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Synthesis of Metal-Based Analogues of Isonicotinoyl and Benzoyl Hydrazones and Their Antioxidant and Cytotoxic Activities

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A series of 20 metal based [Cu(II), Co(II), Ni(II), Mn(II) and Zn(II)] compounds have been synthesized with novel 2,5-dihydroxyacetophenone isonicotinoyl hydrazone ($\mathbf{L^1}$) and 2,5-dihydroxyacetophenone benzoyl hydrazone ($\mathbf{L^2}$) Schiff bases and were screened for antioxidant and cytotoxic activities. All these complexes exhibited strong antioxidant activity against DPPH radical but no nitric oxide radical scavenging activity. Cytotoxicity assay showed toxicity at varied levels, that is, **1**, **3**, **7**, **11**, **15** showed maximum toxicity and **4**, **9**, **10**, **12**, **13**, **16**, **19**, **20** showed minimal cytotoxicity against brine shrimp larvae. Antibacterial activity assay exhibited MIC_{50} values of **2**, **4**, **6**, **8**, **10**, **12**, **14**, **16**, **18**, **20** comparable to standard drugs for both gram-positive and gram-negative bacteria whilst other compounds showed minimal activity against these bacteria. Structure activity relationship shows the role of metal and the kind of complex associated with the said biological activities.

Key Words: Antibacterial activity, Antioxidant activity, Cytotoxicity, Hydrazones, Schiff bases.

INTRODUCTION

Novel analogues of isonicotinoyl hydrazones are currently extensively investigated as a part of the project of novel drug development program. Many *in vitro* as well as *in vivo* studies have revealed a wide variety of their remarkable biological effects such as mobilization of iron from cell, antioxidative and cardioprotective activities, antiproliferative activities and antimicrobial effects[1-5]. Hence, novel aroylhydrazones seem to be promising drug candidates with potential to be used in the treatment of several human pathologies[6]. In order to establish a possible relationship between chemical structure and biolo-gical activity, several attempts are made on the coordination chemistry and biological properties of different metal complexes of isatin and N-alkylisatin of thiosemi-carbazones[7-9], bisthiocarbono-hydrazones[10], semicarbazones[11,12] and hydrazones[13].

Phenolic compounds form an extensive group of secondary metabolites having in common a hydroxyl substituted benzene ring in their structure. Their biological functions in plants are diverse which include UV light protectors used in UV screens and defensive or as signal compounds[14]. Polyphenols exhibit a wide range of biological effects as a consequence of their antioxidant properties[15,16]. Polyphenols therefore help to protect tissues against oxidative stress due to free radicals. Certain polyphenols work as preventative medicines in cardiovascular diseases, cancer, arthritis and autoimmune disorders. Some exhibit antiinflammatory and hepatoprotective effects. They inhibit LDL oxidation *in vitro*[17]. In addition, flavonoids have antithrombotic and antiinflammatory effects[18,19]. The antimicrobial property of polyphenolic compounds has also been well documented[20,21]. Polyphenols also have several industrial applications such as in the production of paints, paper and cosmetics, as tanning agents and in the food industry as additives[22,23].

Keeping in view the promising use of potentially metal-based antimicrobial, antioxidant and antiinflammatory properties, here are reported some metal-based [(Cu(II), Co(II), Ni(II) Mn(II) and Zn(II)] compounds incorporated with the novel 2,5-dihydroxyacetophenone isonicotinoyl hydrazone ($\mathbf{L^1}$) and 2,5-dihydroxyacetophenone benzoyl hydrazone ($\mathbf{L^2}$) Schiff bases.

EXPERIMENTAL

Solvents used were of analytical grade and all the transition metals (II) were used as chloride salts. IR spectra were recorded on 8400-FTIR spectrophotometer using KBr disc method. NMR spectra were recorded on a Bruker-300 MHz NMR spectrophotometer. UV-Visible spectra were obtained in DMSO on a Hitachi U-2000 spectrophotometer. Conductance of the metal

complexes was determined in DMSO on a Hitachi (Japan) YSI-32 model conductometer. Melting points were recorded on a Fischer Johns melting point apparatus and are uncorrected. The complexes were analyzed for their metal contents by EDTA titrations[24].

General procedure of Schiff's bases (L^1, L^2): Preparation of 2,5-dihydroxyacetophenone isonicotinoyl hydrazone (L^1). A solution of isoniazid (3.61 g, 26.35 mmol) and 2,5-dihydroxyacetophenone (4.0 g, 26.35 mmol) in warm ethanol (25 mL) was refluxed for 7-8 h. After the completion of the reaction (monitored by TLC) the solution was cooled and solvent was evaporated. Yellow coloured precipitates were formed which were washed with hot ethanol, filtered and dried to give Schiff base L^1.

