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Antimicrobial metal-based thiophene derived compounds

Shakeela Yasmeen^a, Sajjad Hussain Sumrra^b, Muhammad Safwan Akram^c and Zahid H. Chohan^d

^aInstitute of Chemical Sciences, Bahauddin Zakariya University, Multan, Pakistan; ^bDepartment of Chemistry, University of Gujrat, Gujrat, Pakistan; ^cSchool of Science & Engineering, Teesside University, Middlesbrough, UK; ^dDepartment of Chemistry, University of Sargodha, Bhakkar Campus, Sargodha, Pakistan

ABSTRACT

A novel series of thiophene derived Schiff bases and their transition metal- [Co(II), Cu(II), Zn(II), Ni(II)] based compounds are reported. The Schiff bases act as tridentate ligands toward metal ions via azomethine-N, deprotonated-N of ammine substituents and S-atom of thienyl moiety. The synthesized ligands along with their metal complexes were screened for their *in vitro* antibacterial activity against six bacterial pathogens (*Escherichia coli*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Bacillus subtilis*) and for antifungal activity against six fungal pathogens (*Trichophytonlongifusum*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *Candida glabrata*). The results of antimicrobial studies revealed the free ligands to possess potential activity which significantly increased upon chelation.

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Introduction

The importance and application of Schiff bases has attracted an enormous research interests in the synthesis of metal-based compounds^{1–3}. Many Schiff bases have been reported⁴ to possess significant biological properties such as anti-microbial⁵, anti-bacterial⁶, anti-fungal⁷, anti-tumor⁸, anti-inflammatory⁹, anti-HIV¹⁰, anti-convulsant¹¹, anti-cancer^{12,13}, anti-malarial¹⁴, antiviral¹⁵ and as an analgesic¹⁶. Due to significant structural properties and applications of metals-based compounds in bioactivities, we wish to report a new class of antibacterial and antifungal thiophene derived Schiff bases, *N*-[(1*E*)-1-(thiophen-2-yl)ethylidene]ethane-1,2-diamine (**L**¹), *N*-[(1*E*)-1-(thiophen-2-yl) ethylidene]propane-1,3-diamine (**L**²) and *N*-[(1*E*)-1-(thiophen-2-yl) ethylidene]butane-1,4-diamine (**L**³) (Scheme 1) and their Co(II), Cu(II), Ni(II) and Zn(II) metal chelates (**1**)–(**12**) (Scheme 2). The synthesized Schiff bases and their metal chelates have been screened for their bactericidal/fungicidal activity against a number of bacterial/fungal strains and results have been reported.

Experimental

Materials and methods

All the chemicals used were of analytical grades and purchased from Sigma Aldrich and were used without further purification. All ligand synthetic reactions were carried out in solvents that were purified and dried before use, using standard literature methods. Gallenkamp melting point apparatus was used for recording melting points. The infrared absorption spectra were recorded as KBr pellets on Excalibur FTR-IR 4800 MX spectrophotometer (Bio-Rad Laboratories, Cambridge, MA) in the frequency range 200–4000 cm⁻¹. Elemental analysis was carried out on Perkin Elmer. ¹H and ¹³C NMR spectra were recorded on a Bruker Spectrospin Avance 400 DPX spectrometer using TMS as internal standard and d₆-DMSO as a solvent. Electron impact mass spectra



(EIMS) were recorded on JEOL MS Route instrument. UV–Vis spectra were recorded on ultraviolet spectrometer-1700 in the frequency range of 250–800 nm. Conductivity meter Jen way model 70 was used for measuring the conductivity of metal complexes using 0.001 molar solutions in DMSO at room temperature. A Stanton SM12/S Gouy balance was used to measure the magnetic susceptibility of the metal complexes at room temperature using mercury acetate ligand as a standard. *In vitro* antibacterial and antifungal properties were studied at HEJ Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Pakistan.

Synthesis of ligands

1-(Thiophene-2-yl)ethanone (0.002 M, 0.25 g) dissolved in ethanol (20 mL) was added to a stirred solution of ethane-1,2-diamine/propane-1,3-diamine/butane-1,4-diamine respectively in ethanol (15 mL) in an equimolar ratio followed by 2–3 drops of acetic acid. The reaction mixture was stirred for 8 h with continuous monitoring through TLC and then kept overnight at room temperature. The solid crystallized product was obtained which was filtered, washed with ethanol and dried. It was recrystallized in a mixture of hot ethanol:acetone (1:1).

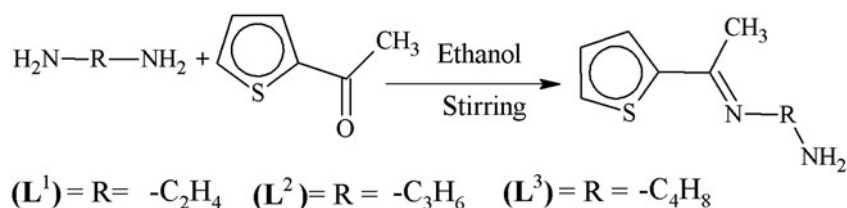
N-[(1*E*)-1-(thiophen-2-yl)ethylidene]ethane-1,2-diamine (**L**¹)

Yield: (1.63 g, 64%); mp 140 °C; color (off white); IR (KBr, cm⁻¹): 3365 (NH₂), 1683 (C=N), 1607 (C=C), 1065 (C–N) and 882 (C–S); ¹H NMR (ppm d₆-DMSO): δ 2.35 (s, CH₃), 3.06 (t, 2H), 3.71 (t, 2H), 5.75 (s, NH₂), 7.05 (d, 1H, *J* = 8.5 Hz), 7.41 (dd, 1H, *J* = 8.5, 2.6 Hz), 7.60 (d, 1H, *J* = 2.6 Hz); ¹³C NMR (DMSO-d₆): δ 14.90 (CH₃), 41.54, 54.18, 123.52, 130.93, 135.18, 137.32 and 156.15; mass spectrum (ESI): [M]⁺ = 168.1. Anal. Calc. for C₈H₁₂N₂S (168.26): C, 57.11; H, 7.19; N, 16.65; S, 19.06. Found: C, 57.05; H 7.15; N, 16.60; S, 19.01.

