

Antimalarial, Antimicrobial, and Acute Toxicity Activities of Mefloquine-Pyrimethamine Metal Complex.

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ABSTRACT

As part of our contribution to the on-going studies in developing a new and effective antimalarial drug. Complexation behavior of mixed Mefloquine (Mef) and Pyrimethamine (Pyr) [Antimalarial drug] with Co(II) have been investigated. The complex of the type ML_1L_2 [where $M=Co(II)$, $L_1= Mef$ and $L_2= Pyr$] were synthesized. The complex is non-electrolytes in methanol. The metal chelate formed was characterized by elemental analysis, magnetic measurements, conductivity measurements, Ultraviolet/Visible, and Infrared spectroscopies. The antimalarial drugs used acts as a tridentate compound with octahedral geometry with metal ion. The biological activities were also carried out viz: antimalarial, antimicrobial and toxicological studies. The results showed that the complex possess antimalarial activities with best clearance of 76.3% at 25mg/kg body weight. Thus they could be promising candidates for novel drugs in pharmaceutical research especially in the area of malaria.

(Keywords: antimalarial, characterization, acute toxicity, antimicrobial)

INTRODUCTION

Mefloquine and Pyrimethamine are used in treatment of malarial due to their Esquizonticide effect. Previous researchers have revealed that efficacies of some therapeutic agents increased upon coordination to transition metals. Incorporation of metallocene into chloroquine and quinine have been reported to yield compounds that were found to be active against both chloroquine sensitive and chloroquine resistant

strains of *plasmodium* and to be safe and effective in mice as well as being non- mutagenic (Obaleye et al., 2009).

Malaria continues to represent a major health problem in tropical countries in terms of spread, high morbidity and severe mortality particularly in children. WHO(1981) According to the world health organization, there are 300 to 500 million clinical cases of malaria each year resulting in 1.5 to 2.7 million death according to World Health Organization in 2009 (WHO, 2009).

Most of the available treatment being decades, old and suffering from limited efficacy or undesirable collateral effects and due to limited arsenal of effective antiparasitic agents and the frequent appearance of chemoresistance. There is an urgent and continuous need to develop new drugs against these ailments (Sanchez-Deigado and Anzellotti, 2004). Metal compounds still offer excellent opportunity to find new molecules against the major protozoan disease such as malaria (Navvaro et al., 2010).

Mefloquine Hydrochloride (First ligand employed in this study) is (\pm)-erythro- α -(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinoline methanol, and it is known for antimalaria activity. The choice of quinoline moiety was as a result of the success with the case of chloroquine. Mefloquine is a white or slightly yellow, crystalline powder, very soluble in water, freely soluble in methanol and alcohol. It melts at about 260°C with decomposition. It shows polymorphism (Ohnmacht et al., 1971).

Pyrimethamine (the second ligand employed in this study) is a 2,4-diaminopyrimidine derivative, an almost white crystalline powder or colorless

crystals. Practically insoluble in water, slightly soluble in alcohol, very slightly soluble in ether. Melting point of 238-243°C. Russel and Hitching (1951) reported a method for the synthesis. The antimalarial activity of pyrimethamine is due to its ability to inhibit plasmodial dihydrofolate reductase enzyme, for which the drug has a pronounced affinity (Ferone et al., 1969).

Pyrimethamine however, displays a markedly greater affinity for the plasmodial enzyme than for the mammalian enzyme, and thus exhibits sufficient selective toxicity towards the parasite. Since the two antimalarial consist of potential binding sites such as nitrogen and oxygen atoms. This work set out to study out the coordination tendencies, characterization after complexing with metals and activities against some microorganisms, malaria parasites (*Plasmodium berghei*) and toxicological studies to determine the margin of safety of the drug.

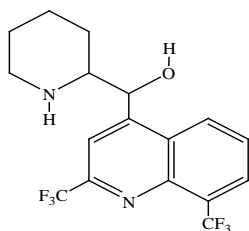


Figure 1: Structure of Mefloquine.

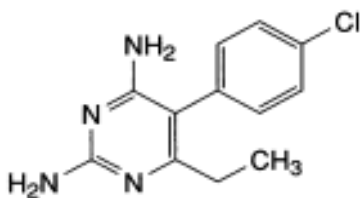


Figure 2: Structure of Pyrimethamine.