2,5-Dihydroxyacetophenone isonicotinoyl hydrazone (L^1): Yield: 63 %. m.p. 296 ºC. IR (KBr, ν_{max}, cm^{-1}): 1679 (CO), 1607 (C=N), 3440 (NH). ^{1}H NMR (DMSO) δ: 2.44 (s, 3H, -N=C-CH_3, C_8-H), 6.77 (m, 2H, phenyl, C_5-H, C_6-H), 7.01 (d, *J* = 2.1Hz, 1H, phenyl, C_3-H), 7.85 (d, *J* = 6 Hz, 2H, phenyl, C_{11}-H, C_{15}-H), 8.8 (d, *J* = 5.7 Hz, 2H, phenyl, C_{12}-H, C_{14}-H), 8.98 (s, 1H, phenyl, 4-OH), 11.55 (s, 1H, phenyl, 1-OH),12.46 (br, 1H, NH) ppm; $C_{14}H_{13}N_3O_3$ EIMS (70 eV) m/z (%): 271 (100, M^+), 254 (39), 165 (7), 150 (38), 135 (11), 123 (45), 106 (61), 91 (6), 79 (47), 51 (16).

General procedure for 2,5-dihydroxyacetophenone benzoyl hydrazone (L^2): To a solution of benzoic acid hydrazide (3.58 g, 0.03 mol) in cold ethanol (15 mL), 2,5-dihydroxyacetophenone (4.0 g, 0.03 mol) was added and the solution was refluxed for 7 h. After the completion of reaction (monitored by TLC) the solution was cooled and solvent was evaporated. Light yellow coloured precipitates were formed which were washed with hot ethanol, filtered and dried to give Schiff base L^2.

2,5-Dihydroxyacetophenone benzoyl hydrazone (L^2): Yield: 73.8 %, m.p. 240 ºC, IR (KBr, ν_{max}, cm^{-1}): 1537 (-NH-), 1607 (-C=N), 1679 (CO); ^{1}H NMR (DMSO) δ: 2.50 (s, 3H, -N=C-CH_3, C_8-H), 6.78 (m, 2H, phenyl, C_5-H, C_6-H), 7.00 (d, *J* = 1.5 Hz, 1H, phenyl, C_3-H), 7.59 (m, 3H, phenyl, C_{12}-H, C_{13}-H, C_{14}-H), 7.94 (d, *J* = 7.2 Hz, 2H, phenyl, C_{11}-H, C_{15}-H), 8.95 (s, 1H, phenyl, 4-OH), 11.30 (s, 1H, phenyl, 1-OH), 12.62 (s, 1H, NH) ppm; $C_{15}H_{14}N_2O_3$ EIMS (70 eV) m/z (%): 270 (36, M^+), 253 (13), 148 (13), 105 (100), 83 (5), 77 (33).

Cobalt(II) complex of L^1 [2:1]: To a turbid solution of 2,5-dihydroxyacetophenone isonicotinoyl hydrazone (0.4 g, 1.48 mmol) in hot 1,4-dioxane (25 mL) was added cobalt(II) chloride (0.176 g, 0.74 mmol) in hot ethanol (2 mL). Green coloured precipitate were formed at once, the reaction mixture was refluxed for 1.5 h and the precipitates were filtered while hot, washed with hot ethanol and dried over silica gel in a vacuum desiccators. All other complexes were as synthesized according to the same method.

Cobalt(II) complex of L^1 [1:1]: To a turbid solution of 2,5-dihydroxyacetophenone isonicotinoyl hydrazone (0.40 g, 1.48 mmol) in hot 1,4-dioxane (25 mL) was added cobalt(II) chloride (0.352 g, 1.48 mmol) in hot ethanol (2 mL). Brown coloured precipitates were formed at once, the reaction mixture was refluxed for 1.5 h and the precipitates were filtered while hot, washed with hot ethanol and dried over silica gel in vacuum desiccators. All other complexes were synthesized according to the same method.

Cobalt(II) complex of L^2 [2:1]: To a refluxed solution of 2,5-dihydroxyacetophenobenzoic acid hydrazide (0.3 g, 0.0011 mol) in hot 1,4-dioxane (5.5 mL), was added cobalt(II) chloride·$6H_2O$ (0.13 g, 0.00055 mol) in hot ethanol (1.5 mL). The clear green colour of the solution appeared. For refluxing about 4 h, sky blue precipitates formed which were filtered while hot, washed with hot 1,4-dioxane and dried to afford 2,5-dihydroxyacetophenonebenzoic acid hydrazide Co(II) complex, which was dried over silica gel in vacuum desiccators. All other complexes were synthesized according to the same method.

