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Synthesis, structural characterization of novel Coumarin derivatives as potential antimalarial agents

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ABSTRACT

*A series of new Coumarin derivatives of aldehydes was synthesized and tested against Plasmodium falciparum strains. Ten new Coumarin analogues showed significant activity in the nano molar range (IC50 = 90–186 nm) against Plasmodium falciparum CQ-resistant strain FcB1. A possible mechanism of interaction implicating binding of these compounds to b-hematin was supported by in vitro tests. Moreover, the importance of the hydrophilic framework attached at the terminal nitrogen atom of the Coumarin and aldehyde joined to the chalcone ring was also explored through molecular dynamic simulations. Seven of the analogues (**SS01, SS02, SS03, SS06, SS07, SS09, SS10**) have exhibited curative antimalarial activity at a dose of 25mg/kg/day×4 and produced suppressive activity at a lower dose of 10 mg/kg/day×4The results of antimalarial activity showed that the ligand **SS-01, SS02** exhibits moderately active while complex SS-0**6, SS09** exhibits good activity for chloroquine sanstive and resistant.*

Keywords: Coumarin, Chloroquine sensitive strain and resistance strain, Antimalarial activity.

INTRODUCTION

Malaria is the most serious, complex and refractory health problems facing humanity. Almost one half of the world's population is exposed to the threat of malaria and the disease is responsible for two million deaths each year, either directly or in association with acute respiratory infections and anaemia and upto 1 million of those deaths are children. Malaria is a leading cause of morbidity and mortality in developing world1. Chloroquine was a mainstream drug in the fight against Plasmodium falciparum, but its efficacy is being eroded by the emergence of resistant parasites. The spread of chloroquine resistance has prompted the re-investigation of the chemistry and pharmacology of alternative antimalarials such as amodiaquine, an other 4- aminoquinoline which proved to be effective against chloroquine-resistant strains.Amodiaquine is a 4-aminoquinoline antimalarial which is effective against many chloroquine resistant strains of P. falciparum. However, clinical use of amodiaquine has been severely restricted because of associations with hepatotoxicity and agranulocytosis 4,5. It has been suggested that the toxicity of amodiaquine is related to the reactive electrophillic metabolites formed. by oxidation of its phenolic side chian, especially to the formation of a quinineimine by cytochrome P-450- catalyzed biological oxidation (Scheme 1). It has been found that amodiaquine is excreted in bile exclusively as the 5´ thioether conjugates (glutathione and cysteinyl) in rats6. This observation indicates that the parent drug undergoes extensive bioactivation in vivo to form amodiaquine quinoneimine (AQQI) or semi quinoneimine (AQSQI) with subsequent conjugation of glutathione7.

The enzyme which catalyzes the formation of ammonia and carbon dioxide by hydrolysis of urea is a nickel based metalloenzyme, is commonly known as urease enzyme. A large variety of prokaryote, most of the plants and a fewer fungi possess this enzyme. There are various negative implications caused by elevation of pH due to high concentration of ammonia produced by such type of reactions, in agriculture and medicine. Such type of negative

implications can be minimized by decreasing the activity of urease enzyme by employing suitable inhibitors. Inhibitors of urease can be broadly classified into two categories: (i) active site directed (substrate-like), (ii) mechanism-based directed. The urease due to its high substrate (urea) specificity can only bind to a few inhibitors with a similar binding mode as urea. [1-12]

The oxidative stress associated with various important diseases and pathogens are controlled by ROS reactive oxygen species which include superoxide anions, hydrogen peroxide, hydroxyl and nitric oxide radicals[13]. The damages caused by free radicals are protected by antimalarias, the radical mediated toxicity and as a result these antimalarias serve as major defense. The prevention and treatment of complicated diseases such as atherosclerosis, stroke, cancer and diabetes is carried out by employing antimalaria agents[13-14].

Ever since the discovery of the first case of chloroquine resistance along the Thai-Combodian border in the late 1950s, Southeast Asia has played an important role as a focus for the development of drug resistance in *Plasmodium falciparum.* Although the first case of quinine resistance had been reported much earlier from South America, the onset of chloroquine resistance marked the beginning of a new chapter in the history of malaria in Southeast Asia and by 1973 chloroquine finally had to be replaced by the combination of sulphadoxine and pyrimethamine (SP) as first line drug for the treatment of uncomplicated malaria in Thailand and more than 10 African countries have also switched their first line drug to SP. In 1985, eventually SP was replaced by mefloquine. The rapid development of resistance to this new drug leads to the introduction of artemisinin as a combination drug in the mid-1990s.

