International Journal Of

Recent Scientific Research

ISSN: 0976-3031
Volume: 7(4) April -2016

NEW METAL BASED ANTIMICROBIAL AND ANTITUBERCULAR AGENTS WITH THEIR CYTOTOXICITY EVALUATION DERIVED FROM 2-AMINO-5-HYDROXYPHENYL-1,3,4-THIADIAZOLES

Lingareddy C., Phaniband M.A., Asma Tatagar., Shashikant Pattan and Javed Moodi

THE OFFICIAL PUBLICATION OF
INTERNATIONAL JOURNAL OF RECENT SCIENTIFIC RESEARCH (IJRSR)
http://www.recentscientific.com/ recentscientific@gmail.com

ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 7, Issue, 4, pp. 10132-10139, April, 2016

International Journal of Recent Scientific Research

Research Article

NEW METAL BASED ANTIMICROBIAL AND ANTITUBERCULAR AGENTS WITH THEIR CYTOTOXICITY EVALUATION DERIVED FROM 2-AMINO-5-HYDROXYPHENYL-1,3,4-THIADIAZOLES

Lingareddy C[1]., Phaniband M.A[2]*., Asma Tatagar[3]., Shashikant Pattan[4] and Javed Moodi[5]

[1]Department of Chemistry, J. T. College, Gadag, Karnatataka, India
[2,3]Department of Chemistry, SDM College of Engineering and Technology, Dharwad, Karnataka, India
[4]Department of Pharmacy, Pravara Pharmacy College, Loni, Pune. India
[5]Department of Chemistry, Kittel Science College, Dharwad, India

ARTICLE INFO

Article History:

Received 15th January, 2015
Received in revised form 21st February, 2016
Accepted 06th March, 2016
Published online 28th April, 2016

Keywords:

Thiadiazole, complexes, microbial, tubercular, cytotoxic activities.

ABSTRACT

Synthesis, spectroscopical characterization and biological studies of Co(II), Ni(II), Cu(II) and Zn(II) complexes of 2-amino-5-hydroxyphenyl-1,3,4-thiadiazoles are described. The ligand coordinates through nitrogen at 4th position of thiadiazole moiety and phenolic oxygen of salicylic acid moiety via deprotonation. The characterization involved elemental analysis, molar conductance, IR, ^{1}H NMR, electronic, magnetic susceptibilities, ESR, FAB-mass and thermal studies. From the above studies it is concluded that the ligands act as a bidenate molecule with stoichiometry being 1:2 (metal:ligand) obeying the general formula [$ML_2.2H_2O$]. Co(II), Ni(II) and Zn(II) complexes possessing an octahedral geometry while Cu(II) complexes have exhibited distorted octahedral geometry. The compounds have been screened in vitro for antibacterial and antifungal activities. Active compounds have been assayed with MIC values. The antitubercular activities for the compounds have been carried out against, $H_{37}Rv$ Strain by middle Brook Method. The brine shrimp bioassay was also carried out to study the in vitro cytotoxicity properties for the ligands and their corresponding complexes.

INTRODUCTION

Sulphur containing ligands have emerged as new medicinal and biochemistrial probes in treatment of several diseases [1]. Pd and Pt based sulphur – containing ligands have shown inhibition against tumors [2]. The antimicrobial studies of metal chelates have revealed new pathways in medicinal chemistry [3]. In vivo studies account to the fact that biologically active compounds become more bacteriostatic and carcinostatic on complexation [4]. Among the various classed of biologically active coordination compounds, complexes with thiadiazoles as ligands have attracted attention. The –N=C-S linkage of thiadiazole ring places this class of compound as an important and interesting biological moiety possessing several microbial activities [5,6]. Its substituents like 2, 5-Disubstituted 1,3,4-thiadiazole moieties have been found to be herbicidal, antitumor, diuretic and bacteriostatic [7,8]. Acetamide derivatives of 2-(benzoyl aminomethyl)-1,3,4-thidiazole have been found to possess antiarrhythmic, antimetastatic, psychoneurosi, schistosomicidal, fungicidal, herbicidal and pesticidal activities [9,10]. Only a few reports on metal complexes of these wonder moieties are available. Iron(II), cobalt(II), nickel(II), copper(II), zinc(II), cadmium(II) and silver(I) with 2-amino-5-phenyl-1,3,4-thiadiazole have been prepared and characterized [11,12]. Bismuth (III), and Silver(I) complexes with 2-amino-5-methyl-1,3,4 thiadiazole [13,14]. Some transition complexes with 2-amino-5-methyl and 2, 5-dimethyl-1,3,4-thiadiazole and some with 5-amino-1,3,4-thiadiazole-2-thiol have been reported [15-16]. Mishra.*et al*. [17] have commented on the synthesis, characterization and biological activity of cobalt(II), nickel(II), copper(II) and zinc(II) complexes with mercapto thiadiazoles. Organotin(IV) adducts synthesis, thermal and biological studies and Ti(IV) complexes derived from 2-amino thiadiazole Schiff bases. In continuation of our work on biologically active ligands [18] and in view of the versatile chelating ability of thiadiazoles, the

*Corresponding author: **Phaniband M.A***
Department of Chemistry, SDM College of Engineering and Technology, Dharwad, Karnataka, India

work was targeted to design novel Co(II), Ni(II), Cu(II) and Zn(II) complexes derived from thiadiazole moiety. The work aims at coordinating behavior of Co(II), Ni(II), Cu(II) and Zn(II) ions with 2-amino-5-hydroxyphenl-1,3,4-thiadiazoles derivatives and its prominent biological applications.

