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RESEARCH ARTICLE

Antibacterial and antifungal metal based triazole Schiff bases

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Abstract

A new series of four biologically active triazole derived Schiff base ligands (**L**^{1}–**L**^{4}) and their cobalt(II), nickel(II), copper(II) and zinc(II) complexes (**1**–**16**) have been synthesized and characterized. The ligands were prepared by the condensation reaction of 3-amino-5-methylthio-1*H*-1,2,4-triazole with chloro-, bromo- and nitro-substituted 2-hydroxybenzaldehyde in an equimolar ratio. The antibacterial and antifungal bioactivity data showed the metal(II) complexes to be more potent antibacterial and antifungal than the parent Schiff bases against one or more bacterial and fungal species.

Keywords: Antibacterial, antifungal, triazole Schiff bases, metal(II) complexes

Introduction

Triazoles and their derivatives occupy a central position[1] amongst the most significant compounds that constitute pharmaceutically and medicinally important drug centers[2]. A group of triazoles such as (Figure 1) terconazole (**1**) has been widely used for the control of vulvovaginal moniliasis caused by *Candida albicans* and other *Candida* species, fluconazole (**2**) is a broad spectrum antifungal[3] and trazodone (**3**) is used as an antidepressant[5]. Similarly, vorozole (**4**), anastrozole (**5**) and letrozole (**6**) potentially inhibit breast cancer[6,7] properties. Many triazole based Schiff bases have also been reported to possess antibacterial[8], antifungal[9], antitumor[10], plant growth regulating[11] and cytotoxic[12] activities. Furthermore, the process of chelation/coordination plays an important role in the biological system[13]. It has been suggested that biological activity of many compounds used as drugs is enhanced[14] upon coordination with the metal atoms. In view of the significant structural and biological applications of triazole compounds, we wish to report the synthesis of a new class of triazole Schiff base derivatives (**L**^{1}–**L**^{4}) and their cobalt(II), nickel(II), copper(II) and zinc(II) metal chelates (**1**–**16**). These compounds have been investigated for *in vitro* antibacterial activity against four Gram-negative (*Escherichia coli, Shigella sonnei, Pseudomonas aeruginosa, Salmonella typhi*) and two Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) bacterial strains, and antifungal activity against six fungal strains (*Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *Candida glabrata)*. *In vitro* Brine shrimp bioassay has also been conducted to study the cytotoxic properties of these compounds.

Materials and methods

All chemicals used were of reagents grade. All metal salts were used as chloride. Melting points were recorded on Fisher Johns melting point apparatus. Infrared spectra were recorded on SHIMADZU FT-IR spectrometer. The C, H and N analyses was carried out using a Perkin Elmer, USA model. The NMR spectra of all compounds were recorded as δ (ppm) in DMSO-d_6 using TMS as internal standard on a Bruker Spectrospin Avance DPX-500 spectrometer. Electron impact mass spectra (EIMS) were recorded on JEOL MS Route Instrument. *In vitro* antibacterial, antifungal and cytotoxic properties were studied at HEJ Research Institute of Chemistry, International Centre for Chemical Sciences, University

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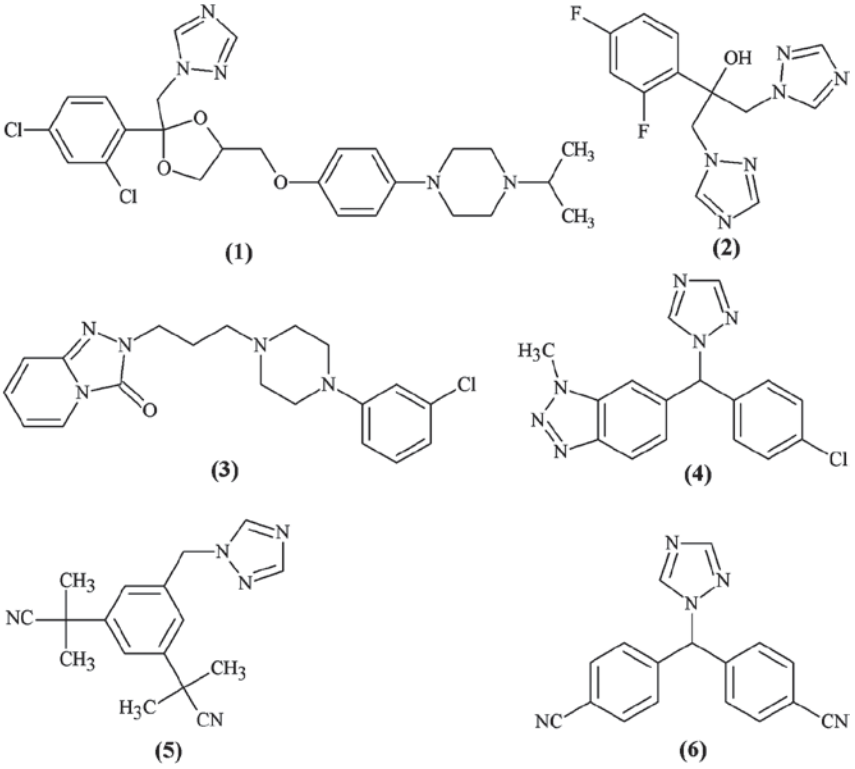


Figure 1. Triazole compounds.

of Karachi, Pakistan and Department of Chemistry, The Islamia University, of Bahawalpur, Pakistan.

General procedure for the synthesis of ligands (L^1–L^4)

2-[(E)-{[5-(methylsulfanyl)-1H-1,2,4-triazol-3-yl]imino} methyl]phenol (L^1)

To a hot magnetically stirred solution of 3-amino-5-methylthio-1*H*-1,2,4-triazole (1.31 g, 10 mmol) in methanol (15 mL) was added a solution of 2-hydroxybenzaldehyde (1.22 g, 1.06 mL, 10 mmol) in methanol (25 mL). The resultant mixture was refluxed for 5 h. The completion of reaction was monitored by TLC. The reaction mixture was cooled to room temperature. On cooling, a light yellow solid product was formed which was filtered and washed with methanol, then with ether and dried. It was recrystallized from a solution mixture of hot ethanol–methanol (1:1). The purity of product was checked by TLC. The same method was applied for the preparation of other ligands.

