Article

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Synthesis of Tetradentate Schiff Base Derivatives of Transition Bimetallic Complexes as Antimicrobial Agents

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A series of Co(II), Cu(II), Mn(II) and Zn(II) bimetallic complexes have been synthesized with the schiff base ligand 2-(bis-2-hydroxyl phenylidene) diimine (L) derived from the condensation of hydrazine and salicylaldehyde. The synthesized ligand and bimetallic complexes were characterized by different spectroscopic techniques. The characterization of ligand was carried out by FT-IR, H^1NMR, C^{13}NMR and MS while the bimetallic complexes were characterized by FT-IR and X-ray crystallographic techniques. The complexes and ligand were employed in vitro for antifungal and antibacterial activities using disc diffusion method. Different fungal strains such as Alternaria Alternate, Aspergillus Flavus and Aspergillus Niger were used to check antifungal activities of bimetallic complexes and ligand. Similarly, the bacterial strains used were Staphylococcus Aureus, Bacillus Subbtilis and Escheria Coli. The biological studies showed that the ligand exhibited lower value of antifungal and antibacterial activities than bimetallic complexes.

Keywords: Schiff base; Transition metals; Hydrazine; Salicylaldehyde; Bacterial strains; Fungal strains.

INTRODUCTION

Schiff bases ligands play an important role as a variety of enzymatic reaction intermediates. Due to the special biological and photophysical properties, the Schiff bases metal complexes are widely used in medicine and in various diagnostic tools. A series of chemical and biological investigations confirms that Schiff bases are very important ligands and take part in many non enzymatic processes. Such Schiff bases are derived from the condensation reaction of amino acids and pyridoxal or by condensing lysine with methylglyoxal[1] respectively. The non enzymatic mechanisms generally proceed in variety of pathogens which involve diseases such as diabetes or the diseases caused by excess of metal ion and by stress. For example, the tissue damage is caused by attack of amino group of proteins on sugar carbonyls.[2] Several unsymmetrical ligands derived from amines and aldehydes have an advantage over symmetrical ligands e.g. the geometry of metal ions, efficiency of enzymes, structural elucidation and peptide linkage are governed by unsymmetrical tetradentate Schiff bases ligands. Some enzymes contain transition metals at active sites and these metalloenzymes catalyzed different reactions. Metalloenzymes were found to be involved in the catalysis of different reactions such as hydrolases, oxidases, oxygenases and isomerases catalyzed hydrolysis, oxidation, reduction and rearrangement reactions respectively.[3]

In previous studies, a tridentate Schiff base formed a stable complex with copper (II).[4] This copper complex was very usefull in various reactions of molecular oxygen involving redox reactions, electron transport and many other biochemical mechanisms. Almost all transition metals form metal complexes with different ligands involving Schiff bases. Besides of all, the Schiff bases derived from salicylaldehyde were also found to have the advantage of the synthesis of a wide variety of metal complexes which are applicable in different industrial, synthetic and biochemical processes.[5]

The Schiff bases and their metal complexes are also considered to be very important due to their interest in a variety of biological mechanisms.[6] Many transition metal complexes were found to exhibit greater stability and are

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very active against different diseases. Depending upon the stability, the transition metals and their complexes have also been used against tumor and cancer treatment.[7] The stability of complexes mainly depends on the chelating and donating powers of the ligand which ultimately facilitates oxidizing and electrophilic reactions. The less stability of metal complexes restricts their applications in some biological processes.[8] Ligands made from salicylaldehyde exhibit higher values of antibacterial activities against different species including S.aureus, P.mirabilis, H.influenza and salmonella spp.[9] Apart from ligands and metal complexes salicylaldehyde is more frequently used as larvicide and fungicides.

In the present research work, a series of Co(II), Cu(II), Mn(II) and Zn(II) bimetallic complexes are synthesized with the Schiff base ligand 2-(bis-2-hydroxyl phenylidene) diimine (L) derived from the condensation of hydrazine and salicylaldehyde. The synthesized ligand and bimetallic complexes with ligand (L) are characterized by different spectroscopic techniques. The ligand characterization is carried out using FT-IR, H^1NMR, C^{13}NMR, MS while the bimetallic complexes are characterized by FT-IR and X-ray crystallographic techniques. Then these complexes and ligand are employed in vitro for antifungal and antibacterial activities by using disc diffusion method. Different fungal strains such as Alternaria alternate, Aspergillus flavus and Aspergillus niger are used to check antifungal activities of bimetallic complexes and ligand.