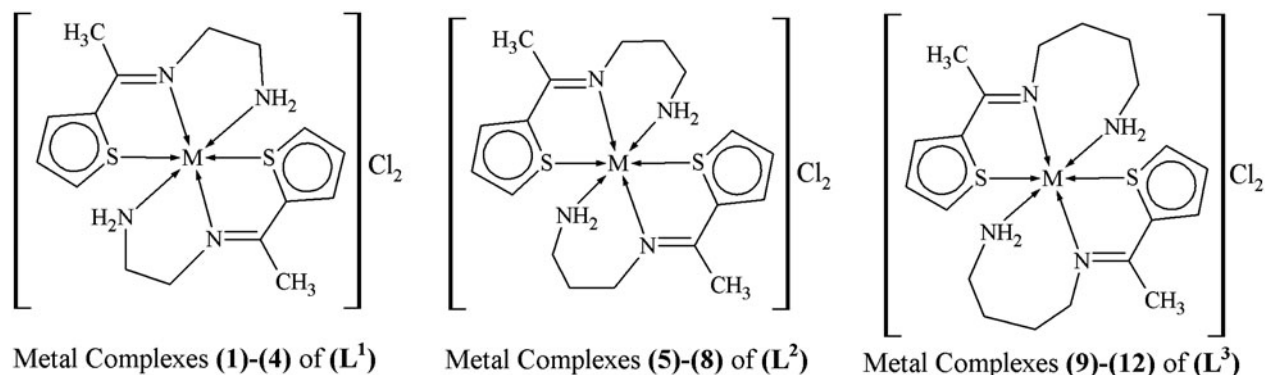
CONTACT Zahid H. Chohan  dr.zahidchohan@gmail.com  Department of Chemistry, University of Sargodha, Bhakkar Campus, Sargodha, Pakistan

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Scheme 1: Preparation of Schiff bases.



Scheme 2: Co(II), Ni(II), Cu(II) and Zn(II) metal complexes (1)–(12) with (L^1) – (L^3) .

N-[(1E)-1-(thiophen-2-yl)ethylidene]propane-1,2-diamine (L^2)

Yield (1.55 g, 57%); mp 160 °C; color (off white); IR (KBr, cm^{-1}): 3348 (NH_2), 1655 (C=N), 1634 (C=C), 1063 (C–N) and 887 (C–S); 1H NMR (ppm d_6 -DMSO): δ 2.31 (s, CH_3), 2.55 (m, 2H), 3.01 (t, 2H), 3.65 (t, 2H), 5.70 (s, NH_2), 7.02 (d, 1H, $J = 8.5$ Hz), 7.38 (dd, 1H, $J = 8.5, 2.6$ Hz), 7.55 (d, 1H, $J = 2.6$ Hz); ^{13}C NMR (DMSO- d_6): δ 14.87 (CH_3), 40.54, 45.31, 55.19, 122.95, 130.22, 134.41, 136.54 and 155.86; mass spectrum (ESI): $[M]^+ = 182.2$. Anal. Calc. for $C_9H_{14}N_2S$ (182.29): C, 59.30; H, 7.74; N, 15.37; S, 17.59. Found: C, 59.23; H 7.70; N, 15.32; S, 17.55.

N-[(1E)-1-(thiophen-2-yl)ethylidene]butane-1,2-diamine (L^3)

Yield (2.64 g, 74%); mp: 98 °C, color (off white); IR (KBr, cm^{-1}): 3387 (NH_2), 1669 (C=N), 1641 (C=C), 1062 (C–N) and 889 (C–S); 1H NMR (ppm d_6 -DMSO): δ 2.33 (s, CH_3), 2.46 (m, 2H), 2.65 (m, 2H), 3.03 (t, 2H), 3.70 (t, 2H), 5.75 (s, NH_2), 7.04 (d, 1H, $J = 8.5$ Hz), 7.40 (dd, 1H, $J = 8.5, 2.6$ Hz), 7.58 (d, 1H, $J = 2.6$ Hz); ^{13}C NMR (DMSO- d_6): δ 14.90 (CH_3), 33.67, 35.25, 41.54, 54.18, 123.45, 130.19, 134.98, 137.18 and 156.09; mass spectrum (ESI): $[M]^+ = 196.1$. Anal. Calc. for $C_{10}H_{16}N_2S$ (196.31): C, 61.18; H, 8.21; N, 14.27; S, 16.33. Found: C, 61.11; H, 8.17; N, 14.21; S, 16.29.

Synthesis of metal(II) complexes

All metal complexes were prepared according to the standard procedure in which ethanol solution (15 mL) of the respective metal(II) salt (5 mmol) was added respectively into a refluxed ethanol solution (30 mL) of the Schiff base ligand (10 mmol). The mixture was refluxed for 3 h, during which a precipitated product was formed. It was then cooled to room temperature, filtered, washed with hot ethanol and finally with diethyl ether. The precipitated product thus obtained was dried and crystallized in a mixture of hot aqueous methanol (1:2) to obtain TLC pure product.