MATERIALS AND METHODS

All chemicals used were of reagent grade and further used without purification. Elemental Analyses (C, H and N) were performed on a Perkin- Elmer 2400 CHN elemental Analyzer Model 1106, Carloerba Strumentazione. The IR spectra of the ligands and their Co (II), Ni(II),Cu(II) and Zn(II) complexes were recorded on a HITACHI-270 IR spectrophotometer in the 4000-250 cm^{-1} region in KBr disks. Molar conductivity measurements were recorded on an ELICO-CM-82 T conductivity bridge with a cell having cell constant 0.51. The electronic spectra of the complexes were recorded in DMF on a VARIAN CARY 50-BIO UV-spectrophotometer in the region of 200-1100 nm. The proton PMR spectra of ligands and their Zn(II) complexes were recorded in $CDCl_3$ on BRUKER 300 MHz spectrometer at room temperature using TMS as an internal reference. FAB mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using Argon/Xenon (6 KV, 10Am) as the FAB gas. The accelerating voltage was 10 KV and the spectra were recorded at room temperature m-Nitrobenzyl alcohol was used as the matrix. The mass spectrometer was operated in the positive ion mode. Thermogravimetric analysis data were measured from room temperature to 1000°C at a heating rate of 10°C/min. The data were obtained by using a PERKIN-ELMER DIAMOND TG/DTA instrument.

Chemistry

General method for the synthesis of ligands (I-III)

A novel series of 2-amino-5-hydroxyphenl-1,3,4-thiadiazoles were synthesized by the reaction of substituted salicylic acid (0.1 mole) and thiosemicarbazide (0.1 mole) in 30 ml $POCl_3$ by refluxing gently for 1 hr. The reaction mixture was cooled and quenched (highly exothermic) with cold water (100 ml). The resulting solution was refluxed for additional 4 hrs and filtered hot. The filtrate was cooled and basified with KOH solution. The solid separated was filtered, washed with water, dried and recrystallized from ethanol. The ligands synthesized were named, 2-amino-5-hydroxyphenl-1,3,4-thiadiazoles(**I**),2-amino-5-cholrohydroxyphenl-1,3,4-thiadiazoles (**II**) and 2-amino-5-nitrohydroxyphenl-1,3,4-thiadiazoles (**III**). The elemental analyses with their molecular formulas are given in (Table I and Fig. 1).

2-amino-5-hydroxyphenl-1,3,4-thiadiazoles (**I**). Nature: Buff colored amorphous solid (methanol); Yield: 85%; m.p. 198-201ºC; IR (KBr, cm^{-1}): 3426 (ν_{OH}), 3324 (ν_{NH2}), 1622 ($\nu_{C=N}$), 1555 ($\nu_{C=C}$) 710 (ν_{C-S}); ^{1}H NMR (DMSO-d6, δppm): 6.35 (s, 2H, NH_2), 7.31-8.1 (4H,m, aromatic), 12.40 (s, 1H, OH);

2-amino-5-cholrohydroxyphenl-1,3,4-thiadiazoles (**II**).Nature : Light Pinkish amorphous solid, Yield 82 %, m.p. 184-186 ºC; IR (KBr, cm^{-1}): 3421 (ν_{OH}), 3331 (ν_{NH2}), 1619 ($\nu_{C=N}$), 1560($\nu_{C=C}$) 708 (ν_{C-S}); ^{1}H NMR (DMSO-d6, δppm): 6.41 (s, 2H, NH_2), 7.42-7.8 (3H,m, aromatic), 12.48 (s, 1H, OH);

2-amino-5-nitrohydroxyphenl-1,3,4-thiadiazoles (**III**) : Light Pinkish amorphous solid, Yield 79 %, m.p. 176-179 ºC; IR (KBr, cm^{-1}): 3424 (ν_{OH}), 3336 (ν_{NH2}), 1623 ($\nu_{C=N}$), 1558($\nu_{C=C}$) 712 (ν_{C-S}); ^{1}H NMR (DMSO-d6, δppm): 6.52 (s, 2H, NH_2), 7.31-7.9 (3H,m, aromatic), 12.58 (s, 1H, OH);

General method for the synthesis of complexes (IV-XV)

Aqueous ethanolic solution of metal chlorides of Co(II), Ni(II), Cu(II) and Zn(II) were added to the hot ethanolic solution of the ligands in (1:2) molar ratios and were refluxed for about 4 hrs on a water bath and the pH of the reaction mixture was adjusted *ca.* 7- 7.5. During the refluxation the metal chelates were separated out. The metal chelates thus separated were filtered, washed successively with ethanol and ether and finally dried over fused $CaCl_2$ in vacuum. The elemental analyses with their molecular formulas are given in Table I. All the complexes (**IV**) – (**XV**) were dark colored except Zn(II) complexes with dirty white color. m.p. > 240 ºC and an yield > 74 %.

Nature: Dark amphormous solid (DMF); Yield: 71%; m.p. Above 300˚C; IR (KBr, cm^{-1}): 3331-3338(ν_{NH2}), 1585-1590 ($\nu_{C=N}$), 1558-1570 ($\nu_{C=C}$) 710-715 (ν_{C-S}), 530-490 (ν_{M-O}), 450-410 (ν_{M-N}); ^{1}H NMR (300 MHz, DMSO-d_6, δppm): 6.52 (s, 2H, NH_2), 7.31-7.9 (4H,m, aromatic);

Biology

The antibacterial activity of the ligands and their Co(II), Ni(II), Cu(II) and Zn(II) metal complexes were assayed against two bacteria namely, *Escherichia Coli* and *Bacillus Subtilis* by with concentration of 100µg/mL by spread plate method. Similar procedure was followed for the antifungal activity of the above said ligands and metal complexes against two fungi namely, *Candida Albicans* and *Fusarium Solani.* The activity was also assayed for the pure solvent DMF and the standard Norfloxacin for each of antibacterial and Grisiofulvin for antifungal cultures. Further for the active compounds MIC values were determined. Final adjustments were made using optical density measurement for bacteria (absorbance 0.05 at a wavelength of 580nm). For the cytotoxicity study brine shrimp (*A.Salina. L)* eggs were hatched in a shallow rectangular plastic dish (22X32cm), filled with artificial seawater. The antitubercular screening was carried out by Middle brook 7H9 agar medium against H_{37}Rv. The DNA cleavage studies have also been carried out for some selected complexes.