EXPERIMENTAL

All solvents and regents (chemicals) including Salicylaldehyde, Cobalt acetate, Copper acetate, Manganese acetate, Zinc acetate, Ethanol, Hydrazine, Acetic acid, Toluene and Ethyl acetate of AR grade were supplied by Sigma-Aldrich (Germany).

Synthesis of Ligand. In order to prepare the ligand (L), condensation reaction was carried out between equimolar of hydrazine (0.01 mole) and salicylaldehyde (0.01 mole). Binary solvent mixture containing toluene and ethanol (1:1) was taken in a flask and stirred for 3 hours. Then both reactants and glacial acetic acid (1 mL) were stirred in 100 mL flask for 6 hours at room temperature. TLC test was employed in a pet. ether-ethylacetate (1:1) solvent system to check the success of the reaction. The reaction flask was put in the refrigerator for 22 hours and no crystal formation was observed. The solvent was removed by rotary evaporator and the product was then subjected to column chromatography by using silica gel and pet. ether-ethylacetate (1:1) solvent system. The final mixture was cooled and solvent was removed by rotary evaporator. The product was dried and 74% productivity was found. Finally purified product was characterized by FT-IR, MS, ^{1}HNMR and ^{13}CNMR techniques.

Synthesis of bimetallic Complexes. All the transition bimetallic complexes were synthesized by the following procedures. Equimolar quantities of metal acetates (Co, Cu, Mn, Zn) and ligand were taken. the metal acetate was dissolved in ethanol/toluene and continuously stirred and heated on hot plate. Whereas, the ligand was stirred in binary solution of toluene and ethanol (1:1). Then both of the solutions were mixed and stirred for 5-6 hours in 250 mL flask. The progress of the reaction was checked by TLC test and the solvent after the completion of the reaction was removed by the rotary evaporator and washed with pet-ether. The crude product was subjected to column chromatography after removing the solvent by rotary evaporator. Then the concentrated solution was put in the refrigerator at low temperature in order to get the crystals but rather cyrstaline, the microcrystalline material was obtained. Finally, the solvent was removed up to dryness and the product was redissolved in a combination of three solvents i.e. ethanol, toluene, and ethyl acetate (TCS by volume 50%, 10% and 40% respectively). The fine crystals were obtained at low temperatures after 3-4 days. The crystals of the bimetallic complex were characterized by FT–IR and X-ray crystallography.

Spectroscipic Analysis. The spectroscopic data of ligand and bimetallic complexes was calculated by using different spectroscopic techniques. FT-IR spectra were taken on a Perkin Elmer FT-IR. Mass spectra of ligand were taken at 70 eV by Electron ionization technique using Perkin Elmer Clarus 680 GC-MS instrument in the R & D section of Zeta Chemical Company (PVT) Ltd, Lahore, Pakistan. H^1NMR and C^{13}NMR were recorded via 60 MHz JNM-ECX60 FT NMR System. Crystal data (X-Ray crystallography) of bimetallic complexes were taken on the single crystal Apex – II Bruker X-Ray diffractometer.

Antimicrobial Activity. The selected strains were *Escheria coli*, *staphylococcus aureus* and *Bacillus Subbtilis*.[10] The ligand and bimetallic complexes were employed for antibacterial activity by using disc diffusion method CLSI, 2007. Nutrient agar was mixed with distilled water and dispersed homogeneously. Sterilization of the medium was carried out by means of autoclave at 121 °C for 20 minutes. The medium was treated with inoculums before it is transferred to petri plates. Hereafter, filter paper discs were placed parallel on growth medium which contains 100 µL (micro liters) of bimetallic complexes and ligand. The incubation of petri plates was carried out for 24 hours at 37 °C for bacterial growth. The complexes and ligand full of antibacterial activities inhibited the growth of bacteria and formed clear zones. Zone reader was employed to measure the inhibition zones in mili meters. The standard drug used was Rifamipicin.[16] Fungal strains were used to test the antifungal activity. The selected strains were *A. flavus*, *A. alternate* and *A. niger.*[10] The growth medium was synthesized, sterilized and then transferred to the petri plates. Petri dishes were incubated for 48 hours at 28 °C for fungus growth. Filter paper discs were cited on growth medium for the growth of fungus. The bimetallic complexes and ligand were applied up to 100 µL (micro liter) on each disc. petri plates were then incubated and the complexes and ligand which showed antifungal activities, they inhibited the growth of fungus and clear zones were produced. The standard drug used for antifungal activity of complexes and ligand was Fluconazol.[11]

