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Synthesis and Characterization of Antiplasmodial Gallium (III) and Iron (III) Complexes of Aminochloroquinoline-3, 4-Hydroxypyridinone Conjugates: Preliminary Studies.

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ABSTRACT

Metallation is a drug optimization strategy that has been used to enhance efficacy as well as pharmacokinetic and pharmacodynamic PK/PD properties in antimalarials. In our earlier studies antiplasmodial aminochloroquinoline-3,4-hydroxypyridinone ACQ-HPO conjugates/hybrid compounds were reported to have remarkable in vitro activity against chloroquine resistant Plasmodia but poor in vivo activity thus the need for their optimization. Herein we report the synthesis of iron (III) and gallium (III) complexes of the ACQ-HPOs which were characterized then evaluated for in vitro antiplasmodial activity. From the preliminary structural elucidation data, the structures of five complexes were found to be ML_3 type while one was a ML_2^+. However, these structural findings remain tentative pending crystallographic analyses. The metal complexation enhanced antiplasmodial activity significantly in the chloroquine resistant CQR strain Dd2. Generally, the metal complexes were more active in resistant strain Dd2 than in the sensitive strain D10. Both the ACQ-HPO ligands and complexes had a lower antiplasmodial activity in both strains than CQ, however the resistance indices, Dd2/D10, of the complexes were 10-fold lower than that of CQ indicating their potential to combat antimalarial drug resistance. Future studies will focus on cytotoxicity tests and crystallography to enable accurate determination of the therapeutic indices of these metallodrugs.

Key words: Aminochloroquinoline, hydroxypyridinone, complexes, Antiplasmodial, metallodrugs, malaria

INTRODUCTION

Malaria remains a major disease burden in tropical developing countries. WHO report of 2023 indicates 263 million cases of malaria globally with 597 000 deaths, 95% of this malaria mortality, occurring in Africa. Furthermore, malaria kills one child every minute globally and is responsible for low birthweights in neonates [1, 2]. The WHO Global Technical Strategy for malaria 2016-2030 and Sustainable Development Goal 2025 and 2030 targets for reduction of malaria morbidity and mortality are unlikely to be met [3]. This is because the 2023 global malaria incidents were nearly three times higher than anticipated. [2].

The emergence and spread, of artemisinin resistance and the threat of resistance to artemisinin combination therapy partner drugs can potentially erode gains made in the treatment of malaria infections. Partial resistance to artemisinins and its partner drugs, of which two are quinolines (i.e. piperaquine and amodiaquine) has been confirmed in Southern America, Eastern Africa and Southern Africa malaria endemic regions, and this requires urgent intervention. In this context the search for antimalarials with new and multiple modes of action continues to be fast tracked. The malaria drug development pipeline therefore needs to be continually populated to enhance malaria control and elimination.

Iron chelation and metal complexation of antiplasmodial ligands is a major front in search of new antimalarial

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

drugs, some of the notable experimental therapeutic compounds being pyridinones and quinolines. Further work continues in the development of quinolines as antimalarials i.e. the quinoline metal complexes and hybrid molecules. One of the most notable success stories is Ferroquine ZY19489 a Zydus product, currently in clinical trials [1]. Metal complexes of chloroquine-based ligands have showed improved activity against CQ sensitive and CQ resistant strains of P. falciparum as well good in vivo activity in animal models [5].

The 3,4-Hydroxypyridinones (3,4- HPOs) is a class of metal chelators mainly derived from maltol and kojic acid with notable therapeutic examples being Deferiprone (DFP), Ferralex G and Ferriprox which are licenced by FDA for iron overload treatment. DFP or N-methyl 3,4-hydroxypyridinone has been evaluated in vitro, in rat models and clinically for antimalarial potential [6,7]. The pyridinone lipophilicity, polarity and hydrogen bonding characteristics have been manipulated to achieve more medicinal applications as well as for bioisosteric replacements for amides, pyridines, pyranones, pyrimidines, pyrazines and phenols. Due to their low BBB permeability, low toxicity, low lipophilicity and high aqueous solubility, 3,4-HPOs have been hybridized with other drugs to enhance multi-targeting and desirable PK and PD properties. Examples of such efforts include Fluorophore-3-hydroxypyridinone antibacterials [9], 3- hydroxypyridin-4-one-coumarin hybrids for the treatment of Alzheimer's disease [10] and 4-aminoquinoline-3-hydroxypyridin-4-ones for antiplasmodial or antitrypanasomal applications [11-13].

Chibale and co-workers synthesized maltol based series of noncytotoxic antiplasmodial 4-aminoquinoline-3-hydroxypyridin-4-one hybrids [12]. These hybrids were synthesized on the basis of the antiplasmodial synergy when their precursors (N-alkyl-3-hydroxypyridin-4-ones) were combined with chloroquine (CQ). It was found that the in vitro antiplasmodial activity of the precursors i.e. N-alkyl-3-hydroxypyridin-4-ones was negated on blocking the chelator moiety via complexation with gallium (III) or benzyl protection [12, 14] and none of the precursors inhibited β-hematin formation. In contrast, majority of the ACQ-HPO hybrids molecules were found to be potent inhibitors of β-hematin formation than CQ, and a correlation between antiplasmodial activity and inhibition of β-hematin formation was also observed.

Among the benzyl protected analogues, the most potent hybrids against K1, 3D7 and W2 strains of P. falciparum had in vitro IC_{50} values in the range of 0.08 - 0.13; 0.004 - 0.01 and 0.02 - 0.1 μM respectively. The most potent deprotected analogue exhibited in vitro IC_{50} values of 0.07, 0.03, and 0.08 μM) against K1, 3D7, and W2, respectively. Overall, the study confirmed that the antiplasmodial activity of the synthesized hybrid molecules depended largely on β-hematin inhibition. More important the metal chelator group was confirmed to enhance activity against resistant strains of P. falciparum and that conjugation with 3,4-HPOs reduced the cytotoxicity of CQ [12]

In subsequent studies by Chibale and coworkers, antiplasmodial ACQ-HPO hybrids derived from kojic acid were synthesized and the most potent compound had an in vitro activity of IC_{50} values of 0.004 μM and 0.03 μM respectively in 3D7 and K1 strains of P. falciparum and this hybrid had the best β-haematin inhibition activity (0.07 IC_{50} equiv. versus 1.91 IC_{50} equiv. for CQ) [11]. In most cases the deprotected compounds in the series had lower resistance indices than the benzyl protected analogues and CQ.

To proof the in vivo efficacy of benzyl-protected 4-aminoquinoline-3-hydroxypyridin-4-one hybrids, the pharmacokinetic properties of one kojic derived hybrid and one maltol derived hybrid were determined using a mouse model infected with Plasmodium berghei in a 4-day Peters' test [15]. When dosed orally the compounds failed, However, when dosed intravenously IV, the two molecules were able to reduce parasitaemia levels in the P. berghei-infected mice, but parasites recrudesced 24 h after the administration of the last dose. Thus there is need to optimize these hybrids for improved pharmacokinetic properties and in vivo efficacy.

