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Metal-based antibacterial and antifungal amino acid derived Schiff bases: their synthesis, characterization and *in vitro* biological activity

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A new series of antibacterial and antifungal amino acid derived Schiff bases and their cobalt(II), copper(II), nickel(II) and zinc(II) metal complexes have been synthesized and characterized by their elemental analyses, molar conductances, magnetic moments, IR and electronic spectral measurements. The spectral data indicated the Schiff base ligands (L_1–L_5) derived by condensation of salicylaldehyde with glycine, alanine, phenylalanine, methionine and cysteine, to act as tridentate towards divalent metal ions (cobalt, copper, nickel and zinc) via the azomethine-N, deprotonated carboxyl group of the respective amino acid and deprotonated oxygen atom of salicylaldehyde by a stoichiometric reaction of M:L (1:2) to form complexes of the type $K_2[M(L)_2]$ [where M = Co(II), Cu(II), Ni(II) and Zn(II)]. The magnetic moments and electronic spectral data suggested that all complexes have an octahedral geometry. Elemental analyses and NMR spectral data of the ligands and their Zn (II) complexes agree with their proposed structures. The synthesized ligands, along with their metal complexes, were screened for their *in-vitro* antibacterial activity against four Gram-negative (*Escherichia coli, Shigella flexeneri, Pseudomonas aeruginosa* and *Salmonella typhi)* and two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) bacterial strains and for *in-vitro* antifungal activity against *Trichophyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani* and *Candida glaberata*. The results of these studies show the metal complexes to be more antibacterial/antifungal against one or more species as compared with the uncomplexed Schiff base ligands. The brine shrimp bioassay was also carried out to study their *in-vitro* cytotoxic properties. Only three compounds (2, 11 and 17) displayed potent cytotoxic activity as $LD_{50} = 8.196 \times 10^{-4}$, 7.315×10^{-4} and 5.599×10^{-4} M/ml respectively, against *Artemia salina*.

KEYWORDS: amino acid; Schiff bases; metal complexes; antibacterial; antifungal; cytotoxicity

INTRODUCTION

The rapid development and understanding of the chemistry of molecular biology and amino acids have created a significant class of compounds that are now helpful in understanding biological functions of macromolecules like proteins. However, some free amino acids also play an important role in many physiological activities of the human body, for example D,L-homocysteic acid (DLH) excites cerebral activities and has been proposed as an agonist of endogenous glutamate receptors in the mammalian central nervous system.[1–4] After determining the efficacy of these compounds, considerable research has been done into the properties of DLH in neuroanatomy,[5] electrophysiology and pharmacology.[6] Amino acids, a significant class of organic-based compounds, contain potential donor sites such as COOH and/or NH_2 which have good ability to coordinate with the metal ions.[7] It is well known that the human body contains essential metaloelements which play important roles and interact with many biological molecules. It would be useful to fully understand the physiological function of such compounds by studying their chemistry coordination and behavior.[8–10] The current research dealing

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with the metal complexes of Schiff bases has expanded enormously and embraces diversified subjects comprising their various aspects in bio-coordination and bio-inorganic chemistry. It is known that the existence of metal ions bonded to biologically active compounds may enhance their activities.[11–15] Our ongoing research has also established[16–19] the fact that non-biologically active compounds become active and less biologically active compounds become more active upon coordination/chelation with the metal ions. In extension to the work available in the literature,[20–24] we wish to report in this paper a series of antibacterial and antiviral amino acid-derived Schiff bases ($\mathbf{L}_1$–$\mathbf{L}_5$) formed by the condensation reaction of salicylaldehyde with amino acids such as glycine, alanine, phenylalanine, methionine and cysteine. These Schiff bases were used to prepare their metal complexes of the type $K_2[M(\mathbf{L})_2]$ [where M = Co(II), Cu(II), Ni(II) and Zn(II)]. All the Schiff base ligands, along with their metal complexes, were screened for their *in-vitro* antibacterial activity against four Gram-negative (*E. coli, S. flexenari, P. aeruginosa and S. typhi)* and two Gram-positive (*B. subtilis* and *S. aureus*) bacterial strains and for *in-vitro* antifungal activity against *T. longifusus, C. albicans, A. flavus, M. canis, F. solani* and *C. glaberata*.

EXPERIMENTAL

Solvents used were analytical grades; all metal(II) were used as their chloride salts. IR spectra were recorded on the Philips Analytical PU 9800 FTIR spectrophotometer. NMR spectra were recorded on Perkin-Elmer 283B spectrometer. UV–visible spectra were obtained in dimethylformamide (DMF) on a Hitachi U-2000 double-beam spectrophotometer. Butterworth Laboratories Ltd (UK) carried out C, H and N analyses. Conductance of the metal complexes was determined in DMF on a Hitachi (Japan) YSI-32 model conduct meter. Magnetic measurements were carried out on solid complexes using Gouy's method. Melting points were recorded on a Gallenkamp (UK) apparatus and are not corrected. The complexes were analyzed for their metal contents by EDTA titration.[25] Antibacterial and antifungal screening was done at HEJ Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Pakistan.