NMR data of the Zn(II) complexes

[Zn(L^1) $_2$] (4): 1H NMR (DMSO- d_6 , δ , ppm): 2.42 (s, CH_3), 3.16 (t, 2H), 3.82 (t, 2H), 5.89 (s, NH_2), 7.15 (d, 1H, $J = 8.5$ Hz), 7.48 (dd, 1H, $J = 8.5, 2.6$ Hz), 7.75 (d, 1H, $J = 2.6$ Hz); ^{13}C NMR (DMSO- d_6): δ 14.98 (CH_3), 42.26, 55.05, 124.64, 131.65, 135.89, 137.96 and 157.25.

[Zn(L^2) $_2$] (8): 1H NMR (DMSO- d_6 , δ , ppm): 2.38 (s, CH_3), 2.70 (m, 2H), 3.28 (t, 2H), 3.83 (t, 2H), 5.99 (s, NH_2), 7.28 (d, 1H, $J = 8.5$ Hz), 7.56 (dd, 1H, $J = 8.5, 2.6$ Hz), 7.76 (d, 1H, $J = 2.6$ Hz); ^{13}C NMR (DMSO- d_6): δ 14.98 (CH_3), 41.82, 46.26, 56.15, 123.89, 130.22, 134.41, 136.54 and 157.16.

[Zn(L^3) $_2$] (12): 1H NMR (DMSO- d_6 , δ , ppm): 2.42 (s, CH_3), 2.52 (m, 2H), 2.70 (m, 2H), 3.15 (t, 2H), 3.84 (t, 2H), 5.90 (s, NH_2), 7.17 (d, 1H, $J = 8.5$ Hz), 7.51 (dd, 1H, $J = 8.5, 2.6$ Hz), 7.73 (d, 1H, $J = 2.6$ Hz); ^{13}C NMR (DMSO- d_6): δ 15.39 (CH_3), 33.76, 35.33, 42.6254, 55.11, 124.55, 130.93, 135.63, 137.74 and 157.14.

Biological activity

Antibacterial activity (in vitro)

Agar-well diffusion method¹⁷ was used to test antimicrobial activity and toxicity of all the newly synthesized acetyl thiophene derived Schiff bases (L^1)– (L^3) and their resulting metal (II) complexes (1)–(12) against four gram-negative (*E. coli*, *S. flexneri*, *P. aeruginosa* and *S. typhi*) and two gram-positive (*S. aureus*, *B. subtilis*) bacterial strains (Figure 1). The wells (6 mm in diameter) were dug in the media with the help of a sterile metallic borer with centers at least 24 mm apart. Bacterial inoculums after 6–8 h of growth approximately having 10⁴–10⁶ colony forming units (CFU)/mL were used. The recommended concentration of the test sample (1 mg/mL in DMSO) was introduced in the respective wells. Other wells supplemented with DMSO and reference antibacterial drug (imipenem) served as a negative and positive control, respectively. The plates were incubated at 37 °C for 24 h. The activity was determined by the measurement of the diameter of zones which showed complete inhibition (mm). Separate studies of DMSO were carried out to rule out any role of DMSO in antibacterial activities but expectedly it showed no activity against any of the bacterial strains used in this study.

Antifungal activity (in vitro)

Antifungal activities of all compounds were studied¹⁷ against six fungal strains (*T. longifusus*, *C. albicans*, *A. flavus*, *M. canis*, *F. solani*

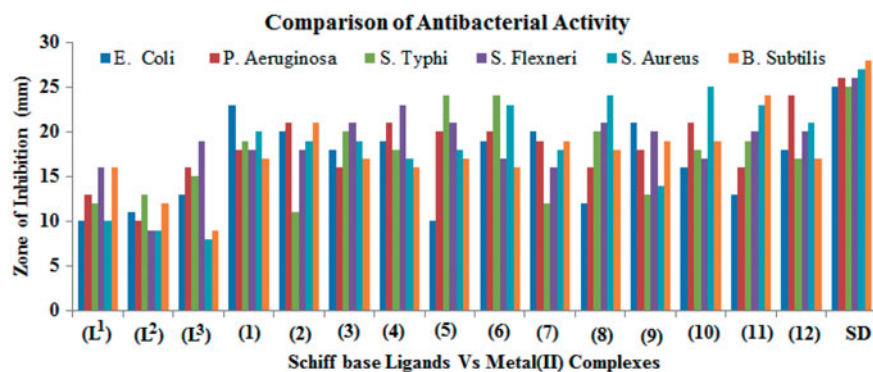


Figure 1. Comparison of antibacterial activity of Schiff bases versus metal(II) complexes.

and *C. glabrata*). Sabouraud dextrose agar (Oxoid, Hampshire, England) was seeded with 10^5 (cfu) mL^{-1} fungal spore suspensions and transferred to petri plates. Discs soaked in 20 mL (200 $\mu\text{g}/\text{mL}$ in DMSO) of the compounds were placed at different positions on the agar surface. The plates were incubated at 32°C for 7 d. The results were recorded as a percentage of inhibition and compared with standard drugs miconazole and amphotericin B.