In addition to the data obtained from antiplasmodial and antimalarial activities of ACQ-HPOs, the success story of Ferroquine (an iron sandwich complex), the studies about non-chelating pyridones binding in plasmodia cytochrome bc1 as a drug target [16 - 18] and the modulation of enzymatic activities on replacement of gallium (III) with iron (III) [19, 20], form extra grounds to justify the study reported herein.

Herein we explore preparation, structural elucidation and in vitro antiplasmodial evaluation of trivalent metal

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

(Gallium and Iron) complexes of selected ACQ-HPOs as a drug optimization strategy.

MATERIALS AND METHODS

Reagents and Purification of solvents

All reagents used for synthesis were purchased from Sigma Aldrich, South Africa as analytical grade reagents and were used without further purification.

Chromatographic separation

Column chromatography was performed using Merck Kieselgel 60: 70 - 230. Reactions were monitored by thin layer chromatography (TLC) using Merck F254 aluminuim backed silica gel 60 pre-coated plates. Detection and visualization of the spots on TLC was by ultra violet light (254/366 nm), charring, 0.125 M ferric ammonium sulfate or 2% ninhydrin in methanol.

Physical and Spectroscopic characterization

Melting points were determined using a Reichert-Jung Thermovar hot stage microscope and are uncorrected. 1H NMR spectra were recorded using a Varian Mercury spectrometer (300 MHz) or on a Bruker spectrometer (400 MHz). All spectra were recorded in deuterated chloroform or dimethylsulfate or methanol with tetramethylsilane as an internal standard. ^{13}C NMR spectra were recorded with the same instruments stated above but at 75 MHz or 100 MHz. Chemical shifts (δ) are reported in parts per million (ppm) downfield from the internal standard tetramethylsilane (TMS). High resolution Mass spectrometry (TOF MS ES or ESI) was performed using a Waters API Q-TOF Ultima at Stellenbosch University, Cape Town. Low resolution mass spectrometry (EI+) was performed on a JOEL GC mate III instrument at the University of Cape Town. Microanalyses were determined on a Fisons EA 1108; C, H, N, S instrument.

Synthesis

Synthesis of the aminochloroquinoline-3,4-hydroxypyridinone ACQ-HPO ligands and their metal complexes followed documented procedures as described elsewhere [11] Hydrogenation experiments were carried out on a Parr instrument at pressures of 1-4 atm and ambient temperature. Compound purification methods included crystallization, solvent extraction, precipitation, freeze drying, column chromatography.

In vitro Antiplasmodial assays

Compounds were tested in duplicate on one occasion against D10 Chloroquine sensitive (CQS) and Dd2 Chloroquine resistant (CQR) strains. Continuous in vitro cultures of asexual erythrocyte stages of P. falciparum were maintained using a modified method as documented by Trager and Jensen [21]. The quantitative assessment of antiplasmodial activity in vitro was determined via the parasite lactate dehydrogenase assay [22], details are in the supplementary information.

RESULTS AND DISCUSSION

The synthesis of the ACQ-HPO ligands **1a, 2a** and **3a** was done as described in our previous studies [11] after which the purified hybrid compounds were used to prepare gallium (III) and iron (III) complexes as depicted in Figure 1.

The procedure involved the reaction of an aqueous solution of iron (III) nitrate nonahydrate or gallium (III) nonahydrate solution with a methanolic solution of the N-(7-chloro-4-quinolyl)-3- (hydroxy)-4(1H)-pyridinone ligand at pH 7 - 8. The equivalents of the reactants were varied to explore possibilities of forming ML_3 and ML_2 type of complexes.

Two iron complexes (**1a, 2a**) and four gallium complexes (**1b**, **2b**, **2c**, **3a**) were prepared (Table 1 and Figure 1). After purification the complexes were characterised by melting point determination, elemental analysis,

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

NMR and mass spectrometry. In the preparation of all the complexes in this study, it was proved that the reaction went on to completion and no remnants of the free ligand were detectable from both proton NMR and analytical Thin Layer Chromatography TLC. In the analytical TLC, Iron (III)chloride or ninhydrin were used as a development reagent to rule out presence of a free ligand.

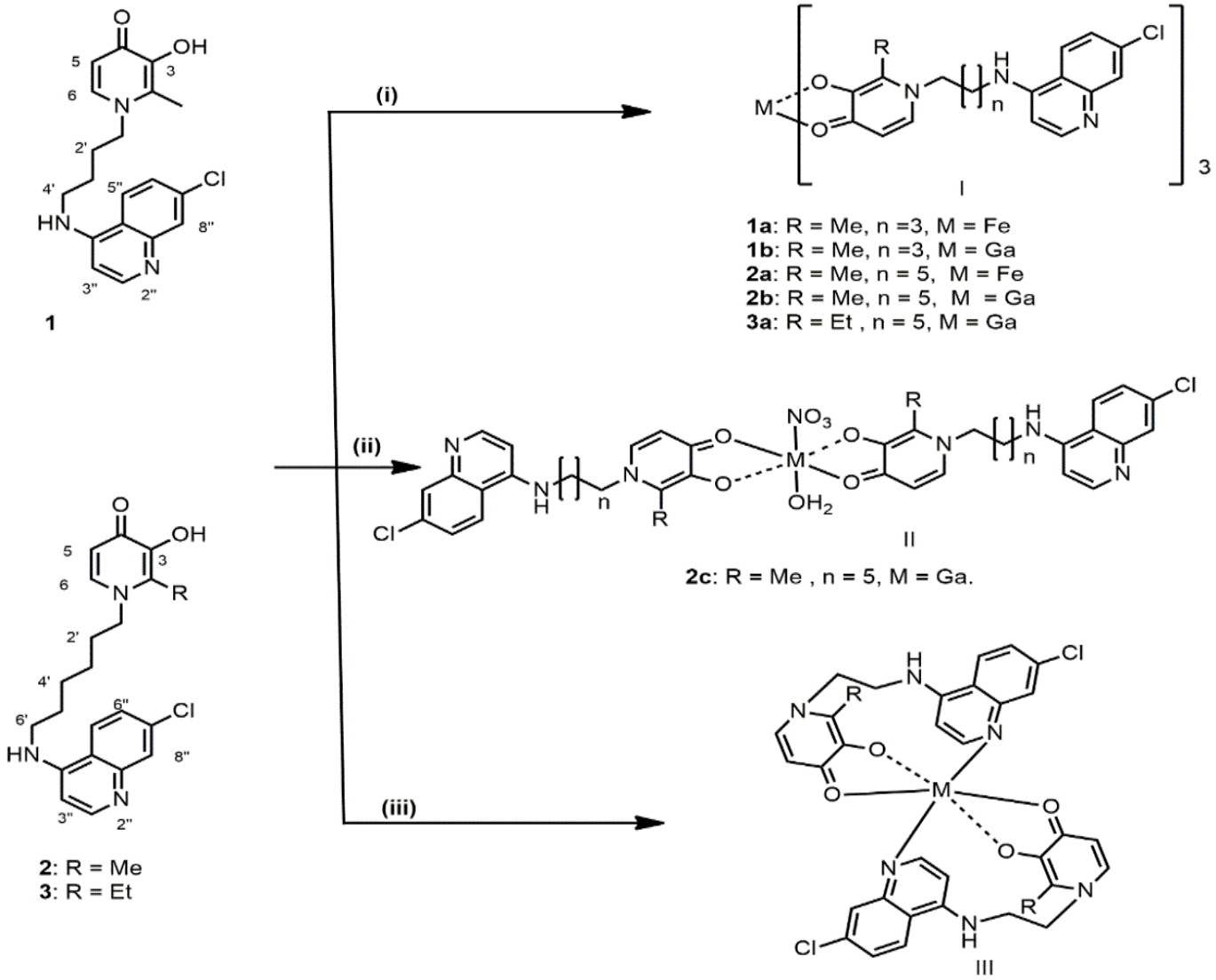

Figure 1: Synthetic routes for the metal complexes

Reagents and conditions: $MNO_3\ 9H_2O$, (M= Ga^{3+} or Fe^{3+}), H_2O/MeOH 1:1, pH 7- 8, reflux 80°C or stir at ambient temperature, (i) 3 eq. ligand , 1 eq. salt (ii) 2 eq. ligand, 1 eq. salt (iii) either i or ii