Cobalt(II) complex of L^2 [1:1]: To a refluxed solution of 2,5-dihydroxyacetophenone benzoic acid hydrazide (0.5 g, 0.0018 mol) in hot 1,4-dioxan (7.5 mL), was added cobalt(II) chloride.$6H_2O$ (0.428 g, 0.0018 mol) in ethanol (2 mL). The bluish green colouration was obtained and resulting reaction mixture was refluxed for 3 h. Bluish green precipitates formed were filtered while hot and washed with hot dioxan and dried to afford 2,5-dihydroxyacetophenonebenzoyl hydrazone Co(II) complex. The crude product was dried over silica gel in vacuum desiccators. All other complexes were synthesized according to the same method.

Antioxidant activity: The stable 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH) was used for the determination of antioxidant activity[25]. Different concentrations of compounds in DMSO were added at an equal volume (5-90 µL) of 100 µM methanolic DPPH in a total volume of 100 µL in 96-well plates. The contents were mixed and incubated at 37 ºC for 0.5 h. The absorbance was measured at 517 nm. Propyl gallate and 3-*t*-butyl-4-hydroxyanisole (*t*-BHT) were used as standard antioxidants. The experiments were carried out in triplicates. IC_{50} value denotes the concentration of the sample which is required to scavenge 50 % of DPPH free radicals. The activity was determined by the following formula.

Per cent scavenging activity

$$= \left[100 - \left(\frac{\text{Abs of test compound}}{\text{Abs of control}} \right) \times 100 \right]$$

NO radical scavenging activity: Sodium nitroprusside in the aqueous solution produce NO radical which turns to nitrite ion that can be determined using Griess reagent[26]. Reaction mixture of 1ml contained 800 µL 50 mM potassium phosphate buffer (pH 7.4), 100 µL 1 mM fresh solution of sodium nitroprusside in water and various concentrations of the test compound. Contents were incubated it at 37 ºC for 2 h followed by the addition of Griess reagent. Absorbance of the pink colour was measured at 540 nm after 0.5 h. NO scavenging activity was calculated by the following formula.

Antioxidant Activity

$$= \left(\frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100 \right)$$

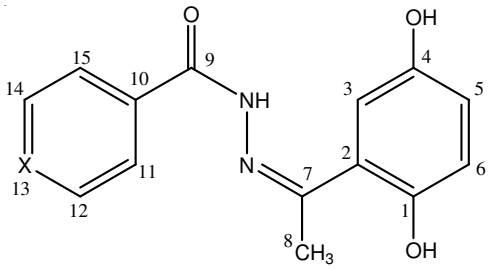
Cytotoxicity assay: The Brine shrimp (*Artemisia salina*) method was used for the cytotoxicity assay[27]. The 2-3 days

old larvae of the brine shrimp after hatching in sea water (36 g/L, pH 7.4) were placed in the test samples. Incubation was done at 30 ± 3 ºC. After 24 h, the number of dead larvae were counted. The results are expressed in terms of % killing of larvae (mortality %) and are taken as mean and standard error of means of three independent experiments. Positive and negative controls were run simultaneously.

Antibacterial activity: The antibacterial activity was performed in sterile 96-wells microplates under aseptic environments. The method is based on the principle that microbial cell number increases as the microbial growth proceeds in the log phase of growth which results in increased absorbance of broth medium[28,29]. The microorganisms were kindly provided by the Department of Biological and Biomedical Sciences, Aga Khan University, Karachi. Four gram-negative (*Shigella sonnei*, *Escherichia coli*) and two gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) were included in the study. The organisms were maintained on stock culture agar. The test samples with suitable solvent and dilution were pipetted into wells (20 µg/well). Overnight maintained fresh bacterial culture after suitable dilution with fresh nutrient broth was poured into wells (180 µL). The initial absorbance of the culture was strictly maintained between 0.12-0.19 at 540 nm. The total volume in each well was kept to 200 µL. The incubation was done at 37 ºC for 16-24 h with lid on the microplate. The absorbance was measured at 540 nm using Synergy HT BioTek® USA microplate reader, before and after incubation and the difference was noted as an index of bacterial growth. The per cent inhibition was calculated using the formula: Inhibition (%) = 100 (X - Y)/X where X is absorbance in control with bacterial culture and Y is absorbance in test sample. Results are mean of triplicate (n = 3, ± sem). Ciprofloxacin, gentamycin and ampicillin were taken as standard. Minimum inhibitory concentration (MIC) was measured with suitable dilutions and results were calculated using EZ-Fit5 Perrella Scientific Inc. Amherst USA software and data expressed as MIC_{50}.

