Iranian Journal of Pharmaceutical Sciences
Autumn 2007: 3(4): 245-250
www.ijps.ir

Original Article

Synthesis and Evaluation of Antimicrobial Activity of Metal Complexes of 4-(2'-Hydroxy Phenyl Imino) Phenyl Sulphonamide

Bharat Bhusan Subudhi*, Prasanna Kumar Panda, Sabuj Sahoo

Department of Pharmaceutical Sciences, Utkal University, Vanivihar, Bhubaneshwar, Orissa, India

Abstract

Keeping in view the promising potential of carbonic anhydrase inhibitor based antimicrobials and enhancement of carbonic anhydrase inhibitory activity by metal complexes of sulfonamides, with an aim to develop better antimicrobial agents we have attempted investigation of antimicrobial activity of metal complexes of 4-(2'-hydroxy phenyl imino) phenyl sulphonamide. Sulfanilamide was taken as the starting material to synthesize 4-(2'-hydroxy phenyl imino) phenyl sulphonamide. Cu (II), Zn (II), Co (II), Ni (II) and Pb (II) complexes were synthesized following reported methods. The *in vitro* screening was carried out using two gram positive bacteria (*S. aureus, E. faecalis*) and two gram-negative bacteria (*E. coli, P. aeruginosa*) by disc diffusion method. Metal complexes were found to enhance the antimicrobial potental of the ligand.

Keywords: Antimicrobial; Carbonic anhydrase inhibitor; 4-(2'-Hydroxy phenyl imino) phenyl sulphonamide; Metal complexes.
Received: June 12, 2007; *Accepted:* August 27, 2007

1. Introduction

Development of carbonic anhydrase (CA) inhibitor based antimicrobials has shown promising results because of the presence of carbonic anhydrases in a multitude of bacteria [1-4]. Metal complexes of sulfonamide CA inhibitors generally act as 10-100 times more potent inhibitors of isozymes CA I, CA II and CA IV compared to the parent sulfonamide from which they were obtained [5, 6]. In this context we have undertaken the antimicrobial evaluation of Zn (II), Cu (II), Pb (II), Ni (II) and Co (II) complexes of 4-(2'-hydroxy phenylimino) phenyl sulphonamide. For this purpose the *in vitro* susceptibility of two gram-positive bacteria (*Staphylococcus aureus, Enterococcus faecalis*) and two gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) to the synthesized compounds was investigated.

*Corresponding author: Bharat Bhusan Subudhi, Department of Pharmaceutical Sciences, Utkal University, Vanivihar, Bhubaneshwar, 751004, Orissa, India.
Tel (+98)533-42387
E.mail:bharatsubudhi@yahoo.co.in

2. Materials and methods

2.1. Chemistry

The purity of the ligand was checked by TLC using chloroform-methanol-DMF

(100:05:05, v/v/v) as developing solvent and iodine as visualizing agent. Melting points were determined in open capillary tubes with the help of melting point apparatus (Sisco) and were uncorrected. The physical constants of compounds are summarized in Table 1. Infrared spectra were recorded on Shimadzu-8400 S spectrophotometer using KBr powder. The conductances of the compounds were determined in DMF with conductivity meter (CM-180, Elico). The electronic spectra were recorded with the help of UV-Visible double beam spectrophotometer (CECIL CE 7200). The complexes were analysed for their metal content by Aanalyst-200 atomic absorption spectrophotometer (Perkin Elmer). Elemental analysis was done with Euro-EA analyzer. The title compounds were synthesized according to the published method starting from sulfanilamide [7-9].

