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ORIGINAL ARTICLE

Design, synthesis, and biological properties of triazole derived compounds and their transition metal complexes

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Abstract

Triazole derived Schiff bases and their metal complexes (cobalt(II), copper(II), nickel(II), and zinc(II)) have been prepared and characterized using IR, ^{1}H and ^{13}C NMR, mass spectrometry, magnetic susceptibility and conductivity measurements, and CHN analysis data. The structure of **L²**, *N*-[(5-methylthiophen-2-yl)methylidene]-1*H*-1,2,4-triazol-3-amine, has also been determined by the X-ray diffraction method. All the metal(II) complexes showed octahedral geometry except the copper(II) complexes, which showed distorted octahedral geometry. The triazole ligands and their metal complexes have been screened for their *in vitro* antibacterial, antifungal, and cytotoxic activity. All the synthesized compounds showed moderate to significant antibacterial activity against one or more bacterial strains. It is revealed that all the synthesized complexes showed better activity than the ligands, due to coordination.

Keywords: *Triazole; metal complexes; antibacterial; antifungal; cytotoxic*

Introduction

Triazoles are widely known to possess broad-spectrum biological activities such as antibacterial[1–4], antifungal[5–8], anticonvulsant[9–11], antitumor[12–14], antitubercular[15–19], antimicrobial[20–24], anticancer[25–27], analgesic[28], cytotoxic[29], insecticidal, herbicidal, plant growth regulatory[30–32], and antiproliferative activities[33,34]. These are extensively used as prospective ligands in a variety of bioinorganic syntheses. Due to their significant biological applications they have gained much attention in bioinorganic and metal-based drug chemistry. In view of its structural and biological importance, we have synthesized a series of triazole derived Schiff bases, *N*-(thiophen-2-ylmethylidene)-1*H*-1,2,4-triazol-3-amine (**L¹**), *N*-[(5-methylthiophen-2-yl)methylidene]-1*H*-1,2,4-triazol-3-amine (**L²**), *N*-[(3-methylthiophen-2-yl)methylidene]-1*H*-1,2,4-triazol-3-amine (**L³**), *N*-[(5-chlorothiophen-2-yl) methylidene]-1*H*-1,2,4-triazol-3-amine (**L⁴**), and *N*-[(5-nitrothiophen-2-yl)methylidene]-1*H*-1,2,4-triazol-3-amine (**L⁵**), from the reaction of 3-amino-1,2,4-triazole and methyl-, chloro-, and nitro-substituted thiophene-2-carboxaldehydes. It coordinates to metal ions such as Cu(II), Co(II), Ni(II), and Zn(II) in different ways depending upon the donor sites of the ligand[35–40]. These compounds have been investigated for their *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 for antifungal activity against *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani*, and *C. glabrata* fungal strains. These studies indicate that all the compounds show moderate to significant activity that increases upon coordination/chelation. These compounds were also checked in the *in vitro* brine shrimp bioassay.

Materials and methods

All reagents and solvents used were of Analar grade. All metals were used as the chloride salts. Melting points were recorded on a Fisher Johns melting point apparatus.

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Infrared (IR) spectra were recorded on a Shimadzu FT-IR spectrometer. C, H, and N analysis was carried out using a PerkinElmer model. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded in dimethylsulfoxide (DMSO)-d_6 using tetramethylsilane (TMS) as internal standard on a Bruker Spectrospin Avance DPX-500 spectrometer. Electron impact mass spectra (EIMS) were recorded on a Jeol MS Route instrument. *In vitro* antibacterial, antifungal, and cytotoxic properties were studied at the HEJ Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Pakistan and the Department of Chemistry, The Islamia University of Bahawalpur, Pakistan.

Synthesis of ligands

N-(Thiophen-2-ylmethylidene)-1H-1,2,4-triazol-3-amine (L1)

A mixture of thiophene-2-carboxaldehyde (1.12 g, 0.93 mL, 10 mmol) and 3-amino-1,2,4-triazole (0.84 g, 10 mmol) in methanol (40 mL) was refluxed for 5 h with monitoring by thin layer chromatography (TLC). The reaction mixture was cooled to room temperature and filtered; within 1 h a fine off-white solid product separated from the clear solution. It was filtered, washed with methanol, dried, and recrystallized from hot ethanol. The same procedure was used for the synthesis of all other ligands.

Physical, analytical, and spectral data of the ligands (L1–L5)

N-(Thiophen-2-ylmethylidene)-1H-1,2,4-triazol-3-amine (L1)

Yield (1.26 g, 71%); m.p. 172°C; IR (KBr, cm^{-1}): 3175 (NH), 1628 (HC=N), 1611 (C=N, triazole), 1570, 1540 (C=C), 1020 (N-N), 960 (C-S); ^{1}H NMR (DMSO-d_6): δ 7.24 (dd, 1H, J = 4.6, 4.0 Hz, C_4-H), 7.76 (d, 1H, J = 4.0 Hz, C_3-H), 7.83 (d, 1H, J = 4.6 Hz, C_5-H), 8.25 (s, 1H, C_6-H), 9.30 (s, 1H, triazole), 13.98 (s, 1H, triazole, NH); ^{13}C NMR (DMSO-d_6): δ 126.7 (C_5), 129.5 (C_4), 132.6 (C_3), 143.6 (C_2), 152.7 (C_8), 156.2 (C_6), 157.9 (C_7); EIMS (70 eV) *m/z* (%): 178 ([M]$^+$, 77), 177 (100), 151 (6), 145 (11), 137 (15), 122 (12), 110 (20), 96 (11), 69 (16); Anal. Calcd. for $C_7H_6N_4S$ (178.21): C, 47.18; H, 3.39; N, 31.44. Found: C, 47.30; H, 3.41; N, 31.35%.

N-[(5-Methylthiophen-2-yl)methylidene]-1H-1,2,4-triazol-3-amine (L2)

Yield (1.40g, 73%); m.p. 168°C; IR (KBr, cm^{-1}): 3185 (NH), 965 (C-S), 1632 (HC=N), 1612 (C=N, triazole), 1575, 1545 (C=C), 1020 (N-N); ^{1}H NMR (DMSO-d_6): δ 2.5 (s, 3H, C_5-CH_3), 6.96 (d, 1H, J = 3.0 Hz, C_4-H), 7.60 (d, 1H, J = 3.0 Hz, C_3-H), 8.20 (s, 1H, C_6-H), 9.20 (s, 1H, triazole), 13.95 (s, 1H, triazole, NH); ^{13}C NMR (DMSO-d_6): δ 15.4 (CH_3), 128.2 (C_4), 130.9 (C_3), 137.6 (C_5), 142.9 (C_2), 153.5 (C_8), 156.5 (C_6), 158.2 (C_7); EIMS (70 eV) *m/z* (%): 192 (M$^+$, 81%), 191 (100), 177 (9), 159 (22), 124 (26), 122 (15), 109 (10), 97 (10), 69 (15); Anal. Calcd. for $C_8H_8N_4S$ (192.24): C, 49.98; H, 4.19; N, 29.14. Found: C, 50.10; H, 4.10; N, 29.20%

N-[(3-Methylthiophen-2-yl)methylidene]-1H-1,2,4-triazol-3-amine (L3)

Yield (1.42 g, 74%); m.p. 172°C; IR (KBr, cm^{-1}): 3180 (NH), 970 (C-S), 1626 (HC=N), 1615 (C=N, triazole), 1568, 1540 (C=C), 1020 (N-N); ^{1}H NMR (DMSO-d_6): δ 2.40 (s, 3H, C_3-CH_3), 7.06 (d, 1H, J = 4.5 Hz, C_4-H), 7.75 (d, 1H, J = 4.5 Hz, C_5-H), 8.20 (s, 1H, C_6-H), 9.25 (s, 1H, triazole), 13.90 (s, 1H, triazole, NH); ^{13}C NMR (DMSO-d_6): δ 14.4 (CH_3), 128.4 (C_2), 129.3 (C_4), 132.9 (C_5), 140.6 (C_3), 153.1 (C_8), 156.8 (C_6), 158.2 (C_7); EIMS (70 eV) *m/z* (%): 192 (M$^+$, 68 %), 191 (40), 177 (100), 150 (13), 134 (15), 124 (31), 123 (40), 109 (21), 97 (22), 80 (11), 70 (14), 65 (13); Anal. Calcd. for $C_8H_8N_4S$ (192.24): C, 49.98; H, 4.19; N, 29.14. Found: C, 49.80; H, 4.00; N, 29.3%.