RESULTS AND DISCUSSION

The Schiff bases (Fig. 1) were prepared by refluxing the mixture of isoniazid and 2,5-dihydroxyacetophenone and benzoic acid hydrazide and 2,5-dihydroxyacetophenone in ethanol. The structures of these Schiff bases formed were established by IR, NMR, Mass spectrometry and micro-analytical data. These Schiff bases were further used for the complexation reaction with Cu(II), Co(II), Ni(II), Mn(II) and Zn(II) metal ions. All of the newly synthesized metal complexes (**1-20**, Table-1) were seemed to be stable in air and moisture. They were prepared by the stoichiometric reaction with the corresponding metal(II) chlorides and the Schiff bases ($\mathbf{L^1}$) and ($\mathbf{L^2}$) in the molar ratios M:L of 1:2 and 1:1. The complexes are amorphous solids, which are decomposed above 100 ºC. These are insoluble in common organic solvents (chloroform, acetone, ethanol and methanol), but soluble in DMSO and DMF. All the chelates exhibited high values of molar conductance (31-116 Ω^{-1} cm^2 mol^{-1}) determined in DMSO which show their ionic and electrolyte nature.



$\mathbf{L^1X}$ = N

$\mathbf{L^2X}$ = CH

Fig. 1. Structure of ligands $\mathbf{L^1}$ and $\mathbf{L^2}$

Infrared spectra: In IR spectra of ligands the disappearance of absorption band at 3420 and 1735 cm^{-1} due to carbonyl

TABLE-1
PHYSICAL AND ANALYTICAL DATA OF THE METAL(II) COMPLEXES (**1-20**)

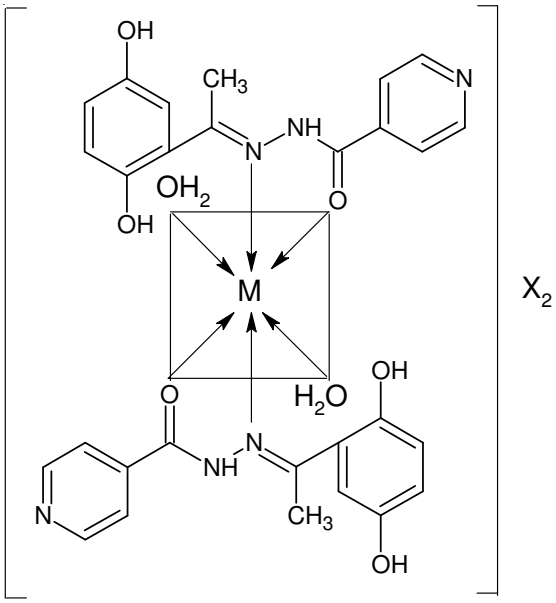
No.	Metal complex/m.f.	D.P.	λ_{max} (nm)	IR (cm^{-1})
1	$[Cu(L^1)_2Cl_2]$ $C_{28}H_{30}Cl_2CuN_6O_8$[713.02]	180	360	1655(CO), 1583(C=N), 3390(NH), 540(M-O), 440(M-N)
2	$[Cu(L^2)_2Cl_2]$ $C_{30}H_{32}Cl_2CuN_4O_8$[711.02]	145	348	1655 (CO), 1583(C=N), 1519(NNH), 540(M-O), 440(M-N)
3	$[Co(L^1)_2Cl_2]$ $C_{28}H_{30}Cl_2CoN_6O_8$[708.41]	235	360	1650(CO), 1593(C=N), 3360(NH), 538(M-O), 420(M-N)
4	$[Co(L^2)_2Cl_2]$ $C_{30}H_{32}Cl_2CoN_4O_8$[706.44]	145	380	1650(CO), 1593(C=N), 1515(NNH), 538(M-O), 420(M-N)
5	$[Ni(L^1)_2Cl_2]$ $C_{28}H_{30}Cl_2NiN_6O_8$[708.17]	140	305	1653(CO), 1588(C=N), 3430(NH), 540(M-O), 440(M-N)
6	$[Ni(L^2)_2Cl_2]$ $C_{30}H_{32}Cl_2NiN_4O_8$[706.20]	155	365	1653(CO), 1588(C=N), 1515(NNH), 540(M-O), 440(M-N)
7	$[Mn(L^1)_2Cl_2]$ $C_{28}H_{30}Cl_2MnN_6O_8$[704.20]	165	305	1654(CO), 1600(C=N), 3440(NH), 540(M-O), 440(M-N)
8	$[Mn(L^2)_2Cl_2]$ $C_{30}H_{32}Cl_2MnN_4O_8$[702.44]	105	340	1654(CO), 1600(C=N), 1510(NNH), 540(M-O), 440(M-N)
9	$[Zn(L^1)_2(CH_3COO)_2]$ $C_{32}H_{36}N_6O_{12}Zn$ [762.05]	200	350	1651(CO), 1600(C=N), 3438(NH), 533(M-O), 440(M-N)
10.	$[Zn(L^2)_2(CH_3COO)_2]$ $C_{34}H_{38}N_4O_{12}Zn$ [760.08]	194	365	1651(CO), 1600(C=N), 1515(NNH), 533(M-O), 440(M-N)
11	$[Co(L^1)Cl_2]$ $C_{14}H_{21}Cl_2CoN_3O_7$[473.17]	215	360	1652(CO), 1588(C=N), 3430(NH), 540(M-O), 420(M-N)
12	$[Co(L^2)Cl_2]$ $C_{15}H_{12}Cl_2CoN_2O_7$[472.18]	115	350	1652(CO), 1588(C=N), 1520(NNH), 540(M-O), 420(M-N)
13	$[Cu(L^1)Cl_2]$ $C_{14}H_{21}Cl_2CuN_3O_7$[477.78]	130	345	1651(CO), 1593(C=N), 3430(NH), 541(M-O), 440(M-N)
14	$[Cu(L^2)Cl_2]$ $C_{15}H_{12}Cl_2CuN_2O_7$[476.80]	173	355	1651(CO), 1593(C=N), 1515(NNH), 541(M-O), 440(M-N)
15	$[Mn(L^1)Cl_2]$ $C_{14}H_{21}Cl_2MnN_3O_7$[469.18]	140	365	1652(CO), 1585(C=N), 3440(NH), 533(M-O), 440(M-N)
16	$[Mn(L^2)Cl_2]$ $C_{15}H_{12}Cl_2MnN_2O_7$[468.19]	120	338	1652(CO), 1585(C=N), 1520(NNH), 533(M-O), 440(M-N)
17	$[Ni(L^1)Cl_2]$ $C_{14}H_{21}Cl_2NiN_3O_7$[472.93]	122	370	1652(CO), 1598(C=N), 3438(NH), 540(M-O), 440(M-N)
18	$[Ni(L^2)Cl_2]$ $C_{15}H_{12}Cl_2NiN_2O_7$[471.21]	105	365	1652(CO), 1598(C=N), 1518(NNH), 540(M-O), 440(M-N)
19	$[Zn(L^1)Cl_2]$ $C_{18}H_{27}N_3O_{11}Zn$[526.81]	210	360	1652(CO), 1589(C=N), 3440(NH), 533(M-O), 440(M-N)
20	$[Zn(L^2)Cl_2]$ $C_{19}H_{18}N_2O_{11}Zn$[525.82]	176	365	1650(CO), 1589(C=N), 1515(NNH), 533(M-O), 440(M-N)