Physical, analytical and spectral data of triazole Schiff bases

2-[(E)-{[5-(methylsulfanyl)-1H-1,2,4-triazol-3-yl]imino} methyl]phenol (L^1)

Yield: 72% (1.7 g). Colour (light-yellow). M.p. 183–185°C. IR (KBr, cm^{-1}): 3265 (OH), 3175 (NH), 1625 (HC=N), 1606 (C=N, triazole), 1030 (N–N), 810 (S–C). ^{1}H NMR 14.10 (s, 1H, triazole NH), 12.00 (s, 1H, OH), 9.35 (s, 1H, azomethine C_7–H), 7.80 (dd, 1H, J = 7.81, 2.35 Hz, ph C_3–H), 7.45 (ddd, 1H, J = 7.90, 7.84, 2.35 Hz, ph C_5–H), 7.1 (dd, 1H, J = 7.84, 2.4 Hz, ph C_6–H), 6.95 (ddd, 1H, J = 7.90, 7.81, 2.4 Hz, ph C_4–H), 2.6 (s, 3H, triazole –SCH_3). ^{13}C NMR 163.22 (C_1), 161.93 (C_7), 158.73 (C_9), 156.23 (C_8), 134.53 (C_5), 131.71 (C_3), 119.58 (C_4), 119.30 (C_2), 116.74 (C_6), 13.88 (C_{10}). EIMS (70eV) m/z (%): 234 ([M]$^+$, 57), 217 (100), 187 (10), 161 (8), 119 (15), 114 (11), 93 (5), 77 (19). Anal. Calcd. for $C_{10}H_{10}N_4SO$ [234.29]: C: 51.27; H: 4.30; N: 23.91; S: 13.69; Found: C: 50.72; H: 4.30; N: 23.88; S: 13.77%.

4-Chloro-2-[(E)-{[5-(methylsulfanyl)-1H-1,2,4-triazol-3-yl] imino}methyl]phenol (L^2)

Yield: 74% (2.0 g). Colour (off-white). M.p. 214–215°C. IR (KBr, cm^{-1}): 3285 (OH), 3180 (NH), 1630 (HC=N), 1607 (C=N, triazole), 1030 (N–N), 825 (C–Cl), 815 (S-C). ^{1}H NMR 14.00 (s, 1H, triazole NH), 11.90 (s, 1H, OH), 9.15 (s, 1H, azomethine C_7–H), 7.70 (d, J = 2.5 Hz, ph C_3–H), 7.30 (dd, 1H, J = 7.94, 2.5 Hz, ph C_5–H), 6.85 (d, 1H, J = 7.92 Hz, ph C_6–H), 2.45 (s, 3H, triazole –SCH_3). ^{13}C NMR 163.24 (C_1), 162.11 (C_7), 158.82 (C_9), 156.23 (C_8), 133.64 (C_5), 131.92 (C_3), 127.58 (C_4), 120.80 (C_2), 118.74 (C_6), 13.90 (C_{10}). EIMS (70 eV) m/z (%): 270/268 ([M]$^+$, 33/54), 253/251 (69/100), 233 (10), 197/195 (5/7), 155/153 (14/21), 114 (11), 77 (19). Anal. Calcd. for $C_{10}H_9N_4SClO$ [268.72]: C: 44.70; H: 3.38; N: 20.85; S: 11.93; Cl: 13.19; Found: C: 44.34; H: 3.34; N: 20.68; S: 12.04; Cl: 13.23%.

4-Bromo-2-[(E)-{[5-(methylsulfanyl)-1H-1,2,4-triazol-3-yl] imino}methyl]phenol (L^3)

Yield: 76% (2.38 g). Colour (greenish-yellow). M.p. 210–212°C. IR (KBr, cm^{-1}): 3275 (OH), 3185 (NH), 1627 (HC=N), 1605 (C=N, triazole), 1025 (N–N), 805 (S–C), 680 (C–Br). ^{1}H NMR 14.15 (s, 1H, triazole NH), 12.20 (s, 1H, OH), 9.35 (s, 1H, azomethine C_7–H), 8.00 (d, 1H, J = 2.44

Hz, ph C_3–H), 7.60 (dd, 1H, *J* = 7.89, 2.44 Hz, ph C_5–H), 6.95 (d, 1H, *J* = 7.89 Hz, ph C_6–H), 2.60 (s, 3H, triazole –SCH_3). ^{13}C NMR 163.36 (C_1), 162.13 (C_7), 158.05 (C_9), 156.37 (C_8), 136.27 (C_5), 135.21 (C_3), 121.42 (C_2), 119.11 (C_6), 115.71 (C_4), 13.95 (C_{10}). EIMS (70 eV) *m/z* (%): 314/312 ([M]$^+$, 46/51), 297/295 (94/100), 267/265 (9/12), 240/238 (8/11), 233 (14), 199/197 (11/15), 174/172 (9/12), 114 (16), 75 (14). Anal. Calcd. for $C_{10}H_9N_4OSBr$ [313.17]: C: 38.35; H: 2.90; N: 17.89; S: 10.24; Br: 25.51; Found: C: 38.33; H: 2.83; N: 17.63; S: 10.19; Br: 25.64%.

***2-[(E)-{[5-(methylsulfanyl)-1H-1,2,4-triazol-3-yl]imino} methyl]-4-nitrophenol* (L^4)**

Yield: 75% (2.1 g). Colour (deep-yellow). M.p. 222–224°C. IR (KBr, cm^{-1}): 3290 (OH), 3182 (NH), 1632 (HC=N), 1606 (C=N, triazole), 1360 (C–NO_2), 1025 (N–N), 815 (S-C). ^{1}H NMR 14.10 (s, 1H, NH), 12.6 (s, 1H, OH), 9.40 (s, 1H, azomethine C_7–H), 8.45 (d, 1H, *J* = 2.45 Hz, ph C_3–H), 8.30 (dd, 1H, *J* = 7.86, 2.45 Hz, ph C_5–H), 7.15 (d, 1H, *J* = 7.86 Hz, ph C_6–H), 2.72 (s, 3H, C_{10}–H). ^{13}C NMR 163.34 (C_1), 162.28 (C_7), 158.44 (C_9), 156.27 (C_8), 140.53 (C_4), 127.71 (C_3), 125.58 (C_5), 120.30 (C_2), 117.74 (C_6), 13.98 (C_{10}). EIMS (70 eV) *m/z* (%): 279 ([M]$^+$, 54), 262 (100), 233 (23), 166 (18), 141 (15), 123 (10), 120 (6), 114 (11), 76 (19). Anal. Calcd. for $C_{10}H_9N_5O_3S$ [279.29]: C: 43.01; H: 3.25; N: 25.08; S: 11.48; Found: C: 42.45; H: 2.98; N: 25.35; S: 11.64%.