Preparation of Schiff bases ($\mathbf{L}_1$–$\mathbf{L}_5$)

To a stirred solution of glycine (20 mmol) in water (20 ml) was added salicylaldehyde (20 mmol) in ethanol (10 ml). The mixture was refluxed for 3 h. During this time the color of the solution turned to orange. The completion of reaction was monitored using TLC. After completion of the reaction, the volume of the reactant mixture was reduced to half *in vacuo*. On cooling a solid product was formed. The solid residue was filtered, washed with ethanol, then with ether, and dried. Crystallization from a mixture of ethanol–propanol (60 : 40) afforded the desired Schiff base ligands. The same method was applied for the preparation of all other ligands using the corresponding salicylaldehyde, working in the same conditions with their respective molar ratio.

[(2-Hydroxyphenyl)methylidene amino]acetic acid ($\mathbf{L}_1$)

Yield 66%; m.p. 196 °C; IR (KBr, cm^{-1}): 3445 (OH), 1703 (COOH), 1610 (azomethine, HC═N), 1325, 1490 (C–N); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.57 (s, 2H, CH_2), 6.93 (s, 1H, azomethine), 7.28–7.79 (m, 4H, Ph), 10.23 (s, 1H, OH), 11.29 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, δ, ppm): 75.48 (CH_2), 115.81, 121.33, 129.86, 130.24, 147.62, 154.85 (*Ph*OH), 150.62 (–CH═N), 181.31 (COOH). Anal. calcd for $C_9H_9NO_3$ (178.0): C, 62.50; H, 5.21; N, 10.33. Found: C, 62.88; H, 5.42; N, 10.54%.

[(2-Hydroxyphenyl)methylidene amino]propanoic acid ($\mathbf{L}_2$)

Yield 68%; m.p. 130 °C; IR (KBr, cm^{-1}): 3455 (OH), 1706 (COOH), 1610 (azomethine, C═N), 1450 (C–N); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.12 (s, 3H, CH_3), 2.30 (t, 1H, CH), 6.93 (s, 1H, azomethine), 7.28–7.79 (m, 4H, Ph), 10.22 (s, 1H, OH), 11.29 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, δ, ppm): 21.63 (CH_3), 77.82 (CH), 115.87, 121.53, 129.82, 130.43, 147.76, 154.92 (*Ph*OH), 150.84 (–CH═N), 181.66 (COOH). Anal. calcd for $C_{10}H_{11}NO_3$ (192.0): C, 60.67; H, 4.49; N, 7.87. Found: C, 60.80; H, 4.16; N, 7.98%.

{[(2-Hydroxyphenyl)methylidene]amino}-3-phenylpropanoic acid ($\mathbf{L}_3$)

Yield 62%; m.p. 182 °C; IR (KBr, cm^{-1}): 3450 (OH), 1708 (COOH), 1590 (azomethine, C═N), 1481 (C–N); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.32 (t, 1H, CH), 2.53 (s, 2H, CH_2), 6.95 (s, 1H, azomethine), 7.16–7.79 (m, 9H, Ph), 10.27 (s, 1H, OH), 11.29 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, δ, ppm): 41.62 (CH_2), 77.46 (CH), 126.73, 128.47, 130.65, 148.53 (–Ph), 115.72, 121.45, 130.26, 130.62, 147.55, 154.91 (*Ph*OH), 150.76 (–CH═N), 181.53 (COOH). Anal. calcd for $C_{16}H_{14}NO_3$ (268.0): C, 71.64; H, 5.22; N, 5.22. Found: C, 71.93; H, 5.54; N, 5.04%.

{[(2-Hydroxyphenyl)methylidene]amino}-3-mercaptopropanoic acid ($\mathbf{L}_4$)

Yield 65%; m.p. 180 °C; IR (KBr, cm^{-1}): 3447 (OH), 1705 (COOH), 1610 (azomethine, C═N), 1420 (C–N), 700 (C–S); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.30 (t, 1H, CH), 2.55 (s, 2H, CH_2), 2.77 (t, 2H, CH_2), 2.85 (s, 3H, CH_3), 6.95 (s, 1H, azomethine), 7.26–7.63 (m, 4H, Ph), 10.27 (s, 1H, OH), 11.29 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, δ, ppm): 30.25 (SCH_3), 47.84 (CH_2), 41.86 (CH_2), 77.81 (CH), 115.62, 121.55, 130.46, 130.73, 147.87, 154.91 (*Ph*OH), 150.84 (–CH═N), 181.67 (COOH). Anal. calcd for $C_{16}H_{14}NO_3$ (252.0): C, 57.14; H, 5.56; N, 5.56. Found: C, 57.44; H, 5.28; N, 5.82%.

{[(2-Hydroxyphenyl)methylidene]amino}-4-(methylthio)butanoic acid ($\mathbf{L}_5$)

Yield 58%; m.p. 85 °C; IR (KBr, cm^{-1}): 3442 (OH), 2660 (SH), 1706 (COOH), 1590 (azomethine, C═N), 1490 (C–N), 756

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(C–S); ^{1}H NMR (DMSO-d$_6$, δ, ppm): 2.15 (s, 3H, CH_3), 2.42 (t, 1H, CH), 2.54 (s, 2H, CH_2), 2.83 (t, 2H, CH_2), 6.94 (s, 1H, azomethine), 7.16–7.79 (m, 4H, Ph), 10.27 (s, 1H, OH), 10.72 (s, 1H, SH), 11.29 (s, 1H, COOH). ^{13}C NMR (DMSO-d$_6$, δ, ppm): 48.42 (CH_2), 77.25 (CH), 115.93, 121.58, 130.14, 130.62, 147.85, 155.31 (*Ph*OH), 150.75 (–CH=N), 181.43 (COOH). Anal. calcd for $C_{12}H_{15}NO_3S$ (224.0): C, 53.57; H, 4.46; N, 6.25. Found: C, 53.37; H, 4.81; N, 6.16%.