RESULTS AND DISCUSSION

Chemistry

Co(II), Ni(II) and Cu(II) complexes are colored, Zn(II) complexes being colorless. They are sparingly soluble in common organic solvents; however, these complexes are soluble to a larger extent in DMF and DMSO. The growth of single crystals of these complexes for X-ray studies is very difficult owing to their amorphous nature and we were unsuccessful in our attempts to do so. The elemental analyses Table.I are consistent with a 1:2 stoichiometry of the type $ML_2.2H_2O$. The molar conductance values in DMF fall in the expected range of 10-30 $cm^2\ \Omega^{-1}\ mol^{-1}$of non-electrolytes [19]. Hence, the complexes may be regarded as non- electrolytes. The molecular weights of the complexes could not be

determined in nitrobenzene because of their insolubility. In order to establish whether the water molecule present in the Co(II), Ni(II), Cu(II) and Zn(II) complexes coordinated to the metal ion, weighed Co(II), Ni(II), Cu(II) and Zn(II) complexes were dried over P_4O_{10} in vacuum for 1 h and weighed again. No loss in weight was observed. This was confirmed by heating the complex for 2 h at 105 ^{0}C and no weight loss was observed. These observations suggest that, water molecules in the complexes are coordinated to the metal ion. This was also proved by DTA and TG analysis.

electronic spectra distorted octahedral geometry around Cu(II) ion is suggested [22]. The electronic spectra of the Zn(II) complexes exhibit only a high intensity band at 27250-28500 cm^{-1} assigned to ligand-metal charge-transfer.

Magnetic data

The magnetic moments obtained at room temperature are listed in (Table I). The Co (II) complexes show magnetic moments in the range of 4.72-5.01 B.M.

Table I Analytical, magnetic and conductance data of the thiadiazole derivatives and their transition metal complexes

Code	Molecular Formula	Found (Calc.) C%	Found (Calc.)H %	Found (Calc.)N%	Found (Calc.) S%	Found (Calc.) Cl%	Found (Calc.) M%	Molar condt. Ohm^{-1} cm^{-2} mol^{-1}	μeff (BM)
I	$C_8H_7N_3OS$	49.51 (49.74)	3.40 (3.63)	21.54 (21.76)	16.27 (16.58)	-	-	-	-
II	$C_8H_6N_3OSCl$	41.97 (42.20)	2.43 2.64)	18.22 (18.46)	13.84 (14.07)	15.39 (15.60)	-	-	-
III	$C_8H_6N_4O_3S$	40.07 (40.34)	2.29 (2.52)	23.19 (23.53)	13.28 (13.46)	-	-	-	-
IV	$[Co(C_8H_7N_3OS)_2(H_2O)_2]$	39.83 (40.10)	2.29 (2.51)	17.31 (17.54)	4.36 (4.52)	-	12.06 (12.31)	16.89	4.78
V	$[Ni(C_8H_7N_3OS)_2(H_2O)_2]$	39.86 (40.11)	3.17 (3.34)	17.37 (17.55)	13.11 (13.37)	-	12.02 (12.26)	19.63	3.27
VI	$[Cu(C_8H_7N_3OS)_2(H_2O)_2]$	39.57 (39.71)	3.52 (3.71)	17.14 (17.37)	12.97 (13.24)	-	12.89 (13.14)	21.8	1.73
VII	$[Zn(C_8H_7N_3OS)_2(H_2O)_2]$	39.24 (39.56)	3.09 (3.30)	17.03 (17.31)	12.89 (13.18)	-	13.23 (13.47)	16.4	Dia
VIII	$[Co(C_8H_6N_3OSCl)_2(H_2O)_2]$	34.78 (35.04)	2.26 (2.56)	15.07 (15.33)	11.43 (11.68)	12.71 (12.96)	10.46 (10.76)	18.2	5.01
IX	$[Ni(C_8H_6N_3OSCl)_2(H_2O)_2]$	34.73 (35.05)	2.29 (2.56)	15.04 (15.34)	11.48 (11.69)	12.74 (12.96)	10.39 (10.72)	22.4	3.31
X	$[Cu(C_8H_6N_3OSCl)_2(H_2O)_2]$	34.41 (34.75)	2.18 (2.53)	14.88 (15.20)	11.31 (11.58)	12.62 (12.85)	11.31 (11.50)	24.1	1.80
XI	$[Zn(C_8H_6N_3OSCl)_2(H_2O)_2]$	34.41 (34.63)	2.30 (2.53)	14.94 (15.15)	11.28 (11.54)	12.57 (12.81)	11.53 (11.79)	17.23	Dia
XII	$[Co(C_8H_6N_4O_3S)_2(H_2O)_2]$	33.54 (33.75)	2.23 (2.46)	19.47 (19.69)	11.01 (11.25)	-	10.09 (10.36)	12.43	4.96
XIII	$[Ni(C_8H_6N_4O_3S)_2(H_2O)_2]$	33.51 (33.76)	2.24 (2.46)	19.48 (19.69)	10.98 (11.25)	-	10.02 (10.32)	13.85	3.14
XIV	$[Cu(C_8H_6N_4O_3S)_2(H_2O)_2]$	33.19 (33.48)	2.20 (2.44)	19.37 (19.53)	10.84 (11.16)	-	10.87 (11.08)	21.34	1.68
XV	$[Zn(C_8H_6N_4O_3S)_2(H_2O)_2]$	33.06 (33.37)	2.17 (2.43)	19.29 (19.47)	10.77 (11.12)	-	11.08 (11.36)	19.87	Dia