N-[(5-Chlorothiophen-2-yl)methylidene]-1H-1,2,4-triazol-3-amine (L4)

Yield (1.47 g, 69%); m.p. 178°C; IR (KBr, cm^{-1}): 3190 (NH), 970 (C-S), 1627 (HC=N), 1610 (C=N, triazole), 1570, 1540 (C=C), 1020 (N-N), 820 (C-Cl); ^{1}H NMR (DMSO-d_6): δ 7.39 (d, 1H, J = 3.5 Hz, C_4-H), 7.70 (d, 1H, J = 3.5 Hz, C_3-H), 8.5 (s, 1H, C_6-H), 9.24 (s, 1H, triazole), 13.99 (s, 1H, triazole, NH); ^{13}C NMR (DMSO-d_6): δ 128.9 (C_3), 132.5 (C_4), 135.6 (C_5), 146.2 (C_2), 153.3 (C_8), 156.9 (C_6), 158.6 (C_7); EIMS (70 eV) *m/z* (%): 212 (M$^+$, 34), 211 (60), 196 (44), 177 (46), 162 (27), 161 (100), 155 (13), 149 (13), 134 (17), 112 (15), 76 (17), 69 (15), 51 (11); Anal. Calcd. for $C_7H_5ClN_4S$ (212.66): C, 39.54; H, 2.37; N, 26.35. Found: C, 39.70; H, 2.34; N, 26.26%.

N-[(5-Nitrothiophen-2-yl)methylidene]-1H-1,2,4-triazol-3-amine (L5)

Yield (1.74 g, 79%); m.p. 203°C; IR (KBr, cm^{-1}): 3175 (NH), 980 (C-S), 1624 (HC=N), 1612 (C=N, triazole), 1560, 1540 (C=C), 1370 (NO_2), 1020 (N-N); ^{1}H NMR (DMSO-d_6): δ 7.72 (d, 1H, J = 4.1 Hz, C_3-H), 7.87 (d, 1H, J = 4.1 Hz, C_4-H), 8.30 (s, 1H, C_6-H), 9.27 (s, 1H, triazole), 14.00 (s, 1H, triazole, NH); ^{13}C NMR (DMSO-d_6): δ 129.9 (C_3), 133.8 (C_4), 141.2 (C_5), 147.0 (C_2), 153.6 (C_8), 157.7 (C_6), 159.1 (C_7); EIMS (70 eV) *m/z* (%): 223 (M$^+$, 75), 182 (10), 177 (100), 150 (30), 136 (17), 123 (15), 109 (27), 95 (33), 82 (10), 69 (23); Anal. Calcd. for $C_7H_5N_5S$ (223.21): C, 37.67; H, 2.26; N, 31.38. Found: C, 37.78; H, 2.34; N, 31.26%.

X-ray structure of N-[(5-methylthiophen-2-yl) methylidene]-1H-1,2,4-triazol-3-amine (L2)

The X-ray structure of one of the ligands, *N*-[(5-methylthiophen-2-yl)methylidene]-1*H*-1,2,4-triazol-3-amine (**L^2**), was determined and published[41] by us elsewhere, and is presented here as Figures 1 and 2 for authentication.

General procedure for the preparation of metal(II) complexes (1–20)

Cobalt(II) complex with N-(thiophen-2-ylmethylidene)-1H-1,2,4-triazol-3-amine (L1)

A warm ethanol (20 mL) solution of Co(II)Cl_2.6H_2O (0.238 g, 1 mmol) was added drop-wise to a magnetically

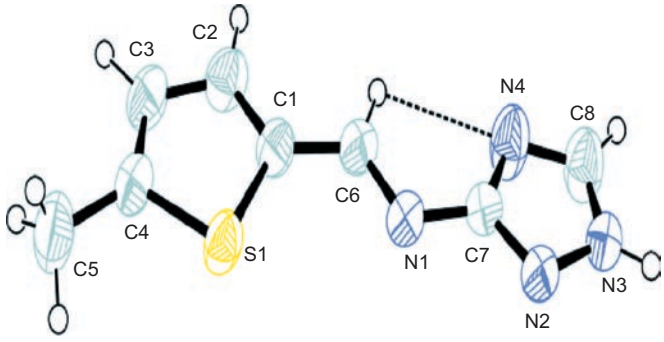


Figure 1. ORTEP diagram of a single molecule in asymmetric unit of **L²**.

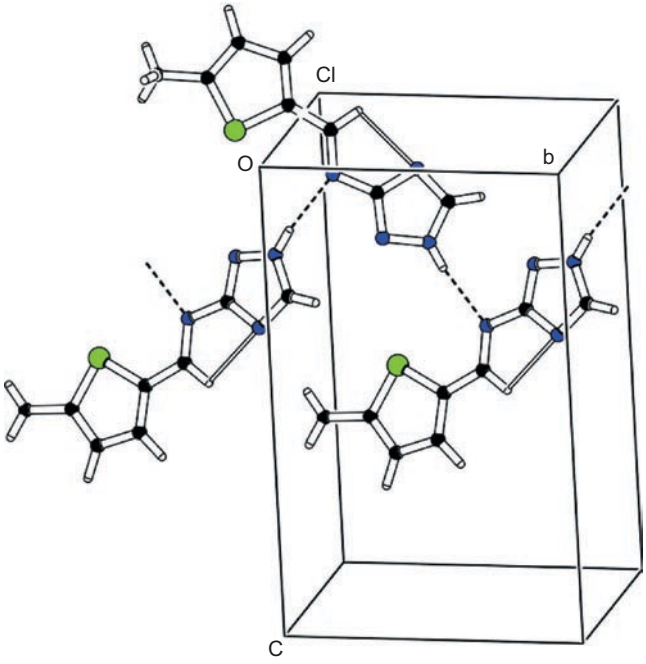


Figure 2. The unit cell packing in **L²**.

stirred solution of *N*-(thiophen-2-ylmethylidene)-1*H*-1,2,4-triazol-3-amine (**L¹**) (0.356 g, 2 mmol) in ethanol (25 mL). The mixture was refluxed for 2 h and cooled to room temperature. On cooling, a colored precipitate product was formed which was filtered, washed with ethanol and then ether, and dried. Crystallization from aqueous ethanol (30:70) gave the desired metal complex. Physical, analytical, and spectral data of Co(II), Ni(II), Cu(II), and Zn(II) complexes are given in Tables 1 and 2. The same method was used for the preparation of all other complexes.

