Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(3):1501-1504

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Novel mixed ligand of 2,5-diamino-1,3,4-thiadiazole schiff base incorporating benzoic acid: Synthesis and antimicrobial activity

Adediji Johnson F, [1*] Adebayo Matthew A,[2] Ajayi Yewande O,[2] and Yusuf Latifat A.[2]

[1]Department of Chemistry, Federal University of Agriculture Abeokuta, Abeokuta, Ogun State, Nigeria
[2]Chemical Science Department, Ajayi Crowther University, Oyo, Oyo State, Nigeria

---

ABSTRACT

As part of our on-going research for more effective antimicrobial drug. A new mixed ligand has been synthesized. The starting material is Bithiourea which was synthesized by the reaction between semicarbazide hydrochloride and potassium thiocynate. The bithiourea produced was cyclised in the presence of 3% Hydrogen peroxide to produce 2,5-diamino-1,3,4-thiadiazole[L]. The new mixed 2,5-diamino-1,3,4-thiadiazole and Benzoic acid[L1] was then synthesized by Mannich reactions(Condensation). The chemical structures were confirmed by means of Elemental analysis, IR, UV/Visible, ^{1}H- and ^{13}C-NMR. Others are thin layer chromatography and conductivity measurement. In-vivo evaluation of antimicrobial activity was carried out against the following micro-organisms: Escherichia Coli, Staphylococcus aureus, and Klebsiella pneumonia (Bacteria species). Aspergillus niger, Aspergillus flavus and Rhizopus species (Fungi species). Our findings, using the chemical, spectroscopic and physical properties showed that the two compounds coordinated in ratio 1:1 stoichiometry. Infrared spectral data also suggest that the ligand behaves as a tridentate ligand with N:S:O donor sequence. The mixed ligand exhibited greater biological activity as compared to the host compound. The difficulty of treating bacterial diseases induced us to assess the antimicrobial properties of this novel compound. This approach might provide interesting compounds with greater biological activity in pharmacological research.

Key words: Mixed ligand complex, Synthesis, Bithiourea, Condensation, Antimicrobial.

INTRODUCTION

There is great interest in synthesis and characterisation of ligands which contain O,N,S-sequence and their metal complexes. The significance of these compounds, apart from their diverse chemical and structural characteristics, stems not only from their potential but also their proved application as biologically active molecules and a wide spectrum of activity.[1,2]

Semicarbazide and thiosemicarbazide derivatives are associated with some important biological activities such as Antitubercular,[3,4,5] anthelmintic, fungicidal, antitumor,[6] antimalarial and antibacterial activity.[7,8] They are found to be pharmacologically and physiologically active.[9] The difficulty of treating bacterial diseases induced us to assess the biological properties of this novel complex. This approach might provide interesting compounds with greater biological activity in pharmacological and metal complexation research.

Thiosemicarbazone and Semicarbazone(Schiff base) especially heterocyclic ones have been the subject of extensive investigation because of their use for the biological applications is very wide, [10,11] as compare to those with the hetero aromatic ring containing substitution as 2 or 4 position, the thiosemicarbazones have less attention. [12,13] Thiosemicarbazone compounds can be converted into complexes by reaction with metal ions and the reaction products have very important uses.[14] There are different substituted amide bonds (-CONH-) in the structure of

these compounds, therefore most of them have good biological activities and there are some reports about their use as herbicides and bactericides. [15]

In order to exploit new type of chelate extracting and biological active compounds, Benzoic acid are used in the present study to react with semicarbazide derivative and the new compound 2,5-diamino-1,3,4-thiadiazolebenzoyl are synthesized, which have not been reported elsewhere to the best of our knowledge. Preparation of the metal complexes, Antitubercular and Antimalaria studies are in progress.

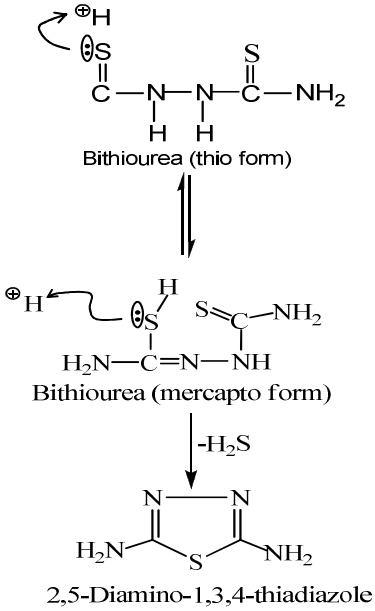
In this paper we wish to report the synthesis, characterisation and biological activities of 2,5-diamino-1,3,4-thiadiazole incorporating Benzoic acid, obtained from cyclisation of bithiourea, a derivative from semicarbazide hydrochloride. The chemical structures were confirmed by means of Elemental analysis, IR, UV/Visible, NMR spectroscopy, thin layer chromatography and Conductivity measurement. The compounds were also screened for antimicrobial activities.