Electronic spectra

The electronic spectra of Co (II) complexes exhibited absorption bands in the region 8000-12000 cm^{-1} and 18000-20000 cm^{-1} corresponding to ν_1 and ν_3 transitions respectively. Which are attributed to the transition $^4T_{1g}$ (F) → $^4T_{2g}$ (F) (ν_1); ^4T_1g (F) → ^4T_1g (P) (ν_3) [20]. These bands are characteristic of high spin octahedral Co (II) complexes. However, ν_2 band is not observed because of its proximity to strong ν_3 _transition. The Ni(II) complexes exhibited three bands at 8000-13000 cm^{-1}; 13000-19000; 24645-25097 cm^{-1} attribute to the transition $^3A_{2g}$ → $^3T_{2g}$ (ν_1); $^3A_{2g}$ → $^3T_{1g}$ (F) (ν_2); $^3A_{2g}$ → $^3T_{1g}$ (P) (ν_3) which indicates octahedral geometry around Ni(II) ion [21]. The Electronic spectra of Cu(II) complexes have displayed three prominent bands. A low intensity broadband in the region 14518-17098cm^{-1} which is assignable to $^2T_{2g}$ ← ^2E_g transition. Another high intensity band in the region 24463-25487 cm^{-1} is due to symmetry forbidden ligand → Metal charge transfer and two sharp bands observed at 31006-31169 cm^{-1} and 36981-37102 cm^{-1} are due to the ligand bands. On the basis of

These values are within the expected range of 4.7-5.2 B.M [23] for octahedral complexes. Hence, the Co (II) complexes may be expected to have octahedral configuration. The Ni (II) complexes show magnetic moments in the range of 3.14-3.31 B.M. It is reported that, the octahedral Ni (II) complexes exhibit magnetic moments in the range of 2.5-3.5 B.M. [24]. The magnetic moments of Cu (II) complexes fall in the range 1.66-1.80 BM. This corresponds to one unpaired electron which account for a slight orbital contribution to the spin only value and the absence of spin –spin interactions in the complexes.

ESR spectra

The EPR spectra of the Cu-(II) complex were recorded on Variant E-4', X-band ESR Spectrometer using cylindrical quartz sample tube at room temperature. Polycrystalline diphenylpicrylhadrazyl (DPPH) was used as 'g' marker. The ESR spectrum of one the representative **XIV** complex as in Figure II was recorded at room temperature (300K) and at liquid nitrogen temperature(77K) which has exhibited

unresolved broad signals giving only one g value, i.e., g_{iso} (g iso at 300K is 2.094 and that at 77K is 2.101 respectively). The shape of ESR lines indicates that the present complexes may have octahedral geometry.[25]

The loss of two water molecules gave a fragment ion $[ML_2+H]^+$ at m/z 443.This species undergoes demetallation to form the species $[L_2]^+$ at m/z of 383 (Fig. IIIb).

Table II Thermal data of one of the representative M_{10}complex

Code	Complex	Temp (°C)	% weight loss	Proposed chemical change	Metal oxide%
IV	$[Co(C_8H_7N_3OS)_2(H_2O)_2]$	115-140	12.80 (12.97)	Two Water molecules	15.93 (16.29)
VII	$[Zn(C_8H_7N_3OS)_2(H_2O)_2]$	275-305	70.48 (70.74)	Two Ligand moieties	
		120-150	12.98 (13.16)	Two Water molecules	
XII	$[Co(C_8H_6N_4O_3S)_2(H_2O)_2]$	280-310	69.33 (69.56)	Two Ligand moieties	16.98 (17.28)
		115-140	12.63 (12.84)	Two Water molecules	15.86 (16.19)
		280-310	70.58 (70.97)	Two Ligand moieties	

Table III Antibacterial and antifungal activity of the compounds (zone of inhibition in mm for 100 µg/mL)

Compuond Code	Antibacterial		Antifungal	
	S..Aurease	*E.coli*	*A.niger*	*C. Albicans*
I	18	17	19	20
II	18	19	20	21
III	16	17	18	20
IV	20	21	23	24
V	17	16	18	21
VI	20	19	21	22
VII	18	18	20	21
VIII	21	21	24	25
IX	19	19	21	22
X	20	21	23	22
XI	19	19	22	21
XII	18	19	22	23
XIII	17	18	19	20
XIV	19	20	21	22
XV	17	18	19	21
Norfloxacin	24	24	--	--
Grisiofulvin	--	--	24	24
DMF	04	04	04	04

Table IV MICs* values of the some active compounds.

Compuond Code	Antibacterial		Antifungal	
	S..Aurease	*E.coli*	*A.niger*	*C. Albicans*
II	12.5	12.5	6.25	6.25
IV	6.25	6.25	3.12	1.56
VI	6.25	12.5	3.12	3.12
VIII	6.25	3.12	1.56	1.56
X	12.5	6.25	3.12	3.12
XII	6.25	6.25	3.12	1.56
XIV	6.25	6.25	3.12	3.12
Grisiofulvin	--	--	1.56	1.56
Norfloxacin	1.56	1.56	--	--

*MICs values were determined as µg / mL active compounds in medium.

FAB-mass spectrum

The FAB spectrum of one of the representative (**I**) ligand shows a molecular ion peak M^+ at m/z 194 which corresponds its molecular mass (Fig. IIIa).

The FAB spectrum of one of the representative (**V**) complex shows a molecular ion peak M^+ at m/z 481 which is equivalent to its molecular weight $[ML_2(H_2O)_2]^+$.

Thermogravimetric study

In the present investigation TGA and DTG studies of Co(II), Ni(II), Cu(II) and Zn(II) complexes have been carried out in static air at a limiting temperature of 1000°C using the heating rate of 10 °C/min. The spectrum of one representative (**X**) complex was presented in (Figure IV). The nature of proposed chem. change with the temperature range and percentage of metal oxide obtained for the Co(II), Ni(II), Cu(II) and Zn(II) complexes are given in the (Table 2). The results are in good agreement with the proposed chemical formulae. The decomposition of the complex proceeds with an endothermic peak at 114 °C. At this temperature it loses two H_2O molecules. In the second stage the temperature range *ca.* 303 °C decomposes the ligand molecule. The final weight corresponded to that of the metal oxide.