NMR data of Zn(II) complexes

[Zn(L1)2Cl2] (4)

^{1}H NMR of Zn(II) complex (DMSO-d_6): δ 7.30 (dd, 2H, *J* = 4.6, 4.0 Hz, C_4-H), 7.80 (d, 2H, *J* = 4.0 Hz, C_3-H), 7.95 (d, 2H, *J* = 4.6 Hz, C_5-H), 8.50 (s, 1H, C_6-H), 9.46 (s, 2H, triazole), 14.0 (s, 2H, triazole, NH); ^{13}C NMR (DMSO-d_6): δ 127.5 (C_5), 129.8 (C_4), 132.8 (C_3), 144.5(C_2), 153.9 (C_8), 158.3, (C_6), 159.4 (C_7).

[Zn(L2)2Cl2] (8)

^{1}H NMR of Zn(II) complex (DMSO-d_6): δ 2.6 (s, 6H, C_5-CH_3), 7.0 (d, 2H, *J* = 3.0 Hz, C_4-H), 7.69 (d, 2H, *J* = 3.0 Hz, C_3-H), 8.43 (s, 2H, C_6-H), 9.38 (s, 2H, triazole), 14.05 (s, 2H, triazole, NH); ^{13}C NMR (DMSO-d_6): δ 15.6 (CH_3), 128.4 (C_4), 131.1 (C_3), 139.1 (C_5), 143.6 (C_2), 154.9 (C_8), 158.7 (C_6), 159.7 (C_7).

[Zn(L3)2Cl2] (12)

^{1}H NMR of Zn(II) complex (DMSO-d_6): δ 2.5 (s, 6H, C_3-CH_3), 7.12 (d, 2H, *J* = 4.5 Hz, C_4-H), 7.95 (d, 2H, *J* = 4.5 Hz, C_5-H), 8.40 (s, 2H, C_6-H), 9.45 (s, 2H, triazole), 14.00 (s, 2H, triazole, NH); ^{13}C NMR (DMSO-d_6): δ 14.5 (CH_3), 129.0 (C_2), 129.4 (C_4), 134.6 (C_5), 140.7 (C_3), 154.2 (C_8), 158.7 (C_6), 159.3 (C_7).

[Zn(L4)2Cl2] (16)

^{1}H NMR of Zn(II) complex (DMSO-d_6): δ 7.44 (d, 2H, *J* = 3.5 Hz, C_4-H), 7.75 (d, 2H, *J* = 3.5 Hz, C_3-H), 8.7 (s, 2H, C_6-H), 9.40 (s, 2H, triazole), 14.05 (s, 2H, triazole, NH); ^{13}C NMR (DMSO-d_6): δ 129.0 (C_3), 132.6 (C_4), 136.7 (C_5), 147.0 (C_2), 154.7 (C_8), 159.2 (C_6), 160.0 (C_7).

[Zn(L5)2Cl2] (20)

^{1}H NMR of Zn(II) complex (DMSO-d_6): δ 7.79 (d, 2H, *J* = 4.1 Hz, C_3-H), 7.95 (d, 2H, *J* = 4.1 Hz, C_4-H), 8.59 (s, 2H, C_6-H), 9.45 (s, 2H, triazole), 14.07 (s, 2H, triazole, NH); ^{13}C NMR (DMSO-d_6): δ 130.0 (C_3), 133.9 (C_4), 142.7 (C_5), 147.8 (C_2), 154.4 (C_8), 159.9(C_6) 160.6 (C_7).

Biological activity

Antibacterial studies

All the newly synthesized compounds (**L¹–L⁵**) and their respective metal(II) chelates **(1–20)** were tested against four Gram-negative (*E. coli*, *S. sonnei*, *P. aeruginosa*, *S. typhi*) and two Gram-positive (*S. aureus*, *B. subtilis*) bacterial strains by the disk diffusion method[42]. The test compounds (ligand/complex) were dissolved in DMSO to obtain 10 mg/mL solutions. A known volume (10 µL) of solution was applied with the help of a micropipette onto sterilized filter paper disks. The disks were dried at room temperature overnight and stored in sterile dry containers. Disks soaked with 10 µL of DMSO and dried in air at room temperature were used as the negative control. The standard antibiotic disks used as the positive control were either purchased from the manufacturer or prepared as

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

No.	M.P (dec.) (°C)	Yield (%)	Found (Calc.) (%) C	H	N
1 $[Co(\mathbf{L}^1)_2]Cl_2$ [486.26] $C_{14}H_{12}N_8S_2Cl_2Co$	230–232	58	34.62 (34.58)	2.42 (2.49)	22.97 (23.04)
2 $[Ni(\mathbf{L}^1)_2]Cl_2$ [486.03] $C_{14}H_{12}N_8S_2Cl_2Ni$	237–239	57	34.66 (34.60)	2.58 (2.49)	23.10 (23.06)
3 $[Cu(\mathbf{L}^1)_2]Cl_2$ [490.88] $C_{14}H_{12}N_8S_2Cl_2Cu$	234–236	60	34.42 (34.25)	2.40 (2.46)	22.95 (22.83)
4 $[Zn(\mathbf{L}^1)_2]Cl_2$ [492.74] $C_{14}H_{12}N_8S_2Cl_2Zn$	244–246	58	34.22 (34.13)	2.50 (2.45)	22.67 (22.74)
5 $[Co(\mathbf{L}^2)_2]Cl_2$ [514.32] $C_{16}H_{16}N_8S_2Cl_2Co$	223–225	59	37.43 (37.36)	3.20 (3.14)	21.66 (21.79)
6 $[Ni(\mathbf{L}^2)_2]Cl_2$ [514.08] $C_{16}H_{16}N_8S_2Cl_2Ni$	231–23	61	37.30 (37.38)	3.06 (3.14)	21.89 (21.80)
7 $[Cu(\mathbf{L}^2)_2]Cl_2$ [518.93] $C_{16}H_{16}N_8S_2Cl_2Cu$	238–240	58	37.14 (37.03)	3.18 (3.11)	21.70 (21.59)
8 $[Zn(\mathbf{L}^2)_2]Cl_2$ [520.77] $C_{16}H_{16}N_8S_2Cl_2Zn$	235–237	58	37.0 (36.90)	3.01 (3.10)	21.60 (21.52)
9 $[Co(\mathbf{L}^3)_2]Cl_2$ [514.32] $C_{16}H_{16}N_8S_2Cl_2Co$	215–217	62	37.31 (37.36)	3.22 (3.14)	21.71 (21.79)
10 $[Ni(\mathbf{L}^3)_2]Cl_2$ [514.08] $C_{16}H_{16}N_8S_2Cl_2Ni$	220–222	59	37.27 (37.38)	3.20 (3.14)	21.86 (21.80)
11 $[Cu(\mathbf{L}^3)_2]Cl_2$ [518.93] $C_{16}H_{16}N_8S_2Cl_2Cu$	228–230	58	36.95 (37.03)	3.02 (3.11)	21.48 (21.59)
12 $[Zn(\mathbf{L}^3)_2]Cl_2$ [520.77] $C_{16}H_{16}N_8S_2Cl_2Zn$	234–236	57	37.05 (36.90)	3.04 (3.10)	21.66 (21.52)
13 $[Co(\mathbf{L}^4)_2]Cl_2$ [555.16] $C_{14}H_{10}N_8S_2Cl_4Co$	222–224	62	30.12 (30.29)	1.86 (1.82)	20.30 (20.18)
14 $[Ni(\mathbf{L}^4)_2]Cl_2$ [554.91] $C_{16}H_{16}N_8S_2Cl_4Ni$	225–227	60	30.05 (30.30)	1.89 (1.82)	20.39 (20.19)
15 $[Cu(\mathbf{L}^4)_2]Cl_2$ [559.77] $C_{16}H_{16}N_8S_2Cl_4Cu$	232–234	58	29.89 (30.04)	1.74 (1.80)	20.30 (20.02)
16 $[Zn(\mathbf{L}^4)_2]Cl_2$ [561.61] $C_{16}H_{16}N_8S_2Cl_4Zn$	229–231	59	30.20 (29.94)	1.89 (1.79)	20.10 (19.95)
17 $[Co(\mathbf{L}^5)_2]Cl_2$ [576.26] $C_{14}H_{10}N_{10}S_2O_4Cl_2Co$	258–260	63	29.04 (29.18)	1.68 (1.75)	24.39 (24.31)
18 $[Ni(\mathbf{L}^5)_2]Cl_2$ [576.02] $C_{14}H_{10}N_{10}S_2O_4Cl_2Ni$	265–267	58	29.37 (29.19)	1.70 (1.75)	24.54 (24.32)
19 $[Cu(\mathbf{L}^5)_2]Cl_2$ [580.87] $C_{14}H_{10}N_{10}S_2O_4Cl_2Cu$	269–271	61	29.12 (28.95)	1.79 (1.74)	24.28 (24.11)
20 $[Zn(\mathbf{L}^5)_2]Cl_2$ [582.71] $C_{14}H_{10}N_{10}S_2O_4Cl_2Zn$	278–280	57	29.01 (28.86)	1.68 (1.73)	23.91 (24.04)