EXPERIMENTAL

All chemicals used were of the purest laboratory grade (Merck) and were used as received. Mefloquine hydrochloride and Pyrimethamine were obtained from SWISS Pharmaceutical Limited, Lagos Nigeria. Carbon Hydrogen and Nitrogen contents were determined using a Perkin-Elmer CHN 2400. The metal estimation was done using an Alpha 4 Atomic Absorption Spectrophotometer with PM 8251 simple pen

recorder. Infrared spectra were recorded on KBr disc in the range 40000-600 cm^{-1} on PUC scientific model 500 FTIR spectrometer. The UV/Visible spectra were on Aquamate spectrophotometer model V4.60. Molar conductivities were carried out using Jenway 4010 conductivity meter. The molar magnetic susceptibilities of the powdered samples were measured using Faraday Balance model 7650 using $\text{Hg}[\text{Co}(\text{SCN})_4]$ calibrant. Thin layer chromatography was carried out using TLC plate coated with silica gel.

Isolates of *Escherichia coli*, *Klebsiella pneumonia* and *Staphylococcus aureus* were obtained from the Department of Microbiology, University of Ilorin, Nigeria. Albino Swiss mice (NK-65) were obtained from the animal house of Nigeria Institute of Medical Research, Lagos. *Plasmodium berghei* used in this study were obtained through the same source.

SYNTHESIS

The complex was prepared following the reported procedure with slight modifications (Adediji et al., 2009). Ethanolic solutions of the metal chlorides were prepared in round bottom flasks (0.01 mole of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$). 0.01mol (4.148 g) of Mefloquine was mixed with 0.01 mol (2.487 g) of Pyrimethamine in a beaker.

The mixed ligands were dissolved in 20 mL of ethanol and added to the solution of the corresponding metal salt dissolved previously in 10 mL of ethanol in a round bottom flask fitted with a condenser. 10% methanolic ammonia solution was used to maintain the pH. The solution was refluxed for 2 h. The metal chelate crystallized, after leaving the reacting solution for about 30 minutes in a refrigerator. The metal complex thus separated were filtered and washed with ethanol and then with distilled water to remove unreacted ligand and metal; finally the solid complex was dried in a desiccator.

Color: Whitish pink.

Nature: powder.

Melting Pt: 201°C. %

Yield: 63.3%.

Soluble in: Hot distilled water, ethanol and methanol.

IR (KBr): 3551.4(N-H)str., 3144.1(O-H) overlapped, 1733.6(C=N), 1137.6(C-N), 724.2(M-L).

UV/Visible data (nm): 193.5, 220.0, 284.5.

Anal. Calcd(%) $\text{Co}(\text{C}_{29}\text{H}_{29}\text{F}_6\text{N}_6\text{OCl})$ M.Wt: 667.0g/mol. C(52.09%), H(4.35%), F(17.09%), N(12.46%), O(2.40%), Cl(2.55%); Found (%) C(52.09), H(4.31), N(12.46). Metal(8.85%) calculated, Found(8.74%). $\mu_{\text{eff}}(\text{BM})=4.62$.

Conductivity ($\Omega^{-1}\text{cm}^{-1}\text{dm}^{-1}$) = 1.09×10^{-5} .

ANTIMICROBIAL ACTIVITY

Antimicrobial Screening of the Ligand and Metal Complex

The stimulatory or inhibitory activity of the ligand and the metal complexes synthesized were determined according to the procedure previously reported by (Obaleye and Famurewa, 1989) as modified by (Mohamed and Abdel-Wahab, 2005). The bacteria species used for this test include clinical sample of *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. The antibacterial activities of the compounds were estimated on the basis of the size of the inhibition zone formed around the wells on sensitivity media. Antifungal activity of each compound was determined using culture of three fungi species; they are *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus species*. They were cultured on potato dextrose agar. The plates were incubated aerobically at $28 \pm 2^\circ\text{C}$ for 96 h.

ANTIMALARIA ACTIVITIES

Treatment of Animals

8-week-old non-infected male Swiss albino mice weighing 18-20 g were used. The animals were housed in standard cages, with standard feed and water given ad libitum, and they were acclimatized for 10 days prior to the experiments. All animal experiments were performed according to the guidelines for experimentation, Nigeria Institute of Medical Research (NIMR).

All the experiments were carried out in a suitable laboratory setting that has ambient illuminations and under controlled temperature of 20°C close to the temperature of where the animals were obtained (Peter and Anatoli, 1998).