A*ntibacterial and antifungal activities* Antibacterial and antifungal activities of synthesized compounds (**I** –**XV**) were tested against two bacteria such as *S.Aurease*, *Escherichia. Coli* and two fungi *A.Niger*, *C.Albicans*, Norfloxacin for bacteria and Grisiofulvin for fungi were used as standard drugs. The zone of inhibition in mm for the ligands and their Co (II), Ni(II), Cu(II) and Zn(II) complexes are presented in Table III. From the data of metal complexes it is clear that the metal chelates exhibit higher antimicrobial activity than that of the free ligand molecules. The compounds were found to be more susceptible towards the fungal strains as compared to the bacterial strains .The cobalt compounds **IV**, **VIII** and **XII** were most active and copper compounds **VI**, **X** and **XIV** were slightly active towards the fungal strains.

The MICs of the active compounds were carried out as described by Clause [26] with minor modifications. Antifungal activities of the yeast were performed by following the guideleines in NCCLs document M27-A using the microdilution broth method [27]. Solutions of the test compounds and reference drug were dissolved in DMF as a concentration of 12.5 µg ml^{-1}. The twofold dilution of the compounds and reference drug were prepared (6.25, 3.12, 1.56, 0.78,) µg ml^{-1}. The broths were maintained at pH 7.2 with an innoclum of (1-2) X 10^3 cells ml^{-1} by the spectrophotometric method and an aliquot of 100 µl was added to each tube of the

serial dilution. The chemical compounds-broth medium serial tuvel dilutions inoculated with each bacterium were incubated on a rotary shaker at 37 ºC for 24 h at 150 rpm. The minimum inhibitory concentrations of the active compounds were recorded as the lowest concentrations of each chemical compounds in the tubes with no growth (i.e. no turbity) of inoculated bacteria and yeast.

Table V Antitubercular Activity*of the compounds. (zone of inhibition in mm)

Code	50 µg/ml	100 µg/ml	150 µg/ml
I	R	R	S
II	R	R	S
III	R	R	R
IV	S	S	S
V	R	R	S
VI	R	S	S
VII	R	R	R
VIII	S	S	S
IX	R	R	S
X	R	S	S
XI	R	R	R
XII	R	S	S
XIII	R	R	R
XIV	R	S	S
XV	R	R	R
Standard	S	S	S

*Standard drug: Streptomycin; **R**-Resistance ; **S**-Sensitive.

Table VI Brine shrimp bioassay data of the some active compounds.

Compound Code	LD_{50} (M/ml)
II	4.687×10^{-3}
IV	7.034×10^{-4}
VI	6.918×10^{-4}
VIII	7.337×10^{-4}
XII	6.121×10^{-4}

The MICs values are shown in Table IV. Only the active compounds **II, IV, VI, VIII, X, XII** and **XIV** were evaluated for their minimum inhibitory concentrations. The compounds have shown potent activity towards the fungal strains as compared to bacterial strains. The compounds are more susceptible towards the fungal strains rather than bacterial strains. The halogenated derivatives have shown have activities. Compound **VIII** was most active exhibiting a MIC value of 1.56 µg / mL active compounds in medium against the fungal strains. The activities of other Cobalt (II) and compounds **IV** and **XII** are in the range of 3.12-1.56 µg / mL for the fungal strains. The Copper (II) compounds **VI, X** and **XIV** have exhibited values of 3.12µg / mL.

The activity of any compound is a complex combination of steric, electronic and pharmacokinetic factors. A possible explanation for the toxicity of the complexes has been postulated in the light of chelation theory. It was suggested that the chelation considerably reduces the charge of the metal ion mainly because of partial sharing of its positive charge with the donor groups and possible π- electron delocalization over the whole chelate ring. This increases the lipophilic character of the metal chelate which favors its permeation through lipoid layers of cell membranes. Furthermore, the mode of action of the compounds may involve the formation of a hydrogen bond through the –N=C group of the chelate or the ligand with the active centers of the cell constituents resulting in interference with the normal cell process. The higher bacteriotoxicity experienced by the compounds may be ascribed to the fact that the ligand and metal ions are more susceptible towards the bacterial cells than fungicidal cells. Thus it can be concluded that although these compounds serve as better fungicides and average good bactericides.

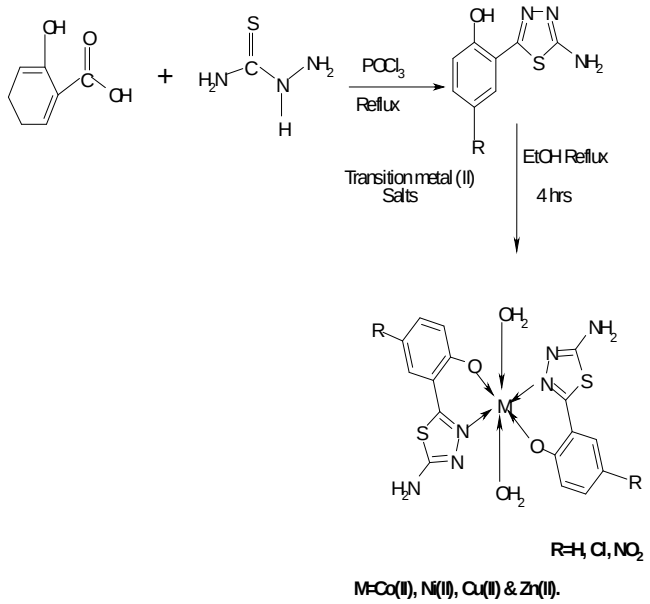

Figure I. Scheme of the work

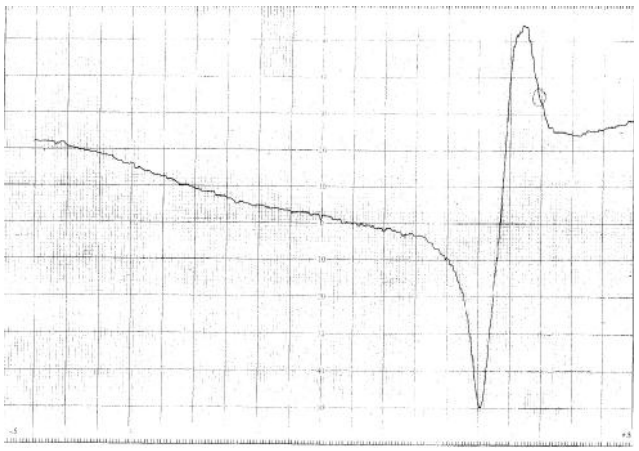

Figure II ESR Spectrum of one of the representative **XIV** complex

Antitubercular assays

The antitubercular screening was carried out by Middle brook 7H9 agar medium against $H_{37}Rv$ Strain [28, 29]. Middle brook 7H9 agar medium containing different derivatives **(I-XV)**, standard drug as well as control. Only compounds **IV** and **VIII** have shown promising activity for this assay. All the other compounds were inactive for this assay (Table V)