*Preparation of metal(II) complexes **1–24***

To a magnetically stirred suspension of the respective amino acid derived Schiff base (0.02 mol) in water (20 ml) was added equimolar KOH (0.02 mol). The mixture was stirred for half an hour. Then ethanol (30 ml) solution of the corresponding metal (II) salt (0.01 M) as chloride was added in this mixture and refluxed for 1 h. The obtained solution was filtered and reduced to half of its volume by evaporation of the solvent *in vacuo*. The solid product thus obtained was washed with ethanol (2 × 15 ml) then with ether and dried. Recrystallization from aqueous–ethanol (20 : 80) gave the desired products. Unfortunately only microcrystalline powders could be obtained, which were impossible to be used for X-ray structural determinations.

Zn(II) complex of [(2-hydroxyphenyl)methylidene amino]acetic acid

^{1}H NMR (DMSO-d$_6$, δ, ppm): 2.69 (s, 2H, CH_2), 7.14 (s, 1H, azomethine), 7.47–7.86 (m, 4H, Ph). ^{13}C NMR (DMSO-d$_6$, δ, ppm): 75.52 (CH_2), 115.83, 121.54, 129.86, 130.36, 147.73, 155.31 (*Ph*O), 151.45 (–CH=N), 181.72 (COO).

Zn(II) complex of [(2-Hydroxyphenyl) methylidene amino]propanoic acid

^{1}H NMR (DMSO-d$_6$, δ, ppm): 2.36 (s, 3H, CH_3), 2.53 (t, 1H, CH), 7.36 (s, 1H, azomethine), 7.43–7.78 (m, 4H, Ph). ^{13}C NMR (DMSO-d$_6$, δ, ppm): 21.75 (CH_3), 78.31 (CH), 116.42, 121.64, 130.22, 130.63, 148.24, 155.45 (*Ph*O), 151.36 (–CH=N), 181.87 (COO).

Zn(II) Complex of {[(2-Hydroxyphenyl) methylidene]amino}-3-phenylpropanoic acid

^{1}H NMR (DMSO-d$_6$, δ, ppm): 2.49 (t, 1H, CH), 2.84 (s, 2H, CH_2), 7.38 (s, 1H, azomethine), 7.42–7.87 (m, 9H, Ph). ^{13}C NMR (DMSO-d$_6$, δ, ppm): 41.75 (CH_2), 77.57 (CH), 126.82, 128.61, 130.68, 148.85 (–Ph), 115.86, 121.67, 130.34, 131.21, 148.22, 155.41 (*Ph*O), 151.46 (–CH=N), 182.24 (COO).

Zn(II) complex of {[(2-Hydroxyphenyl) methylidene]amino}-3-mercaptopropanoic acid

^{1}H NMR (DMSO-d$_6$, δ, ppm): 2.61 (t, 1H, CH), 2.73 (s, 2H, CH_2), 2.98 (t, 2H, CH_2), 2.97 (s, 3H, CH_3), 7.47 (s, 1H, azomethine), 7.48–7.87 (m, 4H, Ph). ^{13}C NMR (DMSO-d$_6$, δ, ppm): 30.43 (SCH_3), 47.97 (CH_2), 42.21 (CH_2), 78.35 (CH), 115.75, 121.67, 130.74, 131.12, 148.22, 155.34 (*Ph*O), 151.56 (–CH=N), 182.23 (COO).

Zn(II) complex of {[(2-Hydroxyphenyl) methylidene]amino}-4-(methylthio)butanoic acid

^{1}H NMR (DMSO-d$_6$, δ, ppm): 2.36 (s, 3H, CH_3), 2.67 (t, 1H, CH), 2.87 (s, 2H, CH_2), 3.18 (t, 2H, CH_2), 7.44 (s, 1H, azomethine), 7.35–7.96 (m, 4H, Ph), 10.71 (s, 1H, SH). ^{13}C NMR (DMSO-d$_6$, δ, ppm): 48.56 (CH_2), 77.42 (CH), 116.21, 121.72, 130.48, 130.83, 148.20, 155.58 (*Ph*O), 151.28 (–CH=N), 181.87 (COO).