2.2. Synthesis of 4-(2'-hydroxy phenyl imino) phenyl sulphonamide

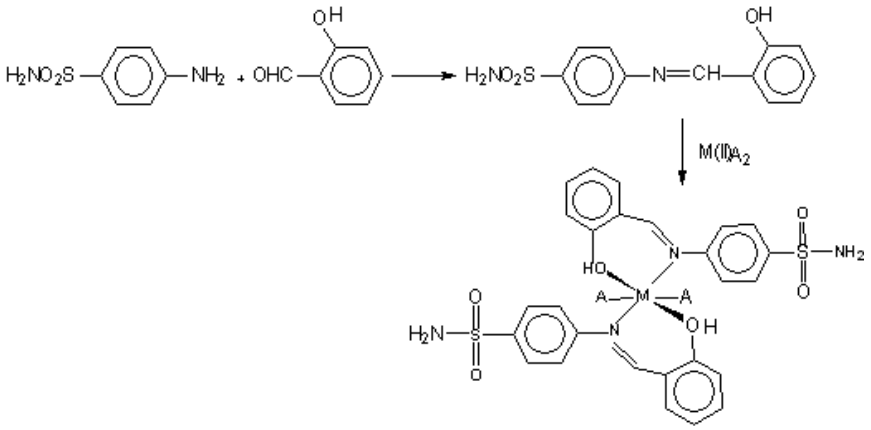
Sulfanilamide (0.05 mol) and salicylaldehyde (0.05 mol) were added into 20 ml of absolute ethanol containing a few drops of glacial acetic acid in a 250 ml round bottomed flask. The reaction mixture was refluxed for 2 h. It was then cooled and ice-cold water was added .The product so formed was filtered, washed, dried and recrystallised from alcohol.

2.3. Synthesis of $[CuL_2(AcO)_2]$

To an ethanolic (20 ml) solution of 4-(2'-hydroxy phenyl imino) phenyl sulphonamide (0.008 mol), an ethanolic solution of the corresponding metal (II) acetate (0.004 mol) was added. The mixture was refluxed for 3 h. The solution was reduced to half of its volume. It was left overnight at room temperature. The product so obtained was filtered, washed repeatedly with water and dried. The Zn (II) and Pb (II) complexes were prepared in a similar manner.

2.4. Synthesis of $[CoL_2Cl_2]$

An ethanolic (10 ml) solution of 4-(2'-hydroxy phenyl imino) phenyl sulphonamide (0.008 mol) was mixed with metal (II) chloride (0.004 mol) in ethanol (10 ml) followed by few drops of acetic acid (pH=6). The mixture was then refluxed for 1 h on a water bath till the complex precipitated. The solid product obtained was filtered, washed with distilled water and dried. In a similar process Ni (II) complex was synthesized.



A=Cl^-, AcO^- ; M=Zn (II), Cu (II), Pb (II), Ni (II) and Co (II)

Scheme 1. Metal complexes of 4-(2'-hydroxy phenyl imino) phenyl sulphonamide.

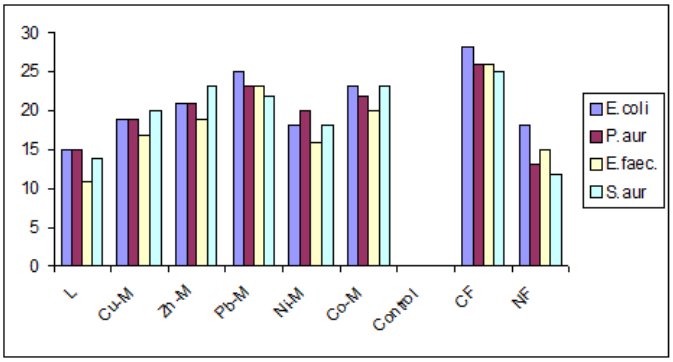


Figure 1. Relative antimicrobial activity of the synthesized compounds.

2.5. Evaluation of antimicrobial activity

The *in vitro* screening was carried out using two gram-positive bacteria (*S. aureus, E. faecalis,*) and two gram-negative bacteria (*E. coli, P. aeruginosa*). Strains were obtained from Post Graduate Department of Microbiolgy, Orissa University of Agricultural Technology, Bhubaneshwar. The organisms were identified following the standard micro-biological methods [10]. The compounds were screened for their antibacterial activity using disc diffusion method [11-13]. The compounds were dissolved in dimethyl formamide (6%), which was previously tested for antibacterial activity against all test bacteria and found to have no antibacterial activity. A solution with a concentration of 30 mg/ml was made for each test compounds and finally sterilized by filtration using 0.45 m millipore filters. The sterile discs (Hi-media, 6 mm in diameter) were impregnated with 10 µl of the test solutions (300 µg/disc) and placed in inoculated agar. The density of the bacterial suspension was standardized by using McFarland standard method [11-13]. Nitrofurantoin (300 µg/disc) and ciprofloxacin (25 µg/disc) were used as standard drugs. The control was prepared using dimethyl formamide. The inoculated plates were incubated at 37 °C for 24 h. The antibacterial activity of test compounds against the bacterial strains is given in Table 3 as zone of inhibition. The relative antibacterial activity of test compounds is illustrated in Figure 1.