RESULTS AND DISCUSSION

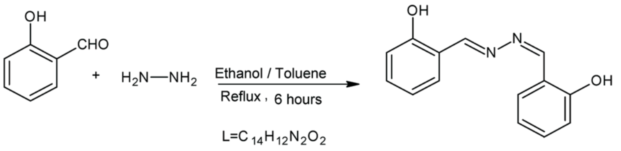
Synthetic route for 4-(bis-4-hydroxyl phenylidene) diimine (L_2) and the bimetallic complexes with L_2

In order to prepare the ligand (L) condensation reaction was carried out between equimolar of hydrazine (6 g, 0.01 moles) and salicylaldehyde (12.2 g, 0.01 moles). Binary solvent mixtures containing toluene and ethanol (1:1) were taken in flask. To this both reactants and glacial acetic acid (1 mL) were added and then refluxed in 100 mL for 6 hours at room temperature. TLC test was employed in a pet. ether-ethylacetate (1:1) solvent system to check the success of the reaction. The reaction flask was put in the refrigerator for 22 hours and no crystal formation was observed. The solvent was removed by rotary evaporator and the product was then subjected to column chromatography by using silica gel and pet. Ether-ethylacetate (1:1) solvent system. The eluent was concentrated upto dryness by rotary evaporator. The product was dried well with vacuum pump and productivity was calculated to be 74%. The final purified product was characterized by FT-IR, MS, ^{1}HNMR and ^{13}CNMR. This newly synthesized ligand was preserved for further use.

Synthesis of bimetallic cobalt complex with Ligand (L)

Equimolar quantities of cobalt acetate tetrahydrate and ligand were reacted. Cobalt acetate tetrahydrate (3.98 g, 0.02 moles) was dissolved in alcohol after continuous stirring. Ligand (L_2) (3.8 g, 0.02 moles) was stirred in binary solution of toluene and ethanol (1:1). Both the solutions were mixed and stirred for 5-6 hours in 250 mL flask. The progress of the reaction was checked by TLC test. The solvent after the completion of the reaction was removed by the rotary evaporator, washed with pet- ether. The crude product was recrystallized from the toluene/pet-ether system. The purified product after filtration was dried well under vacuum. The product was redissolved in minimum quantity of toluene and was put in the refrigerator at low temperature in order to get the crystals but microcrystalline material was obtained. Then the solvent was removed upto dryness and redissolved in combination of three solvents i.e. ethanol, toluene, and ethyl acetate (TCS by volume 50%, 10% and 40%, respectively). The fine crystals were obtained at low temperature after 3-4 days. The crystals of bimetallic cobalt complex were characterized by FT–IR and X-ray crystallography.

Scheme I Synthesis route for 4-(bis-4-hydroxyl phenylidene) diimine



Synthesis of bimetallic copper complex with Ligand (L)

Copper acetate monohydrate and ligand solutions were made by dissolving (3.98 g, 0.02 moles) and (3.8 g, 0.02 moles) in alcohol and binary mixture of toluene and alcohol (1:1), respectively. Both the solutions were heated and stirred separately and then refluxed for 7-8 hours after mixing with each other in 250 mL flask adjusted with stirring system. The success of the reaction was checked by TLC test. The solvent after the completion of the reaction was removed by the rotary evaporator, washed with pet-ether. The crude product was redissolved in the toluene/pet-ether system. The purified product after filtration was dried well under vacuum and yield of the product was calculated upto 71%. The product obtained was characterized by FT-IR.

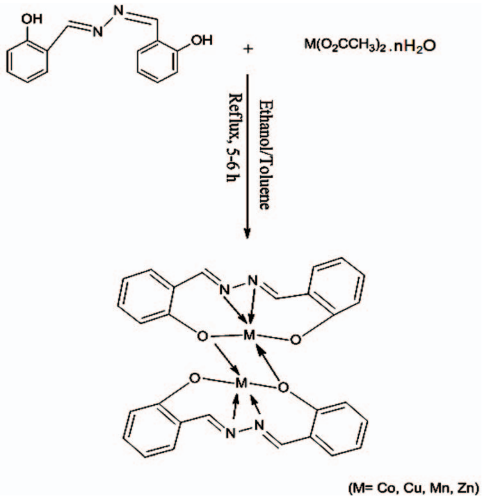
Synthesis of bimetallic manganese complex with Ligand (L)

This bimetallic complex of manganese with ligand 2 was prepared by mixing equimolar quantities of manganese acetate (1.77 g, 0.01 moles) dissolved in ethanol and ligand (2.4 g, 0.01 moles) dissolved in binary mixture of ethanol and toluene (1:1). Both the solutions after mixing were refluxed for 8 hours along with continuous stirring in 250 mL flask adjusted with stirring system. The success of the reaction was checked by TLC test. The solvent after the completion of the reaction was removed by the rotary evaporator, washed with pet-ether. The crude product was redissolved in toluene/pet-ether system. The product after filteration was dried well under vacuum and the yield of the product was calculated upto 64%. The product obtained was characterized by FT-IR.