MATERIALS AND METHODS

2.1 The chemicals and reagents purchased from Merck were used without any alteration. The use of thoroughly washed and oven dried glassware was made sure during the whole work.

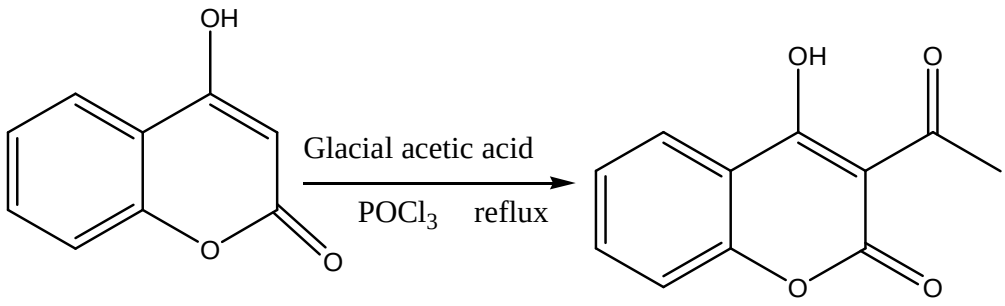
2.2 Physical measurements: The process of weighing was accomplished by using electric Mettler Toledo balance, of AL 204 model. The melting points reported are uncorrected and were taken by using melting point apparatus of Lab India MRVIS10300000. Perkin-Elmer 4400 Series II elemental analyzer was employed for elemental analysis. Jasco 300 FT-IR spectrometer with a range of 400-4000 cm^{-1} was employed for taking IR spectra of the compounds.

2.3 Synthesis of Compunds: The addition of 3-4 drops of conc. H_2SO_4 to a mixture of ethylenediamine (0.01 mol in 60 mL MeOH) and salicylaldehyde (0.02 mole in 60 mL MeOH) was carried out and it was refluxed in water bath for 4 hours at 70 ^oC and then it was kept for cooling in refrigerator. *n*-hexane was used for washing these yellow flakes and recrystallization in absolute methanol was accomplished. Desicator containing P_2O_5 was used for drying purpose.

2.4 Synthesis of PART A: Synthesis of 4-hydroxy 3-acetyl coumarin

Procedure for the synthesis of 3-acetyl coumarin –

4-hydroxy coumarin (0.01 mol) was mixed with glacial acetic acid (15 ml) then Phosphorous oxychloride (60 ml) was added slowly to the mixture and it was heated for 1.5 hr. and then cooled. The reaction mixture was poured into crushed ice and the solid product was separated, washed with water and crystallized from absolute alcohol and found to have m.p. 130 ^oC.

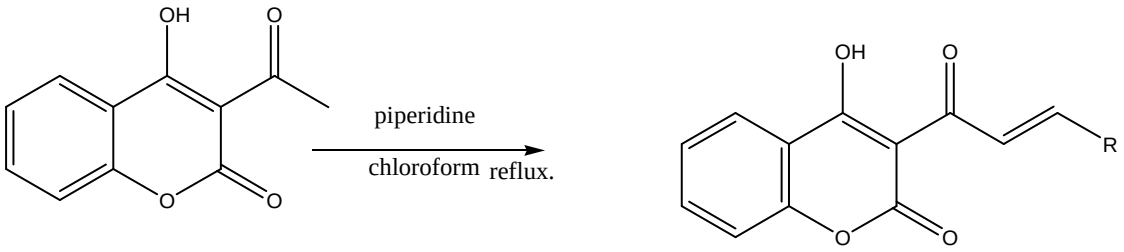


(4-Hydroxy coumarin) **(4-Hydroxy 3- acetyl coumarin)**

PART B: Synthesis of derivatives

General procedure for the synthesis of the Aldehyde derivatives

4-hydroxy 3-acetyl coumarin (0.031 mol) and the substituted aromatic aldehyde (0.03 mol) were dissolved in 6 ml of chloroform. A catalytic amount of piperidine (0.001 mol) was added and the reaction mixture was refluxed for 3 h. The chloroform was distilled out and the product was washed with methanol.