Cytotoxicity Bioassay (in vitro studies)

In the present study brine shrimp (*A.Salina. L)* eggs were hatched in a shallow rectangular plastic dish (22X32 cm), filled with artificial seawater, which was prepared [30] with commercial salt mixture and double distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. 50mg of eggs were sprinkled approximately into the large compartment, which was darkened while the

matter compartment was opened to ordinary light. After two days, nauplii were collected by a pipette from the lighter side. By dissolving 20mg of each compound in 2ml DMF the samples were prepared. From the stock solutions, 500,50,5µg/mL were transferred to vials (three for each dilution were used for each test sample and LD_{50} is the mean of the three values) one vial was used to as a control with only 2mL DMF and another with the above concentrations of *Bleomycin* as a standard. The solvent was allowed to evaporate overnight. After two days, when shrimp larvae were ready, 1mL of seawater and 10 shrimps were added to each vial (25 shrimps/dilution) and the volume was adjusted with seawater to 5 ml per vial. After 24 h, the numbers of survivors were counted. Data were analyzed by Finney computer program to determine the LD_{50} values [31].

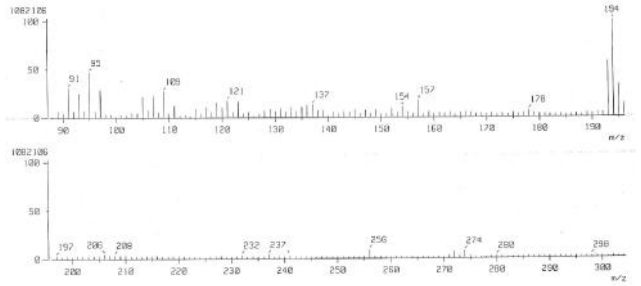

Figure IIIa. FAB-mass Spectrum of one of the representative ligand (**I**).

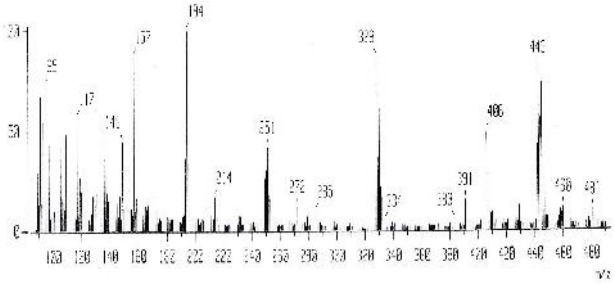

Figure IIIb. FAB-mass Spectrum of one of the representative complex (**V**) .

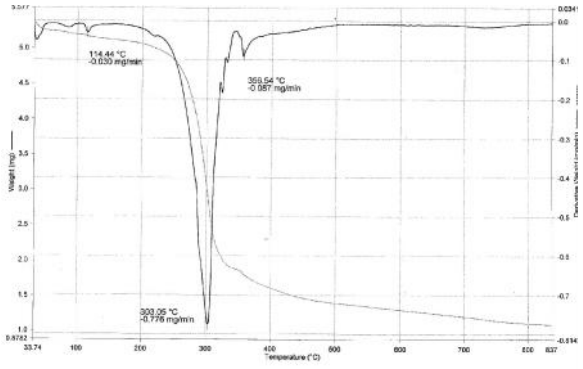

Figure IV TGA/DTG spectrum of one of the representative **X** complex

For the active compounds were screened for their cytotoxicity (brine shrimp bioassay) using protocol of Meyer *et al*. Only four compounds **IV**, **VI**, **VIII** and **XII** have exhibited potent cytotoxic activity against *Artemia salina* while all the other compounds were almost inactive for this assay (Table VI).

DNA cleavage Experiment

*Preparation of Culture media*Nutrient broth (Peptone 10, NaCl 10 and yeast extract 5 g/l) was used for the growth of the *E. coli*. The 50 ml media was prepared, autoclaved for 15 min at 121^0C, 15 lb pressure. The autoclaved media were inoculated with the seed culture and incubated at 37^0C for 24 h.

Isolation of DNA

The fresh bacterial culture (1.5 ml) is centrifuged to obtain the pellet, which is then dissolved in 0.5 ml of lysis buffer (100 mM tris pH 8.0, 50 mM EDTA, 10% SDS). To this 0.5 ml of saturated phenol is added and incubate at 550C for 10 min. Then it is centrifuged at 10,000rpm for 10 min. Then equal volume of chloroform : isoamyl alcohol (24:1) and 1/20th volume of 3M sodium acetate (pH 4.8) is added to this supernatant and centrifuged at 10,000rpm for 10 min. To this supernatant 3 volumes of chilled absolute alcohol is added. The precipitated DNA is Separated by centrifugation. Dry the pellet and dissolve in TE buffer (10 mM tris pH 8.0, 1 mM EDTA) and stored in cold condition.

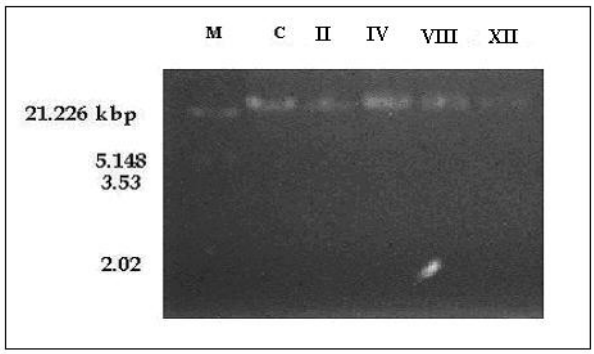

Figure V DNA cleavage experiment showing the gel electrophoresis.