ACUTE TOXICITY STUDY

The antimalarial mixed metal complex was evaluated for their toxicity in *Plasmodium berghei* non-infected mice. Ten mice were randomly divided into two groups of five rats per cage. Before oral administration of a single dose of the metal-drug, the mice were fasted for two hours (OECD, 2001; Peters, 1965). Then the mice in group 1 were given orally 0.2 mL of metal-drug at a single dose of 25 mg/kg body weight. The mice in group 2 (Control group) received 0.5ml of 3% Tween 80. The mice were observed continuously for 24 hours intermittently at interval of 4 hours. They were observed for gross behavioral changes such as feeding, hair erection, lacrimation, mortality and other signs of toxicity manifestations (Lorke, 1983).

PARASITE INOCULATION

Tests were performed in a 4-day suppressive standard test using the methods of David and Lorke at al. (Lorke, 1983; David et al, 2004).

The plasmodium species, that is most widely employed in rodent malaria parasite- *Plasmodium berghei* NK-65 (Chloroquine sensitive strain), obtained from NIMR was used to infect mice for a four day suppressive test.

The *P. berghei* was subsequently maintained in the laboratory by serial blood passage from mouse to mouse.

To infect the mice, blood sample was collected from auxiliary vessels of a donor mouse with a rising parasitaemia of about 30-37%. Then, the blood was diluted in normal saline so that each 0.2mL contained approximately 1×10^6 infected red blood cells. Each animal received inoculum of about 1×10^6 parasite via intraperitoneal route (IP). The inoculated mice were then randomized into five mice per cage and maintained in the animal house on a commercial diet and water ad libitum.

EVALUATION OF SCHIZONTOCIDAL ACTIVITY ON EARLY INFECTION (4-DAY TEST)

The Schizontocidal activity of Co(Mef-Pyr) on early *P. berghei* was evaluated in a four day test. The Peters' and Knight et al. 4-day suppressive test against *P. berghei* infection in mice was employed (Knight and Peters, 1980).

Briefly, adult Swiss male albino mice weighing 18-20 g were inoculated by intra-peritoneal (IP) injection with 1×10^6 infected erythrocytes. The mice were randomly divided into groups of five per cage and treated during 4 consecutive day with daily doses of the metal drug by oral route (5, 15 and 25 mg/kg).

Two control groups were used in each experiment, one treated with Chloroquine at total dose of 25mg/kg while the other group was kept untreated given normal saline as placebo. All experiments were done in triplicate.

On the fifth day blood sample was collected from tail snip of each mouse. Thin smears were prepared and stained with 10% Geimsa solution. Then, each stained slide was examined microscopically (1000xMagnification) power to evaluate the percent suppression of the metal drug with respect to the control groups.

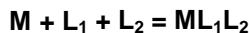
The percent suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those of treated mice. Chloroquine was used as positive control while normal saline was used as a negative control. For each group of mice treated with metal drug or Chloroquine, the mean percentage chemosuppression was then calculated as $100 [(A-B)/A]$, where A was the mean percentage parasitaemia of the mice treated only with saline containing 0.5% Tween-80(the negative control) and B was the mean parasitaemia in the test group.

STATISTICAL ANALYSIS

Data were compared using student's t tests, with a p value of <0.05 being considered statistically significant.

RESULT AND DISCUSSION

The metal chloride salt reacted with the mixed ligands according to the following proposed general equation:



Where; L_1 = Mefloquine, L_2 = Pyrimethamine, $M = Co^{2+}$. The synthesized complex was found to be non-hygroscopic solids with whitish pink color.

The complex is well soluble in DMF and DMSO, and also in ethanol, methanol and water. The complex have sharp melting point, with no decomposition observed at the reported melting point. The average percentage yield is 68.3% R_f , the retention factor values were calculated from the developed single spot for the Complex indicating the purity of the compound (Mohammed and Abdel-Wahab, 2005).

The retention factor of the metal complex was found to be higher than that for the ligand. Comparing the conductivity at room temperature of the ligand with that of its metal complex, it is possible to infer their non-electrolytic nature. The analytical data of the mixed ligand antimalaria metal complex showed that the metal chelate has a 1:1:1 stoichiometry.

The UV/Visible spectra of the ligands and its complex have been interpreted in terms of charge transfer transitions of the metal to the antibonding orbital of the ligand and in terms of the $\pi \rightarrow \pi^*$ transitions of the ligands(William H.D and Fleming I,1980).

Following our recent study of free mefloquine, two absorption bands at 272 and 207 nm were assigned to the $n \rightarrow \pi^*$ and $n \rightarrow \sigma^*$ transitions, respectively. These bands undergo a hypochromic shift in the mixed ligand metal complex due to complexation. Three absorption bands were observed in the spectra of the mixed complex and it has been interpreted charge transfer transitions.

From the UV/Visible data it is possible to infer that there is no $d \rightarrow d$ transitions, and the two ligands used were active in the coordination.