Biological activity

Antibacterial bioassay (in-vitro)

All the synthesized ligands ($\mathbf{L}_1$–$\mathbf{L}_5$) and their corresponding metal (II) complexes (**1–20**) were screened *in-vitro* for their antibacterial activity against four Gram-negative (*E. coli, S. flexenari, P. aeruginosa* and *S. typhi)* and two Gram-positive (*B. subtilis* and *S. aureus*) bacterial strains using the agar well diffusion method.[26] Two to eight hour-old bacterial inoculums containing approximately 10^4–10^6 colony forming units (CFU)/ml were used in these assays. The wells were dug in the media with a sterile metallic borer with centers at least 24 mm apart. The recommended concentration (100 µl) of the test sample (1 mg/ml in DMSO) was introduced into the respective wells. Other wells supplemented with DMSO and reference antibacterial drug, imipenum served as negative and positive controls respectively. The plates were incubated immediately at 37 °C for 20 h. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was compared[27] with the standard drug. In order to clarify any participating role of DMSO in the biological screening, separate studies were carried out with the solutions of DMSO alone and they showed no activity against any bacterial strains.

Antifungal activity (in-vitro)

Antifungal activities of all compounds were studied against six fungal cultures, *T. longifusus, C. albicans, A. flavus, M. canis, F. solani* and *C. glaberata.* Sabouraud dextrose agar (Oxoid, Hampshire, UK) was seeded with 10^5 (cfu) ml^{-1} fungal spore suspensions and transferred to Petri plates. Disks soaked in 20 ml (10 µg/ml in DMSO) of all compounds were placed at different positions on the agar surface. The plates were incubated at 32 °C for 7 days. The results were recorded as zones of inhibition in mm and compared with standard drugs miconazole and amphotericin B.

Minimum inhibitory concentration

Compounds containing antibacterial activity over 80% were selected for minimum inhibitory concentration (MIC) studies (Table 4). The minimum inhibitory concentration was determined using the disk diffusion technique[28] by preparing disks containing 10, 25, 50 and 100 µg/ml of the compounds and applying the protocol.

Cytotoxicity (in-vitro)

Brine shrimp (*Artemia salina* leach) eggs were hatched in a shallow rectangular plastic dish (22 × 32 cm), filled with

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artificial seawater, which was prepared[27] with commercial salt mixture and double-distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened while the matter compartment was opened to ordinary light. After 2 days nauplii were collected using a pipette from the lighted side. A sample of the test compound was prepared by dissolving 20 mg of each compound in 2 ml of DMF. From this stock solutions 500, 50 and 5 μg/ml were transferred to nine vials (three for each dilutions were used for each test sample and LD_{50} is the mean of three values) and one vial was kept as control having 2 ml of DMF only. The solvent was allowed to evaporate overnight. After two days, when shrimp larvae were ready, 1 ml of seawater and 10 shrimps were added to each vial (30 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.[29]

RESULTS AND DISCUSSION

Chemistry, composition and characterization of the ligands

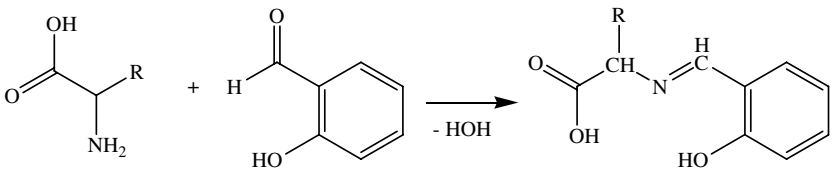
The Schiff bases ($\mathbf{L_1}$–$\mathbf{L_5}$) are stable compounds which were prepared by refluxing an appropriate amount of amino acid with the corresponding salicylaldehyde in methanol, in 1 : 1 molar ratio. The structures (Scheme 1) of the synthesized ligands were established with the help of their IR, NMR and microanalytical data. Certain problems of solubility of the amino acid-derived Schiff bases were encountered due to their reaction with the metal ions, which were resolved by making an equimolar potassium salt of the respective Schiff base ligand and *in-situ* use for the complexation reaction.

Conductance and magnetic susceptibility of the metal (II) complexes

Some physical properties are given in Table 1. All the complexes are intensely colored, air- and moisture-stable amorphous solids which decompose without melting. They are insoluble in common organic solvents and only soluble in water, DMF and DMSO. The molar conductance values (in DMF) fall within the range 173–195 Ω^{-1} cm^2/mol for all complexes, suggesting that these are 2 : 1 electrolytes.[30] The room temperature magnetic moment values of the complexes are given in Table 1. The observed magnetic moment (4.2–4.5 BM) is consistent with half-spin octahedral cobalt (II) complexes. The magnetic moment values (1.7–1.9 BM) measured for the copper (II) complexes lie in the range expected for a d^9-system, which contains one unpaired electron with octahedral geometry.[31] The measured values (3.1–3.4 BM) for the nickel (II) complexes suggest[32] octahedral geometry for these complexes. The zinc (II) complexes were found to be diamagnetic,[33] as expected