(4-Hydroxy 3-acetyl coumarin) **(Substituted aldehyde derivative)**

2.5 Synthesis of Derivatives: 0.01 mole of Etheyl acetate was added slowly with continuous stirring to the ligand **1** (0.01 mole, 100 mL) in equimolar ratio and mixture was then refluxed for 45 minutes. A solution of 1M NaOH was added in dropwise manner and pH was gradually raised to a suitable pH value for complex formation. Maroon coloured crystals were obtained by reluxing the reaction mixture for 90 minutes under vacuum. It was concentrated in a way that the volume of reaction mixture reduced to half of its original volume. The product thus obtained was washed with cooled methanol after filteration. Absolute methanol was used for recrystallization.

2.6 Biological studies: Ferheen *et al.* methodology was adopted for carrying out antimalaria (DPPH scavenging) and chloroquine sensitive strain and resistance strain of these compounds, however a modified method reported by Bibi *et al.* was employed for the measurement of diameter of zone of inhibition for antimalarial assay and then calculation of % inhibition was carried out.

Compound code	R1	R2	IC50 value
SS 01	R_1=R_2= H	Benzyl	62.93
SS 02	R_1=R_2=	Benzylethyl	56.21
SS 03	R_1=R_2=	Methyl cyclopropyl	29.10
SS 04	R_1=R_2=	Methyl ferrocenyl	110.10
SS 05	R_1=R_2=	R_2=4-nitrobenzyl	21.12
SS 06	R_1=H	R_2=4-hydroxybenzyl	18.06
SS 07	R_1=H	R_2=methylferrocenyl	86.10
SS 08	R_1=H	Benzyl	1.21
SS 09	R_1=R_2= H	Benzylethyl	1.04
SS 10	R_1=R_2=	Methylcyclopropyl	0.08
Standard	Chlroquine		0.77

RESULTS AND DISCUSSION

4.1 Synthesis and characterization: Salicylaldehyde upon reaction with ethylenediamine resulted in the formation of Coumarin ligand **1**, however it was resulted in the formation of a complex **2** upon treatment with Ni (II) acetate. A very much clear evidence about the stability of complex **2** was determined by the fact that this compound possesses a high melting point *i.e.* 203 ^{o}C.

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4.2 Infrared spectra: The involvement of azomethine group in complex formation was determined by observing IR spectra of the complex **2** which showed shift in the position of a band at 1618 cm^{-1} due to the presence of ν(-C=N-) (azomethine) in ligand towards lower frequency region *i.e.* 1530 cm^{-1} in the spectrum of complex **2**[22]. The disappearance of distinguishing bands of carbonyl group ν(C=O) group and the amino group indicated the completion of condensation reaction. The presence of a band at 3224 cm^{-1} for the ligand **1** in IR spectrum confirmed the presence of intramolecular hydrogen bonding. The bands at 590 and 465 cm^{-1} were assigned to the vibrations of (Ni-N) and (Ni-O)[23] respectively, in IR spectrum which is the direct evidence for involvement of heteroatoms (*i.e.* oxygen and nitrogen) of ligand in the coordination.

4.3 Suggested structural formula of the complex 2: It has been established by the foregoing discussion that nitrogen atoms of imino group and oxygen atoms of Coumarinligand are involved in complexation. Scheme 1 illustrates the tentative proposed structure for complex **2**.

4.4 Biological studies: The antimalaria, urease inhibition and antimalarial activities of complex **2** and Coumarinligand SS-**1** were conducted by screening these compounds.

The reason for the non-significant antimalaria ability of the compounds **1-2** can be explained by looking into the structures of these compounds. It is well known that the compounds with structures containing one or more functional groups such as –OH, –SH, –COOH, –N, –S–, –O– can show antimalaria activity. But in Coumarinligand **1**, hydroxyls are involved in hydrogen bonding and in case of complex SS-**2**, these are utilized in complex formation. That is why; the compounds have non-significant antimalaria activity.

4.4.1: The *P. falciparum* F-32-Tanzania chloroquine-sensitive strain, FcM29 and FcB1-Columbia chloroquine-resistant strains were cultured according to Trager and Jensen [25], with modifications[26]. The cultures were synchronized by a combination of magnetic concentration and 5% D-sorbitol lysis (Merck, Darmstadt, Germany) [27,28]. The F-32-Tanzania strain was considered to be chloroquine-sensitive (chloroquine IC50: 38 ± 6 nM); the FcM29 and FcB1-Columbia strains were considered to be chloroquine-resistant (chloroquine IC50: 170 ± 32 nM and 230 ± 11 nM, 196 ± 31 nM, and > 100 nM, respectively). Anti-plasmodial activity was determined by the [^{3}H]-hypoxanthine (Amersham-France) incorporation method [29]. The resistance index was calculated as follows: IC_{50} F32/IC_{50} FcB1 and IC_{50} F32/IC_{50} FcM29