EXPERIMENTAL SECTION

All chemicals used in the preparation of the novel compound and in solution studies were of the highest purity grade. Semicarbazide hydrochloride, potassium thiocynate and 3% Hydrogen peroxide were supplied from sigma chemicals. Benzoic acid from BDH was used as supplied. The organic solvents used; absolute Ethanol, Methanol, DMF, and DMSO were also obtained from BDH, Poole, England.

Elemental analyses (C,H,N andS) were carried out using micro-analytical techniques on Heraens-CHN rapid analyser. The IR spectra were recorded using SP3-30 Perkin-Elmer FT-IR spectrometer in the region 4000-400cm^{-1} the spectra were recorded as KBr disks. The ultraviolet/visible analysis was carried out on Genesys.10s v1.200 spectrophotometer. The molar conductance measurements of the compounds were carried out in methanol and DMF using Genway 4200 conductivity meter. Proton and Carbon-13 NMR of the compound was determined using Mercury 200B. Thin layer chromatography was carried out using TLC plate coated with silica gel.

Clinical cultures of the micro-organism used were obtained from the University Teaching Hospital and Department of Microbiology, University of Ilorin, Ilorin, Nigeria.



Scheme 1

Synthesis of 2,5-diamino-1,3,4-thiadiazole (L)
2,5-diamino-1,3,4-thiadiazole was prepared from a reported procedure(Adediji et al; 2011).

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 1 hr 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.

The cyclisation of bithiourea were performed by 3% hydrogen peroxide, H_2O_2, a suggested mechanism of the cyclisation is shown in Scheme 1.

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.

Colour: White. Nature: powder. Melting Pt: 202^{o}C. % yield: 96.4%. Soluble in: Hot distilled water. IR (KBr): 3195(NH_2), and 1430(C-S). UV/Visible data (cm^{-1}): 48780, 42017. Anal. Calcd(%) $C_2H_4N_4S$ M.Wt: 116.00 C(20.69%), H(3.45%), N(48.28%), S(13.79%); Found (%) C(20.68), H(3.42), N(48.21), S(13.76). ^{1}H DMSO-d_6: δ3.51-3.76(m,2H;2,5-H),2.78(S,3H), 2.62-2.75(m,3H; 3,5-H). ^{13}C DMSO- d_6: 160, 131.1, 130.5, 128.9, 114.1

Synthesis of mixed benzoic acid and 2,5-diamino-1,3,4-thiadiazole (L1)

2,5-diamino-1,3,4-thiadiazole L (0.01mole) was dissolved in hot distilled water (double distilled water, 50mL) and ethanolic solution of Benzoic acid (20mL) was added slowly within 5mins, with shaking, and the solution was allowed to stand for 30mins. 10% methanolic ammonia solution was added to maintain the pH. The reaction-mixture was warm gently, filtered and evaporated to half of its volume and kept overnight. The solid thus obtained was filtered and purified by recrystallization from ethanol.

IR(KBr): 2939.13(NH), 1693.30(C=O), 1423.42(C=S), 806.97(Ar-H); ^{1}H NMR(DMSO-d_6:δ9.81(S, 1H;NH), 7.12-8.06(m,8H; 2′,3′,5′,6′,2″,3″,5″,6″-H), 7.19(S,2H; NH_2) ^{13}C-NMR(DMSO-d_6) δppm: 172.67, 134.05, 130.46, 129.59, 128.72, 77.88, 77.24, 76.60. Colour: White. Nature: Crystalline powder. %yield: 70.20%. Melting Point: 122^{o}C. Soluble in: Ethanol, Methanol, Water, DMF, and DMSO. UV/Visible (cm^{-1}): 47619, 39683, 370.37, 34483, 29762, 27624. Anal. Calcd. (%) $C_8H_9N_4OS$ M.Wt: 209.38 C(45.93%), H(4.31%), N(26.79%), S(15.31%); Found(%) C(45.91), H(4.30), N(26.76), S(15.29)

Antimicrobial Screening

The stimulatory or inhibitory activity of the compound and its derivative were determined according to the procedure previously reported, with slight modification.[16,17,19] The bacteria species used for this test include clinical sample of *Echerichia coli*; *Stapphylococcus aureuos* and *Klebsialla pneumonia*. The antibacterial activities of the compound were estimated on the basis of the size of the inhibition zone formed around the wells on sensitive media.[18,21,22,20]

Antifungal activity of each compound was determined using culture of three fungi species; these are: *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus species*. They were cultured on potato dextrose agar. The plates were incubated aerobically at 28±2^{o}C for 96hours. Ofloxacin (60µg/mL) was used as a standard drug for antibacterial activity, and Ketoconazole (60µg/mL) as a standard drug for antifungal activity.

Table I- Antibacterial activity of L and L1

Compound	*Staphylococcus Aureous*		*Escherichia coli*		*Klebsiella pnemoniae*	
	Zone of Inhibition (mm)	% Inhibition	Zone of Inhibition (mm)	% Inhibition	Zone of Inhibition (mm)	% Inhibition
L	13	81.25	12	70.58	11	75.00
L1	11	68.75	11	64.70	12	68.75
Ofloxacin	16	100.0	17	100.0	16	100.0

Table II- Antifungal activity of L and L1

Compound	*Aspergillus niger*		*Aspergillus flavus*		*Rhizopus species*	
	Zone of Inhibition (mm)	% Inhibition	Zone of Inhibition (mm)	% Inhibition	Zone of Inhibition (mm)	% Inhibition
L	12	60.00	16	53.33	13	65.00
L1	11	55.00	14	46.66	12	40.00
Ketoconazole	20	100.0	30	100.0	20	100.0

The zone of inhibition observed around the cups after respective incubation was measured and percentage inhibition of the compounds was calculated.

