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**Journal of Chemical and Pharmaceutical Research, 2012, 4(8):4073-4078**

**Research Article**

**ISSN : 0975-7384**
**CODEN(USA) : JCPRC5**

# Synthesis, effect on alkaline phosphatase and antimicrobial activities of Fe(III) complex of 2,5-diamino-1,3,4-thiadiazole

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**ABSTRACT**

*We reported recently in the Journal of Chemical and Pharmaceutical Research, 2012, 4(3):1511-1518, that Zn(II) coordinated with 2,5-diamino-1,3,4-thiadiazole with enhanced physical and biological properties. As part of our on-going research for more effective antimicrobial drug, the same potentially bioactive ligand, 2,5-diamino-1,3,4-thiadiazole (L), derived from semicarbazide hydrochloride have been prepared and characterized. [($ML_2$)]; M= Fe(III), complex were prepared. Elemental, Infra-red, Ultraviolet/Visible, Magnetic moment, Atomic Absorption, Thin layer chromatography and molar conductance measurements have accomplished characterization of the complex. The IR data reveal that the ligand (L) behave as a tridentate neutral ligand.It coordinated to the metal ion via sulphur and nitrogen of the amines. The molar conductance data reveal that the chelates are non-electrolytes. From the Ultraviolet/Visible spectra and magnetic moment data, octahedral geometrical structure was proposed for the complex. Toxicological studies were carried out on male albino rats [Wistar strain], at the dosage level of 0.60mg/kg body weight thrice daily for 7days on the alkaline phosphatase (ALP) activities on rat kidney, liver and serum. Chelates do not showed toxicity in enzymes activities. In-vivo evaluation of the antimicrobial activities of the metal complex and the ligand showed greater activity, when compared to the parent compound, against some micro-organisms.*

**Key words:** Alkaline phosphatase, Antimicrobial, Characterized, Toxicity

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## INTRODUCTION

Aminourea based derivatives exhibits a range of bioactivities, including anti-angiogenic, anti-tumour, antimalaria, anti-inflammatory and anti-analgesic [1,2], anti-tubercular, anti-glaucoma, anti-HIV, cytotoxic and antimicrobial [3,4] agents.

The synthesis of metal aminourea derivatives had received much attention due to the fact that it is among the first effective chemotherapeutic agents to be employed for the prevention and cure of bacterial infection in humans [5]. The pharmacological activity of these types of molecules is often enhanced by complexation with metal ions [6,7]. The importance of metal ions in biological system is well known [8]. One of the very interesting features of metal coordinated system is concerted spatial arrangement of the ligands around the metal ions.

In continuation to our work on aminourea compounds [9], this article involves the synthesis, characterization and biological studies of Fe(III) complex with 2,5-diamino-1,3,4-thiadiazole had been described. The solid products were characterized by elemental and magnetic measurements, IR, molar conductance, UV/Visible, thin layer chromatography and atomic absorption spectroscopy.

In recent years, several aminourea derivatives have been synthesized and their biological activities have been explored [10], but few experimental data about their toxicological activities have been reported. The present study sought to investigate the physicochemical and toxicological activities of some novel aminourea derivatives.

## EXPERIMENTAL SECTION

All chemicals used in the preparation of the complexes and in solutions studies were of the highest purity available. Semicarbazide hydrochloride, Potassium thiocynanate and 3% hydrogen peroxide were supplied from sigma. Fe(III) hexahydrate (BDH) were used as received. The organic solvents used such as absolute ethanol and methanol were also obtained from BDH.

Elemental analyses (C, H, N, S and M) were performed in the Pontificia Universidade Catholica, Rio de Janeiro, Brazil. The analyses were repeated twice. The IR spectra were recorded using SP3-30 Perkin-Elmer FT-IR spectrometer and in the wave number region 4000-400$cm^{-1}$. The spectra were recorded as KBr disks. The molar magnetic susceptibilities of the powdered samples were measured using Faraday Balance Model 7650 using $Hg[Co(SCN)_4]$ calibrant. The ultraviolet/visible analysis was carried out on Genesys.10S V1.200 spectrophotometer. The molar conductance measurements of the complexes were carried out in DMF using Genway 4200 conductivity meter. Metal contents of the complexes were determined using Alpha4 Atomic Absorption Spectrophotometer with PM8251 simple-pen recorder. Thin layer chromatography was carried out using TLC plate coated with silica gel.