above in the laboratory by applying a known concentration of standard antibiotic solution. Bacterial cultures were grown in nutrient broth medium at 37°C overnight and spread onto solidified nutrient agar medium in Petri plates using sterilized cotton swabs in a standard microbiological working environment[42]. Test and control disks were then applied to the solidified medium surface with the help of sterilized forceps. The plates were incubated at 37°C for 12–15 h. The results were recorded by measuring the zone of inhibition in mm against each compound[42]. Ampicillin was used as the reference compound. Experiments were carried out in triplicate and the values obtained were statistically analyzed.

Antifungal activity (in vitro)

Antifungal activities of all compounds were studied[43] against six fungal strains (*T. longifusus*, *C. albicans*, *A. flavus*, *M. canis*, *F. solani*, and *C. glabrata*). Sabouraud dextrose agar (Oxoid, Hampshire, England) was seeded with 10^5 cfu/mL fungal spore suspensions and transferred to Petri plates. Disks soaked in 20 mL (200 µg/mL in DMSO) of the compounds were placed at different positions on the agar surface[44]. 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.

Minimum inhibitory concentration

Compounds containing significant antibacterial activity (over 80%) were selected for minimum inhibitory concentration (MIC) studies. The minimum inhibitory concentration was determined using the disk diffusion technique by preparing disks containing 10, 25, 50, and 100 µg/mL of the compounds and applying the protocol[44].

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

No.	Ω_M (Ω^{-1} cm^2 mol^{-1})	B.M. (μ_{eff})	λ_{max} (cm^{-1})	IR (cm^{-1})
1	84.6	4.32	8565, 17,545, 29,990	3175 (NH), 1613 (HC=N), 1600 (C=N), 1565, 1540 (C=C), 1020 (N-N), 960 (C-S), 520 (M-N), 365 (M-S)
2	82.8	3.42	9895, 16,105, 29,272	3175 (NH), 1612 (HC=N), 1601 (C=N), 1565, 1540 (C=C), 1020 (N-N), 960 (C-S), 525 (M-N), 370 (M-S)
3	89.5	1.49	14,725, 25,350	3175 (NH), 1610 (HC=N), 1600 (C=N), 1565, 1540 (C=C), 1020 (N-N), 960 (C-S), 523 (M-N), 367 (M-S)
4	85.9	Dia	28,530	3175 (NH), 1610 (HC=N), 1600 (C=N), 1565, 1540 (C=C), 1020 (N-N), 960 (C-S), 520 (M-N), 364 (M-S)
5	87.8	4.4	8610, 17,580, 29,910	3175 (NH), 1617 (HC=N), 1602 (C=N), 1565, 1540 (C=C), 1020 (N-N), 965 (C-S), 520 (M-N), 364 (M-S)
6	83.0	3.28	10,130, 16,210, 29,355	3175 (NH), 1616 (HC=N), 1601 (C=N), 1565, 1540 (C=C), 1020 (N-N), 965 (C-S), 522 (M-N), 368 (M-S)
7	88.6	1.43	14,670, 25,427	3175 (NH), 1613 (HC=N), 1599 (C=N), 1565, 1540 (C=C), 1020 (N-N), 965 (C-S), 526 (M-N), 365 (M-S)
8	88.0	Dia	28,628	3175 (NH), 1616 (HC=N), 1600 (C=N), 1565, 1540 (C=C), 1020 (N-N), 965 (C-S), 528 (M-N), 363 (M-S)
9	78.6	4.55	8590, 17,520, 30,150	3180 (NH), 1611 (HC=N), 1602 (C=N), 1565, 1540 (C=C), 1020 (N-N), 970 (C-S), 529 (M-N), 369 (M-S)
10	82.5	3.37	10,105, 16,185, 29,491	3180 (NH), 1610 (HC=N), 1603 (C=N), 1565, 1540 (C=C), 1020 (N-N), 965 (C-S), 522 (M-N), 364 (M-S)
11	87.00	1.60	14,670, 25,540	3180 (NH), 1609 (HC=N), 1604 (C=N), 1565, 1540 (C=C), 1020 (N-N), 965 (C-S), 525 (M-N), 366 (M-S)
12	86.00	Dia	28,680	3180 (NH), 1611 (HC=N), 1601 (C=N), 1565, 1540 (C=C), 1020 (N-N), 965 (C-S), 527(M- N), 360 (M-S)
13	79.3	4.35	8675, 17,610, 30,180	3190(NH), 1608(HC=N), 1599 (C=N), 1560, 1540 (C=C), 1020 (N-N), 970 (C-S), 820 (C-Cl), 531 (M- N), 367 (M-S)
14	81.0	3.5	9960, 15,988, 29,375	3190 (NH), 1609 (HC=N), 1598 (C=N), 1560, 1540 (C=C), 1020 (N-N), 970 (C-S), 825 (C-Cl), 529 (M-N), 369 (M-S)
15	78.7	1.64	14,910, 25,350	3190 (NH), 1611 (HC=N), 1599 (C=N), 1560 1540 (C=C), 1020 (N-N), 970 (C-S), 824 (C-Cl), 527 (M -N), 360 (M-S)
16	81.2	Dia	28,465	3175 (NH), 1612 (HC=N), 1597 (C=N), 1560, 1540 (C=C), 1020 (N-N), 970 (C-S), 830 (C-Cl), 523 (M-N), 364 (M-S)
17	82.6	4.55	8502, 17,710, 29,970	3175 (NH), 1610 (HC=N), 1602 (C=N), 1560, 1540 (C=C), 1370 (C-NO_2), 1020 (N-N), 980 (C-S), 520 (M-N), 362 (M-S)
18	78.8	3.48	9980, 15,875, 29,472	3175(NH), 1609(HC=N), 1596 (C=N), 1560, 1540 (C=C), 1375 (C-NO_2), 1020 (N-N), 980 (C-S), 525(M- N), 370 (M-S)
19	83.00	1.59	14,815, 25,445	3175 (NH), 1605 (HC=N), 1599 (C=N), 1560, 1540 (C=C), 1372 (C-NO_2), 1020 (N-N), 980(C-S), 521 (M-N), 360 (M-S)
20	89.5	Dia	28,538	3175 (NH), 1608 (HC=N), 1600 (C=N), 1560, 1540 (C=C), 1372 (C-NO_2), 1020 (N-N), 980 (C-S), 529 (M-N), 366 (M-S)