Table 1. Physicochemical data of the synthesized compounds.

Compound	Molecular formula	Molecular weight	Melting point	Color/ solubility	Yield (%)
Ligand	$C_{12}H_{11}O_3N_2S$	263	212	Yellowish/ ethanol	75
$[CuL_2(AcO)_2]$	$C_{30}H_{30}CuN_4O_{10}S_2$	733.55	214	Brown/ DMF	65
$[ZnL_2(AcO)_2]$	$C_{30}H_{30}ZnN_4O_{10}S_2$	715.19	192	Grey/ ethanol	62
$[PbL_2(AcO)_2]$	$C_{30}H_{30}PbN_4O_{10}S_2$	877.20	148	Brown/ DMF	54
$[NiL_2\ Cl_2]$	$C_{26}H_{24}Cl_2NiN_4O_6S_2$	681.7	210	Grey/ ethanol	55
$[CoL_2\ Cl_2]$	$C_{26}H_{24}Cl_2CoN_4O_6S_2$	681.9	211	Soil color/ ethanol	55

The results obtained are expressed in mean ± standard deviation of three determinations.

3. Results and discussion

The IR band at 1674.27 cm^{-1} (Table 2) due to C=O stretching of salicylaldehyde shifted to 1626 cm^{-1} (C=N) in the spectrum of the ligand suggesting formation of Schiff base of salicylaldehyde and sulfanilamide. Low molar conductivities (5-11 $ohm^{-1}.cm^{-2}.mol^{-1}$) in DMF solutions measured for 1:2 (M:L) complexes indicate the non-electrolyte nature. The band for C=N stretching for ligand was observed at lower frequency by 9-35 cm^{-1} in the metal complexes, indicating participation of the azomethine nitrogen in the complexation. The shifting of the OH band at 3394.83 cm^{-1} in the spectrum of free ligand to 3338-3346 cm^{-1} in the spectra of metal complexes also indicate coordination of the oxygen of OH group to the metal ions. These suggest the bidentate coordination of ligand to the metal that is further supported by the appearance of weak, low frequency new bands at 530-550 cm^{-1} (M-N) and 440-450 cm^{-1} (M-O). The electronic spectra of these complexes are also consistent with an octahedral environment around the Co (II) ion. The spectra displayed band at, 29455 cm^{-1} (339.5 nm) attributed to $^4T_{1g} \rightarrow {}^4T_{2g}$ transitions, in a low-spin octahedral geometry. The electronic spectra of Cu (II) complex showed band at 28169 cm^{-1} (355 nm) assigned

Table 2. IR (KBr; ν , cm^{-1}), Electronic (nm), conductance and elemental data.