ν(C=O) and ν(NH_2) stretching vibrations and a new band appeared at *ca.* 1607 cm^{-1} assigned[30] the azomethine ν(HC=N) linkage indicate that amino and aldehydic moieties of the starting reagents have been converted into their corresponding Schiff's bases. The ν(NH)-amide, ν(C=O)-amide and ν(NNH)-imino stretching frequencies were present at 3440, 1679 and 1607 cm^{-1}, respectively. A comparison[31] of the IR spectra of the Schiff bases to their metal (II) chelates (Table-1), indicated that the Schiff bases are coordinated to the metal atom mainly in two ways, thus the ligands are acting in a bidentate manner. The band absorption at 1607 cm^{-1} corresponding to azomethine group was shifted to lower frequencies by *ca.* 20 cm^{-1} indicating[32] participation of the azomethine nitrogen is coordinated. A new medium strong band appearing at 540-533 cm^{-1} is assigned[33] to ν(M-O). This demonstrated that oxygen of the C=O-amide has formed a coordinate bond with the metal ions in an enolic form. A weak band at 440, 420 cm^{-1} is assigned to ν(M-N). This further confirms that the nitrogen of the HN-imino group bonds to the metal atom. All of the data establishes that a conjugate chelate ring formed by ligand enolization exists in the complexes.

NMR spectra: The NMR spectra of ligands were determined in DMSO. The ^1H NMR spectral data are reported along with the possible assignments. All the protons were found to be in their expected regions[34,35].