M: Standard Molecular weight Marker**; C-** *E. coli*- Control DNA of *E. coli***;**

Agarose gel electrophoresis

Cleavage products were analyzed by agarose gel electrophoresis method. Test samples (1mg/ml) were prepared in DMF. The samples (25µg) were added to the isolated DNA of *E. coli*and *A. niger*. The samples were incubated for 2 hour at 37_C and then 20 µl of DNA sample (mixed with bromophenol blue dye at 1:1 ratio) were loaded carefully into the electrophoresis chamber wells along with standard DNA marker containing TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 ltr) and finally loaded on agarose gel and pass the constant 50 V of electricity for around 30 min. Remove the gel and stained with 10.0 μg/mL ethidium bromide for 10-15 min and the bands observed under UV transilluminator and photographed to determine the extent of DNA cleavage and the results are compared with standard DNA marker.

Electrophoretic analysis

The Schiff base (II) and its Co(II), Ni(II) Cu(II) and Zn(II) complexes **IV,V,VI** and **VII** were studied for their DNA cleavage activity by agarose gel electrophoresis method and presented in the Figure V. The gel after electrophoresis clearly revealed that, only the Co(II) complex (**IV**) is acted on DNA as there was molecular weight difference between the control and the treated DNA samples. The difference was observed in the bands (Lane 2 and 6) compared to the control DNA of *E.coli*

and *A.niger*. This shows that the control DNA alone does not show any apparent cleavage where as Co (II) complex shown. However, the nature of reactive intermediates involved in the DNA cleavage by the complexes has not been clear. The results indicated the important role of metal in these isolated DNA cleavage reactions. As the compound **IV** was observed to cleave the DNA, it can be concluded that the compound inhibits the growth of the pathogenic organism by cleaving the genome.

CONCLUSIONS

With help of various physico-chemical techniques, geometries of the newly synthesized compounds have been proposed. The compounds **IV**, **VIII** and **XII** were found most active and susceptible towards the fungal strains. The compounds **IV** and **VIII** have shown promising activity against $H_{37}Rv$ Strain for antitubercular assay. The compounds **IV**, **VI**, **VIII** and **XII** have exhibited potent cytotoxic activity against Artemia salina with lower LD_{50} values. The complex **IV** has cleaved the DNA inhibits the growth of the pathogenic organism by cleaving the genome.

Due to insolubility in water and common organic solvents and in fusibility at higher temperatures all the complexes are thought to be polymeric in nature. The tentative structures for the complexes (Fig. I) were based on elemental analyses, IR, ^{1}H NMR, electronic, magnetic measurements, thermal and Mass spectral studies.

Acknowledgements

Authors are thankful to Director, University Sophisticated Instrumentation Centre (USIC) for the recording the IR and ^{1}H NMR spectral data. Authors extend their thanks to Central Drug Research Institute, Lucknow for providing FAB mass spectra. Thanks are to Sophisticated Test and Instrumentation Centre (STIC) Cochin, for providing the thermal and analytical data. Authors thank IIT Powai, Mumbai for ESR spectra. Authorities of Pravara Pharmacy College, Loni, Pune for providing facilities to carry out the invitro biological studies. BioGenics Research Centre, Hubli for providing facilities to carry out DNA cleavage studies.