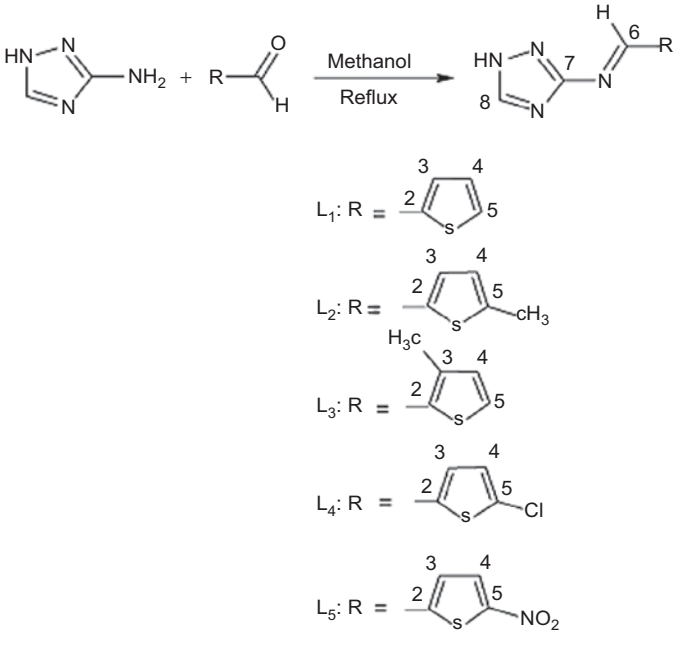
In vitro cytotoxicity

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 a commercial salt mixture and double distilled water[45]. 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 smaller compartment was opened to ordinary light. After 2 days, nauplii were collected by a pipette from the light side. A sample of test compound was prepared by dissolving 20 mg of each compound in 2 mL of DMSO. From this stock solution, 500, 50, and 5 µg/mL were transferred to nine vials (three for each dilution 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. The solvent was allowed to evaporate overnight. After 2 days, when the shrimp larvae were ready, 1 mL of sea-water 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 using Finney's computer program to determine the LD_{50} values[46].

Results and discussion

Chemistry

The Schiff base derivatives of triazole **(L^1–L^5)** were prepared by refluxing an appropriate amount of 3-amino-1,2,4-triazole with a series of methyl-, chloro-, and nitro-substituted thiophene-2-carboxaldehydes, as shown in Scheme 1. All triazole derivatives were only soluble in methanol, ethanol, dioxane, dimethylformamide, and dimethylsulfoxide. The compositions were consistent with their microanalytical and mass spectral data. The metal(II) complexes **(1–20)** were prepared in a stoichiometric (metal:ligand, 1: 2) molar ratio.



Scheme 1. Preparation of ligands.

Cobalt, copper, nickel, and zinc were used as the chlorides. Physical measurements and analytical data of complexes **1–20** are given in Tables 1 and 2.

Conductance and magnetic susceptibility measurements

The electrolytic nature of the metal complexes **(1–20)** was indicated by molar conductance values (in dimethylformamide, DMF) (Table 2), which fell in the range 78.6–89.5 Ω^{-1} cm^2 mol^{-1}, showing an electrolytic nature[47]. The magnetic moment values of the solid metal complexes obtained at room temperature are also given in Table 2. The magnetic moment values for Co(II) and Ni(II) complexes were found to be in the ranges of 4.32–4.55 B.M. and 3.28–3.50 B.M., respectively, indicative of three and two unpaired electrons for Co(II) and Ni(II) ions in an octahedral environment[48]. The magnetic moment values 1.43–1.64 B.M. for Cu(II) complexes are indicative of one unpaired electron per Cu(II) ion, which suggests that the structures of copper(II) complexes had spin-free distorted octahedral geometry[49]. All the Zn(II) complexes were found to be diamagnetic.

IR spectra

The characteristic bands of IR spectra of the ligands **(L^1–L^5)** and their metal complexes are given in "Materials and methods" above and in Table 2. The presence of a strong new band in the ligands at 1624–1632 cm^{-1} gave a clue of condensation of the carbonyl υ(C=O) group of thiophene-2-carboxaldehyde with the amino (NH_2) group of triazole to develop an azomethine (HC=N) linkage[50], which is also supported by the absence of spectral bands at 1715 and 3325 cm^{-1} originally assigned to carbonyl υ(C=O) and amine $\upsilon(NH_2)$ stretching vibrations. The IR spectral bands of the ligands at 1610–1615 cm^{-1} and 1540–1575 cm^{-1} were assigned to triazole (C=N) and thiophene (C=C), respectively. The IR bands of the ligands at 1624–1632 and 1610–1615 cm^{-1} assigned to the azomethine (HC=N) vibration and triazole (C=N), respectively, were shifted to lower frequencies at 1603–1616 and 1596–1604 cm^{-1}, by 15–20 cm^{-1} [51], indicating the coordination of azomethine (HC=N) and (C=N) of triazole with the metal(II) ions. In addition to this, new weak bands appeared in all complexes at lower frequency regions 360–370 cm^{-1} and 520–532 cm^{-1}, which indicated the coordination of metal–sulfur (M-S) and metal–nitrogen (M-N), respectively. The IR spectra of the ligands **(L^1–L^5)** and their metal(II) complexes confirmed coordination of the ligands with the metal(II) ions tridentately through the sulfur of thienyl and nitrogens of azomethine (HC=N) and triazole (C=N)[52].

1H NMR spectra

^{1}H NMR spectral data of the free ligands (**L^1–L^5**) and their Zn(II) complexes are recorded in "Materials and methods." The exhibited signals of all protons of the Schiff bases due to heteroaromatic/aromatic groups were found to be in their expected regions[53]. ^{1}H NMR spectra of the Schiff bases displayed protons due to azomethine (C_6-H) and triazole (C_8-H) at δ 8.20–8.50 and δ 9.20–9.30 respectively, as singlets. The

^{1}H NMR spectrum of **L^1** exhibited thienyl C_3-H and C_5-H as a doublet at 7.76 ppm and 7.83 ppm, respectively. However, the thienyl C_4-H of **L^1** appeared as a double doublet at 7.24 ppm. The peaks appearing at 6.96 ppm and 7.60 ppm were assigned to the protons C_4-H and C_3-H, respectively, of the thienyl ring as a doublet of **L^2** [54]. The spectrum of **L^3** showed thienyl C_4-H and C_5-H as a doublet at 7.06 ppm and 7.75 ppm, respectively. ^{1}H NMR spectra of ligands **L$_2$** and **L$_3$** exhibited methyl protons C_3-CH_3 and C_5-CH_3 as singlets at δ 2.4–2.5. In the case of compounds **L$_2$** and **L$_3$**, as C_4-H was shielded from the electron-donating effect of methyl (CH_3) at the 3- and 5-positions, these appeared upfield at 6.96 ppm and 7.06 ppm as compared to **L^1** at 7.24 ppm. The ^{1}H NMR spectrum of **L^4** displayed C_3-H and C_4-H as a doublet at 7.70 ppm and 7.39 ppm, respectively. The thienyl proton of C_3-H and C_4-H appeared as a doublet at 7.72 ppm and 7.87 ppm of compound **L^5**, respectively. Due to the electron-withdrawing effect of the NO_2 group in compound **L^5**, thienyl C_4-H appeared downfield at 7.87 ppm as compared to the thienyl C_4-H at 7.24 ppm in ligand **L^1** [55]. A broad singlet at δ 13.90–14.0 displayed the NH proton of triazole in all the ligands, which disappeared on exchangement with D_2O. By comparing ^{1}H NMR spectra of diamagnetic Zn(II) complexes with the free ligands, the proton signals of the triazole ring (C_8-H) and azomethine (CH=N) were assigned a downfield shift by 0.20–0.40 ppm upon coordination. All other protons underwent a downfield shift by 0.11–0.24 ppm due to the increased conjugation[56].