Synthesis of bimetallic zinc complex with Ligand (L)

This bimetallic complex was synthesized by reacting equimolar quantities of zinc acetate dihydrate and ligand. The ligand (3.8 g, 0.02 moles) was dissolved in binary mixture of toluene and ethanol (1:1). Zinc acetate dihydrate (3.38 g, 0.02 moles) was dissolved in ethanol. Stirring and heating of ligand and zinc acetate was done on separate hot plates. Subsequently both solutions were mixed and refluxed for 7 hours. The progress of the reaction was checked by TLC test and the solvent after the completion of the reaction was removed by the rotary evaporator, washed with pet- ether. The crude product was recrystallized from toluene-pet-ether system. The purified product after filtration was dried well under vacuum and the yield of the product was calculated upto 73%. The product obtained was characterized by FT-IR.

Scheme II Synthesis route for bimetallic complexes with L_2



Physical properties of Ligand (L) and the bimetallic complexes with ligand (L)

The Ligand (L) and bimetallic complexes with ligand (L) showed sharp melting points and were soluble in most of the organic solvents as expressed in Table 1.

Crystallization of bimetallic complexes with ligand (L) in triple component solvent system

We successfully adopted a combination of three solvents system in order to get excellent crystals of the bimetallic complexes. Three different individual solvents were employed for the crystallization of the said bimetallic complexes but none of them was good enough to dissolve the compounds and ultimately weigh down the crystallization of the complexes. A three component solvent system (Ethanol, toluene, ethyl acetate: 50%, 10% and 40% respectively) was used to significantly enhance the solubility.[12] The results were inspiring because the solubility was increased when 0.5 g of the individual bimetallic complex was experienced which may be due to strong intermolecular interactions between the solvents and the solute particles as shown in Table 2.

Table 1. Physical properties of ligand (L) and complexes of (L)

Sr #	Comp.	Mol. Weight	Mol. formula	Melting point
1	L	240	$C_{14}H_{12}O_2N_2$	102 °C
2	Cu(L)	602	$Cu_2(C_{14}H_{12}O_2N_2)_2$	>350 °C
3	Co(L)	594	$Co_2(C_{14}H_{12}O_2N_2)_2$	347 °C
4	Mn(L)	586	$Mn_2(C_{14}H_{12}O_2N_2)_2$	328 °C
5	Zn(L)	606	$Zn_2(C_{14}H_{12}O_2N_2)_2$	>350 °C

Characterization of ligand and bimetallic complexes

FT-IR was used to confirm the bimetallic complexes and ligand's functional groups. The range used for the FT-IR spectra was 400-4000 cm^{-1} as in our previous work.[13] NMR spectra (H^1NMR and C^{13}NMR) of the ligand were recorded by using – ECX60 FT NMR (60 MHz) and solvent used was $CDCl_3$. Reference standard used was TMS. The ligand was analyzed for molecular mass on the basis of previous reported results the data was elucidated and confirmed.[14] Apex – II Bruker X-Ray diffractometer was used to establish the structure, bond angles and lengths of bime-

Table 2. Solubility of ligand (L) and complexes with ligand (L)

Sr #	Comp.	Solubility			
		DMSO	*Chloroform*	*Ethyl alcohol*	*Mixed solvent system*
1	L	+	+	+	+
2	Cu(L)	Slightly soluble	Slightly soluble	Slightly soluble	+
3	Co(L)	Slightly soluble	Slightly soluble	Slightly soluble	+
4	Mn(L)	Slightly soluble	Slightly soluble	Slightly soluble	+
5	Zn(L)	Slightly soluble	Slightly soluble	Slightly soluble	+

tallic complexes.

Antimicrobial activity analyses

The antimicrobial activity was determined by using disc diffusion method against different species of fungi and bacteria. The results confirmed that the activity of bimetallic complexes is considerable high against bacteria and fungus as compared to ligand (Table 4.27). The synthesized ligand and complexes were tested for their activity against *B. subtilis, S.aureus, E. coli* using Rifamipicin as positive control.[10] The ligand showed less activity as compared to bimetallic complexes. The highest activity was shown against *B. subtilis* with zone 14.5 ± 0.577 mm. The activity against *S. aureus* and *E.coli* were average with zones 11 ± 0.154 mm and 12.25 ± 0.062 mm respectively. All results are given in comparison with standard drug (Rifamipicin) which gave the zone 24.5 ± 0.577 mm (Table 3 & Fig. 1).