General procedure for the synthesis of complexes (1–16)

***Preparation of cobalt(II) complex with 2-[(E)-{[5-(methylsulfanyl)-1H-1,2,4-triazol-3-yl]imino}methyl]phenol* (L^1) (1)**

To a hot magnetically stirred solution of 2-[(E)-{[5-(methylsulfanyl)-1H-1,2,4-triazol-3-yl]imino}methyl] phenol **L^1** (1.87 g, 8 mmol) in ethanol (30 mL) was added dropwise a solution of $CoCl_2 \cdot 6H_2O$ (0.95 g, 4 mmol) in ethanol (20 mL). The resultant mixture was refluxed for 2 h. The colored precipitated product formed during refluxing was collected by filtration, thorough washing with ethanol followed by ether and dried. Recrystallization in hot aqueous–ethanol (1:2) afforded TLC pure product. The same method was used for the preparation of all other complexes. Physical, analytical and spectral data are given in Supplementary Tables S1 and S2.

NMR Data of Zn (II) Complexes

***[Zn (L^1-H)$_2$]* (4)**

^{1}H NMR of Zn (II) complex; 2.72 (s, 6H, triazole –SCH_3), 7.12 (ddd, 2H, *J* = 7.90, 7.81, 2.4 Hz, ph C_4–H), 7.24 (dd, 2H, *J* = 7.84, 2.4 Hz, ph C_6–H), 7.58 (ddd, 2H, *J* = 7.90, 7.84, 2.35 Hz, ph C_5–H), 7.98 (dd, 2H, *J* = 7.81, 2.35 Hz, ph C_3–H), 9.69 (s, 2H, C_7–H), 14.21 (s, 2H, triazole NH). ^{13}C NMR of Zn(II) complex; 14.03 (C_{10}), 117.04 (C_6), 119.77 (C_2), 119.79 (C_4), 131.99 (C_3), 134.77 (C_5), 158.29 (C_8), 159.98 (C_9), 163.35 (C_7), 164.17 (C_1).

***[Zn (L^2-H)$_2$]* (8)**

^{1}H NMR of Zn (II) complex; 2.49 (s, 6H, triazole -SCH_3), 7.03 (d, 2H, *J* = 7.94 Hz, ph C_6–H), 7.48 (dd, 2H, *J* = 7.94, 2.5 Hz, ph C_5–H), 7.86 (d, 2H, *J* = 2.5 Hz, ph C_3–H), 9.49 (s, 2H, C_7–H), 14.11 (s, 2H, triazole NH). ^{13}C NMR of Zn(II) complex; 14.13 (C_{10}), 118.99 (C_6), 121.15 (C_2), 127.85 (C_4), 132.25 (C_3), 133.92 (C_5), 157.59 (C_8), 160.0 (C_9), 163.55 (C_7), 164.33 (C_1).

***[Zn (L^3-H)$_2$]* (12)**

^{1}H NMR of Zn (II) complex; 2.72 (s, 6H, triazole –SCH_3), 7.12 (d, 2H, *J* = 7.89 Hz, ph C_6–H), 7.79 (dd, 2H, *J* = 7.89, 2.44 Hz, ph C_5–H), 8.18 (d, 2H, *J* = 2.44 Hz, ph C_3–H), 9.74 (s, 2H, C_7–H), 14.26 (s, 2H, triazole NH). ^{13}C NMR of Zn(II) complex; 14.12 (C_{10}), 115.96 (C_4), 119.39 (C_6), 121.74 (C_2), 135.53 (C_3), 136.66 (C_5), 158.00 (C_8), 159.51 (C_9), 163.66 (C_7), 164.87 (C_1).

***[Zn (L^4-H)$_2$]* (16)**

^{1}H NMR of Zn (II) complex; 2.83 (s, 6H, triazole –SCH_3), 7.30 (d, 2H, *J* = 7.86 Hz, ph C_6–H), 8.47 (dd, 2H, *J* = 7.86, 2.45 Hz, ph C_5–H), 8.61 (d, 2H, *J* = 2.45 Hz, ph C_3–H), 9.81 (s, 2H, C_7–H), 14.21 (s, 2H, triazole NH); ^{13}C NMR of Zn(II) complex; 14.1 (C_{10}), 117.99 (C_6), 120.59 (C_2), 125.83 (C_5), 127.99 (C_3), 140.84 (C_4), 157.42 (C_8), 159.59 (C_9), 163.78 (C_7), 164.75 (C_1).

Biological activity

Antibacterial studies

The synthesized Schiff bases **L^1**–**L^4** and their respective metal(II) complexes **1–16** were tested against four Gram-negative (*Escherichia coli, Shigella sonnei, Pseudomonas aeruginosa, Salmonella typhi*) and two Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) bacterial strains by the disk diffusion method[15]. The test compounds (ligand/complex) were dissolved in DMSO to get 10 mg/mL solution. A known volume (10 µL) of the solution was applied with the help of a micropipette onto the sterilized filter paper discs. The discs were dried at room temperature over night and stored in sterilized dry containers. Discs soaked with 10 µL of DMSO and dried in air at room temperature were used as the negative control. The standard antibiotic discs used as positive control were prepared as mentioned above in the laboratory by applying a known concentration of the standard antibiotic solution. Ampicillin was used as standard antibiotic. Bacterial culture was grown in nutrient broth medium at 37°C overnight and spread onto the solidified nutrient agar medium in Petri plates using sterilized cotton swabs. Test and control disks were then applied to the medium surface with the help of sterilized forceps. The plates were incubated at 37°C for 24–48 h. The results were recorded by measuring the zone of inhibition in millimeter against each compound[15]. The experiments were carried out in triplicate and the values obtained were statistically analyzed.