The infrared data showed the results of the most informative and indicative region. The assignments have been interpreted based on

literature values obtained for similar structural compounds (Obaleye et al., 1999).

The shifts observed in the absorption bands between Mefloquine, Pyrimethamine and its metal complex showed that there is coordination. Metal-Ligand bands were observed in the ranges of 610 - 950 cm^{-1} in the metal complex. The Co (II) complex shows a μ_{eff} value of 4.62 BM, which corresponds to high spin (octahedral) stereochemistry (Kamaruddin and Roy, 2001).

The *in vivo* studies of the ligands and the corresponding mixed ligand metal complex gave the antimicrobial activity of the compound. Mixed ligand metal complex as shown in Figures 1 and 2 below were found to be more active at higher (1.0 g/dm^3) concentrations than their corresponding ligands. The synthesized complex was active against the three bacteria used, while they were found to be active against only two fungi (*A. niger* and *A. flavus*). Reports have shown that the metal salt used [$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$] have no inhibitory activity on bacterial and fungi species used (Adediji et al., 2009).

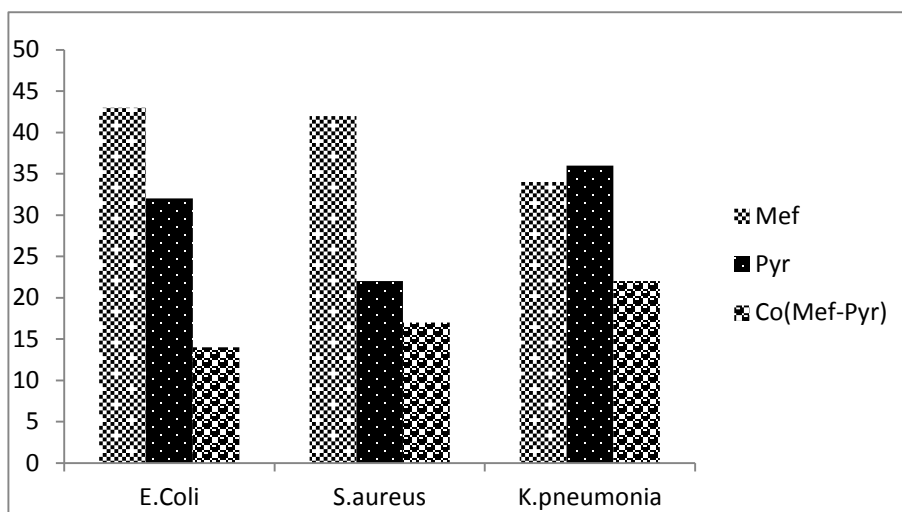


Figure 1: Inhibitory Activity of the Ligand and Metal Complexes against *E. coli*, *S. aureus*, and *K. pneumonia*.

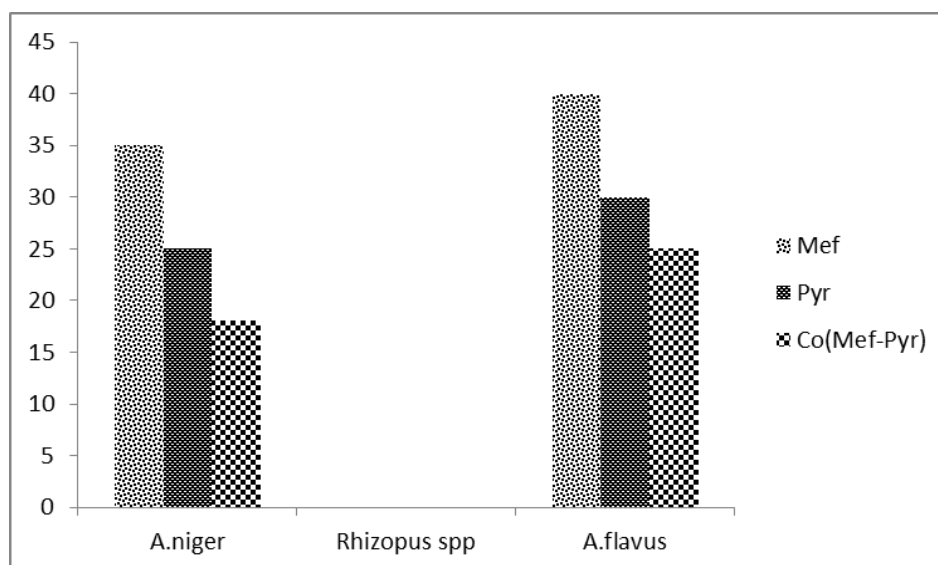


Figure 2: Inhibitory Activity of the Ligand and Metal Complexes against *A. niger*, *Rhizopus sp.*, and *A. flavus*.

