Chem. Soc. Dalt. Trans.*, no. 23, pp. 4426–4433, 2002.

[61] M. A. L. Blackie, P. Beagley, K. Chibale, C. Clarkson, J. R. Moss, and P. J. Smith, "Synthesis and antimalarial acti v ity in v itro of new heterobimetallic complexes : Rh and Au deri v ati v es of chloroquine and a series of ferrocenyl-4-amino-7-chloroquinolines," vol. 688, no. 3, pp. 144–152, 2003.

[62] J. L. Vennerstrom *et al.*, "N , N -Bis (7-chloroquinolin-4-yl) heteroalkanediamines," *J. Med. Chem*, vol. 41, no. 22, pp. 4360–4364, 1998.

[63] K. Raynes, M. Foley, I. L. Tilley, and L. W. Deady, "Novel Bisquinoline Antimalarials SYNTHESIS, Antimalarial Activity, and Inhibition of Haem Polymerisation," *Biochem. Pharmacol.*, vol. 52, pp. 551–559, 1996.

[64] K. M. Liebman *et al.*, "Unsymmetrical Bisquinolines with High Potency against P. falciparum Malaria," *Molecules*, vol. 25, no. 2251, pp. 1–15, 2020.

[65] N. Chavain *et al.*, "Antimalarial activities of ferroquine conjugates with either glutathione reductase inhibitors or glutathione depletors via a hydrolyzable amide linker," *Bioorg. Med. Chem.*, vol. 17, no. 23, pp. 8048–8059, 2009.

[66] C. Tyagi *et al.*, "Targeting the intersubunit cavity of Plasmodium falciparum glutathione reductase by a novel natural inhibitor : Computational and experimental evidence," *Int. J. Biochem. Cell Biol.*, vol. 61, pp. 72–80, 2015.

[67] G. N. Sarma, S. N. Savvides, K. Becker, M. Schirmer, R. H. Schirmer, and P. A. Karplus, "Glutathione Reductase of the Malarial Parasite Plasmodium falciparum : Crystal Structure and Inhibitor Development," *J. Mol. Biol.*, vol. 2836, no. 03, pp. 893–907, 2011.

[68] H. Insburg and J. Golenser, "Glutathione is involved in the antimalarial action of chloroquine and its modulation affects drug sensitivity of human and murine species of Plasmodium," *Redox Rep. Commun. Free Radic. Res.*, vol. 0002, pp. 276–279, 2013.

[69] E. Davioud-charvet *et al.*, "A Prodrug Form of a Plasmodium falciparum Glutathione

ISSN 0795 - 22066

Reductase Inhibitor Conjugated with a 4-Anilinoquinoline," *J. Med. Chem.*, vol. 44, pp. 4268–4276, 2001.

[70] C. Biot *et al.*, "Double-drug development against antioxidant enzymes from Plasmodium falciparum," *Redox Rep. Commun. Free Radic. Res.*, vol. 8, no. 5, pp. 280–283, 2013.

[71] C. Biot *et al.*, "Insights into the mechanism of action of ferroquine. Relationship between physicochemical properties and antiplasmodial activity," *Mol. Pharm.*, vol. 2, no. 3, pp. 185–193, 2005.

[72] C. Supan *et al.*, "Pharmacokinetics of ferroquine, a novel 4-aminoquinoline, in asymptomatic carriers of Plasmodium falciparum infections," *Antimicrob. Agents Chemother.*, vol. 56, no. 6, pp. 3165–3173, 2012.

[73] F. Dubar *et al.*, "The ferroquine antimalarial conundrum: Redox activation and reinvasion inhibition," *Angew. Chemie - Int. Ed.*, vol. 52, no. 30, pp. 7690–7693, 2013.

[74] F. Dubar *et al.*, "Deciphering the Resistance-Counteracting Functions of Ferroquine in *Plasmodium falciparum* - Infected Erythrocytes," *ACS Med. Chem. Lett.*, vol. 3, no. 6, pp. 480–483, 2012.

[75] F. Dubar *et al.*, "The antimalarial ferroquine: Role of the metal and intramolecular hydrogen bond in activity and resistance," *ACS Chem. Biol.*, vol. 6, no. 3, pp. 275–287, 2011.

[76] C. Biot, F. Nosten, L. Fraisse, J. Khalife, and D. Dive, "The antimalarial ferroquine: from bench to clinic," *Parasite*, vol. 18, pp. 207–214, 2011.

[77] J. S. Mccarthy *et al.*, "A Phase II pilot trial to evaluate safety and efficacy of ferroquine against early Plasmodium falciparum in an induced blood - stage malaria infection study," *Malar. J.*, vol. 469, no. 15, pp. 1–9, 2016.

[78] Y. Adoke *et al.*, "A randomized , double - blind , phase 2b study to investigate the efficacy , safety , tolerability and pharmacokinetics of a single - dose regimen of ferroquine with artefenomel in adults and children with uncomplicated Plasmodium falciparum malaria," *Malar. J.*, vol. 222, no. 20, pp. 1–25, 2021.

[79] K. S. Madhusoodanan and F. Murad, "NO-cGMP signaling and regenerative medicine involving stem cells," *Neurochem. Res.*, vol. 32, no. 4–5, pp. 681–694, 2007.

[80] V. S. Sharma and D. Magde, "Activation of Soluble Guanylate Cyclase by Carbon Monoxide and Nitric Oxide : A Mechanistic Model," vol. 505, pp. 494–505, 1999.

[81] G. Jaouen, A. Vessières, S. Top, G. Jaouen, A. Vessières, and S. Top, "Ferrocifen type anti cancer drugs," vol. 44, no. 24, pp. 8802–8817, 2015.

[82] S. Nobre, D. Seixas, C. C. Roma, and M. Saraiva, "Antimicrobial Action of Carbon Monoxide-Releasing Compounds □," vol. 51, no. 12, pp. 4303–4307, 2007.

ISSN 0795 - 22066