Characterization

The melting points of the iron (III) and gallium (III) complexes were observed to be much higher than that of the ligands and in some cases 2-fold, this confirmed the formation of the complexes as reported elsewhere [11, 13, 24]. The high melting points of the complexes can be attributed to the crystal packing effects present in coordination complexes. Proton NMR was used to confirm the presence and sites of metal chelation. The proton NMR spectra of the iron (III) complexes (**1a** and **2a**) exhibited peak broadening and merging with only two peaks observed. This phenomenon can be attributed to the paramagnetic nature of iron ion (Figure 2) [25]. The two broad singlets at δ9 – 6 ppm and δ4 – 2 ppm were assigned to the aromatic and aliphatic protons respectively. For the Gallium (III) complexes, the presence of Coordination Induced shifts CIS of signals associated with protons neighboring metal coordinated functional groups indicated complex formation (Figure 3). The Pyridinone hydroxyl and carbonyl groups as well as the quinoline -N were expected to coordinate to the metal centres. Initial assumption was that the N-1 of the quinoline can participate in the coordination to give a complex with the proposed structure type C (Figure 1).

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

Table1. Melting points of ACQ-HPO hybrids and their respective Ga (III) and Fe (III) complexes

Complex	M(III)	n	R	Complex type ML_x	MP °C Complex	MP °C Ligand
1a	Fe	3	Me	ML_3	225 - 226	124 -125
1b	Ga	3	Me	ML_3	215 - 216	124 -125
2a	Fe	5	Me	ML_3	196 - 200	134 -138
2b	Ga	5	Me	ML_3	194 - 196	134 -138
2c	Ga	5	Me	ML_2	183 - 184	134-138
3a	Ga	5	Et		202 - 204	resin

However, the spectroscopic data reported herein ruled out coordination via the quinoline nitrogen and confirmed the sole participation of the 3,4-hydroxypyridinone group in the coordination. The main piece of evidence for this was the absence of significant CIS in the proton NMR signals of the quinoline protons. Generally, when the quinoline moiety participates in complex formation the main coordination point is via the quinoline N-1 thus the H-2", 3" and 8" are expected to show reasonable CIS on complexation [26]. From literature the expected CIS values indicative of coordination are 1.8 - 0.5 ppm for H-8" and > 0.6ppm for H-2" and 3" [26]. These CIS values were not observed in the ACQ-HPO complexes except for **1b** (Table 2).

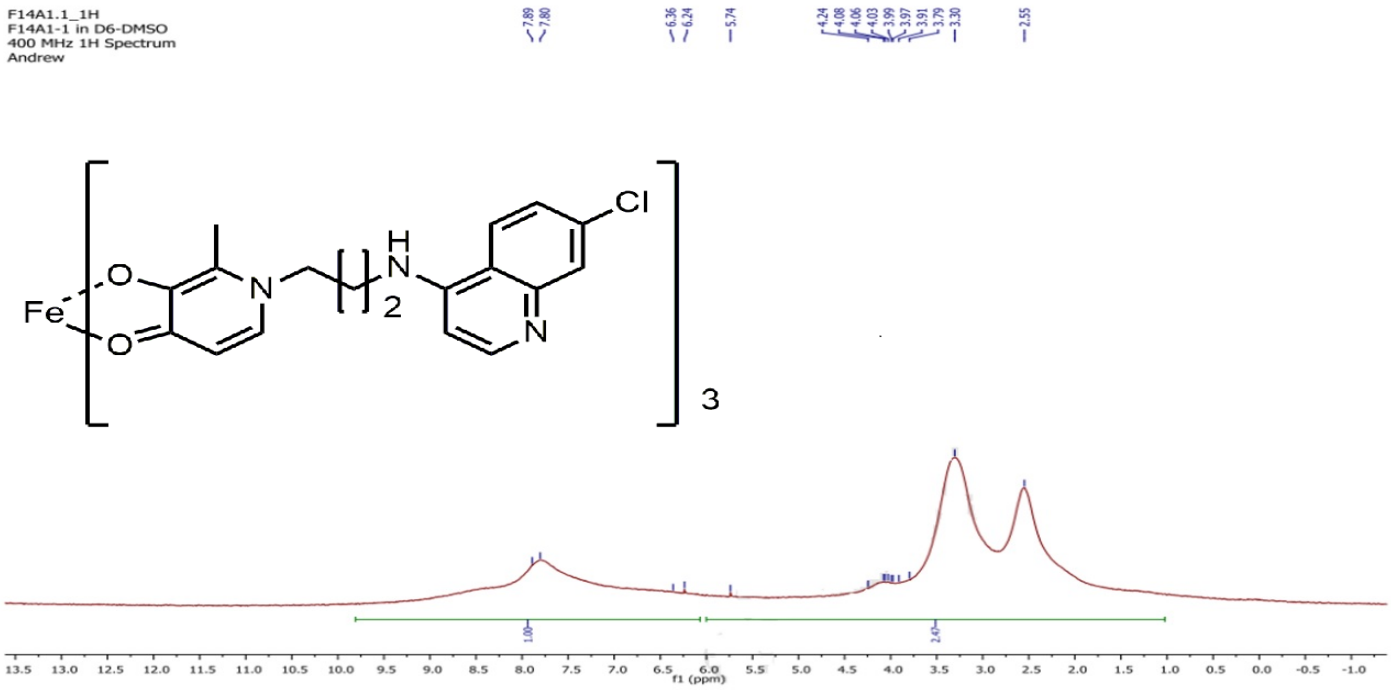

Figure 2. Proton NMR spectrum of the iron (III) complex 1a

This implied that in these complexes, the quinoline was just a pendant group and not involved in metal chelation. In the case of **1b**, the CIS Δppm for H-8" was significantly higher (0.58ppm) relative to other complexes (> 0.1 ppm), see Table 2, however this CIS value is on the extreme lower end of the expected range of 1.8 – 0.5 ppm. Since the other key quinoline protons in **1b** (H-2'', 3'') did not experience any significant CIS one can rule out coordination via the quinoline N. Therefore 0.58ppm CIS for H-8'' in **1b** could be due to strong long-range electronic disturbance enhanced by the relatively shorter alkyl linker (4 carbon chain) between the chelating moiety (3,4-HPO) and the quinoline moiety. The Mass Spectrometry data of **1b** showed an ML_3 peak and this meant complexation via N-1 did not occur because all the six coordination sites on the metal centre are assumed to be occupied by three HPO moieties.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