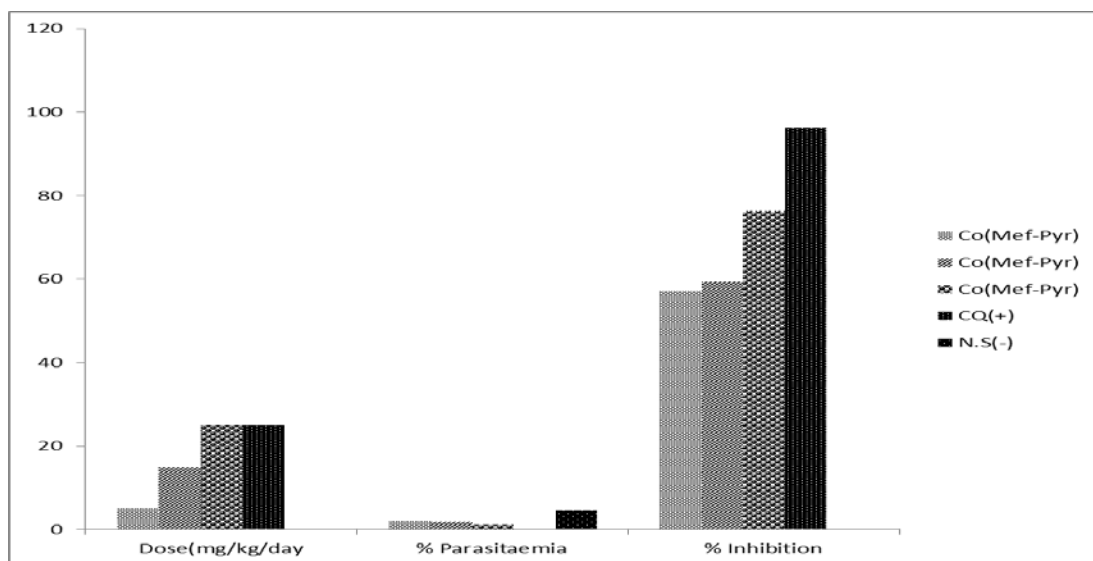


Figure 3: Antimalarial Activity of Co(Mef-Pyr) Complex and Chloroquine in Mice Infected with *Plasmodium berghei*.

In the acute toxicity tests, all the mice administered antimalaria metal complex at 5-25 mg/kg exhibited insignificant signs of toxicity, ranging from writhing and gasping (LD_{50} of >25 mg/kg) to decreased respiratory rate, decreased limb tone, and death. The LD_{50} was calculated to be >25 mg/kg. The present results indicate that metal-drug possesses useful blood Schizontocidal when used at doses that cause no marked toxicity in mice.

The metal drug complex used in this study was observed to show some antimalarial activity judging by its percentage chemosuppression in comparison with Chloroquine in the 4-day suppressive test (Figure 3).

Treatment of mice infected with *p.berghei* with metal drug showed a dose-dependent chemosuppression in comparison with Chloroquine treated controls with the 25mg/kg treated group of mice showing the highest percent chemosuppression. The activity might be attributed to presence of antimalarial properties of the ligands (Mefloquine and Pyrimethamine) enhanced by coordination of cobalt ion in the complex.

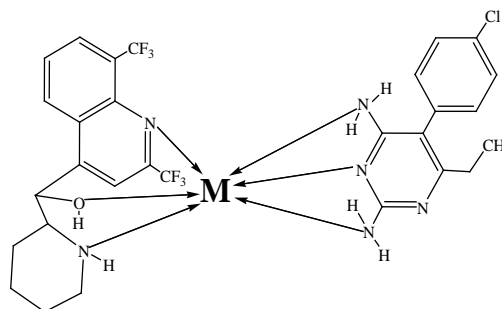


Figure 4: The Proposed Coordination Mode of the Metal Complex.

CONCLUSION

The Mefloquine (Mef) and Pyrimethamine (Pyr) coordinates to the Co(II) ion using the N:N:O donor atoms in the compound. The assignment of octahedral geometry proposed has been based on the information obtained by magnetic and infrared measurements. In the absence of no suitable crystal for single crystal X-ray structure, the proposed coordination modes of the complexes are presented in Figure 4. The metal complex possesses greater physical and biological properties compare to their parent compound. The metal complexes possess antimalaria properties with best clearance of 76.3% at 25mg/kg body weight.

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