ALP assay kits were obtained from Randox Laboratories Limited, Antrim, United Kingdom. Albino rats (*Wistar Strain*) were obtained through the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria[9].

### Treatment of animals

Male albino rats (Wistar strain), weighing between 160 - 180 g were obtained commercially from Ilorin, Kwara state, Nigeria and housed in the animal house. They were kept in wire meshed cages and fed with commercial rat chow (Bendel Feeds Nigeria Ltd) and supply water *ad libitum*. Twelve rats were divided into three groups of 6 rats per group. The first group was used as control and received distilled water. The second group of rats was treated with free ligand (2,5-diamino-1,3,4-thiadiazole) while the third group were subdivided into three groups treated with metal complex $[Fe(L)_2]$. The distilled water, ligand and solution of metal complex were administered orally to the rats of various groups three times daily for seven days at the dose of 0.60 mg/Kg body weight. The animals were sacrificed 24 hrs after the last treatment.[9]

### Preparation of serum and tissue homogenates

The method described by Yakubu *et al*. [11] was used to prepare the serum. The rats were sacrificed by cervical dislocation. Blood samples were collected by cardiac punctures into clean, dry centrifuge tube after which they were left for 10 min at room temperature. The tubes were then centrifuged for 10 min at 3000 x g in an MSC (Essex, UK) bench centrifuge. The clear supernatant (serum) was aspirated using a Pasteur pipette into clean, dry sample bottles and then frozen overnight before use.

The liver and kidney excised from rat, blotted of blood stains was rinsed in 1.15% KCl and homogenized in 4 volumes of ice-cold 0.01 mol $dm^{-3}$ potassium phosphate buffer (pH 7.4). The homogenates were centrifuged at 12,500 x g for 15 min at 4°C and the supernatants, termed the post-mitochondrial fractions (PMF) were aliquoted and used for enzyme assays.

### Determination of serum and tissue ALP activities

Serum and tissue's ALP activities were determined using Randox diagnostic kits.. ALP activity determination was based on the method of Wright *et al* [12]. The yellow colour p-nitrophenol formed was monitored at 405 nm. Protein determination of serum and all fractions was estimated by the method of Lowry *et al.* [26] as modified by Yakubu *et al.* [11] using bovine serum albumin as standard.

### Statistical analysis

The data were analysed using one way ANOVA followed by Duncan multivariable post-hoc test for comparison between control and treated rats in all groups. Values of p less than 0.05 were considered statistically significant.

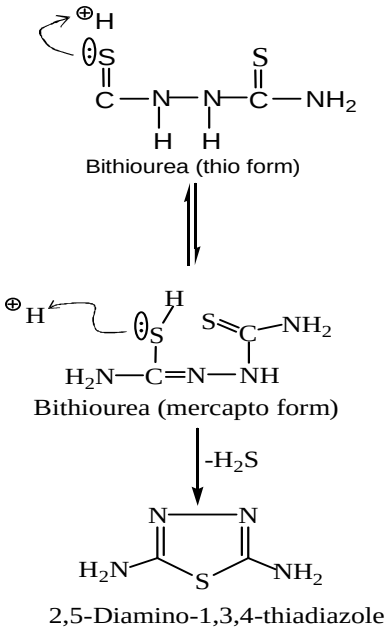
### Antimicrobial screening of the ligand and metal complex

The stimulatory or inhibitory activity of the ligand and the metal complex synthesized were determined according to the procedure previously reported by Obaleye and Famurewa (1989) [13] as modified by Mohamed and Abdel-Wahab (2005).[14] The bacteria species used for this test include clinical sample of *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia*. 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 ±2°C for 96 h.

**Synthesis of the ligand: mechanism of the reaction**

Based on previous reported procedure by Adediji *et al* [9]. The cyclisation of bithiourea were performed by 3% hydrogen peroxide, $H_2O_2$, the probable mechanisms of this cyclisation is as follows:




**Reaction Scheme**

The bithiourea undergoes tautomerism in the mercapto form and by protonation; a molecule of hydrogen sulphide is detached. This gives a positively charged carbon nucleus with a lone pair of electrons on the second sulphur atom which makes cyclisation possible.