Table 2. Proton NMR Coordination Induced shift values Δ (CIS) for Gallium complexes

Proton	**CIS for 1b** (ppm)	**CIS for 2b** (ppm)	**CIS for 2c** (ppm)	**CIS for 3a** (ppm)
2”	0.09	0.05	0.04	0.07
3’’	0.59	0.12	0.16	0.54
5”	0.58	0.16	0.15	0.10
6”	0.24	0.04	0.16	0.04
8”	0.58	0.02	0.06	0.02
5	0.78	0.70	0.57	0.34
6	1.10	0.74	0.49	0.72

These double drugs hence behaved as bidentate chelators by using the 3,4- hydroxypyridinone moieties to chelate the metal centres. Significant CIS were observed in the proton NMR of the HPO protons specifically H-5 and H-6 (Table 2 and Figure 3) proofing the complexation via the hydroxypyridinone.

The 13 carbon NMR was used an extra piece of evidence to proof complexation whereby significantly high CIS values due to deshielding were noted**.** CIS values of about 9.8 ppm and 11.0 ppm were observed in the carbon NMR spectra of **1b** and **2c** respectively for carbonyl carbon. Longer spectral acquisition time was required to achieve well resolved

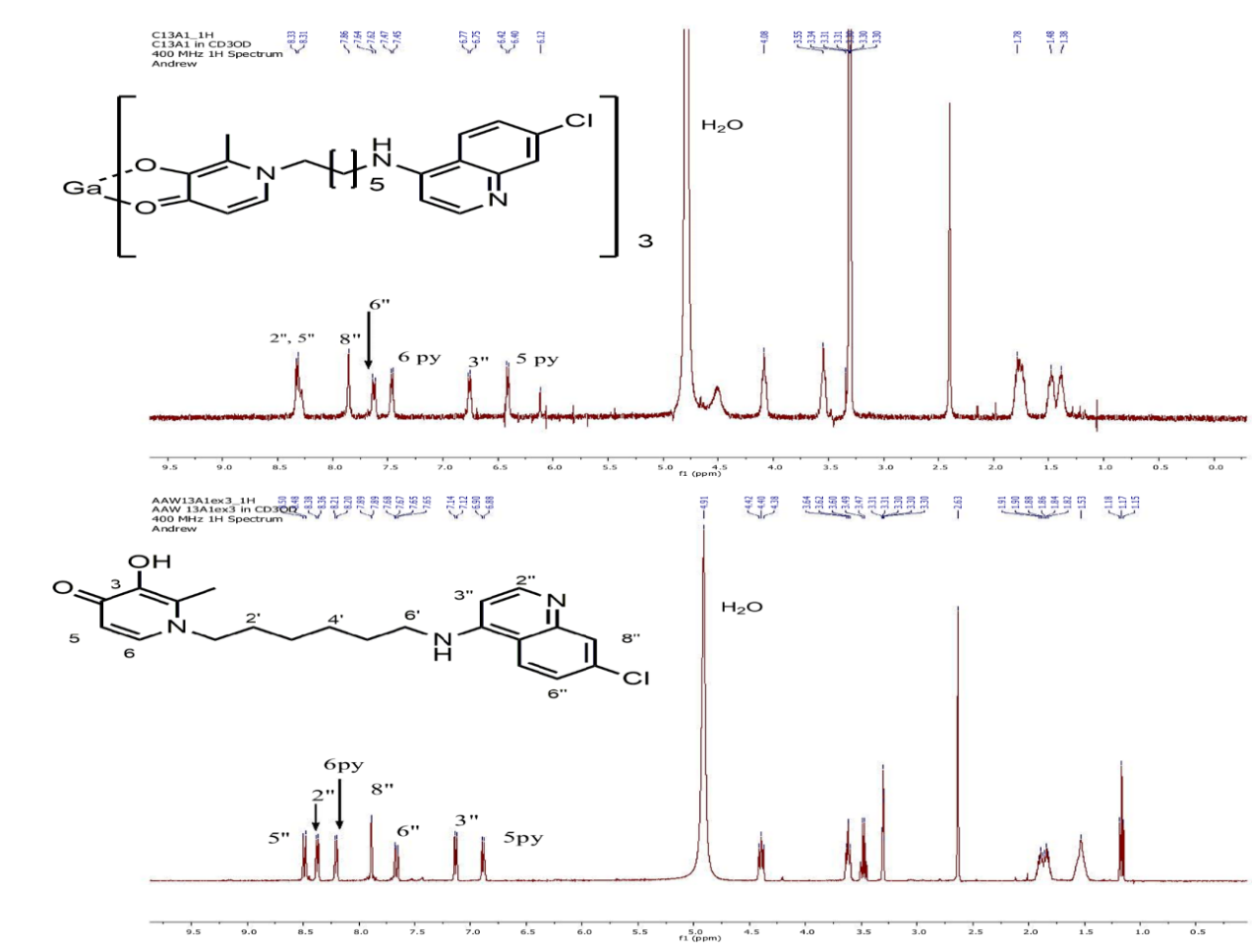

Figure 3. Proton NMR spectra of ligand 2 and its gallium complex 2b

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

carbon-13 NMR spectra for these complexes but for most them this was not achieved except for **1b** and **2c**.

Collectively, elemental analysis, NMR, MS, ion exchange tests and melting point evidence showed all the metal complexes prepared were ML_3 type except for one, **2c** which was found to be ML_2 type.

All the other complexes had a negative ion exchange reaction with the phosphorous hexaflouride anion PF_6^- except for complex **2c** indicating **2c** was cationic. The negative reaction between **2b** and the PF_6^- salt as well as the significant difference in the melting point values of **2b** and **2c** (10°C, Table 1) further confirms that the two complexes are structurally different despite containing the same ligand and metal core. The CIS for the pyridinone protons in these two closely related complexes, **2c** and **2b**, differ by a factor of 2 implying the H-5 and H-6 of the respective complexes are not in similar chemical environments. It is thus postulated that the pyridinone protons in **2c** are less shielded due to the (presumed) presence of the nitrate NO_3^-, an electron withdrawing group.

It has been reported elsewhere that Ga (III) and iron (III) complexes of 3,4-HPOs have a tendency to form $[ML_2]^+$, ML^{2+} and ML_3 species in solution [27] hence **2c** can be assumed to have formed via a $[ML_2]^+$ species and the empty coordination sites filled by the available water molecule as a neutral ligand and the NO_3^- as the anionic ligand to give an $[ML\ H_2O.NO_3]$ complex.

The MS data of the two iron (III) complexes (**1a**, **2a**) and that of **1b** showed m/z corresponding to ML_3 which is the expected molecular ion peak. The mass spectra of **2b** and **3a** did not show the molecular ions for ML_3 however, peaks corresponding to ML_2 were observed which can be attributed to the fragmentation of the ML_3 complex.