The results are presented in **Table I and Table II**. The novel compound showed potent activity against all the micro-organism used, when compared with standard drugs used. It was also found to be effective against all micro-organisms at the same concentration. Thus, it is concluded from the screening results that L1 were most effective against all micro-organism at a concentration of 60µg/mL.

RESULTS AND DISCUSSION

The new Schiff base, L1 would be a good precursor for metal complexes like Lanthanide series and other metal like Co, Cr, Mn, Mg, Pd, Ni, Fe, Zn, Ca, Hg, and As in bidentate or tridentate respectively.

We disclosed analytical data like elemental analysis, ^{1}H NMR, ^{13}C NMR, IR, UV/Visible for new Schiff base ligand. More work is going on for preparation of metal complexes and biological activities viz: Antimalaria, Antitubercular and Toxicological studies.

CONCLUSION

The new Schiff base would be good precursors for metal complexes like Lanthanide series and other metals, synthesis were confirmed by elemental analysis, 1H and 13C NMR, IR, UV/Visible; The novel compound might also be a potent compound in pharmacological research.

REFERENCES

[1] DL Klayman; JP Scovill; JF Bartosevich and JJ Bruce, *J. Med. Chem*., **1983**, 26: 39 – 39.
[2] D Kovala-Demertzi; MA Demertzis; JR Miller; C Papadopoulou; C Dodorou and G Filousis, *J. Inorg. Biochem.* **2001**,
[3] T Samba; Y Funahasi; N Ono; Y Yamamoto; NH Sagi; M Asada; K Yoshimatsu and T Wakabayashi, *Clin. Cancer Res*., **2004**, 10: 1430 – 1438.
[4] J Slawinski and M Gdaniek,. *Eur. J. Med. Chem*., **2005**, 40: 377 – 389.
[5] Q Chen; PNP Rao and EE Knaus, *Bioorg. Med. Chem*., **2005**, 13: 2459 – 2468.
[6] AK Gadad; MN Noolvi and RV Karpoormath, *Bioorg. Med. Chem*., **2004**,12: 5651 – 5659.
[7] MJ Nieto; FL Alovero; RH Manzo and MR Mazzieri,. *J. Med. Chem.* **2005**, 361 – 369.
[8] JN Domínguez; C León; J Rodrigues; NG de Domínguez; J Gut and PJ Rosenthal, *II Farmaco*, **2005**, 60: 307 – 311.
[9] KG Mallikarjun, *E-J Chem*., **2005**, 2(6), 58 – 61.
[10] JR Dimmock SC Vashishtha and JP Stables, *J.Med Chem*., **2000**, 35,241-248.
[11] T Klimova; EI Klimova; GM Mertinez Mendez S; JM Stivalet and LR Ramirez, *J. Organomet Chem*; **2001**, 6333, 137-142.
[12] M Baldini; M Belicchi-Ferrari; F Bisceglie; G Pelosi; S Pinelli. and P Trasconi, *Bioorganic and Medicinal Chemistry Letters;* **2003**.
[13] Liu Guang-fei., Liu Lang., Hu Xin, Jia Dian-Ze and Yu Kai-Bei. *Chinese. J .Struct Chem.,* **2006**. 25(10), 1233-1237.
[14] K Rehse and U Muller. XXVII Inhibition of Leucocytes Adherence to Endothelium by the Oligoamine RE 1492C and the NO-Donor RE 2047. **1995**
[15] KR Arjun; RP Nayan; RD Horish and SN Sunil, *E-J. Chem*. **2009**, 6(3), 747-752.
[16] JF Adediji; ET Olayinka; MA Adebayo and O Babatunde, *Int. J. Phys. Sci*., **2009**, 4: 529 – 534.
[17] GG Mohamed and ZH Abdel-Wahab,. S*pectrochimica Acta. Part A: Mol. & Biomol. Spec.*, **2005**, 9: 2231 – 2238.
[18] AL Barry. (Lea and Febiger, Philadelphia, USA), *Biol Abstr*, **1976**, 64, 25183.
[19] M Amuthaselvi; P Jothi; A Dayalan; V Duraipandiyan and S Ignacimuthu, *J.Chem. Pharm. Res*, **2011**, 3((1): 382-387
[20] Rakesh Tada, Naimish Chavda and k Manish Shah, *J.Chem. Pharm. Res*.**2011**, 3(2):290-297
[21] SA Aly, *J.Chem. Pharm. Res*; **2011**, 3(6): 1028-1036
[22] K Shashikala Devi; M Ramaiah; GK Vanita; K Veena and VP Vaidya, *J.Chem. Pharm. Res*; **2011**, 3(1): 445-451.