IR spectra

The selected IR spectra of the ligands and its metal complexes along with their tentative assignments are reported in the Experimental and in Table 1, respectively. The IR spectra of all the ligands show the absence of bands at 3245 and 1745 cm^{-1} due to the $\nu(HN_2)$ group of amino acids and of ν(HC═O) of salicylaldehyde. Instead, a new band at 1590–1610 cm^{-1} due to azomethine ν(C═N) linkage appeared in all the ligands, indicating[34] that condensation between aldehyde of salicylaldehyde and that of amino group of amino acid has taken place, resulting in the formation of the desired Schiff base ligands ($\mathbf{L_1}$–$\mathbf{L_5}$). Comparison of the IR spectra of the ligands with their metal(II) complexes showed[35] a major shift in azomethine ν(C═N) linkage to lower wavenumbers by 15–20 cm^{-1} and a new band at 1575–1590 cm^{-1}, suggesting[36] involvement of the azomethine-N with the metal ion. Also, disappearance of the stretching vibrations at 3444–3450 and 1700–1708 cm^{-1} assigned to ν(OH) and ν(COOH) and appearance of a new band at 1335–1350 cm^{-1} in turn gave a clue of the deprotonation and coordination of O in ν(C–O) and ν(COO) with the metal atom, respectively. This data overall suggests that in the azomethine-N, deprotonated-O of ν(OH) and deprotonated-O of ν(COOH) groups are involved in coordination[37] with the metal ion in all the metal (II) anionic complexes (**1–20**) in which potassium is present as a counter



L_1: R = H
L_2: R = CH_3
L_3: R = CH_2-C_6H_5
L_4: R = CH_2-CH_2-SCH_3
L_5: R = CH_2-SH

Scheme 1. Reaction used for synthesis of the starting acids ($\mathbf{L_1}$–$\mathbf{L_5}$).

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Table 1. Physical, spectral and analytical data of the metal (II) complexes (**1–20**)

No.	Metal chelate	MP (°C)	Yield (%)	BM (μ_{eff})	IR (cm^{-1})	λ_{max} (cm^{-1})	Calcd (found), %		
							C	H	N
1.	$K_2[Co(\mathbf{L}_1)_2]$ [491.13] $C_{18}H_{14}CoN_2O_6K_2$	218–220	75	4.2	1575 (C═N), 1335 (C–O), 425 (M–O), 390 (M–N)	17553, 21739, 29215	43.98 (44.01)	2.85 (2.91)	5.70 (5.63)
2.	$K_2[Cu(\mathbf{L}_1)_2]$ [495.74] $C_{18}H_{14}CuN_2O_6K_2$	216–218	77	1.7	1580 (C═N), 1350 (C–O), 425 (M–O), 390 (M–N),	15245, 30235	43.57 (43.44)	2.82 (2.52)	5.85 (5.45)
3.	$K_2[Ni(\mathbf{L}_1)_2]$ [490.89] $C_{18}H_{14}NiN_2O_6K_2$	232–234	75	3.1	1585 (C═N), 1345 (C–O), 425 (M–O), 390 (M–N),	12897, 16585, 24490, 30215	44.00 (43.81)	2.85 (2.98)	5.70 (5.99)
4.	$K_2[Zn(\mathbf{L}_1)_2]$ [495.59] $C_{18}H_{14}ZnN_2O_6K_2$	208–210	77	Dia	1590 (C═N), 1340 (C–O), 425 (M–O), 390 (M–N),	28445	43.58 (43.13)	2.82 (2.62)	5.65 (5.11)
5.	$K_2[Co(\mathbf{L}_2)_2]$ [519.13] $C_{20}H_{18}CoN_2O_6K_2$	278–280	76	4.4	1582 (C═N), 1335 (C–O), 425 (M–O), 390 (M–N)	18100, 22325, 28565	46.23 (46.73)	3.47 (3.55)	5.39 (5.13)
6.	$K_2[Cu(\mathbf{L}_2)_2]$ [523.74] $C_{20}H_{18}CuN_2O_6K_2$	260–262	75	1.7	1585 (C═N), 1345 (C–O), 425 (M–O), 390 (M–N)	15795, 30380	45.82 (45.46)	3.44 (3.64)	5.35 (5.12)
7.	$K_2[Ni(\mathbf{L}_2)_2]$ [518.89] $C_{20}H_{18}NiN_2O_6K_2$	242–244	76	3.1	1575 (C═N), 1340 (C–O), 425 (M–O), 390 (M–N)	13233, 16590, 25000, 29815	46.25 (46.17)	3.47 (3.12)	5.40 (5.45)
8.	$K_2[Zn(\mathbf{L}_2)_2]$ [523.59] $C_{20}H_{18}ZnN_2O_6K_2$	234–240	75	Dia	1585 (C═N), 1345 (C–O), 425 (M–O), 390 (M–N)	28680	45.84 (45.48)	3.44 (3.50)	5.35 (5.23)
9.	$K_2[Co(\mathbf{L}_3)_2]$ [671.13] $C_{32}H_{26}CoN_2O_6K_2$	234–236	75	4.5	1580 (C═N), 1350 (C–O), 425 (M–O), 390 (M–N)	17750, 21995, 29210	57.22 (57.23)	3.87 (3.55)	4.17 (4.23)
10.	$K_2[Cu(\mathbf{L}_3)_2]$ [675.74] $C_{32}H_{26}CuN_2O_6K_2$	244–246	77	1.8	1577 (C═N), 1340 (C–O), 425 (M–O), 390 (M–N)	15490, 30355	56.83 (56.47)	3.85 (4.18)	4.14 (4.85)
11.	$K_2[Ni(\mathbf{L}_3)_2]$ [670.89] $C_{32}H_{26}NiN_2O_6K_2$	226–228	75	3.3	1580 (C═N), 1335 (C–O), 425 (M–O), 390 (M–N)	12915, 16585, 24685, 30335	57.24 (57.38)	3.88 (3.34)	4.17 (4.23)
12.	$K_2[Zn(\mathbf{L}_3)_2]$ [675.59] $C_{32}H_{26}ZnN_2O_6K_2$	210–212	78	Dia	1585 (C═N), 1345 (C–O), 425 (M–O), 390 (M–N)	28525	56.84 (56.43)	3.85 (3.97)	4.14 (4.66)
13.	$K_2[Co(\mathbf{L}_4)_2]$ [583.13] $C_{20}H_{18}CoN_2O_6S_2K_2$	216–218	76	4.3	1590 (C═N), 1340 (C–O), 425 (M–O), 390 (M–N)	17855, 21925, 28960	41.16 (41.66)	3.09 (3.53)	4.80 (4.72)
14.	$K_2[Cu(\mathbf{L}_4)_2]$ [587.74] $C_{20}H_{18}CuN_2O_6S_2K_2$	235–237	75	1.9	1587 (C═N), 1345 (C–O), 425 (M–O), 390 (M–N)	15515, 30290	40.83 (40.54)	3.06 (3.13)	4.76 (4.57)
15.	$K_2[Ni(\mathbf{L}_4)_2]$ [582.89] $C_{20}H_{18}NiN_2O_6S_2K_2$	222–224	76	3.2	1580 (C═N), 1350 (C–O), 425 (M–O), 390 (M–N)	13130, 16597, 24880, 30270	41.17 (41.62)	3.09 (3.57)	4.80 (4.66)
16.	$K_2[Zn(\mathbf{L}_4)_2]$ [587.59] $C_{20}H_{18}ZnN_2O_6S_2K_2$	218–220	75	Dia	1585 (C═N), 1340 (C–O), 425 (M–O), 390 (M–N)	29140	40.84 (41.06)	3.06 (3.81)	4.77 (4.98)
17.	$K_2[Co(\mathbf{L}_5)_2]$ [639.13] $C_{24}H_{26}CoN_2O_6S_2K_2$	242–244	76	4.5	2660 (SH), 1575 (C═N), 1335 (C–O), 425 (M–O), 390 (M–N)	17985, 22125, 29175	45.06 (45.68)	4.07 (4.16)	4.38 (4.82)
18.	$K_2[Cu(\mathbf{L}_5)_2]$ [643.74] $C_{24}H_{26}CuN_2O_6S_2K_2$	231–233	75	1.8	2660 (SH), 1585 (C═N), 1350 (C–O), 425 (M–O), 390 (M–N)	15750, 30360	44.74 (44.36)	4.04 (4.56)	4.35 (4.73)
19.	$K_2[Ni(\mathbf{L}_5)_2]$ [638.89] $C_{24}H_{26}NiN_2O_6S_2K_2$	220–222	76	3.4	2660 (SH),1580 (C═N), 1344 (C–O), 425 (M–O), 390 (M–N)	13215, 16575, 24910, 30320	45.08 (45.64)	4.07 (4.38)	4.38 (4.16)
20.	$K_2[Zn(\mathbf{L}_5)_2]$ [643.59] $C_{24}H_{26}ZnN_2O_6S_2K_2$	216–218	75	Dia	2660 (SH), 1590 (C═N), 1340 (C–O), 425 (M–O), 390 (M–N)	29145	44.75 (45.38)	4.04 (4.15)	4.35 (4.62)