*Antifungal activity (*in vitro*)*

Antifungal activities of all compounds were studied against six fungal strains (*Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium*

solani and *Candida glabrata),* according to literature protocol[16]. Sabouraud dextrose agar (Oxoid, Hampshire, England, UK) was seeded with 10^5 (cfu) mL^{-1} fungal spore suspensions and transferred to Petri plates. Discs soaked in 20 mL (200 µg/mL in DMSO) of test 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 percentage of inhibition and compared with standard drugs miconazole and amphotericin B[15].

Minimum inhibitory concentration (MIC)

Compounds containing significant antibacterial activity (over 80%) were selected for minimum inhibitory concentration (MIC) studies. The minimum inhibitory concentration was determined using the disc diffusion technique by preparing discs containing 10, 25, 50 and 100 µg/mL of the test compounds and applying the protocol[17].

In vitro *cytotoxicity*

In vitro cytotoxic activity of all synthesized ligands and their metal(II) complexes were studied using the protocol of Meyer et al[18]. Brine shrimp (*Artemia salina* leach) eggs were hatched in a shallow rectangular plastic dish (22 × 32 cm), filled with artificial seawater, which was prepared with commercial salt mixture and double distilled water[18]. 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, in dark while the matter compartment was opened to ordinary light. After two days, Nauplii were collected by a pipette from the side in ordinary light. A sample of the test compound was prepared by dissolving 20 mg of each compound in 2 mL of DMSO. From this stock solutions, 500, 50 and 5 µg/mL were transferred to 9 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 DMSO only to determine its participating role. The solvent was allowed to evaporate overnight. After 2 days, when shrimp larvae were ready, sea water (1 mL) and 10 shrimps were added to each vial (30 shrimps/dilution) and the volume was adjusted with sea water to 5 mL per vial. After 24 h, the number of survivors was counted. Data were analyzed by Finney computer program to determine the LD_{50} values[18].

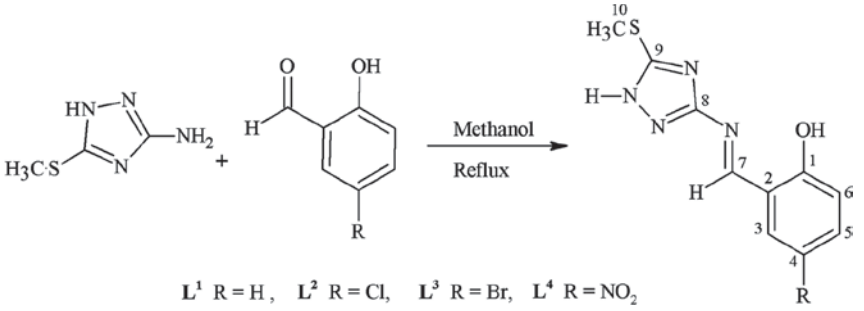
Results and discussion

Chemistry

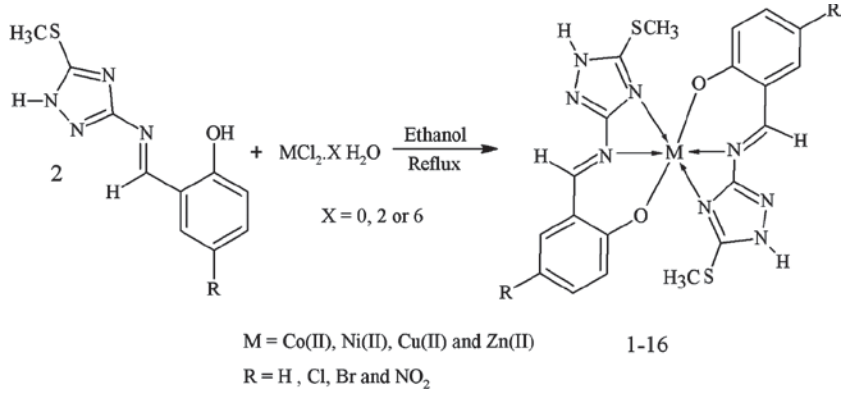
The triazole derived Schiff bases (**L^1**–**L^4**) have been prepared by the reaction of 3-amino-5-methylthio-1*H*-1,2,4-triazole with a series of chloro-, bromo-, and nitro- substituted 2-hydroxybenzaldehyde under reflux (Scheme 1).

All derivatives were soluble in ethanol, dioxane, DMF, and DMSO at room temperature and in methanol on heating only. The metal complexes (**1**–**16)** were obtained by stoichiometric reaction of the corresponding ligand with metal [Co(II), Ni(II), Cu(II) and Zn(II)] as chlorides in a molar ratio M:L (1:2) (Scheme 2).

All metal complexes were air and moisture stable. They were insoluble in common organic solvents and only soluble in DMF and DMSO. Physical and analytical



Scheme 1. Preparation of the ligands **L^1**–**L^4**.



Scheme 2. Proposed structure of the metal(II) complexes.

data of the complexes (**1**–**16**) are given in Supplementary Tables S1 and S2.

Molar conductivity and magnetic properties

The molar conductance values of the metal complexes (**1**–**16**) in DMF (Supplementary Table S2) fall in the range 11.7–19.8 $\Omega^{-1}\cdot cm^2\cdot mol^{-1}$ showing their non-electrolytic nature[19]. The room temperature magnetic moment values of the Co(II), Ni(II) and Cu(II) complexes are given in Supplementary Table S2. The observed magnetic moment of Co(II) complexes were in the range of 4.43–4.74 B.M indicating the high-spin Co(II) complexes with three unpaired electrons in octahedral environment[20]. The Ni(II) complexes showed μ_{eff} values in the range of 3.24–3.51 B.M indicating two unpaired electrons per Ni(II) ion suggesting their octahedral geometry[21]. The measured values 1.39–1.74 B.M for Cu(II) complexes were indicative of one unpaired electron per Cu(II) ion for d^9-system suggesting distorted octahedral[20] configuration. All the Zn(II) complexes were found to be diamagnetic[21] as expected.