Minimum inhibitory concentration (MIC)

Compounds showing promising antibacterial (>80%) activity were selected for minimum inhibitory concentration (MIC) studies¹⁸. Disc diffusion method was applied to determine minimum inhibitory concentration by preparing discs containing 10, 25, 50 and 100 μg mL^{-1} concentrations of the compounds along with standards at the same concentrations.

Results and discussion

Chemistry

The condensation reaction of 1-(thiophene-2-yl) ethanone with ethane-1,2-diamine, propane-1,3-diamine and butane-1,4-diamine yielded three Schiff bases (**L**¹)–(**L**³) (Scheme 1) in an equimolar ratio. The synthesized ligands were stable in air and moisture. They were colorless crystalline solids showing their melting points in the range of 98 – 160°C . All of them were soluble in methanol, ethanol, dioxane, DMSO and DMF. These ligands exhibited their tridentate character and reacted readily with Co(II), Cu(II), Ni(II) and Zn(II) metals as their in ethanol to form their respective metal(II) complexes (Scheme 2).

All the synthesized metal(II) complexes were microcrystalline in nature and were intensely colored except the Zn(II) complexes, which were off-white in color. The metal(II) complexes decomposed without melting and were soluble in ethanol, methanol, DMSO and DMF. The analytical data of the complexes indicated a 1:2 (metal: ligand) stoichiometry. The spectral data as well as elemental analysis of the synthesized ligands and their metal(II) complexes were in good agreement with their structure, indicating the high purity of all the compounds.

IR spectra

The IR spectra of Schiff bases reveal presence of the active donor sites like azomethine-N ($\text{C}=\text{N}$), aliphatic ammine ($\text{N}-\text{H}_2$) and C–S moieties which are responsible for chelation with the transition metal atoms. The Schiff bases (**L**¹)–(**L**³) possessed typical

azomethine ($\text{C}=\text{N}$) stretching¹⁹ at 1655 – 1683 cm^{-1} , hence confirming production of the required product. One of each amino group remained unchanged during condensation reaction and their bands were also observed at 3348 – 3387 cm^{-1} , respectively, as they did not take part in the reaction. All the ligands showed C–N and C–S stretching²⁰ at 1062 – 1065 and 882 – 889 cm^{-1} , respectively. The IR spectral comparison of the Schiff bases (**L**¹)–(**L**³) with their metal(II) complexes (**1**)–(**12**) indicated that the Schiff bases were principally coordinated to the metal(II) ions in a tridentate manner. The vibrations of azomethine group in all the metal complexes (**1**)–(**12**) shifted to lower frequency (10 – 20 cm^{-1}) at 1638 – 1668 cm^{-1} demonstrating the coordination of the azomethine nitrogen²¹ with the metal(II) atoms. Shifting of ($\text{N}-\text{H}_2$), C–N and C–S vibrations at 3348 – 3387 , 1062 – 1065 and 882 – 889 cm^{-1} to 3330 – 3375 , 1040 – 1045 and 868 – 877 cm^{-1} , respectively, supported the coordination of ligands with metal atoms. Appearance of the new bands at 543 – 580 and 460 – 471 cm^{-1} could be assigned²² to $\nu(\text{M}-\text{N})$ and $\nu(\text{M}-\text{S})$ vibrations in their metal complexes, which were actually absent in their ligands also confirmed the mode of chelation. Therefore, these clues supported the evidence of the participation of azomethine-N, thienyl-S in the coordination. This data provides good evidence for the coordination of the metal(II) ions to the synthesized Schiff base ligands.

¹H NMR spectra

¹H NMR spectra of the Schiff bases (**L**¹)–(**L**³) and their diamagnetic Zn(II) complexes were recorded in DMSO- d_6 and spectral data is provided in the experimental section. The ¹H NMR spectra of Schiff bases displayed²³ characteristic amino (NH_2) protons at 5.70 – 5.75 ppm as a singlet which provided evidence for the condensation of only one amino group of diamines with acetyl thiophene. All of the ligands (**L**¹)–(**L**³) showed methyl (CH_3) protons at 2.31 – 2.35 ppm as a singlet and thienyl protons at 7.02 – 7.60 ppm as a doublet and double of doublet. The methylene (CH_2) protons of ligand (**L**¹) were observed at 3.06 – 3.71 ppm as a triplet, propylene protons of ligand (**L**²) were found at 2.55 – 3.65 ppm as a triplet and multiplet. Similarly, butylene protons of ligand (**L**³) appeared at 2.46 – 3.70 ppm as a triplet and multiplet. The complexation of the azomethine ($\text{C}=\text{N}$) nitrogen was proven by the downfield shifting of the methyl protons in their Zn(II) complexes, which could be attributed to the discharging of electronic cloud toward the Zn(II) ion. All protons underwent downfield shift by 0.06 – 0.18 ppm due to the increased conjugation on coordination with the zinc metal atom. Largely, the number of protons calculated from the integration curves²⁴ and obtained values of the CHN analysis agreed were found to be in agreement.