The significant differences in melting points indicate the presence of relatively stronger bonds in complexes that enabled intact ML_3 ion peaks in Mass spectral data (**1b**, **1a** and **2a**) unlike complex **2b**. Consequently, the relatively low Mp can further be used to explain the absence of the molecular ion ML_3 peaks or presence of ML_2 in the mass spectra of **2b** due to ease of fragmentation.

It is known that the ML_2 complex species of 3-4-HPOs possess a single charge unlike the neutral ML_3, hence the ML_2 fragments for complexes like **2b** did not require further protonation or cationization in order to be detected in mass spectrometry [28].

Recrystallization of the complexes was attempted for purification purposes and for X-ray crystallographic studies but this was unsuccessful. Crystallization by vapour diffusion using the following solvent pairs was attempted; methanol/diethylether, methanol/ethylacetate, methanol/heptane, methanol/dichloromethane, dry methanol/diethylether, methanol/acetonitrile, DMSO/dichloromethane, but were unsuccessful.

To make the elemental analysis data meaningful, the following assumptions were made. Firstly, that traces of $NaNO_3$ with varied moles of water of hydration were pressumed to exist in most of the metal complexes [24, 28]. This is in line with previous reports on Galium and metal complexes of *N*-alkylpyridinones in which the following types of species were verified from elemental analysis and mass spectrometry i.e. ML_3 +Na + H, ML_3 + Na, ML_3 +H_2O, ML_3 +$NaNO_3$, M_2L_3 [13, 24]. Secondly, high equivalents of water of hydration were assumed to exist in these complexes' crystal lattices. This corresponds to earlier report by Nelson and co-workers who proved via X-Ray crystallography the formation of dodecahydrates of N-alkyl-HPO gallium (III) complexes i.e. $ML_3.20H_2O$. The waters of crystallization in these dodecahydrates were in form of hydrogen bonded water-host network.

From elemental analysis the following was therefore deduced based on the two assumptions stated above: Compound **1a** as $ML_3.10H_2O$. $NaNO_3$; compound **1b** as $ML_3.10H_2O$. $NaNO_3$; **2a** as $ML_3.7H_2O$; **2b** as $ML_3.10H_2O$. $NaNO_3$; **2c** as $[ML_2\ H_2O\ NO_3]$ $3H_2O.NaNO_3$ and **3a** as ML_3 $12H_2O$ $2NaNO_3$.

All the complexes in this study exhibited poor solubility in water, methanol and ethanol thus a likelihood to cause precipitation in bioassay media and impact negatively on accurrate evaluation of biological activities. Similar observations on solubility of metal complexes have been reported elsewhere by Puerta and co-workers [29]. These complexes were however found to be soluble in a mixture of dichloromethane and methanol (2:8)

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

as well as in DMSO, a solvent that was used in the in vitro antiplasmodial assays.

Antiplasmodial activity

To confirm the effect of metal complexation on the antiplasmodial activity of ACQ-HPO ligands we evaluated the antiplasmodial activity of the gallium (III) and iron (III) complexes of the ACQ-HPOs in sensitive D10 and resistant Dd2 strains (Figure 4)

The complexes and their ligands were found to be as active as CQ in the resistant Dd2 strain, but less active than CQ (IC_{50} = 0.0155µM) in the sensitive strain D10. The antiplasmodial activity against CQ resistant Dd2 of the parent ACQ-HPO ligands (**1** and **2**) was found to be lower than some of their metal complexes indicating complexation enhanced antiplasmodial activity. Generally, the metal complexes showed high selectivity for CQR strain than in the CQS strain implying pre-complexation potentially enhances selectivity against resistant strains. These findings are similar to our earlier reports [11] where chelating hybrids (deprotected analogs) exhibited higher activity in CQR strains than in CQS strains, a contrast to benzyl and methoxy protected analogues.

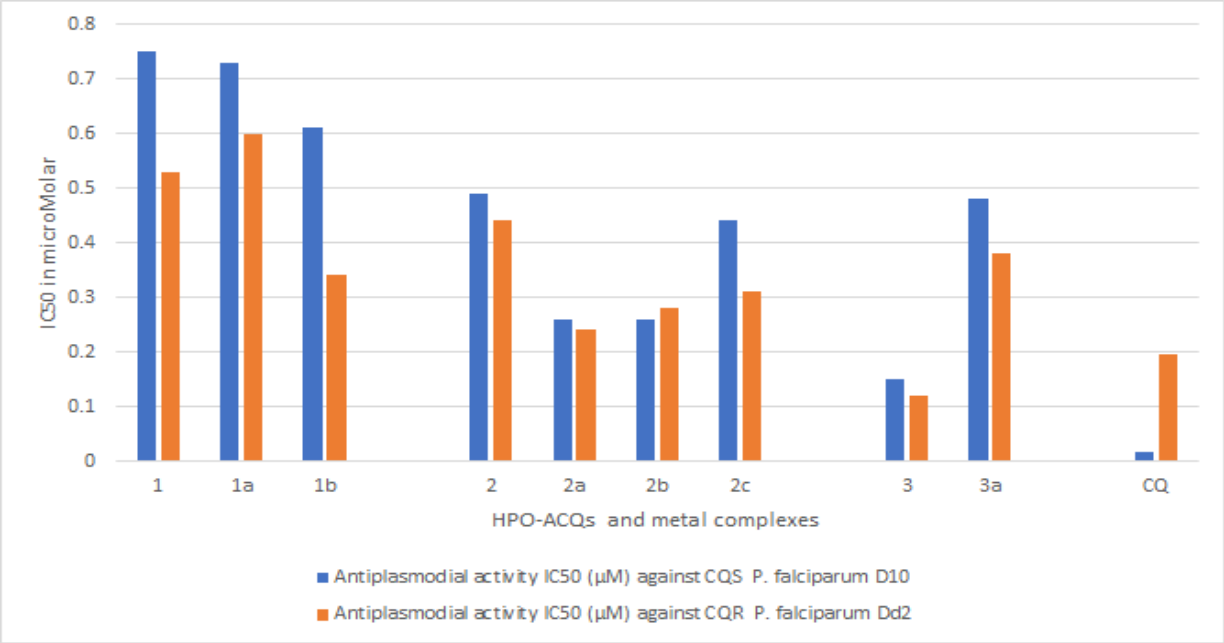

Figure 4. Comparison of in vitro antiplasmodial activities of gallium (III) and iron (III) ACQ-HPO complexes and their ligands

The complexes and the free ligands in this study were conspicuously less active in both strains than CQ except for compound **3** (with respect to the CQR strain). Interestingly, the compounds had significantly lower Resistance Indices than CQ i.e. upto 10 - fold, thus these complexes have potential to combat drug resistant malaria (supporting Information Table S1)

Apart from acting through hemozoin inhibition, it is envisaged that the deprotected ACQ-HPO hybrids (the ligands in this study) may act against P. falciparum by one or both of the following two possible mechanisms. Firstly, these hybrid compounds may complex with labile iron hence abrogating this essential nutrient via the HPO moiety (Heppner 1988) and or secondly, by forming parasite toxic iron complexes via coordination using available electron donor groups [30 – 31]. In this study pre-complexation is done prior to in vitro testing thus these complexes may be acting against the parasite via the second mechanism.