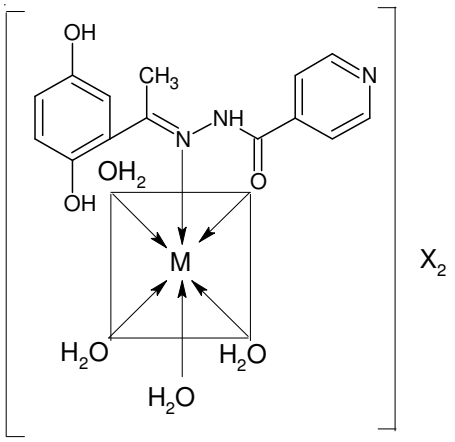
Mass spectrometry: Mass spectra were determined by the instrument JEOL MS Route and MAT 312 that gives us different fragments of the Schiff bases. Molecular ion peaks were appeared at same value of the molecular weight of Schiff bases.

The conclusions drawn from these studies further support to the mode of bonding discussed in their IR spectra. It was also observed that DMSO did not have any coordinating effect on the spectra of ligands or their metal complexes. Thus, the suggested structures of the complexes are given in Figs. 2-5.



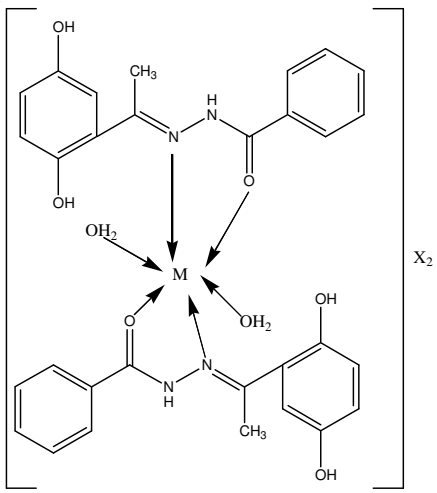
M = Cu, Co, Ni, Mn, Zn; X = Cl, CH_3COO

Fig. 2. Structure of 2,5-dihydroxyacetophenone isonicotinoyl hydrazone transition metal complex [2:1]



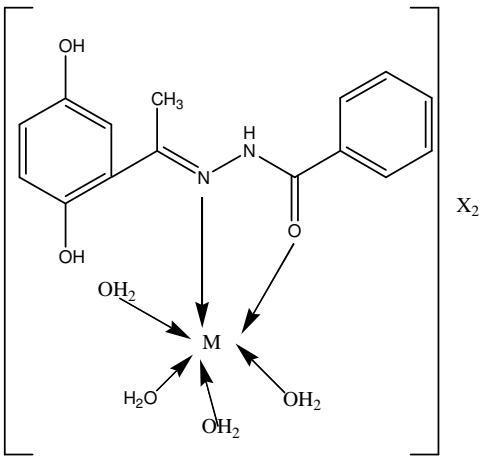
M = Cu, Co, Ni, Mn, Zn; X = Cl, CH_3COO

Fig. 3. Structure of 2,5-dihydroxyacetophenone isonicotinoyl hydrazone transition metal complex [1:1]



M = Cu, Co, Ni, Mn, Zn; X = Cl, CH_3COO

Fig. 4. Structure of 2,5-dihydroxyacetophenone benzoyl hydrazone transition metal complex [2:1]



M = Cu, Co, Ni, Mn, Zn; X = Cl, CH_3COO

Fig. 5. Structure of 2,5-dihydroxyacetophenone benzoyl hydrazone transition metal complex [1:1]

Biological activity of complexes: Antioxidant and cytotoxic activities of complexes are given in Table-2. DPPH scavenging activities were shown by all complexes except **9**

TABLE-2
ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF METAL (II) COMPLEXES (n = 3, MEAN ± SEM)