IR spectra

The characteristic bands of IR spectra of the ligands and their metal complexes are reported in experimental section and in Supplementary Table S2. The IR spectra of all the ligands exhibited[22] the bands at 3175–3185, 1605–1607, 1025–1030 and 805–815 cm^{-1} respectively, due to (N–H), (C=N), (N–N) and (–S–C) vibrations of triazole moiety. The stretching bands in the spectra of the ligands at 1715 and 3325 cm^{-1} due to carbonyl ν(C=O) and amino $\nu(NH_2)$ groups disappeared and in turn, a new strong band at 1625–1632 cm^{-1} assigned to azomethine (–C=N) linkage[22] appeared providing a clue for condensation of amino (NH_2) group of triazole with carbonyl (C=O) group of aldehydes. The vibration of ν(–OH) at low values, reflected the existence[22] of intramolecular hydrogen bonding between (–OH) and azomethine (–C=N) group. The ligands, **L²** and **L³** exhibited the vibrations at 825 and 680 cm^{-1} assigned[22] to ν(C–Cl) and ν(C–Br), respectively. The ligand, **L⁴** showed[22] band at 1360 cm^{-1} owing to vibrations of $\nu(C–NO_2)$ group. A strong band appeared in the spectra of all metal complexes at 1612–1617 cm^{-1} due to azomethine (HC=N) group that shifted to lower frequency by 10–17 cm^{-1} indicating the participation[22] of the azomethine nitrogen with the metal ion. Similarly, the triazole ring (C=N) band originally appearing at 1605–1607 cm^{-1} in the spectra of the ligands shifted to lower frequency at 1590–1594 cm^{-1} in the spectra of the metal complexes by 12–17 cm^{-1} indicative[22] of the involvement of triazole ring nitrogen in the complexes. In all the metal(II) complexes, a new band appeared[22] at 525–535 cm^{-1} due to ν(M–N) vibrations. However, the band appearing at 1025–1030 cm^{-1} due to ν(N-N) mode of triazole ring in all the ligands remained unchanged in the spectra of the metal(II) complexes indicating non-involvement of the nitrogen of (N–N) of triazole ring with the metal ion. The disappearance[22] of broad band of ν(OH) at 3265–3290 cm^{-1} in spectra of all metal complexes and appearance of new band of (C–O) at 1380–1385 cm^{-1} revealed deprotonation and coordination of hydroxyl-O to the metal atom. This metal to oxygen coordination was further justified by the appearance of new band at 450–465 cm^{-1} due to (M–O).

^{1}H NMR Spectra

The ^{1}H NMR spectral data of the ligands (**L¹**–**L⁴**) and their diamagnetic Zn(II) complexes are recorded in the experimental part. The exhibited signals of all the protons due to heteroaromatic/aromatic groups were found[23] as to be in their expected region. The spectra of all the Schiff base ligands displayed a singlet[23], due to proton of azomethine C_7–H and hydroxyl group (–OH) of phenol moieties at 9.15–9.40 and 11.90–12.60 ppm, respectively. The strong down field shift of the hydroxyl proton indicated[23] its participation in intramolecular hydrogen bonding. The Schiff base ligands **L²**–**L⁴** displayed C_3–H and C_6–H protons of phenol as doublet at 7.70–8.45 and 6.85–7.15 ppm, respectively and the same ligands showed C_5–H proton of phenol as double of the doublet at 7.30–8.30 ppm. The **L¹** exhibited the phenol C_4–H and C_5–H protons as doublet of the double doublet at 6.95 and 7.45 ppm, respectively. However, the phenol C_3–H and C_6–H protons appeared as double of the doublet at 7.80 and 7.1 ppm. Moreover, all the Schiff base ligands displayed (–S–CH_3) methyl protons of triazole as singlet at 2.45–2.72 ppm. A broad singlet at 14.00–14.15 ppm displayed[23] due to tautomerism of NH proton of triazole in all the ligands. The coordination of the azomethine (CH=N) nitrogen are assigned[23] by the downfield shifting of azomethine (C_7–H) proton signal from 9.15–9.40 ppm in the free ligands to 9.49–9.81 ppm in their zinc(II) complexes. Furthermore, signal of hydroxyl (OH) protons appearing at 11.90–12.60 ppm in the spectra of free ligands, disappeared in spectra of their corresponding zinc complexes indicating[23] deprotonation and coordination of the hydroxyl-O with zinc metal. All other protons overall underwent downfield shift by 0.11–0.25 ppm due to the increased conjugation[23] in the spectra of the Zn(II) complexes.

^{13}C NMR Spectra

The ^{13}C NMR spectra of the Schiff base ligands **L¹**–**L⁴** and their Zn(II) complexes were done in DMSO-d_6. The ^{13}C NMR spectral data are reported along with their possible assignments in the experimental section and all the carbons were found in the expected regions[23]. The conclusion obtained from these studies provides further support to the mode of bonding explained in their IR and ^{1}H NMR spectral data. The ^{13}C NMR spectra of all the Schiff base ligands, displayed[23] the azomethine carbon (C_7) and methyl carbon (C_{10}) of triazole (–S–CH_3) at 161.93–162.28 and 13.88–13.98 ppm, respectively. The carbons of phenol moiety of all the Schiff base ligands were appeared[23] in the range from 115.71–163.36 ppm. The downfield peak in all the ligands at 163.22–163.36 ppm is due to (C_1). This downfield shift of (C_1) signal is

due to attachment of hydroxyl (OH) group at (C_1) position. Similarly, the ligand **L²** showed downfield peak at 127.58 ppm of (C_4) owing to the electronegative effect of chloro group at this position, as compared to ligand **L¹** which showed upfield peak at 119.58 ppm due to absence of chloro group at this position. The triazole carbons (C_8) and (C_9) of all the ligands appeared[23] in the region of 156.23–158.82 ppm. The spectra of Zn(II) complexes of all the ligands exhibited[23] a downfield shifting of azomethine carbon (C_7) from 161.93–162.28 ppm in free ligands to 163.35–163.78 ppm in the zinc(II) complexes, revealing the coordination of azomethine nitrogen to the Zn(II) ion. Also the downfield shifting of triazole carbon (C_9) from 158.05–158.82 ppm in Schiff base ligands to 159.51–160.0 ppm in Zn(II) complexes, displaying[23] the coordination of triazole nitrogen to the zinc ion. Similarly, the phenol carbon (C_1) of the ligands existed near the coordination sites (C_1–O–M) showed downfield shift by 0.9–2.1 ppm from 163.22–163.36 ppm in the spectra of ligands to 164.17–164.87 ppm in the spectra of the zinc(II) complexes. Furthermore, all other carbons of the all the ligands in Zn(II) complexes underwent[23] downfield shifting by 0.24–0.8 ppm due to the increased conjugation and coordination with the metal atoms.