13C NMR spectra

^{13}C NMR spectra of the free Schiff bases and their diamagnetic zinc(II) complexes were recorded in DMSO-d_6. The ^{13}C NMR spectral data of the free Schiff bases and their zinc(II) complexes along with possible assignments are reported in "Materials and methods." The carbons were found to be in their expected regions[53]. These studies were well supported by the IR and ^{1}H NMR spectral data. The azomethine carbon (C_6) of all the Schiff bases appeared in the region of δ 156.2–157.7 ppm[55]. The ^{13}C NMR spectrum of **L^1** showed that the thienyl carbons C_2, C_3, C_4, and C_5 were found at 143.6, 132.6, 129.5, and 126.7 ppm, respectively. All thienyl carbons in all ligands were present in the region of δ 126.7–147.0. All the ligands showed triazole carbons at δ 152.7–159.1. The methyl groups of ligands **L^2** and **L^3** appeared in the region of δ 14.4–15.5. Downfield shifting of the azomethine carbon (C_6) from 156.2–157.7 ppm in the triazole Schiff bases to 158.3–159.9 ppm in the metal(II) complexes revealed coordination of the azomethine to the metal atom. Similarly, the carbon atom attached to the N of the triazole ring that participated in the coordination of carbon also showed a downfield shift by 0.24–2.40 ppm[54].

Mass spectra

The electron impact mass spectra (EIMS) gave compositions: $C_7H_6N_4S$, 178.0 (calcd. 178.21); $C_8H_8N_4S$, 192.0 (192.24); $C_8H_8N_4S$, 192.2 (192.24); $C_7H_5ClN_4S$, 212.2 (212.6); and $C_7H_5N_5O_2S$, 223.2 (223.21). **L^1** showed a base peak at 177 of fragment $[C_7H_5N_4S]^+$; for **L^2** this was observed at 191 of fragment $[C_8H_7N_4S]^+$; for **L^3** at 177.2 of fragment $[C_7H_5N_4S]^+$; for **L^4** at 177.2 of fragment $[C_7H_5N_4S]^+$; and for **L^5** at 177.2 of fragment $[C_7H_5N_4S]^+$; these are the most expected stable fragments. The most probable fragmentation pattern was the cleavage of C=N (exocyclic as well as endocyclic), C=C, C-C, and C-S bonds.

Electronic spectra

The electronic spectral values of Co(II), Ni(II), Cu(II), and Zn(II) complexes are recorded in Table 2. The electronic spectra of Co(II) complexes generally showed three absorption bands in the regions 8502–8675, 17,520–17,710, and 29,910–30,180 cm^{-1}, which may be assigned to $^4T_{1g} \rightarrow {}^4T_{2g}(F)$, $^4T_{1g} \rightarrow {}^4A_{2g}(F)$, and $^4T_{1g} \rightarrow {}^4T_g(P)$ transitions, respectively, and are suggestive of octahedral geometry[49,55] around the Co(II) ion. The electronic spectral data of Ni(II) complexes showed d–d bands in the regions 9960–10,130, 15,875–16,210, and 29,272–29,491 cm^{-1}, assignable respectively to the transitions $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$, and $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$, which are characteristic of Ni(II) in octahedral geometry[48,56]. The electronic spectra of Cu(II) complexes showed an absorption band in the region 14,670–14,910 cm^{-1}, which may be assigned to the transition $^2E_g \rightarrow {}^2T_{2g}$. The high-energy band at 25,350–25,540 cm^{-1} is due to forbidden ligand → metal charge transfer. On the basis of the electronic spectra, a distorted octahedral geometry around the Cu(II) ion is suggested[49,57]. The Zn(II) complexes were diamagnetic; they did not show any d–d transition and their spectra were dominated[58] only by the charge transfer band at 28,465–28,728 cm^{-1}.

Biological activity

Antibacterial bioassay (in vitro)

Antibacterial activity of the title Schiff bases and their metal chelates was determined against four Gram-negative (*E. coli*, *S. sonnei*, *P. aeruginosa*, *S. typhi*) and two Gram-positive (*S. aureus*, *B. subtilis*) bacterial strains (Table 3) according to the literature protocol[42]. The results for all the synthesized compounds were compared with those of the standard drug ampicillin (Figures 3 and 4). The ligands showed varying degrees of inhibitory effect: low, moderate, and significant, on the growth of the different tested strains, while their metal complexes had only moderate to significant inhibitory effects on the growth of the different tested strains (Table 3). The Schiff base ligand **L^1** possessed significant activity (53–64%) against (c), (d), and (e), moderate activity (45–46%) against (a) and (b), and weaker activity (24%) against (f). The antibacterial activity of compound **L^2** was found to be significant (56–69%) against (a), (c), and (f), moderate (48–50%) against (b) and (e), and weaker (24%) against (d). Significant activity (64–66%) was observed for ligand **L^3** against (b) and (d), moderate (44–48%) against (c), (e), and (f), and weaker (30%) against (b). The ligand **L^4** showed good antibacterial activity (53–66%) against (d), (e), and (f), moderate (46%) activity against (a) and (c), and weaker (33%) against (b).

Table 3. Antibacterial bioassay (concentration used 1 mg/mL of DMSO) of ligands and metal(II) complexes.

	Zone of inhibition (mm)						
	Gram-negative				Gram-positive		
Compound	(a)	(b)	(c)	(d)	(e)	(f)	SA
L^1	12	11	17	18	16	07	3.50
L^2	18	12	18	09	14	17	3.33
L^3	08	16	15	18	12	14	2.56
L^4	12	08	15	17	16	17	2.67
L^5	13	06	17	14	18	16	3.00
1	12	12	19	21	17	12	3.50
2	13	17	18	19	13	14	2.33
3	17	16	23	20	17	13	2.56
4	18	17	17	22	16	11	2.22
5	20	16	19	12	17	13	2.50
6	21	12	18	18	18	15	2.33
7	19	16	25	17	16	21	2.67
8	21	12	18	13	17	24	3.50
9	13	19	14	20	13	18	2.83
10	12	20	13	23	16	20	3.67
11	12	18	15	21	14	19	2.83
12	13	17	18	20	19	20	1.89
13	17	12	19	19	17	21	2.17
14	18	11	19	20	19	19	2.22
15	16	16	18	22	20	21	2.17
16	20	16	15	17	18	24	2.44
17	16	12	19	18	19	20	2.22
18	16	16	15	21	23	17	2.67
19	19	11	22	17	21	19	2.78
20	17	16	23	18	20	15	2.22
SD	26	24	32	28	27	29	2.00

Note. (a), *E. coli*; (b), *S. sonnei*; (c), *P. aeruginosa*; (d), *S. typhi*; (e), *S. aureus*; (f), *B. subtilis*. <10, weak; >10, moderate; >16, significant. SD, standard drug (ampicillin); SA, statistical analysis.