¹³C NMR spectra

The ¹³CNMR spectra of the Schiff bases and their diamagnetic Zn(II) complexes were recorded in DMSO-d₆ and data is reported along with their possible assignments in the experimental section and all the carbons were found in their expected regions. The ¹³CNMR spectra of the Schiff bases (**L**¹)–(**L**³) displayed methyl carbons at 14.87–14.90 ppm. Similarly, carbons of ethylene, propylene and butylene were found at 33.67–55.19. However, the distinctive azomethine (C=N) carbons were observed at 155.86–156.15 ppm. The thienyl carbons of hetero-aromatic rings were observed at 122.95–137.32 ppm. Downfield shifting of the azomethine carbons were found in the uncomplexed Schiff bases from 155.86–156.15 to 157.14–157.25 ppm in their Zn(II) complexes, which can be attributed to the shifting of electronic density toward the Zn(II) ion. Similarly, all carbons of methylene, ethylene, propylene, butylene groups and aromatic rings being near to the coordination sites also showed downfield shifting by 0.6–1.3 ppm due to the increased conjugation and coordination with the metal atoms. The downfield shifting also confirmed the coordination of the azomethine to the zinc metal atom. Moreover, the presence of the number of carbons is well in agreement with the expected values from the literature²⁴. Furthermore, the conclusions drawn from these studies present further support to the modes of bonding discussed in their IR and ¹H NMR spectra.

Mass spectra

The mass fragmentation pattern of the ligands (**L**¹)–(**L**³) followed the cleavage of C=N, C–N, N–H, C–C, C=C and C–S bonds. The mass spectral data and the most stable fragmentation values of the ligands were depicted in the experimental section. All the ligands showed pronounced molecular ion peaks. The mass spectra of ligand (**L**¹) displayed a molecular ion peak at *m/z* 168.1

(calc. 168.26) of [C₈H₁₂N₂S]^{•+}, which loses a methyl group (CH₃) as a radical to give the most stable fragment at *m/z* 153 of [C₇H₉N₂S]⁺. Similarly, the molecular ion peak of ligand (**L**²) was observed at *m/z* 182.2 (calc. 182.29) of fragment [C₉H₁₄N₂S]^{•+} and most stable fragment [C₈H₁₁N₂S]⁺ was found at *m/z* 167. In the same way, the molecular ion peak [C₁₀H₁₆N₂S]^{•+} of ligand (**L**³) appeared at 196.2 (calc. 196.31) and its base peak [C₉H₁₃N₂S]⁺ was observed at *m/z* 181. The mass spectral data of the Schiff bases strongly confirmed the best possible structure of ligands and also their bonding pattern.

Molar conductances and magnetic measurements

The molar conductance of the metal complexes was studied when dissolved in DMSO. The data of molar conductance was (86.8–65.2 ohm⁻¹ cm² mol⁻¹) found to be in the range which indicated the electrolytic²⁵ nature of the complexes. The magnetic moment (B.M.) values of all the metal(II) complexes (**1**)–(**12**) were measured at room temperature. The Co(II), Ni(II) and Cu(II) complexes were found to be paramagnetic and their magnetic susceptibilities were 4.2–4.5, 3.1–3.4 and 2.2–2.5 B.M., respectively, thus indicative of one, two and three unpaired electron suggesting octahedral^{26,27} geometry. All the Zn(II) complexes were found to be diamagnetic²⁸ in nature (Table 1).

Electronic spectra

The electronic spectral values of the metal(II) complexes are recorded in Table 2. The electronic spectra of Co(II) complexes displayed well resolved absorption bands in the region 7590–7645, 17 490–17 642 and 23 134–23 252 cm⁻¹ which may be assigned to ⁴T_{1g} → ⁴T_{2g}(F), ⁴T_{1g} → ⁴A_{2g}(F) and ⁴T_{1g} → ⁴T_g(P) transitions, respectively²⁹. A high-energy band at 29 858–30 030 cm⁻¹ was due to metal → ligand charge transfer. These bands suggest

Table 1. Physical measurements and analytical data of metal(II) complexes (1)–(12).

No.	Structure	Yield (%)	MW/formula	M.P. (°C)	Elemental analysis (%) Calc. (found)			
					C	H	N	M
(1)	[Co(L ¹) ₂]	75	[466.36] C ₁₆ H ₂₄ N ₄ S ₂ Cl ₂ Co	311–312	41.21 (41.14)	5.19 (5.16)	12.01 (11.96)	12.64 (12.60)
(2)	[Ni(L ¹) ₂]	72	[466.12] C ₁₆ H ₂₄ N ₄ S ₂ Cl ₂ Ni	315–316	41.23 (41.17)	5.19 (5.15)	12.01 (11.97)	12.59 (12.55)
(3)	[Cu(L ¹) ₂]	68	[470.97] C ₁₆ H ₂₄ N ₄ S ₂ Cl ₂ Cu	300–301	40.80 (40.74)	5.14 (5.11)	11.90 (11.85)	13.49 (13.44)
(4)	[Zn(L ¹) ₂]	65	[472.81] C ₁₆ H ₂₄ N ₄ S ₂ Cl ₂ Zn	305–306	40.64 (40.58)	5.12 (5.09)	11.85 (11.81)	13.56 (13.50)
(5)	[Co(L ²) ₂]	69	[494.41] C ₁₈ H ₂₈ N ₄ S ₂ Cl ₂ Co	309–311	43.73 (43.66)	5.71 (5.68)	11.33 (11.27)	11.92 (11.88)
(6)	[Ni(L ²) ₂]	70	[494.17] C ₁₈ H ₂₈ N ₄ S ₂ Cl ₂ Ni	305–307	43.75 (43.70)	5.71 (5.66)	11.34 (11.30)	11.88 (11.82)
(7)	[Cu(L ²) ₂]	75	[499.02] C ₁₈ H ₂₈ N ₄ S ₂ Cl ₂ Cu	321–322	43.32 (43.28)	5.66 (5.63)	11.23 (11.19)	12.73 (12.68)
(8)	[Zn(L ²) ₂]	72	[500.87] C ₁₈ H ₂₈ N ₄ S ₂ Cl ₂ Zn	319–321	43.16 (43.08)	5.63 (5.60)	11.19 (11.15)	12.80 (11.75)
(9)	[Co(L ³) ₂]	78	[522.46] C ₂₀ H ₃₂ N ₄ S ₂ Cl ₂ Co	305–306	45.98 (45.91)	6.17 (6.13)	10.72 (10.68)	11.28 (11.23)
(10)	[Ni(L ³) ₂]	68	[522.22] C ₂₀ H ₃₂ N ₄ S ₂ Cl ₂ Ni	325–326	46.00 (46.94)	6.18 (6.15)	10.73 (10.68)	11.24 (11.18)
(11)	[Cu(L ³) ₂]	65	[527.08] C ₂₀ H ₃₂ N ₄ S ₂ Cl ₂ Cu	329–331	45.57 (45.51)	6.12 (6.08)	10.63 (10.58)	12.06 (12.02)
(12)	[Zn(L ³) ₂]	71	[528.92] C ₂₀ H ₃₂ N ₄ S ₂ Cl ₂ Zn	327–329	45.42 (45.35)	6.10 (6.06)	10.59 (10.55)	12.36 (12.30)