The synthesized ligand and bimetallic complexes were tested for their activity against various fungus (*A. alternata, A.niger, A. flavis*) by using disc diffusion method. Fluconazol is used as standard drug. The highest activity was shown by bimetallic complexes. The activity against *A. flavus* and *A. niger* was highest with zone 11 ± 0.154 mm and 11 ± 0.154 mm respectively. *A. alternata* with zones 14.5 ± 0.577 showed the average activity. All above results are in comparison with standard drug (Fluconazol) which gave the zone 24.0 ± 0.577 mm (Table 4 & Fig. 2).

Table 3. Antibacterial activity data of ligands and complexes

Sr#	Comps.	Tested Microorganism Diameter of Inhibition Zone		
		E. coli	*S. aureus*	*B. subtilus*
1	(L)	Nil	4.25 ± 0.957	6 ± 0.816
2	Co (L)	10.25 ± 0.500	11 ± 0.154	14.5 ± 0.577
3	Cu (L)	11 ± 0.154	9.5 ± 0.577	12.5 ± 1.732
4	Mn (L)	4.25 ± 0.957	6 ± 0.816	3.25 ± 0.500
5	Zn(L)	12.25 ± 0.062	6.24 ± 0.957	14.5 ± 0.577
6	Standard	23.25 ± 2.060	24.5 ± 0.577	23 ± 0.816

Table 4. Antifungal activity data of ligands and complexes

Sr#	Comps.	Tested Microorganism Diameter of Inhibition Zone		
		A. niger	*A. flavous*	*A. alternate*
1	(L)	8.25 ± 1.500	5.25 ± 0.957	6 ± 0.816
2	Co (L)	7.25 ± 0.957	9.5 ± 1.000	9 ± 0.816
3	Cu (L)	11 ± 0.154	9.5 ± 0.577	12.5 ± 1.732
4	Mn (L)	4.75 ± 0.500	3.25 ± 0.500	9.75 ± 0.957
5	Zn(L)	10.25 ± 0.500	11 ± 0.154	14.5 ± 0.577
6	Standard	24.5 ± 0.577	23.25 ± 2.060	23 ± 0.816

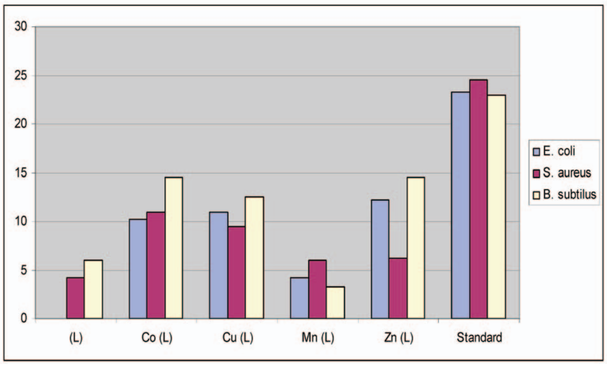


Fig. 1. Antibacterial activity data of Ligand (L) and complexes with Ligand.

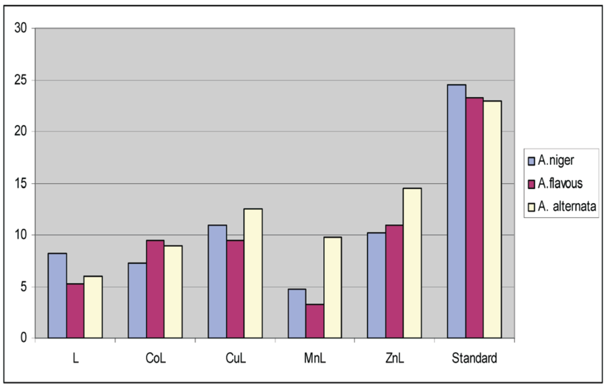


Fig. 2. Antifungal activity data of Ligand (L) and complexes with ligand L.

It is recommended that the antimicrobial activity of the complexes is due to either by killing the microbes or inhibiting their development by jamming their active sites. The values designated that most complexes have higher antimicrobial activity than the free ligands. The enhanced activity of metal complexes can be explained on the basis of chelation. On chelation, the polarity of the metal ion will be decreased to a greater extent due to the overlap of the ligand.[15]

CONCLUSIONS

From the antimicrobial activity results, it was concluded that metal complexes have higher values of antibacterial and antifungal activities than the parental synthesized ligand and ultimately, this is due to the double chelating effect of transition metals with ligand in bimetallic complexes.

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