**Procedure**

30 g (0.2mol) of bithiourea was introduced into a 250 $cm^3$ round bottomed flask and 40 $cm^3$ of 3% $H_2O_2$ was added. The mixture was refluxed at 50 - 60 °C for one hour with continuous stirring. The product was then filtered under vacuum and dried at 100 °C in the oven and the percentage crude yield was determined. It was thereafter recrystallised from boiling water.

**Synthesis of the metal complex**

The complex was prepared based on previous reported procedures with slight modifications [15]. An aqueous or ethanolic solution of the metal salt ($FeCl_3.6H_2O$) was mixed with an aqueous ethanolic solution of 2,5-diamino-1,3,4-thiadiazole (which was dissolved in minimum amount of the solvent) in 0.01 mol each. The reaction mixture was heated in a 250 $cm^3$ round bottomed flask for 15 min on a water bath and there was change of colouration, indicating the precipitates of the complex appearing. The reaction mixture was reduced to about one third when the metal complex separated out on cooling. The complex formed was recovered from the solution by filtration. It was washed and recrystallised from ethanol and then dried in vacuum over $CaCl_2$.

## RESULTS AND DISCUSSION

The metal chloride salt react with ligand L (L = 2,5-diamino-1,3,5-thiadiazole) according to the following proposed general equation [$ML_2$], where M = $Fe^{3+}$ metal salt. The complex synthesized was found to be non-hygroscopic solid with brown colour as shown in Table1. The complex is well soluble in DMSO and DMF and hot distilled water. It has sharp melting point, there was no decomposition observed. The average percentage yield was 60.2%. The retention factor ($R_f$) values was calculated from the developed single spot for the complex indicating the purity of the compound [14]. The retention factor of the metal complex was found to be higher than the ligand. Comparing the conductivity of the ligand with that of the metal complex, at room temperature suggest its non-electrolytic nature. The analytical data of the metal complex showed 1:2 stoichiometry.

**Table 1: some physical properties of L and its metal complex**

| Parameters | L | $Fe(L)_2$ |
|---|---|---|
| Melting point (°C) | 202 | 210 |
| Colour | White | Brown |
| % yield | 96.4 | 60.2 |
| Conductivity ($\Omega^{-1}$ $cm^{-1}$ $dm^{-3}$) | - | $1.4 \times 10^{-6}$ |
| Empirical formula | $C_2H_6N_4S_2$ | $Fe(C_2H_6N_4S_2)_2$ |
| Formula weight | 116.00 | 288.00 |
| $\mu_{eff}$ (BM) | - | 6.60 |
| % Metal content Found (calculated) | - | 19.42 (19.49) |

**Infrared spectra and mode of bonding**.

The IR spectra of the free ligand and their metal complex were carried out in the range of 4000-400$cm^{-1}$ and are listed in Table 2. The assignments have been carried out based on literature values obtained for similar structural compounds [16].

The important IR frequencies (in KBr) of the ligand, L and the metal complex with their tentative assignments are given. Both the free ligand and the metal complex are characterized by V(N−H), $\delta(NH_2)$, v(C−S) and v(C=S) bands [17,18,19]. The absorption patterns look quite similar to that of the free ligand which is in agreement with coordination through nitrogen atom. The band around 3400-3100$cm^{-1}$ is assigned to v(NH) and is supported by the presence of $\delta(NH_2)$ deformation bands around 1600-1500$cm^{-1}$. A blue shift was observed in the v(C−S) frequency of the complexes, in comparison to the free ligand, which indicates coordination through the sulphur atom. Bands between 800-900$cm^{-1}$ which were absent in the free ligand are assigned to M-L i.e metal-ligand coordination. Therefore, from the IR spectra, it is concluded that the ligand L behaves as a neutral tridentate ligand. It coordinated to the metal ions via the nitrogen of the amines and sulphur atom.

**Table 2: IR spectral assignment and UV/Visible data of L and its metal complex.**

| Ligand/complex | υ (NH) $cm^{-1}$ | υ (C=S) $cm^{-1}$ | $\delta(NH_2)$ $cm^{-1}$ | Wavelength nm ($cm^{-1}$) |
|---|---|---|---|---|
| L | 3407.8, b | 769.0, s | 1566.0, s | 205(48780)<br>238(42170) |
| $Fe(L)_2$ | 3231.2, b | 746.9, s | 1529.0, w | 214(46729)<br>235(42553) |

**Molar Conductance data**

The molar conductance of the solid complexes ($\Lambda_m$, $\Omega^{-1}$ $cm^2$ $mol^{-1}$) was calculated. The DMF solubility of the above complexes made calculations of the molar conductivity ($\Lambda_m$) of $10^{-3}$M solution at 25°C possible. The data in Table 1 in supplementary data show that the molar conductance are of relatively low values for Fe(III), indicating the non-electrolytic nature of the complex. Therefore, the molar conductance data confirm the results of the elemental analyses and IR spectra data.