Table 2. Conductivity, magnetic and spectral data of metal(II) complexes (1)–(12).

No.	Ω _M (Ω ⁻¹ cm ² mol ⁻¹)	B.M. μ _{eff}	λ _m (cm ⁻¹)	IR (cm ⁻¹)
(1)	88.3	4.2	7622, 17 490, 23 134, 29 858	3348 (NH ₂), 1663 (C=N), 1045 (C–N), 868 (C–S), 574 (M–N), 462 (M–S)
(2)	88.9	3.1	9875, 15 263, 25 743, 29 586	3350 (N–H), 1665 (C=N), 1044 (C–N), 870 (C–S), 565 (M–N), 460 (M–S)
(3)	92.7	2.2	15 713, 19 415, 29 967	3352 (NH ₂), 1666 (C=N), 1045 (C–N), 869 (C–S), 547 (M–N), 468 (M–S)
(4)	87.4	Dia	29 926	3355 (NH ₂), 1668 (C=N), 1043 (C–N), 872 (C–S), 548 (M–N), 470 (M–S)
(5)	93.1	4.3	7590, 17 587, 23 252, 30 030	3330 (NH ₂), 1645 (C=N), 1043 (C–N), 875 (C–S), 576 (M–N), 466 (M–S)
(6)	86.8	3.2	9939, 15 379, 25 865, 29 690	3336 (NH ₂), 1642 (C=N), 1045 (C–N), 874 (C–S), 561 (M–N), 465 (M–S)
(7)	92.3	2.4	15 681, 19 617, 29 935	3332 (NH ₂), 1638 (C=N), 1044 (C–N), 873 (C–S), 573 (M–N), 463 (M–S)
(8)	87.0	Dia	29 898	3335 (NH ₂), 1640 (C=N), 1045 (C–N), 871 (C–S), 546 (M–N), 470 (M–S)
(9)	91.2	4.5	7645, 17 642, 23 162, 29 961	3374 (NH ₂), 1649 (C=N), 1040 (C–N), 877 (C–S), 580 (M–N), 469 (M–S)
(10)	95.2	3.4	9914, 15 290, 25 812, 29 635	3375 (NH ₂), 1656 (C=N), 1041 (C–N), 876 (C–S), 568 (M–N), 463 (M–S)
(11)	90.5	2.5	15 802, 19 585, 29 892	3370 (NH ₂), 1652 (C=N), 1042 (C–N), 875 (C–S), 548 (M–N), 468 (M–S)
(12)	94.8	Dia	29 902	3372 (NH ₂), 1654 (C=N), 1043 (C–N), 874 (C–S), 543 (M–N), 471 (M–S)

presence of a high-spin octahedral geometry around the Co(II) ion. The electronic spectral data of Ni(II) complexes exhibited³⁰ the d-d bands in the region of 9875–9939, 15 263–15 379 and 25 743–25 865 cm⁻¹, which could be assigned to the spin allowed transitions of ³A_{2g}(F) → ³T_{2g}(F), ³A_{2g}(F) → ³T_{1g}(F) and ³A_{2g}(F) → ³T_{1g}(P), respectively, consistent with the octahedral geometry. A high-intensity band was also observed at 29 585–29 690 cm⁻¹ due to metal → ligand charge transfer. The spectra of Cu(II) complexes demonstrated two weaker bands at 15 681–15 802 and 19 415–19 617 cm⁻¹, which represent the transitions ²B_{1g} → ²A_{1g} and ²B_{1g} → ²E_g, respectively, indicating their octahedral geometry. The high-energy band was also observed at 29 892–29 967 cm⁻¹ assigned to the metal → ligand charge transfer. On the basis of electronic spectra, octahedral geometry around the Cu(II) ion was suggested³¹. The Zn(II) complexes did not show any d-d transition thus showing diamagnetic nature and their spectra were dominated only by a charge transfer band³² at 29 898–28 926 cm⁻¹.