ion. Furthermore, stretching vibration due to SH at 2660 cm^{-1} in sulfur containing amino acid derived ligand ($\mathbf{L}_5$) remains unchanged, indicating that the SH group is not involved in coordination. The far IR spectra of the metal complexes (Table 1) exhibited[38] new bands which are not present in the spectra of the ligands. These bands are located at 425 and 390 cm^{-1}, which were assigned to the ν(M–O) of salicyl-O and ν(COO) of amino acid and, ν(M–N) of azomethine nitrogen supporting the bonding of the salicyl-O, amino acid-O and that of azomethine-N atoms to the metal ion.

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According to the above mentioned data, the ligands ($\mathbf{L}_1$–$\mathbf{L}_5$) behave as tridentate towards metal (II) ions, via two deprotonated oxygen atoms, one each of the salicylaldehyde and carboxylic acid moieties and one of the azomethine-N, forming 5- and 6-membered stable chelate rings around the central metal atom thus giving overall constancy to the metal complex.

NMR spectra

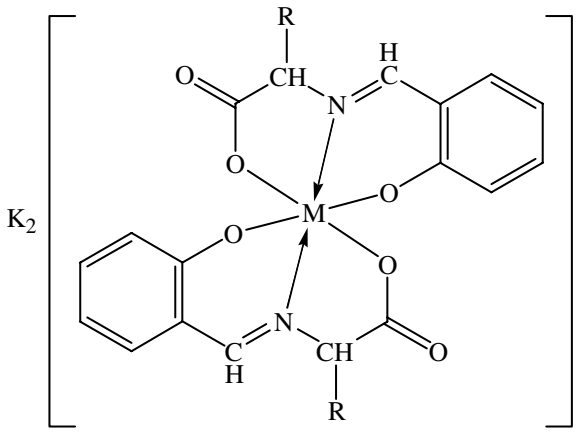
The ^{1}H NMR spectral data are reported along with the possible assignments in experimental. All the protons were found to be in their expected region.[39] The conclusions drawn from these studies lend further support to the mode of bonding discussed in their IR spectra. In the spectra of diamagnetic Zn(II) complexes, these signals shifted downfield due to the increased conjugation and coordination to the metal atoms.[40] The number of protons calculated from the integration curves, and those obtained from the values of the expected CHN analyses, agree with each other. It was observed that DMSO did not have any coordinating effect either on the spectra of the ligands or on its metal complexes.