**UV/Visible Spectra and Magnetic moments**

Absorption bands Table 2 of 2,5-diamino 1,3,4-thiadiazole and its metal complexes showed that all metal complexes gave strong absorption bands within the range of 205 nm - 364 nm. These bands are tentatively assigned to charge transfer and intra-ligand transitions respectively on other relative intensities and position[16,20].

UV/Visible spectra of this ligand and its complexes have been interpreted in terms of charge transfer transitions from the metals to the antibonding orbital of the ligand and of the $\pi \rightarrow \pi^*$ transition. The ultraviolet spectrum of the free 2,5-diamino-1,3,4-thiadiazole showed two absorption bands at 205 nm and 238nm. These transitions involve energies of 48780 $cm^{-1}$and 42017 $cm^{-1}$. These bands are assigned to the $n \rightarrow \sigma^*$ and $n \rightarrow \pi^*$ transition respectively. These bands undergo hypsochromic shifts in the metal complex due to Complexation ions of the ligand.[21]

Fe-DT has an electronic configuration of $d^5$ and a spectroscopic ground state term symbol of $^6S$. S-orbital here is a non-degenerate state and cannot be split by either an octahedral or a tetrahedral field.[22] Hence no d-d transition is expected in the spectrum of this $d^5$ complex. Absorptions at 214 nm, 235 nm and 319 nm which involve energies of 46729 $cm^{-1}$, 42553 $cm^{-1}$ and 31348 $cm^{-1}$ are due to transitions of the chromophoric groups in the coordinated ligand. The Fe (III) complex is coloured inspite of the fact that they have $d^5$ electronic configuration and the colour may be attributed to charge transfer band at 31348 $cm^{-1}$.[22]

Figures 1 show the results of antibacterial and antifungal activities of free ligand and metal complex. The studies of the ligand and its metal complex gave the antimicrobial activity of the compounds. The Metal complex was found to be more active at higher ($1.0g/dm^3$) concentration than its corresponding ligand. The synthesized complex was active against the three bacteria used, while they were found to have no activity against the fungi used. Reports have shown that $FeCl_3.6H_2O$ has no inhibitory activity on bacteria and fungi species. [15]