Compound	Analytical data
Salicylaldehyde	**IR:** 3238.59(O-H-str, broadened and centered), 3045.7(C-H-aromatic), 1674.27(C=O str) 1444(C=C-str), 1249.91(C-O str)
Ligand	**IR:** 3394.83(O-H-str), 3255.95(N-H-str), 1626.05(C=N-str), 1346.36(S=O) asymmetric-str), 1190.12(S=O) symmetric-str), 2982.05(C-H-aromatic), 1433.16(C=C-str). 692.47(C-Sstr). **Electronic (nm):** 256.5,294, 420.**C.H.N.(%);Calc.(Found):** 54.75(53.84), 4.18 (4.41), 10.64 (10.7)
$[CuL_2(AcO)_2]$	**IR:** 3338.89 (O-H- str), 3246.31(N-H- str), 1604.83 (C=N-str), 1313.57 (S=O) asym metric-str), 1153.47 (S=O) symmetric-str), 3064.99 (C-H-aromatic), 1456.30(C=C-str), 667.39(C-S str). 457.14(M-O-str), 553.59(M-N-str). Conductance(ohm-1cm-2mol-1): 8.5.Electronic(nm): 355,402.5nm.Cu(%); Calc.(Found): 8.65 (8.1) C.H.N. (%); Calc. (Found): 49.07(49.84), 4.08(6.2), 7.63(7.52)
$[ZnL_2(AcO)_2]$	**IR:** 3329.24 (O-H- str), 3236.53 (N-H- str), 1606.16 (C=N-str), 1313.49 (S=O) asym metric - str), 1153.47 (S=O) symmetric -str), 3064.99 (C-H-aromatic), 1445.63,1508.38(C=C-str), 668.25 (C-S str). 457.14(M-O-str), 553.59(M-N-str). Conductance(ohm-1cm-2mol-1):7.4. Electronic(nm):271.5, 339.5nm. Zn (%); Calc.(Found):9.14(8.93) C.H.N.(%);Calc.(Found): 45.75(46.2), 3.52(3.34),8.21(7.96)
$[PbL_2(AcO)_2]$	**IR:** 3346.61 (O-H- str), 3246.31(N-H- str), 1591.33,1620.26 (C=N-str), 1313.57 (S=O) asymmetric-str), 1157.33 (S=O) symmetric-str), 3084.28 (C-H-aromatic), 1454.38, 1529.60 (C=C-str). 630.74 (C-S str). 457.14(M-O-str), 543.94 (M-N-str) Conductance(ohm-1 cm-2 mol-1):9.4. Electronic(nm): 257, 295.5, 355, 400.5nm. C.H.N.(%); Calc.(Found): 50.33(50.94), 4.19(4.35),7.82(7.24)
$[NiL_2\ Cl_2]$	**IR:** 3338.89 (O-H- str), 3246.31(N-H- str), 1604.83 (C=N-str), 1313.57 (S=O) asym metric-str), 1153.47 (S=O) symmetric-str), 3064.99 (C-H-aromatic), 1456.30, 1508.38 (C=C-str). 667.39(C-S str). 457.14(M-O-str), 553.59 (M-N-str). Conductance(ohm-1 cm-2 mol-1):5.7.Electronic (nm): 295.5, 352.5,408.5.Ni (%); Calc.(Found):8.61(7.87)
$[CoL_2\ Cl_2]$	**IR:** 3344.68 (O-H str), 3246.31(N-H str), 1618.33 (C=N-str), 1313.57 (S=O) asymmetric-str), 1153.47 (S=O) symmetric-str), 3059.20 (C-H-aromatic), 1456.30, 1527.67(C=C-str). 659.68 (C-S str). 457.14(M-O-str), 543.94 (M-N-str) Conductance (ohm-1 cm-2 mol-1): 11.5.Electronic (nm): 250.5,298,355,399nm Co (%); Calc.(Found):8.63(8.24).

Table 3. *In vitro* antibacterial activities of the synthesized compounds against pathogens by disc diffusion method.

Compound	Concentration µg/disc	*E. coli*	*P. aeruginosa*	*E. faecalis*	*S. aureus*
Ligand(L)	300	14.8±1.3	15.0±0.8	10.5±1.4	13.7±0.7
[CuL2(AcO)2]	300	18.6±0.5	18.5±0.5	17.0±0.5	19.9±1.2
[ZnL2(AcO)2]	300	21.0±0.7	20.8±0.1	19.0±1.5	22.5±2.7
[PbL2(AcO)2]	300	25.0±0.7	23.3±1.4	23.4±2.2	22.0±3.1
[NiL2 Cl2]	300	17.5±2.4	19.6±2.1	16.3±1.2	17.8±0.7
[CoL2Cl2]	300	23.2±0.8	22.4±1.4	19.7±0.9	22.6±0.7
Control	-	-	-	-	-
CF	25	28.0±0.15	26.4±0.45	26.4±0.45	25.3±0.15
NF	300	18.4±0.45	12.6±0.65	15.3±0.37	11.8±0.75

"-" Indicates no zone of inhibition. All the values are mean ± SD of three determinations. Values showed significant difference from solvent control at $p<0.001$.

to charge transfer bond, supporting octahedral geometry of the complex. The quantitative assay of elements and metals generated values close to the theoretical values of the metals and elements in the proposed complex. The analytical data in the study are in agreement with previously reported metal complexes [7] and the proposed structure.