Electronic spectra

The Co(II) complexes exhibited well-resolved bands at 17 553–18 100 cm^{-1} and a strong high-energy band at 21 739–22 325 cm^{-1} (Table 1) and are assigned[41] to the transitions $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ for a high-spin octahedral geometry. A high-intensity band at 28 565–29 215 cm^{-1} was assigned to the metal to ligand charge transfer. The magnetic susceptibility measurements for the solid Co(II) complexes are also indicative of three unpaired electrons per Co(II) ion, suggesting[42] consistency with their octahedral environment.

The electronic spectra of the Cu(II) complexes (Table 1) showed two low-energy weak bands at 15 245–15 797 cm^{-1} and a strong high-energy band at 30 255–30 420 cm^{-1}. The low-energy band in this position typically is assigned to the transition $^2Eg \rightarrow {}^2T_{2g}$ and expected for an octahedral configuration. The strong high-energy band, in turn, is assigned[43] to metal → ligand charge transfer. Also, the magnetic moment values (1.7–1.9 BM; Table 1) for the copper (II) are indicative of anti-ferromagnetic spin-spin interaction through molecular association.

The electronic spectra of the Ni (II) complexes showed d–d bands in the region 24 490–25 000, 16 585–16 597 and 12 897–13 233 cm^{-1}. These are assigned[42] to the spin-allowed transitions $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$, respectively, consistent with their well-defined octahedral configuration. The band at 29 815–30 335 cm^{-1} was assigned to metal → ligand charge transfer. The magnetic measurements (3.1–3.4 BM) showed two unpaired electrons per Ni (II) ion, also suggesting[41] an octahedral geometry for the Ni (II) (Figure 1) complexes. The Zn (II) complexes exhibited only a high-intensity band at 28 445–29 145 cm^{-1} and is assigned[43] to a ligand–metal charge transfer.



M = Co (II), Cu (II), Ni (II) or Zn (II)

Figure 1. Proposed structure of the metal (II) complex **(1–20)**.

Biological activity

Antibacterial activity

The antimicrobial activity data of all synthesized compounds are summarized in Tables 2 and 3 and show that the newly synthesized compounds ($\mathbf{L}_1$–$\mathbf{L}_5$) and their metal complexes (**1–20**) possess biological activity. These new derivatives obtained by condensation of the amino group of amino acid with salicylaldehyde were screened for their antibacterial activity against *E. coli, B. subtillis, S. flexenari, S. aureus, P. aeruginosa* and *S. typhi.* The antibacterial screening results exhibited marked enhancement in activity on coordination with the metal ions against one or more testing bacterial strains. The zinc complexes showed more activity than other metal complexes. This enhancement in the activity is rationalized on the basis of the structures of the ligands by possessing an additional azomethine (C═N) linkage which is important in elucidating the mechanism of transamination and resamination reaction in biological system.[44,45] It has also been suggested[46–49] that the ligands with nitrogen and oxygen donor systems might inhibit enzyme production, since the enzymes which require these groups for their activity appear to be especially more susceptible to deactivation by the metal ions upon chelation. Chelation reduces the polarity[50–53] of the metal ion, mainly because of the partial sharing of its positive charge with the donor groups and possibly the π-electron delocalization within the whole chelate ring system thus formed during coordination. This process of chelation thus increases the lipophilic nature of the central metal atom, which in turn favors its permeation through the lipoid layer of the membrane.[54–56] This is also responsible for increasing the hydrophobic character and liposolubility of the molecule in crossing the cell membrane of the microorganism and hence enhances the biological utilization ratio and activity of the

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Table 2. Results of antibacterial bioassay (concentration used 1 mg/ml of DMSO)

	Compound (zone of inhibition in mm)																								
Bacteria	L_1	L_2	L_3	L_4	L_5	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	SD[a]
Gram-negative																										
(a)	15	16	17	15	16	17	17	19	19	20	18	20	20	23	19	20	22	20	16	16	21	19	18	19	22	30
(b)	09	07	09	06	08	10	11	11	12	10	10	11	11	10	12	11	14	10	11	11	12	09	10	10	11	27
(c)	15	17	18	14	16	18	15	18	19	17	16	19	20	20	18	19	21	17	17	18	20	20	18	16	21	26
(d)	11	15	17	14	17	18	17	8	20	20	17	18	21	20	20	19	22	19	17	18	20	19	18	20	22	27
Gram-positive																										
(e)	17	15	16	17	17	18	18	18	19	19	19	20	21	18	19	19	21	19	17	18	20	18	19	19	20	30
(f)	17	17	18	14	15	17	18	19	20	19	19	19	20	19	20	19	21	19	18	17	20	20	19	19	22	28

(a) = *E. coli*, (b) = *S. flexenari*, (c) = *P. aeruginosa*, (d) = *S. typhi*, (e) = *S. aureus*, (f) = *B. subtilis.* >10: weak; >10: moderate; >16: significant.
[a] SD = standard drug (imipenum).