The sensitivity of different stages of *P. falciparum* was tested using the FcB1 strain. Serial dilutions of nitidine, which were close to its IC_{50} determined previously on this strain, were prepared. After synchronization over a six-hour period (time between magnetic collection of previous stages and sorbitol lysis after invasion), the parasites were plated at ring stage in 24-well plates. The drugs (nitidine and chloroquine as a control) were added, and the plates were incubated for 8 h; the corresponding wells were then washed while the drugs were added into the new wells for another eight-hour incubation. The cultures were then incubated until the end of the erythrocytic cycle plus an additional 24 h. Giemsa-stained thin smears were made, and parasitaemia was confirmed by the numeration of at least 10,000 erythrocytes [30].

4.9.2 Mode of action: Although the exact biochemical mechanism is not completely understood, the mode of action of antimicrobials may involve various targets in the microorganisms. These targets include the following: (i) The higher activity of the metal complexes may be due to the different properties of the metal ions upon chelation. The polarity of the metal ions will be reduced due to the overlap of the ligand orbitals and partial sharing of the positive charge of the metal ion with donor groups. Thus, chelation enhances the penetration of the complexes into lipid membranes and the blockage of metal binding sites in the enzymes of the microorganisms. (ii) Tweedy's chelation theory predicts that chelation reduces the polarity of the metal atommainly because of partial sharing of its positive charge with donor groups and possible electron delocalization over the entire ring. This consequently increases the lipophilic character of the chelates, favoring their permeation through the lipid layers of the bacterial membrane[32]. (iii) Interference with the synthesis of cellular walls, causing damage that can lead to altered cell permeability characteristics or disorganized lipoprotein arrangements, ultimately resulting in cell death. (iv) Deactivation of various cellular enzymes that play a vital role in the metabolic pathways of these microorganisms. (v) Denaturation of one or more cellular proteins, causing the normal cellular processes to be impaired. (vi) Formation of a hydrogen bond through the azomethine group with the active centers of various cellular constituents, resulting in interference with normal cellular processes [31-33].

In vitro antimalarial effects of the investigated compounds were tested against activity against chloroquine sensitive strain and resistance strain. The results showed that the AA-5 exhibits moderate activity, but complex AA-**10** exhibits good activity for chloroquine sensitive strain and resistance strainspecies and significant for Gram-negative species