Similar to the trends observed in the ACQ-HPO ligands [10, 11] the antiplasmodial activity of the Ga (III) and Fe (III) complexes of HPOs were reported to increase with lipophilicity or increase in alkyl linker chain. The data in Figure 4 (or Table S1 in supporting information), shows that complexes with ligands with the longest alkyl linker chains (n-hexyl linker **2a, 2b, 3a, 2c**) are the most active and those with shortest alkyl linker chain (n-propyl **1a, 1b**) are the least active.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

On comparing the activities of iron (III) versus gallium (III) complexes, the nature of the hydroxypyridinone's 6-alkyl group (being ethyl or methyl) seem not to affect the antiplasmodial activity of the complex.

From the limited data presented the type of metal core seems not to have any significant influence on the antiplasmodial activities of the complexes (Figure 4 and Table S1, in supporting information). However, if one considers compounds **2a, 2b, 3a**: the iron (III) complex **2a** appears to be more active than the Ga complexes (**2b** and **3a**). This observation contrasts the comparison of **1a** (iron III complex) and **1b** (gallium III complexes), whereby the gallium complex **1b** is relatively more active. This close semblance of biological activity between HPO-gallium (III) and HPO-iron (III) complexes of related ligands corresponds to findings from similar studies [18, 31]

Eventhough preceding studies by Lipunova and co-workers show that the biological activity of quinoline complexes is governed mainly by the metal nature [33], in the current report the change in metal core from Ga (III) to Iron (III) did not affect the antiplasmodial activity significantly implying coordination via the quinoline N was unlikely, furthermore the CIS value for the H adjacent to the quinoline N were too small to confirm any coordination via the quinoline-N as earlier discussed.

In the previous studies [11] it was observed that complexation with gallium greatly reduced the antiplasmodial activity of *N*-alkyl-3,4-hydroxypyridinones, however in this study, the complexation of ACQ-HPOs with gallium (III) and iron (III) causes slight improvement or no change in antiplasmodial activity (Figure 4, Table S1 in supporting information). This implies that unlike the N-alkyl-3,4-HPOs, iron chelation is not the major mode of antimalarial activity for the ligands of these complexes. Therefore, other mechanisms of antiplasmodial action predominate even after complexation. These findings supports our previous findings that indicated benzylprotected ACQ-HPO compounds have higher antiplasmodial activity than deprotected analogues and their antiplasmodial activity correlated significantly with beta hematin inhibition. Therefore, hemozoin inhibition remains a major mode of antiplasmodial action given the presence of the 7-chloroquinolines substructure in these complexes.

From our earlier studies, deprotected analogues had improved selectivity against CQR strain, however pre-complexation of these deprotected analogues with Fe(III) and Ga (III) in this study, seems to further enhance selectivity for resistant strains, this is clear when one compares the IC_{50} values of a ligand and its respective metal complex (Figure 4; Table S1 in Supporting information).

CONCLUSİON

This study explores the potential contribution of metal chelation towards the antiplasmodial activity of the ACQ-HPOs. The tendative structural elucidation of the synthesised metal complexes indicated formation ML_3 alongside ML_2^+ complexes. Therefore future work will aim at the preparation of X-Ray quality crystals of the complexes to enable complete structural elucidation. Complete structural elucidation and future cytotoxicity studies data will be key to enabling more accurate determination of in vitro antiplasmodial activity before progression to in vivo studies. Metallation of the quinoline heterocyclic N-1 which is known to enhance potency against resistant strains may be pursued for these hybrids with or without concurrent complexation of the hydroxypyridinone group. Considering the data reported here, complexes **2a** and **2b** presents good candidates for optimization in the fight against CQR strains. The data and discussion presented herein is preliminary since the structures of the ACQ-HPO gallium (III) and iron (III) complexes have not been fully elucidated thus the conclusion stated remains tentative.

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SUPPORTİNG INFORMATİON

_N_-(7-chloro-4-quinolyl)-1-(4-aminobutyl)-3-(hydroxy)-2-methyl-4(*1H*)-pyridinone dihydrogen chloride (1)

Brown crystals (100 mg, 70%) from hot Et_2O; R_f (50% MeOH/ CH_2Cl_2) 0.3; 1H NMR (400MHz, DMSO) δ_H

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

9.9 (1H, *m*, NH), 8.87 (1H, *d*, *J* 9.3, H-5"), 8.5 (1H, *d, J* 7.2, H-2"), 8.36 (1H, *d, J* 6.9, H 6), 8.1 (1H, *d, J* 2.1, H-8"), 7.7 (1H, *dd, J* 1.8, 9.0, H-6"), 7.34 (1H, *d, J* 6.9, H-5), 6.86 (1H, *d, J* 6.9, H-3"), 4.43 (2H, *t, J* 7.2, H 1‟‟), 3.58 (2H, *q, J* 6.0, H-4‟‟), 2.5 (3H, *s*, CH_3-2) 1.92-1.77 (4H, *m*, H-2‟‟, 3‟‟); 13C NMR (100MHz, DMSO) δ_C 159, 156, 143.6, 143.3 142, 139, 138.8, 127 (2C), 126.9, 119, 116, 111, 99, 70.8, 56, 42.9, 24.8, 13.2.; MS. *m/z* calculated for $C_{19}H_{21}N_3O_2Cl$ [M+H] 358.13 found 358.1 (100%)

N-(7-chloro-4-quinolyl)-1-(6-aminohexyl)-3-(hydroxy)-2-methyl-4(1H)-pyridinone dihydrogen chloride (2)

White powder (179 mg, 90%), from hot Et_2O; Mp 134-138°C R_f (50% MeOH/ CH_2Cl_2) 0.61; 1H NMR (400MHz, CD_3OD) δ_H 8.48 (1H, *d, J* 9.2, H-5"), 8.37 (1H, *d, J* 7.2, H 2"), 8.2 (1H, *d, J* 6.8, H-6), 7.88 (1H, *d, J* 2, H-8"), 7.66 (1H, *dd, J* 2.0, 9.2 H-6"), 7.1 (1H, *d, J* 6.8, H-5), 6.88 (1H, *d, J* 7.2 H-3"), 4.4 (2H, *t, J* 8.0, H-1‟‟), 3.6 (2H, *t, J* 7.2, H 6‟‟), 2.6 (3H, s, CH_3-2), 1.9 – 1.8 (4H, m, H-2‟‟,5‟‟) 1.56 – 1.48 (4H, m, H-3‟‟, 4‟‟); 13C NMR (100MHz, CD_3OD) δ_C 158.3, 156.4, 143.9, 142.5, 142, 138.8, 138, 127.4 (2C), 125, 119.8, 115.7, 110.7, 98.6, 65.7, 56.7, 43.6, 30, 27.7, 25.8, 11.6.; MS. *m/z* calculated for $C_{21}H_{25}N_3O_2Cl$ [M+H] 386.16 found 386.0 (100%); Anal. Calcd. (found) for C54.97 (54.87), H 5.71 (5.94), N 9.16 (9.35), $C_{21}H_{26}Cl_3N_3O_2$

N-(7-chloro-4-quinolyl)-1-(6-aminohexyl)-3-(hydroxy)-2-ethyl-4(1H)-pyridinone dihydrogen chloride (3)