Table 3. Results of antifungal bioassay (concentration used 200 μg/ml)

	Compound (% inhibition)																								
Organism	L_1	L_2	L_3	L_4	L_5	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	SD[a]
(a)	00	00	00	20	00	00	00	00	00	00	18	00	00	00	20	00	24	00	25	00	05	00	00	00	27	A
(b)	00	07	00	00	00	00	00	00	00	00	20	00	00	00	00	00	00	00	20	00	00	00	00	00	00	B
(c)	15	00	00	00	00	00	00	00	18	00	20	00	00	00	10	00	19	00	00	00	28	00	00	00	00	C
(d)	10	00	00	15	00	00	14	00	21	00	00	00	20	00	00	00	00	00	35	00	30	00	00	00	30	D
(e)	00	15	00	00	00	00	00	00	21	00	20	00	00	27	00	00	00	00	25	00	00	00	00	00	00	E
(f)	00	00	00	25	00	00	00	00	00	00	26	00	00	00	00	28	00	00	28	00	28	00	00	00	00	F

(a) = *T. longifucus*, (b) = *C. Albicans*, (c) = *A. flavus*, (d) = *M. canis*, (e) = *F. Solani*, (f) = *C. glaberata*. SD = standard drugs MIC μg/ml; A = miconazole (70 μg/ml: 1.6822×10^{-7} M), B = miconazole (110.8 μg/ml: 2.6626×10^{-7} M), C = amphotericin B (20 μg/ml: 2.1642×10^{-8} M), D = miconazole (98.4 μg/ml: 2.3647×10^{-7} M), E = miconazole (73.25 μg/ml: 1.7603×10^{-7} M), F = miconazole (110.8 μg/ml: 2.66266×10^{-7} M).

testing drug/compound. Certain metal complexes, however, did not show much enhancement in activity as compared with the uncomplexed ligands, which may be explained by the extent of their solubility which is directly proportional to the permeability through the lipoid layer of the micro-organism.

Antifungal activity

All the synthesized compounds were screened for antifungal activity against six fungi, *T. longifusus, C. albicans, A. flavus, M. canis, F. solani* and *C. glaberata.* The findings indicated (Table 3) that most of the tested compounds were found to be inactive. The present investigations suggest that metal complexation does play a role in enhancing the activity. On the basis of these studies, it is established that those ligands which showed week and/or moderate activity, on complexation/coordination, displayed enhanced antifungal profile. Amongst the series, compounds **14, 16** and **20** proved to be the most active members.

Minimum inhibitory concentration

The minimum inhibitory concentration was determined for two compounds (**12** and **20**) against *P. aeruginosa* and *S. typhi*. These two compounds primarily showed antibacterial activity more than 80%; therefore these were selected for MIC. The results (Table 4) indicated that compound **20** proved to be the most active against the organisms tested at the concentrations used.

Table 4. Results of minimum inhibitory concentration (M) of the selected compounds (**12**) and (20) against selected bacteria

No.	**12**	**20**
P. aeruginosa	8.342×10^{-8}	3.916×10^{-8}
S. typhi	4.171×10^{-8}	3.916×10^{-8}

Cytotoxic bioassay

All the synthesized compounds were screened for their cytotoxicity (brine shrimp bioassay) using the protocol of Meyer *et al*.[28] From the data recorded in Table 5, it is evident that only three compounds, **2, 11** and **17**, displayed potent cytotoxic activity, $LD_{50} = 8.196 \times 10^{-4}$, 7.315×10^{-4} and 5.599×10^{-4} M/ml, respectively, against *Artemia salina*, while all other compounds were almost inactive for this assay.

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Table 5. Brine shrimp bioassay data of the ligands (L_1–L_5) and their metal (II) complexes (**1–20**)

Compound	LD_{50} (M/ml)
L_1	$>5.618 \times 10^{-3}$
L_2	$>5.208 \times 10^{-3}$
L_3	$>3.731 \times 10^{-3}$
L_4	$>3.968 \times 10^{-3}$
L_5	$>4.464 \times 10^{-3}$
1	$>1.946 \times 10^{-3}$
2	8.196×10^{-4}
3	$>1.947 \times 10^{-3}$
4	$>1.929 \times 10^{-3}$
5	$>2.058 \times 10^{-3}$
6	$>2.038 \times 10^{-3}$
7	$>2.059 \times 10^{-3}$
8	$>2.039 \times 10^{-3}$
9	$>1.681 \times 10^{-3}$
10	$>1.668 \times 10^{-3}$
11	7.315×10^{-4}
12	$>1.668 \times 10^{-3}$
13	$>1.730 \times 10^{-3}$
14	$>1.717 \times 10^{-3}$
15	$>1.731 \times 10^{-3}$
16	$>1.717 \times 10^{-3}$
17	5.599×10^{-4}
18	$>1.566 \times 10^{-3}$
19	$>1.578 \times 10^{-3}$
20	$>1.566 \times 10^{-3}$

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