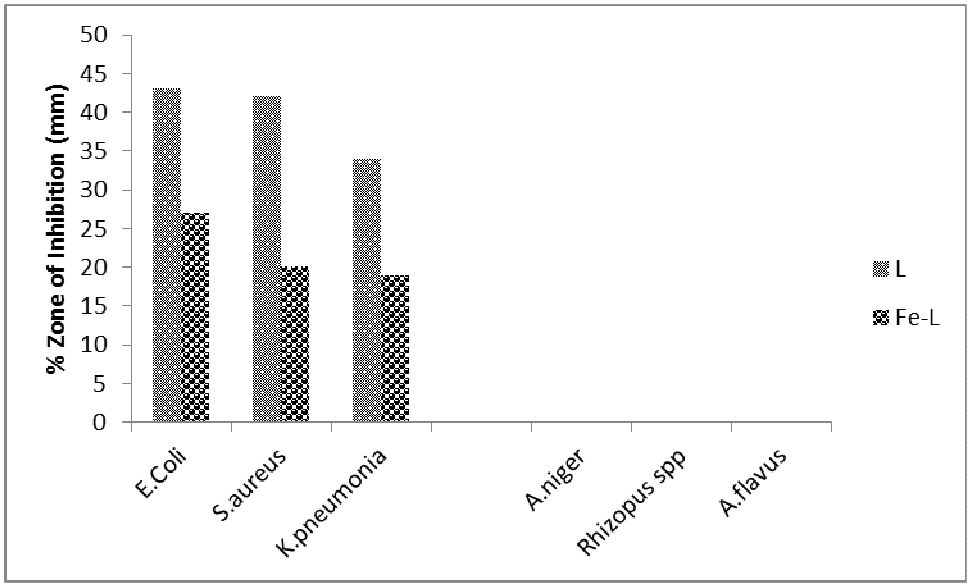



**Figure 1. Inhibitory activity of the ligand and metal complex against microorganisms**

Figs 2. show the results of ALP activities on the serum, kidney and liver. There was no significant reduction ($p<0.05$) in serum ALP activities of 2,5-diamino-1,3,4-thiadiazole and its metal complex compared with control, this suggests that the integrity of the plasma membrane of the cells in the various tissues might have not been adversely affected. This is because ALP is a membrane-bond enzyme often used to assess the integrity of the plasma membrane and endoplasmic reticulum[9]. The observed significant increase in the ALP activities in the liver and kidney of the rat administered with metal complex suggests an enhancement of the activities of the existing enzymes by the drugs and their metabolites. The increase may be as a result of stress imposed on the tissue by the drug, which may lead to loss of the enzyme molecule through leakage into extra-cellular fluid, which has been significantly noticed in the serum. In a bid to offset this stress, the tissue may increase the de novo synthesis of the enzyme, thus accounting for the increase in activities in these tissues [24] Overall, the integrity of the cell membranes of the various tissues (especially kidney and liver) was not adversely affected by the metal complex.

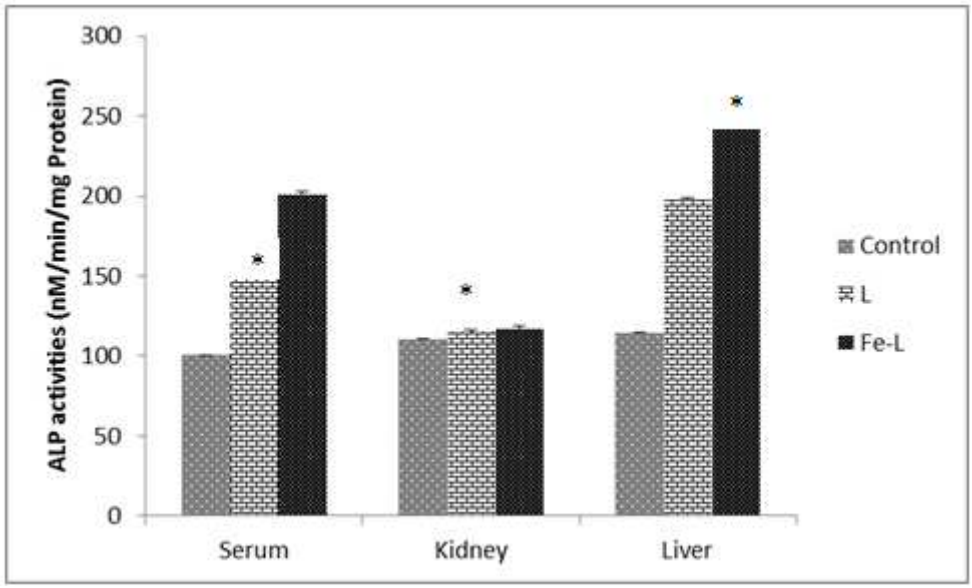



**Fig. 2: Effect of administration of ligand and the metal complex on the activities of alkaline phosphatase of rat serum, kidney and liver.**
** Significantly different from the control ($p<0.05$)*

**Structural Interpretation**

The structural information from this complex is in agreement with the data reported in this paper based on the elemental, IR and UV/visible spectra, molar conductance, and magnetic moment measurements.

The 2,5-diamino-1,3,4-thiadiazole coordinate via nitrogen of the amines and sulphur atom forming three binding chelating sites.

The Fe(III) complex shows a $\mu_{eff}$ value of 6.60BM, which corresponds to high spin (octahedral) stereochemistry (Kamruddin and Roy, 2001) [25,27]

## CONCLUSION

It is established from combined results of the chemical and physical analysis and from previous reports that the ligand (2,5-diamino-1,3,4-thiadiazole) employed in this work coordinated with Fe(III). The metal complex possesses better physical properties than the parent compound. Based on antimicrobial activities reported and toxicological activities observed from the above data. Metal complex of 2,5-diamino-1,3,4-thiadiazole would be a better therapeutic drug for antibacterial treatment.

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