No	Metal complex	Antioxidant activity IC_{50} (μg/mL)	Per cent NO inducing activity (%)	Cytotoxicity (% mortality) SEM ≤ 6.5
1	$[Cu(L^1)_2Cl_2]C_{28}H_{30}N_6O_8Cl_2Cu$	54.2 ± 0.95	-3.17 ± 0.55	100
2	$[Cu(L^2)_2Cl_2]C_{30}H_{32}N_4O_8Cl_2Cu$	156.3 ± 5.62	2.85 ± 0.17	40
3	$[Co(L^1)_2Cl_2]C_{28}H_{30}N_6O_8Cl_2Co$	59.5 ± 1.21	31.07 ± 1.18	100
4	$[Co(L^2)_2Cl_2]C_{30}H_{32}N_4O_8Cl_2Co$	56.3 ± 0.73	55.64 ± 1.11	10
5	$[Ni(L^1)_2Cl_2]C_{28}H_{30}N_6O_8Cl_2Ni$	38.6 ± 0.81	35.23 ± 1.19	60
6	$[Ni(L^2)_2Cl_2]C_{30}H_{32}N_4O_8Cl_2Ni$	57.2 ± 1.52	12.27 ± 0.59	40
7	$[Mn(L^1)_2Cl_2]C_{28}H_{30}N_6O_8Cl_2Mn$	32.2 ± 0.87	88.13 ± 2.56	100
8	$[Mn(L^2)_2Cl_2]C_{30}H_{32}N_4O_8Cl_2Mn$	33.5 ± 0.66	63.07 ± 1.19	60
9	$[Zn(L^1)_2(CH_3COO)_2]C_{32}H_{36}N_6O_{12}Cl_2Zn$	> 200	61.64 ± 1.85	10
10.	$[Zn(L^2)_2(CH_3COO)_2]C_{34}H_{38}N_4O_{12}Cl_2Zn$	33.7 ± 0.32	15.6 ± 0.29	10
11	$[Co(L^1)Cl_2]C_{14}H_{21}N_3O_7Cl_2Co$	52.1 ± 1.33	27.33 ± 1.13	100
12	$[Co(L^2)Cl_2]C_{15}H_{12}N_2O_7Cl_2Co$	47.0 ± 0.82	37.67 ± 1.23	10
13	$[Cu(L^1)Cl_2]C_{14}H_{21}N_3O_7Cl_2Cu$	59.8 ± 0.98	71.22 ± 2.25	10
14	$[Cu(L^2)Cl_2]C_{15}H_{12}N_2O_7Cl_2Cu$	72.8 ± 3.12	3.47 ± 1.97	70
15	$[Mn(L^1)Cl_2]C_{14}H_{21}N_3O_7Cl_2Mn$	31.8 ± 0.56	62.27 ± 1.68	100
16	$[Mn(L^2)Cl_2]C_{15}H_{12}N_2O_7Cl_2Mn$	> 200	18.67 ± 0.55	10
17	$[Ni(L^1)Cl_2]C_{14}H_{21}N_3O_7Cl_2Ni$	36.9 ± 0.77	39.73 ± 0.95	50
18	$[Ni(L^2)Cl_2]C_{15}H_{12}N_2O_7Cl_2Ni$	36.4 ± 1.33	64.46 ± 1.12	50
19	$[Zn(L^1)Cl_2]C_{18}H_{27}N_3O_{11}Cl_2Zn$	92.8 ± 1.52	147.33 ± 2.97	10
20	$[Zn(L^2)Cl_2]C_{19}H_{18}N_2O_{11}Cl_2Zn$	34.5 ± 0.43	14.53 ± 0.35	10
3-*t*-BHA (standard antioxidant)		44.12 ± 2.31	–	–
Propyl gallate (standard antioxidant)		32.21 ± 0.83	–	–

and **16**. All these complexes showed antioxidant activity comparable to that of the standard. However, when nitric oxide (NO) scavenging activity was assayed, all these complexes showed NO-inducing activity. The complexes **1**, **3**, **7**, **11**, **15** killed all brine shrimp larvae and showed 100 % mortality that indicates that these complexes are highly cytotoxic. On the other hand, complexes **4**, **9**, **10**, **12**, **13**, **16**, **19**, **20** showed the least cytotoxicity against the larvae whilst remaining complexes were moderate in cytotoxic properties.

Antibacterial activity assay exhibited MIC_{50} values of **2**, **4**, **6**, **8**, **10**, **12**, **14**, **16**, **18**, **20** which are highly acceptable when compared to standard drugs for both gram-positive and gram-negative bacteria. These results indicate that the compounds are effective against both gram-positive and gram-negative bacteria. However, other compounds **1**, **3**, **5**, **7**, **9**, **11**, **13**, **15**, **17**, **19** (Table-4) showed minimal or no activity against all these bacteria as determined by the per cent growth inhibition. Compounds **5**, **17**, **19** showed some antibacterial activities against bacteria. These results demonstrate a potential of compounds in Table-3 for further determinations in antibacterial assays on a wide range of gram-positive and gram-negative bacteria.

With respect to structure activity relationship (SAR), the 2,5-dihydroxyacetophenone benzoyl hydrazone ($\mathbf{L^2}$) metal complexes **2**, **4**, **6**, **8**, **10**, **12**, **14**, **16**, **18**, **20** have more antioxidant activity as compared to 2,5-dihydroxyacetophenone isonicotinoyl hydrazone ($\mathbf{L^1}$) metal complexes **1**, **3**, **5**, **7**, **9**, **11**, **13**, **15**, **17**, **19** (Table-2) whereas $\mathbf{L^1}$ metal complexes are much more cytotoxic as compared to the $\mathbf{L^2}$ metal complexes (Table-2). Moreover, the mono-ligand metal complexes (ML^2) **12**, **14**, **16**, **18**, **20** shows less cytotoxicity as compared to their respective *bis*-ligand metal complexes (ML^2_2) **2**, **4**, **6**, **8**, **10**. The antibacterial activities (MIC_{50} μ mol) of less cytotoxic metal complexes (ML^2 and ML^2) were then tested against both