Mass spectra

The mass spectral data and fragmentation pattern of all the Schiff base ligands **L¹**–**L⁴** clearly justify the formation of the ligands possessing proposed structures and their bonding pattern. The mass spectra of ligand **L¹** showed molecular ion peak *m/z* 234 (calcd. 234.29) of $[C_{10}H_{10}N_4SO]^{\cdot+}$, which loses a hydroxyl group (OH) as radical to give most stable fragment at *m/z* 217 of $[C_{10}H_9N_4S]^+$. Similarly the mass spectra of ligand, **L²** showed molecular ion peak at *m/z* 268 (calcd. 268.72) of $[C_{10}H_9N_4SOCl]^{\cdot+}$, with base peak fragment of $[C_{10}H_8N_4SCl]^+$ at *m/z* 251. The ligand, **L³** exhibited molecular ion peak at *m/z* 314.0/312 (calcd. 313.17) of $[C_{10}H_9N_4SOBr]^{\cdot+}$ with base peak fragment $[C_{10}H_8N_4SBr]^+$ at *m/z* 298/296. In the same way the ligand, **L⁴** displayed molecular ion peak at *m/z* 279.0 (calcd. 279.29) of $[C_{10}H_9N_5SO_3]^{\cdot+}$, with stable fragment $[C_{10}H_8N_5SO_2]^+$ as base peak at *m/z* 262. The fragmentation pattern followed the cleavage of C=N (exocyclic as well as endocyclic), C–OH, C–C, C–N and C–S bonds. Fragmentation pattern of one ligand **L¹** is shown as Figure 2.

Electronic spectra

The electronic spectral values of Co(II), Ni(II), Cu(II) and Zn(II) complexes are recorded in Supplementary Table S2. The Co(II) complexes in general, exhibited three absorption bands in the region at 8560–8880, 17,680–17,945 and 29,684–29,940 cm^{-1} are respectively assigned to the transitions $^4T_{1g} \rightarrow {}^4T_{2g}(F)$, $^4T_{1g} \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g} \rightarrow {}^4T_g(P)$, showing an octahedral geometry[24] around the Co(II) ion. The spectra of Ni(II) complexes showed bands in the region at 9694–10,095, 15,860–16,213 and 29,456–29,750 cm^{-1} which are assigned[24] due to the transitions of $^3A_{2g} \rightarrow {}^3T_{2g}(F)(\nu 1)$, $^3A_{2g} \rightarrow {}^3T_{1g}(F)$ ($\nu 2$) and $^3A_{2g} \rightarrow {}^3T_{1g}(P)$ (ν_3), respectively, consistent with their well-defined octahedral configuration. The electronic spectra of Cu(II) complexes exhibited low-energy absorption band at 14,670–14,880 cm^{-1} assigned to the transitions $^2E_g \rightarrow {}^2T_{2g}$. The high-energy band at 25,590–25,860 cm^{-1} is due to forbidden ligand → metal charge transfer. On the basis of which a distorted octahedral geometry is suggested for Cu(II) complexes[24]. The diamagnetic Zn(II) complexes did not show any d–d transitions and their spectra were dominated[24] only by the charge transfer band at 28,675–28,930 cm^{-1}.

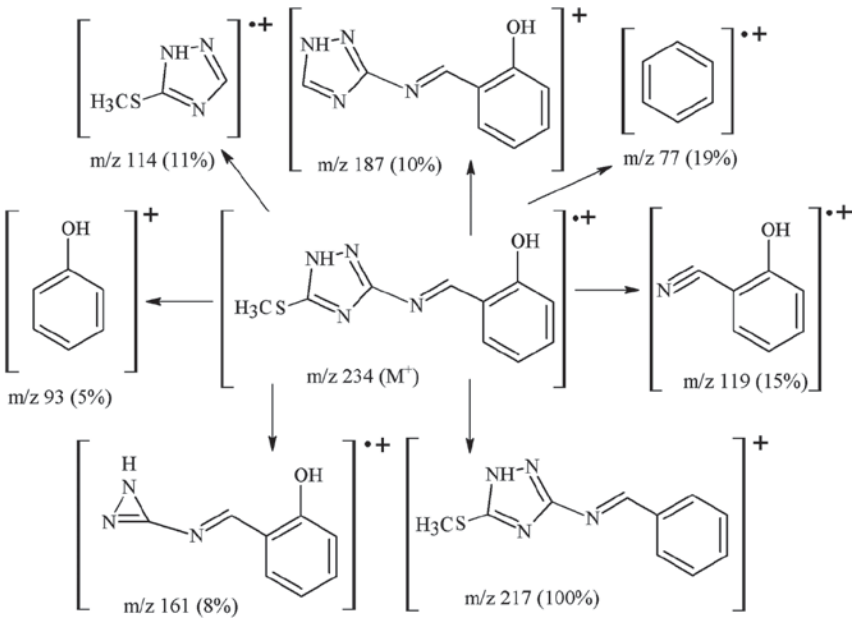


Figure 2. Proposed mass fragmentation pattern of **L¹**.