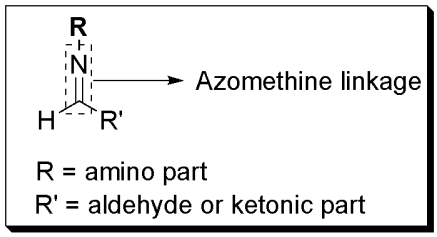

Figure. 1 General Representation of the structure of a Coumarin base

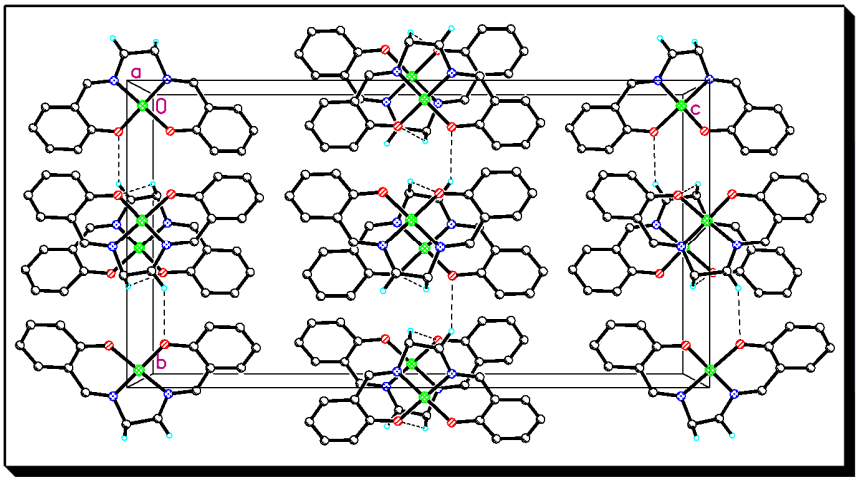

Figure. 2 ORTEP diagram of Ni (II) complex 2

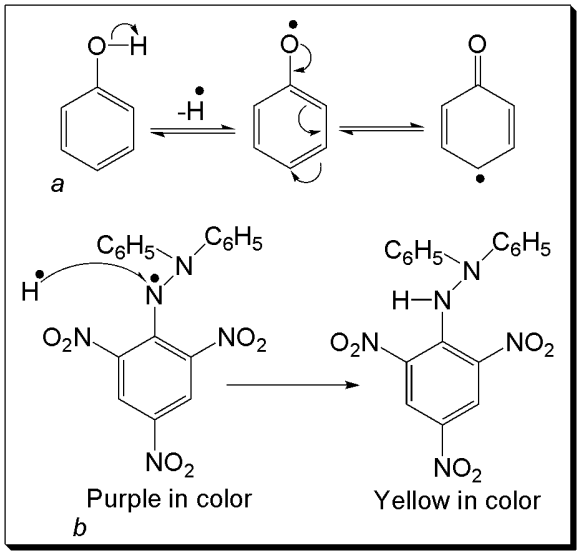

Figure. 3 Packing of the Ni (II) complex 2. Intermolecular C—H···O hydrogen bonds are shown as dashedlines

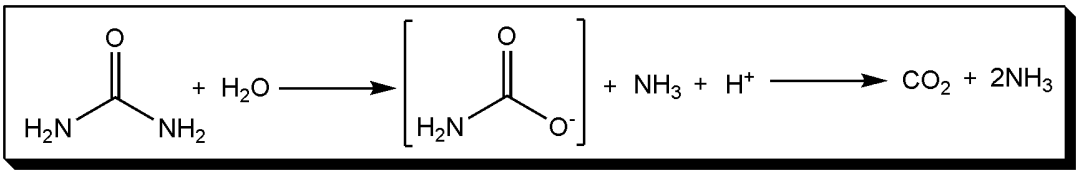

Figure. 4 (a) Removal of a hydrogen atom from the compound. (b) Hydrogen atom donated to DPPH radical by compound

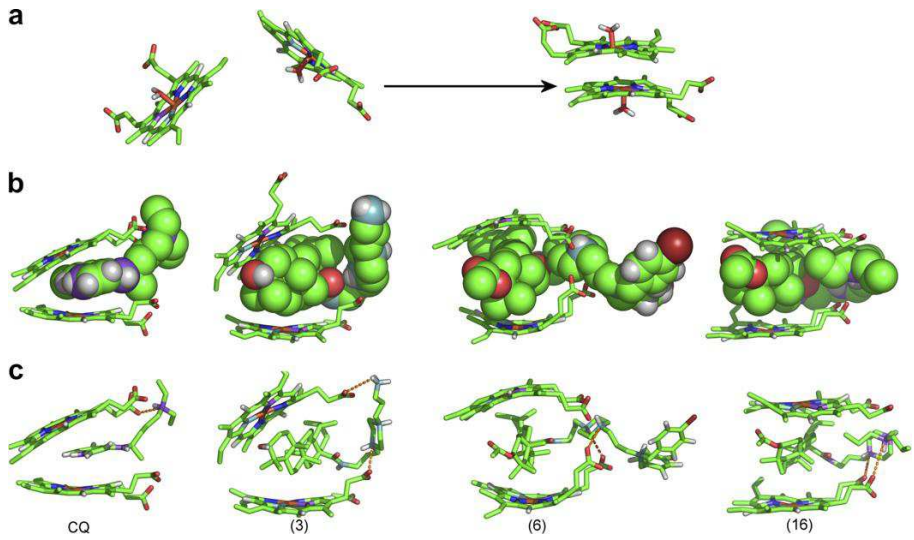

Figure. 5) Spontaneous association of heme molecules in aqueous solution during a MD simulation. (b and c) Complexes between heme and chloroquine (CQ), 3, 6, and 16, obtained after spontaneous di usion in solvent shown in space filing and tube representations, respectively

CONCLUSION

On the basis of this work, it is concluded that Coumarinl ligand **SS-5** and its complex **SS-10** are non-significant as compared to standard BHA and CoumarinAA-5 has significant result while the complex SS-**5** shows non-significant urease inhibition as compared with thiourea. *In vitro* antimalarial effects of the investigated compounds showed that the ligand SS-0**1, SS02** exhibits moderately active while complex SS-0**6, SS09** exhibits good activity, but complex **SS-03 and SS-06** exhibits good activity for chloroquine sensitive strain and resistance strain species and significant for Gram-negative species

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