TABLE-3
ANTIBACTERIAL ACTIVITIES (MIC_{50} μ mol) OF METAL (II) COMPLEXES (n = 3, MEAN ± SEM)

Compound No.	Gram negative				Gram positive	
	Escherichia coli	*Shiglla sonnei*	*Salmonella typhi*	*Pseudomonas aeroginosa*	*Staphylococcus aureus*	*Bacillus subtilis*
2	10.98 ± 0.49	13.12 ± 0.10	14.11 ± 0.51	12.36 ± 0.32	14.67 ± 0.27	13.19 ± 0.17
4	13.37 ± 0.31	8.61 ± 0.48	14.64 ± 0.32	9.43 ± 0.21	16.74 ± 0.27	11.19 ± 0.27
6	15.54 ± 0.05	16.00 ± 0.54	13.66 ± 0.23	18.81 ± 0.83	17.75 ± 0.46	11.11 ± 0.11
8	16.94 ± 0.47	13.19 ± 0.11	15.39 ± 0.01	16.57 ± 0.28	20.58 ± 0.45	10.01 ± 0.22
10	14.69 ± 0.35	14.56 ± 0.15	13.61 ± 0.31	12.16 ± 0.11	6.38 ± 0.33	13.31 ± 0.11
12	11.66 ± 0.29	7.89 ± 0.29	12.71 ± 0.18	8.41 ± 0.29	15.50 ± 0.35	11.36 ± 0.52
14[	14.28 ± 0.31	12.28 ± 0.25	13.22 ± 0.43	10.81 ± 0.20	7.38 ± 0.98	14.08 ± 0.13
16	13.76 ± 0.95	12.12 ± 0.18	13.26 ± 0.21	13.68 ± 0.21	9.34 ± 0.34	10.81 ± 0.22
18	15.64 ± 0.65	13.12 ± 1.49	11.86 ± 0.56	13.60 ± 0.21	21.57 ± 0.255	12.20 ± 0.04
20	20.69 ± 0.02	17.11 ± 0.13	10.51 ± 0.09	12.31 ± 0.34	22.57 ± 0.03	16.19 ± 0.37
Ciprofloxacin	8.21 ± 0.02	7.31 ± 0.08	11.66 ± 0.13	10.33 ± 0.21	9.42 ± 0.11	8.36 ± 0.13
Gentamycin	9.29 ± 0.02	9.31 ± 0.18	11.21 ± 0.31	10.89 ± 0.11	8.42 ± 0.12	10.36 ± 0.13
Ampicilin	11.32 ± 0.13	11.98 ± 0.13	10.85 ± 0.16	12.33 ± 0.15	10.69 ± 0.06	11.66 ± 0.14

TABLE-4
ANTIBACTERIAL ACTIVITIES (% GROWTH INHIBITION AT 100 µg/mL) OF METAL (II) COMPLEXES (n = 3, MEAN VALUES ONLY)

Compound No.	Gram negative				Gram positive	
	Escherichia coli	*Shiglla sonnei*	*Salmonella typhi*	*Pseudomonas aeroginosa*	*Staphylococcus aureus*	*Bacillus subtilis*
1	-67.49	-46.4	-16.78	-14.04	46.52	-27.46
3	47.3	-32.8	-4.26	0.79	-45.38	-12.92
5	39.92	-26.4	50.79	50.40	36.77	-16.06
7	-3.7	4.5	10.81	17.65	34.91	-13.40
9	-50.06	-66.2	-41.31	-16.93	-21.16	-45.31
11	16.13	-7.1	31.35	24.79	-103.2	23.91
13	27.71	-9.4	-4.42	9.77	52.00	8.03
15	11.75	-2.5	17.88	13.85	-57.1	-13.40
17	-72.18	17.0	35.66	41.46	29.29	27.42
19	-18.76	57.8	52.42	52.42	19.47	36.11
Ciprofloxacin	93.65	96.32	91.23	89.63	91.24	94.77
Gentamycin	89.34	87.32	84.66	81.45	89.41	89.61
Ampicilin	92.47	91.25	92.08	89.51	91.63	94.12

gram-positive and gram-negative bacteria and results showed the outstanding activity comparable with standard drugs (Table-3). Keeping in view all the bio-assay data, it is obviously clear that the complexes having carbocyclic unit in the ligand are less toxic and highly active as compared to the complexes bearing heterocyclic moiety in the ligand.

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