Biological studies

Antibacterial activity (in vitro)

The synthesized novel Schiff bases (L¹)–(L³) and their metal(II) complexes (1)–(12) were screened for their *in vitro* antibacterial activity against *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella typhi* (*S. typhi*), *Shigella flexneri* (*S. flexneri*), *Staphylococcus aureus* (*S. aureus*) and *Bacillus subtilis* (*B. subtilis*) according to the standard procedure¹⁷ and results are depicted in Table 3. The results were compared with imipenem, a standard broad-spectrum β lactam antibiotic. Ligand (L¹) exhibited significant (16 mm) activity against *S. flexneri* and *B. subtilis* and rest of the strains showed moderate (10–13 mm) activity. Ligand (L²) displayed weaker (9 mm) activity against *S. flexneri* and *Staphylococcus aureus*, and against rest of the strains demonstrated moderate (10–13 mm) activity. Ligand (L³) also demonstrated significant (16–19 mm) activity against *P. aeruginosa* and *S. flexneri*, moderate (13–15 mm) activity against *E. coli* and *S. typhi* and weaker activity (8–9 mm) against the remaining two strains. The transition metal complexes (1), (3), (4), (6), (10) and (12) showed overall significant (16–25 mm) activity against all the strains. The complexes (2), (7) and (9) exhibited an overall

Table 3. Antibacterial activity of ligands (L¹)–(L³) and metal(II) complexes (1)–(12).

Compounds	[Zone of inhibition (mm)]							Average
	Gram-negative				Gram-positive			
	(a)	(b)	(c)	(d)	(e)	(f)	(SA)	
(L ¹)	10	13	12	16	10	16	2.71	12.83
(L ²)	11	10	13	09	09	12	1.63	10.67
(L ³)	13	16	15	19	08	09	3.66	13.33
(1)	23	18	19	18	20	17	2.14	19.17
(2)	20	21	11	18	19	21	3.78	18.33
(3)	18	16	20	21	19	17	1.87	18.50
(4)	19	21	18	23	17	16	2.61	19.00
(5)	10	20	24	21	18	17	4.35	18.33
(6)	19	20	24	17	23	16	3.19	19.83
(7)	20	19	12	16	18	19	2.94	17.33
(8)	12	16	20	21	24	18	4.18	18.50
(9)	21	18	13	20	14	19	3.28	17.50
(10)	16	21	18	17	25	19	3.27	19.33
(11)	13	16	19	20	23	24	4.17	19.17
(12)	18	24	17	20	21	17	2.74	19.50
SD	25	26	25	26	27	28	1.07	26.17

Average activity of ligand (L¹)–(L³) = 12.28 mm; average activity of complexes (1)–(12) = 18.71 mm; (a) *E. coli*, (b) *P. aeruginosa*, (c) *S. typhi*, (d) *S. flexneri*, (e) *S. aureus* and (f) *B. subtilis*; SD: standard drug (imipenem); weaker = 0–09 mm; moderate = 10–15 mm; above 16 mm = significant; SA: statistical analysis.

significant (16–21 mm) activity against all strains except *S. typhi* which possessed low to moderate (11–13 mm) activity. Rest of the complexes (5), (8) and (11) demonstrated significant (16–24 mm) activity against all bacterial strains except *E. coli*, which showed moderate (10–13 mm) activity. The data depicted in Table 3 clearly indicates that (L³) exhibited overall good bacterial activity as compared to the other three ligands. The Ni(II) complex (6) of (L²) was found to be the most active complex. The metal(II) complexes showed³³ higher activity results upon chelation rather than their non-coordinated Schiff bases.

Antifungal activity (in vitro)

The newly synthesized compounds were screened for antifungal activity against *Trichophytonlongifusum*, *Candida albicans* (*C. albicans*), *Aspergillus flavus* (*A. flavus*), *Microsporium canis* (*M. canis*), *Fusarium solani* (*F. solani*) and *Candida glabrata* (*C. glabrata*) fungal strains Table 4 according to the available protocols in the relevant literature¹⁷. The obtained results in percentage inhibition were compared with the results obtained from standard drugs, miconazole and amphotericin B. The ligand (L¹) displayed significant (57%) activity against *Trichophytonlongifusum* fungal strain, moderate (38–50%) activity against *C. albicans*, *A. flavus*, *M. canis* and *F. solani* but showed weaker (31%) activity against *C. albicans*. Moreover, the ligand (L²) exhibited significant (56–60%) activity against *C. albicans* and *Fusarium solani*, moderate (38–41%) activity against *Trichophytonlongifusum*, *A. flavus* and *C. glabrata* but complete lack of activity against *M. canis*. Furthermore, the ligand (L³) possessed moderate (36–51%) activity against *Trichophytonlongifusum*, *C. albicans*, *M. canis* and *F. solani* and interestingly demonstrated inactivity against the remaining strains. The metal complexes (1)–(4) exhibited overall significant (55–74%) activity against all the fungal strains. Similarly, the complex (5)–(8) displayed significant (55–75%) activity against all the strains except *M. canis* where they showed low (20–31%) activity. The compounds (9)–(12) demonstrated significant (55–78%) activity against *Trichophytonlongifusum*, *C. albicans*, *M. canis* and *Fusarium solani*, moderate (35–42%) against *A. flavus* and weaker (17–31%) activity

Table 4. Antifungal activity of ligands (L¹)–(L³) and metal(II) complexes (1)–(12).