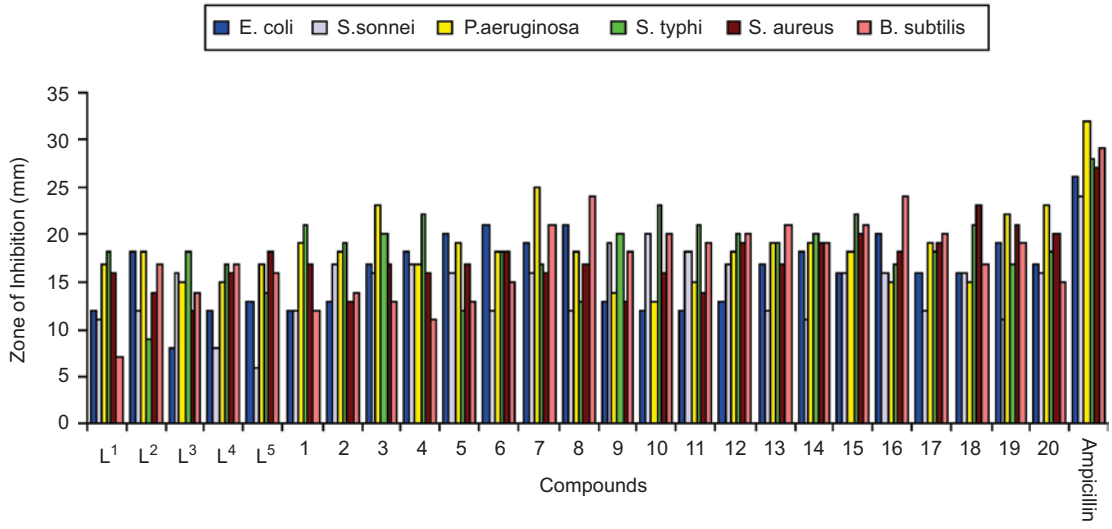


Figure 3. Comparison of antibacterial activity.

Ligand **L^5** also showed significant activity (53–66%) against (c), (e), and (f), moderate activity (50%) against (a) and (d), and weaker (25%) against (b). Compounds **1–20** showed overall significant activity (53–85%) against (a), (b), (c), (d), (e), and (f). However, moderate activity (37–50%) was observed for compounds **1**, **2**, **9**, **10**, **11**, and **12** against (a), **1**, **6**, **8**, **13**, **14**, **17**, and **19** against (b), **9**, **10**, **11**, and **16** against (c), **5** and **8** against (d), **2**, **9**, and **11** against (e), and **1–6** and **20** against (f). The antibacterial results (Table 3) evidently show that the activity of the Schiff base

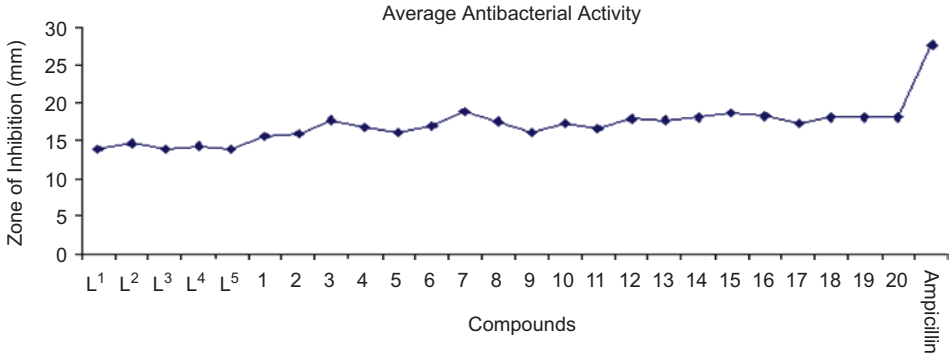


Figure 4. Average antibacterial activity.

Table 4. Antifungal bioassay (concentration used 200 µg/mL) of ligands and metal(II) complexes.

	% Inhibition						
Compound	(a)	(b)	(c)	(d)	(e)	(f)	SA
L^1	29	08	53	42	54	21	18.07
L^2	46	22	29	10	39	54	13.00
L^3	33	46	00	50	57	48	15.00
L^4	24	42	08	62	39	47	14.00
L^5	32	24	48	59	66	18	16.50
1	38	12	59	34	59	29	13.67
2	41	22	38	48	61	36	09.00
3	24	26	57	51	44	39	10.50
4	35	16	56	44	55	33	11.83
5	39	38	43	16	49	67	11.00
6	54	40	58	12	43	46	10.78
7	44	27	37	24	45	56	09.50
8	53	35	39	14	53	45	10.50
9	20	42	39	57	46	50	08.67
10	14	46	44	49	25	67	14.22
11	41	54	10	54	23	54	15.22
12	24	49	25	50	52	53	11.78
13	33	43	20	72	45	43	10.78
14	38	57	09	63	41	29	14.17
15	29	34	14	56	43	52	10.67
16	39	43	28	59	38	53	08.44
17	35	29	53	59	74	20	17.17
18	39	21	37	65	59	31	13.33
19	41	29	44	67	69	14	16.00
20	34	15	36	70	68	23	18.67
SD	A	B	C	D	E	F	

Note. (a), *T. longifusus*; (b), *C. albicans*; (c), *A. flavus*; (d), *M. canis*; (e), *F. solani*; (f), *C. glabrata*. SD, standard drug; MIC: 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, 2.6626×10^{-7} M/mL). SA, statistical analysis.

compounds was enhanced on coordination with the metal ion. Enhancement in activity of the Schiff bases upon coordination can be explained on the basis of chelation theory. Chelation reduces the polarity of the metal ion to a significant extent due to overlapping with donor groups. Further, the delocalization of π-electrons over the whole chelate ring is increased, which in turn enhances the lipophilicity of the complexes[59]. The increased lipophilicity in turn enhances the penetration of complexes into lipid membranes, thus killing more bacteria.

In vitro antifungal bioassay

The antifungal screening of all compounds was carried out against *T. longifusus*, *C. albicans*, *A. flavus*, *M. canis*, *F. solani*, and *C. glabrata* fungal strains (Table 4) according to the literature protocol[43]. Some of the Schiff base derivatives of triazole showed moderate to significant degrees of inhibitory effect on the growth of the tested strains, whereas some showed either low or no inhibitory effect. However, the Schiff base **L^1** showed significant activity (53–54%) against (c) and (e), **L^2** showed significant activity (54%)

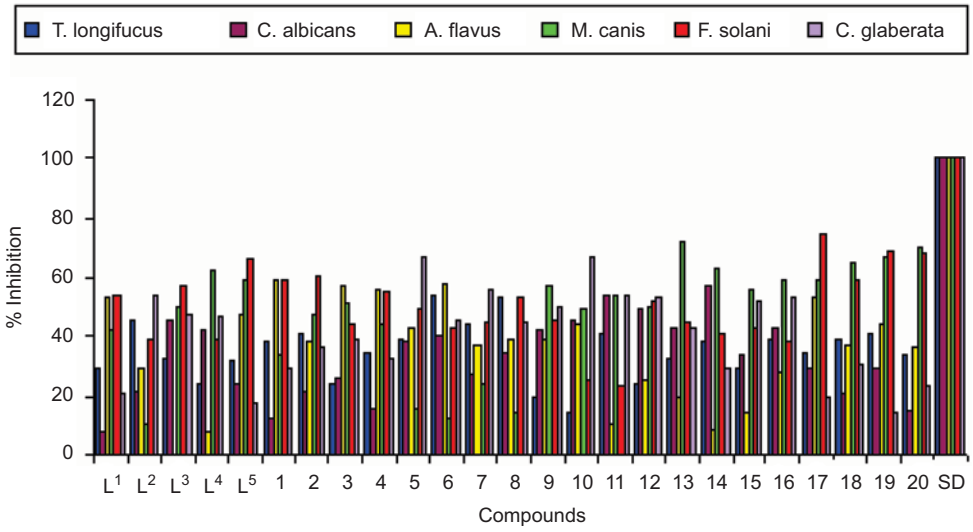


Figure 5. Comparison of antifungal activity.