Brown resin (169 mg, 88%); R_f (50% MeOH/CH_2Cl_2) 0.64; 1H NMR (400MHz, CD_3OD) δ_H 8.4 (1H, *d, J* 9.3, H-5"), 8.37 (1H*, d, J* 7.2, H-2"), 8.18 (1H, *d, J* 6.9, H-6), 7.87 (1H, *d, J* 1.5 H-8"), 7.68(1H, *d, J* 8.8, H-6"), 7.1 (1H, *d, J* 7.2, H-5), 6.94 (1H, *d, J* 7.2, H-3"), 4.39 (2H, *t, J* 7.5, H-1‟‟), 3.62 (2H, *d, J* 7.5, H-6‟‟), 3.06 (2H, *q, J* 7.5, CH_3CH_2 -2), 1.97-1.79 (4H, m, H-2‟‟, 5‟‟), 1.55-1.15 (4H, m, H-3‟‟, 4‟‟), 1.29 (3H, *t, J* 7.5, CH_2CH_3-2); 13C NMR (100MHz, DMSO) δ_C 159, 156, 146.7, 143.6, 143.4, 139.1, 138.9, 138.7, 127.5 (2C), 126.2, 119.8, 111.7, 99.3, 56.1, 43.9, 31.3, 28.2, 26.7, 26.2, 20.3, 12.6; MS. *m/z* calculated for $C_{22}H_{26}N_3O_2Cl$ [M+H] 400.18 found 400.2 (100%)

The metal complexes were prepared as described below. Hydrogenation was performed on a Parr instrument at pressures of 1-4 atm. at ambient temperature. Complex purification included crystallization, precipitation, freeze drying.

Tris(3-hydroxy-2-methyl-N-(7-chloro-4-quinoinlyl)-1-(6-aminobutyl)-3-(hydroxy)-2-methyl-4(1H)-pyridinonato iron (III) (1a)

A solution of iron (III) nitrate nonahydrate (20.2 mg, 0.05 mmol) in 1ml deionised water was added to a stirred solution of **1** (67.8 mg, 0.15 mmol), in 2ml MeOH at pH 8 (2 M NaOH). The mixture was refluxed at 80 °C for 4h. The resultant precipitate was washed with water, MeOH/H_2O 5:1 and cold MeOH in that order then freeze dried for 3h at - 57°C to obtain a brick red solid. Analogous procedure was applied to synthesize other complexes **(1b, 2a**, **2b** and **3a)** using respective ligand **(1,2, 3)** and metal salt [iron (III) nitrate nonahydrate, gallium (III) nitrate nonahydrate]

Brick red solid (48 mg, 72%); mp. 225-226 °C; R_f 0.04 (MeOH/CH_2Cl_2 1:1) 1H NMR ($DMSOd_6$, 400MHz), δ_H 9-6 (br.s – quinolinyl, pyridinone C-H), 4-2 (br.s, aliphatic C-H); δ_C ($DMSOd_6$, 100MHz) no peaks observed (30°C); Anal. Calcd (found) for $C_{57}H_{57}Cl_3FeN_9O_6$ $10H_2O$ $NaNO_3$. C 48.71 (49.73), H 5.52 (5.00), N 9.97 (9.46); MS. *m/z* found 1173 ([ML_3 +2Na+H, 25%), 1171 ([ML_3 +2Na - H], 100%), 858.7 ([ML_2 2Na - H], 18%)

Tris (*N*-(7-chloro-4-quinolinyl)-1-(6-aminobutyl)-3-(hydroxy)-2-methyl-4(1H)-pyridinonato gallium (III) (1b)

Dark brown solid (117 mg, 81%); mp. 215 – 216 °C; R_f 0.14 (MeOH/ CH_2Cl_2,1:1). 1H NMR ($DMSOd_6$, 400MHz) δ_H 8.41 (1H, *d, J* 4.8, H-2"), 8.29 (1H, *d, J* 7.8, H-5"), 7.52 (1H, s, H8"), 7.46 (2H, *m*, H-6, 6"), 6.56 (1H, *m*, H-5), 6.27 (1H, *d, J* 7.6, H-3"), 4.18 (2H, *m*, H‟‟), 3.38 (masked by H_2O, H-4‟‟), 2.31 (3H, s, CH_3-py), 1.79 (4H, *m,* H-2", 3‟‟), δ_C ($DMSOd_6$, 100MHz) , 168.5, 167.1 (2C), 154.0 (3C), 152.6, 152.5 (2C), 151.9 (2C), 151.7 (2C), 151.4 (2C), 148.9 (2C), 148.7, 135.1 (2C), 134.7, 131.6 (3C), 129.5, 127.6 (2C), 127.3,

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

125.1 (6C), 118.1 (2C), 107.8 (2C), 106.9, 99.5 (3C), 55.1 (2C), 54.7, 42.8 (2C), 42.7, 28.7, 28.5 (2C), 25.5, 25.4 (2C), 12.7 (3C).. Anal. Calcd (found) for $C_{57}H_{57}Cl_3GaN_9O_6$ $NaNO_3$ $12H_2O$. C 47.50 (47.63), H 5.66 (5.04), N 9.72 (9.53); MS. *m/z* found 1140.28 (ML_3, 42%), 783.13 (ML_2 +2H, 100%),

Tris(N-(7-chloro-4-quinolinyl)-1-(6-aminohexyl)-3-(hydroxy)-2-methyl-4(1H)-pyridinonato iron (III) (2a)

Brick red solid (90 mg, 66%); 196 – 200°C; R_f 0.1 (MeOH/ $CH_2Cl_2$1:1) 1H NMR ($DMSOd_6$, 400MHz), 9-6 (br.s – quinolinyl, pyridinone C-H), 4-2 (br.s, aliphatic C-H); δ_C ($DMSOd_6$, 100MHz) no peaks observed (30 °C); Anal. Calcd (found) for $C_{63}H_{69}Cl_3FeN_9O_6$ $7H_2O$. C 56.61 (56.34), H 6.26 (5.81), N 9.43(10.06); MS. *m/z* found 1233 ([ML_3 +2H + Na, 100%).

Tris-(N-(7-chloro-4-quinolinyl)-1-(6-aminohexyl)-3-(hydroxy)-2methyl-4(1H)-pyridinonato gallium (III) (2b)

Pink shiny solid (101 mg, 81%); mp. 194 – 196 °C; R_f 0.14 (MeOH/ CH_2Cl_2,1:1) δ_H (CD_3OD, 400MHz) 8.32 (2H, *m*, H-2", 5"), 7.86 (1H, *s*, H-8"), 7.62 (1H, *d, J* 8.7, H-6"), 7.46 (1H, *d, J* 5.2, H-6 py), 6.76 (1H, *d, J* 6.0, H-3"), 6.40 (1H, *d, J* 5.4, H-5 py), 4.08 (2H, *m,* H-1"), 3.55 (2H, *m*, H-6"), 2.40 (3H, *s,* CH_3 py), 1.78 (4H, *m*, H-2", 5"), 1.43 (4H, s, H-3", 4"). Anal. Calcd (found) C 50.80 (50.77), H 6.02 (5.91), N 9.40 (10.20) for $C_{63}H_{69}Cl_3GaN_9O_6$ $10H_2O$; $NaNO_3$. MS. *m/z* found 946 (ML_2 + 2Na + NO_3, 100%). ML_3 (absent).