Compounds	[% Inhibition (mm)]						(SA)	Average
	(a)	(b)	(c)	(d)	(e)	(f)		
(L ¹)	57	40	45	50	38	31	8.42	43.50
(L ²)	41	60	39	00	56	38	19.39	39.00
(L ³)	36	44	00	46	51	00	21.32	29.50
(1)	74	62	67	62	60	56	5.71	63.50
(2)	68	55	59	65	61	59	4.26	61.17
(3)	71	59	56	71	59	58	6.21	62.33
(4)	69	66	70	62	57	60	4.73	64.00
(5)	60	75	61	29	69	57	14.51	58.50
(6)	56	67	57	20	74	61	17.16	55.83
(7)	65	76	60	31	61	55	13.69	58.00
(8)	68	72	58	29	70	62	14.58	59.83
(9)	59	57	42	70	78	27	16.95	55.50
(10)	55	60	35	71	71	17	19.61	51.50
(11)	66	64	40	60	62	31	13.35	53.83
(12)	60	58	38	58	69	30	13.57	52.17
SD	A	B	C	D	E	F	–	–

Average activity of ligands (L¹)–(L³) = 37.33%; average activity of complexes (1)–(12) = 58.01%; (a) *T. longifusum*, (b) *C. albicans*, (c) *A. flavus*, (d) *M. canis*, (e) *F. solani* and (f) *C. glabrata*; SD: standard drugs; MIC μg/mL; A = miconazole (70 μg/mL: 1.6822 × 10⁻⁷ M/mL), B = miconazole (110.8 μg/mL: 2.6626 × 10⁻⁷ M/mL), C = amphotericin B (20 μg/mL: 2.1642 × 10⁻⁸ M/mL), D = miconazole (98.4 μg/mL: 2.3647 × 10⁻⁷ M/mL), E = miconazole (73.25 μg/mL: 1.7603 × 10⁻⁷ M/mL) and F = miconazole (110.8 μg/mL: 2.6626 × 10⁻⁷ M/mL); weaker = 0–33%; moderate = 34–54%; 55–100% = significant; SA: statistical analysis.

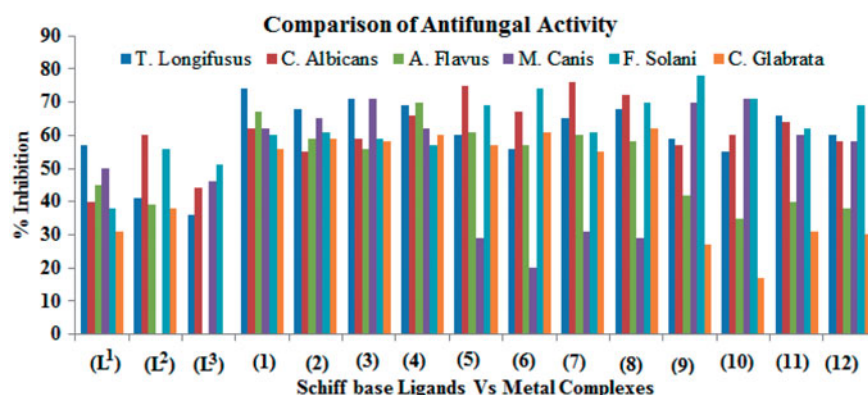


Figure 2. Comparison of antifungal activity of Schiff bases versus metal(II) complexes.

Table 5. Minimum inhibitory concentration ($\mu\text{g/mL}$) of the metal complexes (1)–(12).

Compounds	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. typhi</i>	<i>S. flexneri</i>	<i>S. aureus</i>	<i>B. subtilis</i>
(1)	18.42	–	–	–	–	–
(2)	23.69	–	–	–	–	–
(3)	–	–	33.93	24.32	–	–
(4)	–	38.86	–	19.23	–	–
(5)	–	–	36.61	–	–	–
(6)	–	–	30.08	–	19.55	–
(7)	38.22	–	–	–	–	–
(8)	–	–	26.43	39.91	22.39	–
(9)	31.52	–	–	–	–	–
(10)	–	33.35	–	–	32.36	–
(11)	–	–	–	–	26.31	19.42
(12)	–	36.73	–	–	–	–

against *C. glabrata*. It is obvious from the data reported in Table 4 that ligand (L^1) displayed overall good fungal activity as compared to the other three ligands. The Zn(II) complex (4) of (L^1) was found to be the most active complex. The metal(II) complexes showed enhanced³⁴ activity results compared to their non-coordinated Schiff bases upon complexation (Figure 2).

Minimum inhibitory concentration (MIC)

The transition metal(II) complexes which displayed promising anti-bacterial activity (more than 80%) were selected for MIC studies and results are presented in Table 5. MIC values of the metal complexes fell in the range of 18.42–39.91 $\mu\text{g/mL}$. Amongst these, the complex (1) was found to be the most active possessing maximum inhibition 18.42 $\mu\text{g/mL}$ against bacterial strain *E. coli*.

Conclusions

The newly synthesized Schiff bases acted as tridentate ligands for chelation with the Co(II), Cu(II), Ni(II) and Zn(II) metal atoms. Physical (magnetic and molar conductance), spectral (IR, NMR and electronic) and analytical (C, H, N, Co, Cu, Ni and Zn%) data established that the Schiff base ligands are coordinated with the metal ions through azomethine-N, thienyl-S and ammine-N presenting an octahedral geometry. The obtained results of antibacterial and antifungal activities revealed that the metal complexes are found to be more biological active against one or more bacterial and/or fungal strains as compared to their parent non-coordinated Schiff base ligands. Usually, it is claimed that the functional groups like azomethine-N, thienyl-S and ammine-N present in the compounds are responsible for the enhancement of bacterial and fungal activities. These findings propose that the synthesized metal-based

compounds have the potential to be developed therapeutic agents.

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Disclosure statement

The authors declare that there is no conflict of interests regarding the publication of this article and are responsible for the contents and writing of the article.

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