Biological activity

Antibacterial bioassay

Antibacterial activity data of newly synthesized Schiff bases and their corresponding metal(II) complexes was determined against four Gram-negative (*Escherichia coli, Shigella sonnei, Pseudomonas aeruginosa, Salmonella typhi*) and two Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) bacterial strains (Table 1) according to literature protocol[15]. The obtained results were compared with standard drug ampicillin and 3-amino-5-methylthio-1*H*-1,2,4-triazole (Supplementary Figures S1 and S3). The percentage of activity was compared with the activity of the standard drug (ampicillin) considering its activity as 100 %. The obtained results indicated that the Schiff base ligand, $\mathbf{L^1}$ exhibited moderate antibacterial activity (46–53 %) against all bacterial strains whereas the Schiff base ligand, $\mathbf{L^2}$ showed significant activity (55.17 %) against **(f)** and moderate activity (46–53 %) against **(a)**–**(e)**. The antibacterial results from Table 1 indicated that the Schiff base ligand, $\mathbf{L^3}$ possessed significant activity (57.14 %) against **(d)** and moderate activity (43–52 %) against **(a)**–**(c)**, **(e)** and **(f)** while the ligand, $\mathbf{L^4}$ exhibited significant activity (55.17–61.53 %) against **(a)** and **(f)** and moderate activity (48–52 %) against **(b)**–**(e)**. The antibacterial data of metal(II) complexes indicated that metal(II) complexes, **1**, **3–8**, **10** and **12–14** exhibited significant activity (54–82 %) against all observed bacterial strains. However, the metal(II) complexes, **2** and **15** showed significant activity (57–81 %) against **(a)** and **(c)**–**(f)** and moderate activity (53 %) against **(b)**. Similarly, the metal(II) complexes, **9** possessed significant activity (59–79 %) against **(b)** and **(d)**–**(f)** and moderate activity (47–52 %) against **(a)** and **(c)**, compound **11** also showed significant activity (61–71 %) against **(a)**, **(b)** and **(d)**–**(f)** and moderate activity (47 %) against **(c)**. Compound **16** displayed significant activity (57–75 %) against **(a)-(d)** and **(f)** and moderate activity (52 %) against **(e)**. The comparative studies of data, exhibited that the Schiff base ligands $\mathbf{L^1}$-$\mathbf{L^4}$ were more active than 3-amino-5-methylthio-1*H*-1,2,4-triazole against tested bacterial strains which provided evidences that the activity of 3-amino-5-methylthio-1*H*-1,2,4-triazole increased upon Schiff base preparation. Moreover, the synthesized Schiff base ligands possessed smaller average activity values (14.3 mm) than the average values (17.9 mm) of metal(II) complexes which showed that the activity was enhanced[25] upon coordination.

Antifungal bioassay

In vitro *antifungal bioassay*

Antifungal screening of all the newly synthesised Schiff base ligands, and their metal(II) complexes, was carried out against *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani*

Table 1. Antibacterial bioassay (concentration used 1 mg/mL of DMSO) of ligands $\mathbf{L^1}$–$\mathbf{L^4}$ and the metal(II) complexes **1**–**16**.

	Zone of inhibition (mm)							
	Gram-negative			Gram-positive				
Compounds	**(a)**	**(b)**	**(c)**	**(d)**	**(e)**	**(f)**	SA	Average
$\mathbf{L^1}$	14	12	15	14	13	15	1.17	13.8
$\mathbf{L^2}$	14	13	15	15	14	16	1.05	14.5
$\mathbf{L^3}$	13	13	14	16	14	15	1.17	14.2
$\mathbf{L^4}$	16	12	17	14	13	16	1.97	14.7
1	18	17	17	19	16	19	1.21	17.7
2	19	13	19	16	19	21	2.86	17.8
3	16	18	18	19	16	17	1.21	17.3
4	17	16	20	17	17	18	1.38	17.5
5	16	17	18	18	18	24	2.81	18.5
6	19	16	20	17	17	19	1.55	18.0
7	17	18	21	17	16	18	1.72	17.8
8	18	20	19	16	17	24	2.83	18.6
9	14	19	15	21	16	18	2.64	17.2
10	19	17	19	18	16	19	1.26	18.0
11	16	17	15	20	17	18	1.72	17.2
12	18	17	18	23	18	19	2.14	18.8
13	19	17	20	16	18	19	1.47	18.2
14	19	16	18	17	16	24	3.01	18.0
15	21	13	20	18	17	17	2.80	17.7
16	19	18	22	16	14	20	2.86	18.2
A	10	10	12	10	11	11	0.82	10.7
SD	26	24	32	28	27	29	2.73	27.7

Average of Ligands $\mathbf{L^1}$–$\mathbf{L^4}$ = 14.3 mm; average of Complexes **1–16** = 17.9 mm. Activity <10 = weak; >10 = moderate; > 16 = Significant.
(a), *E. coli;* **(b)**, *S. sonnei;* **(c)**, *P. aeruginosa;* **(d)**, *S. typhi;* **(e)**, *S. aureus;* **(f)**, *B. subtilis;* **A,** 3-Amino-5-methylthio-1*H*-1,2,4-triazole; **SD**, standard drug (Ampicillin).
SA = Statistical Analysis.

and *Candida glabrata* fungal strains according to the literature[16] protocol and their results were compared with standard drugs Miconazole and Amphotericin B. The antifungal data (Table 2) exhibited that the Schiff base ligand, **L**[1] showed significant activity (57–61 %) against **(b)** and **(c)**, moderate (38–46 %) against **(a)**, **(d)** and **(f)** and weak (33 %) against **(e)** fungal strains. The Schiff base ligands, **L**[2] and **L**[4] showed significant activity (57–60 %) against **(c)** and **(b)**, while the ligand **L**[2] exhibited moderate activity (43–48 %) against **(b)** and **(f)**, and weak (33 %) against **(a)** and **(d)** fungal strains. Schiff base ligand **L**[4] showed moderate activity (39–51 %) against **(a)**, **(d)** and **(f)** and weak (33 %) against **(b)** fungal strains. The activity of Schiff base ligand, **L**[3] was found to be significant (54–67 %) against **(e)** and **(f)** moderate (38–47 %) against **(a)**-**(c)** and weak (33 %) against **(d)** fungal strains. The results of antifungal activity exhibited that the metal(II) complexes **10**, **13**, **14** and **16** showed significant activity (54–57 %) against **(a)** the complexes **1–4** showed significant activity (57–66 %) against **(b)** and the complexes **1–8** and **13–16** showed significant activity (56–66 %) against **(c)** fungal strains. Similarly, the metal(II) complexes, **5–9** and **11–16** possessed significant activity (56–65 %) against **(e)** and the complexes **9–12** showed significant activity (69–76 %) against **(f)** fungal strains. The metal(II) complexes **1**-**5**, **8**, **9**, **11**, **12** and **15** showed moderate activity (34–53 %) against **(a)**, and the same activity shown by complexes **5–12** against **(b)**, **9–12** against **(c)** and **1–5**, **8**, **13–16** against **(d)**. In the same way, the metal(II) complex **10** showed moderate activity (34–53 %) against **(e)** and the complexes **1–8** and **12–16** possessed same activity against **(f)** fungal strains. The metal (II) complexes, **1–4** and **6** showed weaker activity (19–31 %) respectively against **(e)** and **(a)** and the complex **7** also showed weaker activity (29–30 %) against **(a)** and **(d)** fungal strains. Similarly the metal (II) complexes, **9–12** showed weaker activity (20–24 %) against **(d)**, and also the complexes, **13** and **16** possessed weaker activity (30–31 %) against **(b)** fungal strains. The average activity data comparison showed that the metal(II) complexes exhibited greater average activity value (47.4 %) than the average activity value of the ligands (44.4 %). The above discussions concluded that the antifungal activity of the Schiff base ligands increased[26] upon chelation/coordination with the metal ions. The comparative activity data of the ligands and their metal(II) complexes is presented in Supplementary Figures S2 and S4.