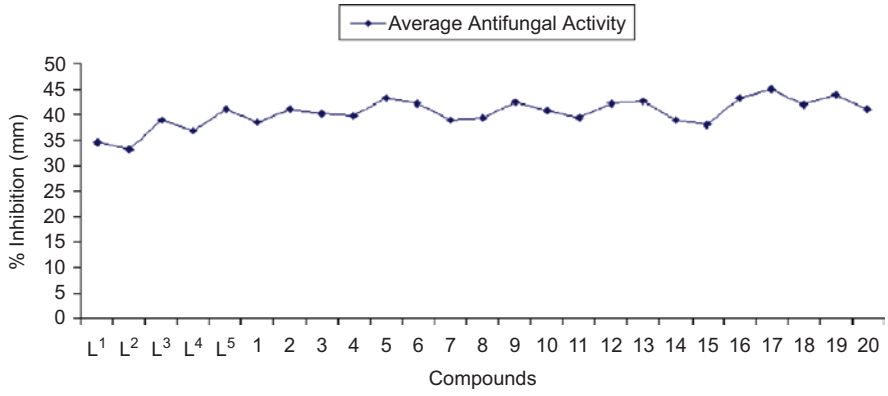


Figure 6. Average antifungal activity.

Table 5. Minimum inhibitory concentration (μg/mL) of selected compounds **6**, **8**, **10**, **16**, and **18** against selected bacteria.

	6	**8**	**10**	**16**	**18**
Gram-negative					
E. coli	60.66	44.37	—	—	—
S. sonnei	—	—	50.12	—	—
S. typhi	—	—	59.63	—	—
Gram-positive					
S. aureus	—	—	—	—	35.48
B. subtilis	—	56.76	—	70.66	—

against (f), **L**^{3} showed significant activity (57%) against (e), **L**^{4} showed significant activity (62%) against (d), and **L**^{5} showed significant activity (59–66%) against (d) and (e). The results given in Table 4 show that compounds **6** and **8** possessed significant activity (53–54%) against (a), **11** and **14** showed significant activity (54–57%) against (b), **1**, **3**, **4**, **6**, and **17** had significant activity (54–59%) against (c), **9**, **11**, and **13–20** possessed significant activity (54–72%) against (d), **1**, **2**, **4**, **8**, and **17-20** had significant activity (53–74%) against (e), and **5**, **7**, **10**, **11**, **12**, and **13** showed significant activity (53–67%) against (f). Most of the other compounds showed moderate and only a few possessed weaker activity against *T. longifusus*, *C. albicans*, *A. flavus*, *M. canis*, *F. solani*, and *C. glabrata* fungal strains. The results of inhibition were compared with the results for the standard drugs, miconazole and amphotericin B[44] (Figures 5 and 6).

Minimum inhibitory concentration (MIC)

The antibacterial results for all the synthesized compounds obtained after preliminary screening showed that

Table 6. 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]	$>8.15\times10^{-4}$
L[2]	$>8.66\times10^{-4}$
L[3]	$>3.64\times10^{-4}$
L[4]	$>4.51\times10^{-4}$
L[5]	$>4.46\times10^{-4}$
1	$>7.54\times10^{-4}$
2	$>9.39\times10^{-4}$
3	4.47×10^{-5}
4	5.80×10^{-5}
5	5.48×10^{-4}
6	$>4.48\times10^{-4}$
7	1.92×10^{-4}
8	9.44×10^{-4}
9	$>6.32\times10^{-4}$
10	$>4.86\times10^{-4}$
11	$>8.16\times10^{-4}$
12	$>5.78\times10^{-4}$
13	$>9.08\times10^{-4}$
14	2.52×10^{-4}
15	4.61×10^{-5}
16	$>4.51\times10^{-4}$
17	$>4.94\times10^{-4}$
18	$>3.72\times10^{-4}$
19	$>4.59\times10^{-4}$
20	9.60×10^{-5}

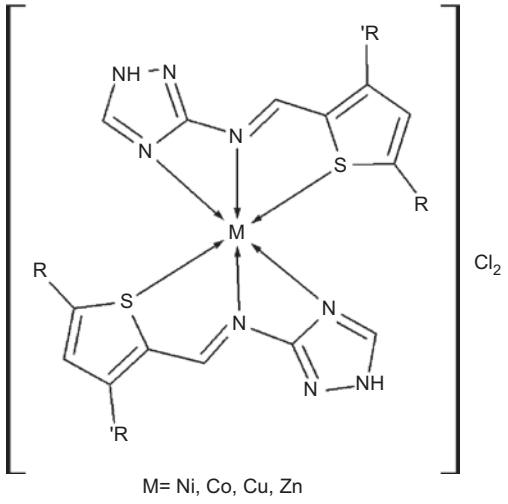


Figure 7. Proposed structure of the metal complexes.

compounds **6**, **8**, **10**, **16**, and **18** were the most active (above 80%). These five compounds were therefore selected for minimum inhibitory concentration (MIC) studies (Table 5). The MIC of these compounds was in the range 35.48–70.66 µg/mL. The MIC results in Table 5 show that compound **18** was the most active. It inhibited the growth of *S. aureus* at 35.48 µg/mL.

In vitro cytotoxic bioassay

The synthesized ligands **(L**[1]**–L**[5]**)** and their metal(II) complexes **(1–20)** were screened for their cytotoxicity (brine shrimp bioassay) using the protocol of Meyer *et al.*[45]. The cytotoxic data recorded in Table 6 reveal that only six compounds, **3**, **4**, **7**, **14**, **15**, and **20**, displayed potent cytotoxic activity, $LD_{50} = 4.47\times10^{-5}$ to 2.52×10^{-4} M, against *Artenia salina*, while all other compounds can be considered as almost inactive in this assay. It was interesting to note that the metal complexes showed potent cytotoxicity as compared to the ligands. The values of LD_{50} [46] of the synthesized compounds from Table 6 show that the cytotoxic activity of the copper complexes is better than of the other metal complexes. This activity relationship may help to serve as a basis for future direction toward the development of certain cytotoxic agents for clinical application.

Conclusions

The target compounds were achieved and characterized successfully (Figure 7). Their screening results revealed that the antibacterial and antifungal activity increases upon chelation/coordination. Chelation reduces the polarity of the metal ion, which in turn increases the lipophilic nature of the metal. This lipophilic character experienced by the metal ions further enhances effective penetration through the lipid layer of the cell membrane of the microorganism by killing the bacteria more efficiently. Further, it has been suggested that some functional groups such as azomethine (HC=N), or heteroatoms present in the compounds, may play an important role in increasing the biological activity of the synthesized compounds.

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Declaration of interest

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