References

1. Geronikaki A, Litina DH, Zablotskaya A, Segal I. Organosilicon-Containing thiazole derivatives as potential lipoxygenase Inhibitors and Anti-inflammatory agents. Bioinorganic Chemistry and Applications. 2007; Article ID 92145: 1-7.
2. Patil BG, Havinale BR, Shallom JM, Chitnis MP. Syntheses and spectroscopic studies of potential antitumor copper (II) complexes with 5-phenylazo-3-methoxy salilcylidene thiosemicarbazone and N' substituted thiosemicarbazones. *Journal of Inorganic Chemistry*. 1989; 36:107-113.
3. Hassan MU, Chohan AH, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors schiff's bases of aromatic and heterocyclic sulfonamides and their metal complexes. Journal of Enzyme Inhibition and Medicinal Chemistry. 2004; 19:263-267.
4. Chohan ZH, Arif M,. Akhtar MA, Supuran CT.Metal-based antibacterial and antifungal agents: synthesis, characterization, and in vitro biological evaluation of Co(II), Ni(II), Cu(II) and Zn(II) complexes with amino acid derived compounds. Bioinorganic Chemistry and Applications. 2006; Article ID 83131:1-13.
5. Al-Shihry SS. Synthesis and spectral studies of Schiff bases of 2-amino-5-mercapto-1, 3, 4-thiadiazole. Scien. J. King Faisal Univ. 2005; 6 (2):1426
6. Mamo A, Pappalardo A, Clasadonte MT. Sulfur Bridged Multidentate Ligands Based on (Bi)pyridyl-(Bi)-1,3,4-Thiadiazolyl Conjugates. Molecules 2003; 8: 310-317.
7. Fadayan M, Kulkarni YD, Pakdaman AS.Synthesis of Some New 5[[{(Substituted amino) methyl benzimidazole-2-yl}thio] methyl-N-[(Substituted phenyl) methylene]-1,3,4-thiadiazoles-2- amino as potential Anthelmintic Drug.. Asian J. Chem.1993;5: 282-286
8. Abdel-Hamid MK, Atef A, El-Koussi NA, Mahfouz NM. Quantative structure-activity relationship studies on a series of 1, 3, 4-thiadiazole-2-thione derivatives as tumor-associated carbonic anhydrase IX inhibitors. J Enzy Inhibtn and Med Chem. 2008; 1: 1-8.
9. Cho NS, Young YH, Lee CH. Convergent Synthesis of Macrocycles Composed of 5-Amino-2*H*-1, 2, 4-thiadiazolin-3-one or 5-Amino-2*H*-1,2,4-thiadiazoline-3-thione and 1,3-Benzenedimethanethiol. *Bull. Korean Chem. Soc*. 2004; 25(10):1581-1584.
10. Supuran CT. Complexes with biologically active ligands: Synthesis of coordination compounds of diazoxide with transition and Main Group cations. Metal Based Drugs. 2 1996; 3:25-30.
11. Gupta ML. Stability Constants of 2N-(3,5-Diiodo salicylidene)-5-phenyl 1,3,4-Thiadiazole Complexes with Cu(II), Ni(II) and Fe(III). Asian J. Chem. 1993; 5: 470-472.
12. Fabretti AC, Franchini GC, Peyronel G. Pseudo tetrahedral Co (II) complexes of 2,5-dimethyl-1,3,4 thiadiazole. J Coord Chem. 1979; 9:111-115.
13. Fabretti AC, Franchini GC, Malavasi W, Peyronel G.Palladium(II)halide complexes with 2,5-dimethyl-2-amino-5-methyl-2-ethylamino thiadiazoles. Polyhedron. 1985; 4(6):989-992.
14. Nunokawa K, Okazaki K, Onaka S, Ito M, Sunahara T, Ozeki T, Imai H, Inoue K. Synthesis and X-ray structure study on new Au (I) polymer architectures based on multi-sulfur tentacles. J Org Met Chem. 2005; 690 (5):1332-1339.
15. Lucarini M, Pedulli GF. An EPR study of the reaction of tetrabutylammonium borohydride with N-Heterocycles. J Org Met Chem. 1995; 494(1):123-131
16. Simao D, Alves H, Santos IC, Gama V, Almeida M. Synthesis and crystal structure of copper and gold complexes of 1, 2,5-thiadiazole-3,4-dithiolate.Charge transfer salt. Inorg. Chem Communs.2003; 6(5):565-568.
17. Srivastava AK, Pandey OP, Sengupta SK. Synthesis, Spectral and Antimicrobial studies of Bis (cyclopentadienyl) titanium (IV) derivatives with Schiff bases derived from 2-amino-5-phenyl-1,3,4-thiadiazole. Bioinorg Chem and Applicns. 2005; 3: 289-297.
18. Phaniband MA, Dhumwad SD. Synthesis, Characterization and Biological studies of Co(II),Ni(II),

Cu(II) and Zn(II) complexes of Schiff bases derived from 4-substituted carbostyrils [quinolin2(1H)–ones]. Trans Met Chem. 2007; 32:1117-1125.
19. El-Bindary AA. Spectral studies on metal-ligand bonding in complexes of 1-acetyl-2-(coumariniminecarboxamide-3-yl) hydrazine. Spectrochimica Acta Part A 2001; 57: 49-56.
20. Ahmed IT. Synthesis, spectral, thermal and electrochemical characterization of metal (II) complexes with 1-S-methylcarbodithioate-4-substituted thiosemi-carbazides. J Coord Chem. 2008;61:1730–1743.
21. Demir I, Bayrakci M, Mutlu K, Pekacar AI. Synthesis and Characterization of a Novel Iminooxime Schiff Base Ligand and Its Complexation with Copper (II), Nickel (II), Zinc(II), Cadmium(II), and Cobalt(II). Acta Chim Slov 2008; 55: 120–124.
22. Mincinione G, Scozzafava A., Supuran C.Inhibitors of carbonic andydrase isozymes I, II and IV with trifluoromethylsulfonamide derivatives and their Zn (II) and Cu (II) complexes. Metal Based Drugs. 1997; 4(2): 27-34.
23. Bentiss F, Lagrenee M, Wignacourt JP, Holt EM. Complexes of Co (II), Ni (II) and Cu(II) with a thia ligand2,5 bis(2-pyridyl)-1,3,4-thiadiazole. Polyhedron. 2002; 21 (4): 403-408.
24. Asianidis P, Cox PJ, Divanidis S, Karagiannidis P. Trans-1,2-bis(diphenylphos phine)ethene as bridging ligandin thione-S-ligated dimeric copper(I) chloride complexes. Inorg Chim Acta. 2004; 357(4):1063-1076.
25. Prasad RN, Mathur M. Synthesis characterization of Cr(III), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) complexes of 2,12-dimethyl-3-13-di-*n*-propyl-l,4,11,14-tetraazacycloeicosa- 1,13,11,13-tetraene. *Journal of the Serbian Chemical Society* 2002; 67: 825–832.
26. G.W. Clause, Understanding Microbes: A Laboratory Textbook for Microbiology, W.H. Freeman and Company. New York. USA, 1989.
27. National Committe for Clinical Laboratory Standards. Refernce method for broth dilution antifungal suceptibility testing for yeasts; approved standard Document M27 –A, Villanova, National Committee for Clinical Laboratory Standards 1997; 17:1-29.
28. Elmer WK, Stephen DA, William MJ, Paul CS, Washing Jr CW, Text book of Diagnostic Microbiology, 5th ed; Lippincot-Pub; 2002.
29. Ellen JB, Lancer RP, Sydney MF, Bailey and Scott's Diagnostic Microbiology, 9th ed; 2000.
30. Sakyan I, Logoglu E, Sari N, Akiyan ,N. Antimicrobial activities of N-(2-hydroxy-1-naphthalidene) amino acid Schiff bases and their manganese (III) complexes. Biometals 2004; 17:115- 120.
31. Finney DJ. Probit Analysis, 3rd ed. Cambridge, UK: Cambridge University Press 1971.

****

How to cite this article:

Phaniband M.A *et al*.2016, New Metal Based Antimicrobial And Antitubercular Agents With Their Cytotoxicity Evaluation Derived From 2-Amino-5-Hydroxyphenyl-1,3,4-Thiadiazoles. *Int J Recent Sci Res.* 7(4), pp. 10132-10139

TSSN 0976-3031