Minimum inhibitory concentration (MIC)

The synthesized Schiff base ligands and their metal(II) complexes, having antibacterial activity more than 80 % were selected for MIC studies and obtained results are recorded in Table 3. It was investigated from the data that the synthesized compounds exhibited varying degree of inhibitory effect on the growth of tested bacterial strains.

Table 2. Antifungal bioassay (concentration used 200 μg/mL) of ligands **L**[1]–**L**[4] and the metal(II) complexes **1**–**16**.

	% Inhibition (mm)							
Compounds	**(a)**	**(b)**	**(c)**	**(d)**	**(e)**	**(f)**	SA	Average
L[1]	35	61	57	39	18	46	15.70	42.7
L[2]	28	43	60	33	57	48	12.76	44.8
L[3]	47	40	38	19	54	67	16.21	44.2
L[4]	51	27	59	39	57	43	12.11	46.0
1	39	65	60	44	19	50	16.46	46.2
2	37	62	59	40	22	48	14.91	44.7
3	40	57	62	43	23	49	13.85	45.7
4	35	66	58	45	25	39	15.13	44.6
5	34	47	64	37	60	50	11.99	48.7
6	31	45	62	33	58	52	12.85	47.2
7	29	46	63	30	62	49	14.81	46.5
8	35	43	61	35	59	51	11.48	47.3
	51	44	39	23	59	74	17.48	48.3
10	55	39	43	21	50	69	16.18	46.2
11	47	42	40	24	56	76	17.45	47.5
12	53	43	45	20	57	75	18.16	48.8
13	54	30	64	43	65	48	13.32	50.7
14	57	29	56	44	59	44	11.47	48.2
15	52	33	62	42	60	40	11.66	48.2
16	55	31	65	37	62	49	13.60	49.8
SD	A	B	C	D	E	F	–	–

Average of ligands **L**[1]–**L**[4] = 44.4%; Average of complexes **1–16** = 47.4%.

(a), *T. longifucus;* **(b)**, *C. albicans;* **(c)***A. flavus;* **(d)**, *M. canis;* **(e)**, *F. Solani;* **(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); F, Miconazole (110.8 μg/mL: 2.66266 × 10^{-7} M/mL). SA, statistical analysis.

Table 3. Minimum inhibitory concentration (μg/mL) of the selected compounds **5**, **8**, **12**, **14** and **15** against selected bacteria

Bacterial strains	(5)	(8)	(12)	(14)	(15)
Gram negative					
E. coli	–	–	–	–	79.46
S.sonnei	–	58.62	–	–	–
S. typhi	–	–	60.62	–	–
Gram positive					
B. subtilis	90.33	67.2	–	70.75	–

The preliminary antibacterial screening results of all the compounds exhibited that the metal(II) complexes **5**, **8**, **12**, **14**, and **15** were found to be the most (above 80%) active compounds. Therefore, these compounds were selected for minimum inhibitory concentration (MIC) studies (Table 3). The MIC values of these compounds fall in the range 58.62–90.33 μg/mL. The MIC result, however, showed that the compound **8** was the most active showing maximum inhibition against bacterial strain *S.sonnei*.

In vitro cytotoxic bioassay

The synthesized ligands and their metal (II) complexes were screened for their cytotoxicity (brine shrimp bioassay) using the protocol of Meyer et al[18]. The **c**ytotoxic data recorded in Supplementary Table S3, revealed that only two compounds, **3** and **11** displayed potent cytotoxic activity having LD_{50} as 1.10×10^{-4}–1.67×10^{-4} M against *Artemia salina* while all other compounds can be considered as almost inactive in this assay. It was interesting to note that metal complexes showed potent cytotoxicity as compared to ligands. This activity relationship can serve as a useful tool for future studies in designing and development of cytotoxic agents.

Conclusion

All the newly synthesized Schiff bases **(L^1–L^4)** act as tridentate ligands through the coordination of triazole (C=N) and azomethine (CH=N) nitrogens and, phenolic oxygen with the metal ion. The bonding of ligands to the metal ion and octahedral geometry observed by all the metal complexes was confirmed by the analytical, IR, ^{1}H NMR, electronic and magnetic studies. From the *in vitro* antibacterial and antifungal activity data shown against representative bacterial and fungal strains is evident that the Schiff bases and their Co(II), Ni(II), Cu(II) and Zn(II) complexes were found to possess good antibacterial and antifungal activities however, the metal complexes showed more activity as compared to the simple Schiff bases.The average activity data (Tables 1 and 2) of the Schiff base ligands revealed that halo- and nitro-substituted ligands showed overall more activity than the unsubstituted ligand. Amongst the substituted ones, the nitro-substituted ligand **(L^4)** possessed higher activity than the chloro-substituted ligand **(L^2)**, which in turn shows more activity than the bromo-substituted ligand **(L^3)**. It was also observed that amongst the metal ions, the Zn(II) complexes showed overall more antibacterial activity than other metals. Similarly, Co(II) complexes were overall found to be more antifungal than the other metal ions.

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Declaration of interest

The authors report no conflict of interest and are responsible for the contents.

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