The antimicrobial activity of the ligand and its metal complexes were significantly different from that of solvent control ($p<0.001$). All the metal complexes showed better zones of inhibition than nitrofurantoin (NF). The activity of Pb (II) and Co (II) complexes were equivalent to that of ciprofloxacin (CF). The Pb (II) complex was most active against *E. coli, P. aeruginosa* and *E. faecalis*. The Co (II), Zn (II) and Pb (II) complexes were equally active against *S. aureus* (Table 3). The Cu (II) and Ni (II) complexes were more potent than NF but the zones of inhibition were less compared to that of other metal complexes. From the relative antimicrobial action (Figure 1), it is evident that complexation of the ligand with metals has enhanced their antimicrobial potential. The enhancement was more pronounced in case of Pb (II), Co (II) and Zn (II) complexes. Further study to elucidate the pharmacokinetic behavior of these complexes and their *in vivo* action are to be made to prove their therapeutic utility.

Acknowledgement

The authors are thankful to HOD, Department of Chemistry, Utkal University for providing instrumental facility and HOD, Department of Microbiology, OUAT for providing the pure bacterial strains.

References

[1] Chirica LC, Elleby B, Jonsson BH, Lindskog S. The complete sequence, expression in *Escherichia coli*; purification and some properties of carbonic anhydrase from *Neisseria gonorrhoeae*. *Eur J Biochem* 1997; 244: 755-60.

[2] Eickhoff TC, Nelson MS. *In vitro* activity of carbonic anhydrase inhibitors against *Neisseria meningitidis*. *Antimicrob Agents Chemother* 1966; 6: 389-92.

[3] Nafi BM, Miles RJ, Butler LO, Carter ND, Kelly C, Jeffery S. Expression of carbonic anhydrase in *Neisseriae* and other heterotrophic bacteria. *J Med Microbiol* 1990; 32: 1-7.

[4] Smith KS, Ferry JG. Prokaryotic carbonic anhydrases. *Microbiol Rev* 2000; 24: 335-66.

[5] Supuran CT. Carbonic anhydrase inhibitors; complex-type mechanism-based inhibitors. *Rev Roum Chim* 1992; 37: 849-55.

[6] Supuran CT, Scozzafava A, Casini A. Carbonic anhydrase inhibitors. *Med Res Rev* 2003; 23: 146-89.

[7] Ul-Hassan M, Scozzafava A, Chohan ZH, Supuran CT. Carbonic anhydrase inhibitors: Metal complexes of a sulfanilamide derived Schiff base and their interaction with isozymes II, IV, and I. *J Enz Inhib* 2001; 16: 499-505.

[8] Singh K, Tyagi P. Synthesis, characterization and biological studies of Co (II), Ni (II), Cu (II) and Zn (II) complexes with bidentate Schiff bases derived by heterocyclic ketone. *Eur J Med Chem* 2006; 1: 147-53.

[9] Raman N, Ravichandran S, Thangaraja C. Copper (II), cobalt (II), nickel (II) and zinc (II) complexes of Schiff base derived from benzil-2, 4-dinitrophenylhydrazone with aniline. *J Chem* Sci 2004; 116: 215-9.

[10] Collins CH, Lyne PM, Grange *JM. Microbiological Methods*. 6th ed. London: Butterworths, 1989; pp. 127-9.

[11] Jorgensen JH, Turnidge JD, Washington JA. Antibacterial susceptibility tests: Dilution and disk diffusion methods. In: Murray PR, Pfaller MA, Tenover FC, Baron EJ, Yolken RH,(editors). *Mannual of clinical microbiology*. 7th ed. Washington DC: ASM Press, 1999; pp.1526-43.

[12] Ringertz S, Rylander M and Kronvall G. Disk diffusion method for susceptibility testing of *Neisseria gonorrhoeae*. *J Clin Microbiol* 1991; 29: 1604-9.

[13] Drew WL, Barry AL, Toole RO, Sherris JC. Reliability of the Kirby-Bauer disc diffusion method for detecting methicillin-resistant strains of *Staphylococcus aureus*. *Appl Microbiol* 1972; 24: 240-7.