Aqua-bis-(N-(7-chloro-4-quinolinyl)-1-(6-aminohexyl)-3-(hydroxy)-2-methyl-4(1H)-pyridinonato-nitrato- gallium (III) (2c)

A solution of gallium (III) nitrate nonahydrate (31.2mg, 0.075mmol) in 1ml deionised water, was added dropwise to a solution of compound **2** (66mg, 0.15mmol), in 2ml MeOH, pH was adjusted to 8 (2M NaOH). Since a precipitate had formed immediately after the addition of reactants, there was no need for refluxing. This is unlike what was observed in preparation of other complexes. The resultant precipitate was washed with water, MeOH/H_2O 5:1 then cold MeOH and freeze dried for 1.5h at -57 °C to obtain a pale pink solid. To confirm anion exchange in **2c**, an aqueous solution of sodium hexafluoride phosphate was added to a hot solution of the complex in DMSO, a precipitate formed afterwards. This test was repeated for the other complexes and none of them formed a precipitate.

Pale pink solid (40 mg, 50%); mp. 183 – 184 °C; R_f 0.14 (MeOH/ CH_2Cl_2,1:1); δ_H ($DMSOd_6$, 300MHz) 8.41 (1H, *d, J* 5.7, H-2"), 8.33 (1H, *d, J* 9, H-5"), 7.82 (1H, *d, J* 1.5, H-8"), 7.71 (1H, *d, J* 5.4, H-6 py), 7.50 (1H, *dd, J* 9, 1.8, H-6"), 6.72 (1H, *d, J* 6.4, H-3"), 6.53 (1H, *m,* H-5 py) , 4.13 (2H, *t, J* 7.2, H-1"), 3.44 (obscured by H_2O, H-6"), 2.44 (3H, *s*, CH_3 py), 1.69 (4H, *m,* H-2'', 5''), 1.40 (4H, *s*, H-3", 4"). δ_C ($DMSOd_6$, 75MHz) 169.3, 151.8, 146.8, 143.1, 135.9, 134.3, 130.8, 126.5, 126.2, 122.8, 120.9, 117.2, 106.9, 98.5, 54.5, 39.7, 29.9, 27.5, 25.9, 25.4, 12.2. Anal. Calcd (found) for [$C_{42}H_{46}Cl_2GaN_6O_4^+$ NO_3^-] $3H_2O.NaNO_3$. C 48.48 (48.52), H 5.04 (5.62), N 10.77(10.04); HRMS (TOF MS ES or ESI). *m/z* calculated for $C_{42}H_{46}Cl_2GaN_6O_4$ [ML_2+H] 837.2213 found 837.2210. ML_3 absent.

Tris(N-(7-chloro-4-quinolyl)-1-(6-aminohexyl)-3-(hydroxy)-2-ethyl-4(1H)-pyridinonato gallium (III) (3a)

A solution of gallium (III) nitrate nonahydrate (21mg, 0.05mmol) was added dropwise while stirring to an aqueous solution of **3** (60 mg, 0.15mmol). After pH adjustment to 7 (1M NaOH), the mixture was refluxed for 4h at 85°C, then left to cool overnight. TLC indicated all the ligand had been used up in the reaction. Drying by rotary evaporation afforded an orange powder, which was washed with water to give orange crystals, which were further dried under vacuum. **3a** did not show a positive reaction with PF_6^- implying the formation of a neutral complex presumably ML_3 type complex.

Orange crystals (10 mg, 24%), from H_2O; mp. 200 – 204 °C; R_f (50% MeOH/ CH_2Cl_2) 0.24; δ_H (400MHz, CD_3OD) 8.3 (2H, *m*, H2"-5"), 7.85 (1H, *d, J* 0.8, H-8"), 7.64 (1H, *dd, J* 1.6, 9.0, H-6"), 7.46 (1H, *d, J* 6.8, H-6), 6.76 (1H, *d, J* 6.8, H-5), 6.4 (1H, *d, J* 6.8, H3"), 4.09 (2H, *t, J* 8.7, H-1"), 3.56 (2H, *t,* J 7.6, H-6"), 3.41 (2H, *m,* CH_2CH_3-Py), 2.4 (3H, *br.s,* CH_2CH_3-Py), 1.8–1.72 (4H, m, H-2",5"), 1.5–1.38(4H, m, H-3", 4"); MS.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

m/z found 974 (ML_2 +2Na + NO_3, 100%), ML_3 (absent); Anal. Calcd (found) for $C_{66}H_{75}Cl_3GaN_9O_6$ $12H_2O$ $2NaNO_3$. C 47.97 (48.04), H 6.04 (5.67), N 9.32 (8.47).

In vitro Antiplasmodial assays

Compounds were tested in duplicate on one occasion against D10 Chloroquine sensitive (CQS) and Dd2 Chloroquine resistant (CQR) strains. Continuous in vitro cultures of asexual erythrocyte stages of P. falciparum were maintained using a modified method of Trager and Jensen (1976). The quantitative assessment of antiplasmodial activity in vitro was determined via the parasite lactate dehydrogenase assay using a modified method by Makler (1993). The samples were prepared to a 2mg/ml stock solution in 10% DMSO or 10% methanol and were sonicated to enhance solubility. The samples were tested as a suspension if not completely dissolved. The stock solutions were stored at -20ºC and further dilutions were prepared on the day of the experiment. Chloroquine (CQ) was used as the reference drug in all experiments. A full dose-response was performed for all compounds to determine the concentration inhibiting 50% parasite growth (IC_{50} value). Compounds were tested at a starting concentration of 100µg/ml, which was then serially diluted 2-fold in complete medium to give 10 concentrations; with the lowest concentration being 0.2µg/ml. The same dilution technique was used for all the samples. CQ was tested at a starting concentration of 100ng/ml. The highest concentration of solvent to which the parasites were exposed had no measurable effect on parasite viability (data not shown). The IC_{50} values were obtained using a non-linear dose response curve fitting analysis via GraphPad Prism v.4.0 software.

Table S1. In vitro antiplasmodial activities of the ACQ-HPO Ga(III) and Fe(III) complexes and respective ligand

Ligand/metal complex	**Core metal**	**number of C in linker**	**Antiplasmodial activity IC_{50} (µM) against CQS *P. falciparum* D10**	**Antiplasmodial activity IC_{50} (µM) against CQR *P. falciparum* Dd2**	**Resistance index Dd2/D10**
1	n/a	4	0.75	0.53	0.70
1a	Fe	4	0.73	0.6	0.80
1b	Ga	4	0.61	0.34	0.50
2	n/a	6	0.49	0.44	0.90
2a	Fe	6	0.26	0.24	0.90
2b	Ga	6	0.26	0.28	1.1
2c	Ga	6	0.44	0.31	0.7
3	n/a	6	0.15	0.12	0.78
3a	Ga	6	0.48	0.38	0.78
CQ	n/a		0.0155	0.194	12.5

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