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Synthesis and characterization of biologically active new Schiff bases containing 3-functionalized 1,2,4-triazoles and their zinc(II) complexes: crystal structure of 4-bromo-2-[(*E*)-(1H-1,2,4-triazol-3-ylimino)-methyl]phenol

Zahid H. Chohan* and M. Hanif

Biologically active triazole Schiff bases (L^1–L^3) derived from the reaction of 3-amino-1,2,4-triazole with chloro-, bromo- and nitro- substituted salicylaldehydes and their Zn(II) complexes (1–3) have been synthesized and characterized by their physical, spectral and analytical data. Triazole Schiff bases potentially act as tridentate ligands and coordinate with the Zn(II) metal atom through salicylidene-O, azomethine-N and triazole-N. The complexes have the general formula $[M(L\text{-}H)_2]$, where M = zinc(II) and L = (L^1–L^3), and observe an octahedral geometry. The Schiff bases and their Zn(II) complexes have been screened for *in-vitro* antibacterial, antifungal and brine shrimp bioassay. The biological activity data show the Zn(II) complexes to be more potent antibacterial and antifungal than the parent simple Schiff bases. Copyright © 2011 John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this article.

Keywords: triazole Schiff bases; zinc(II) complexes; antibacterial; antifungal activity

Introduction

Compounds containing triazole moiety have been revealed to exhibit a wide variety of interesting biological properties such as anticancer,[1,2] antifungal,[3–6] antimicrobial,[7–9] analgesic,[10] anti-inflammatory,[11–14] antibacterial,[15–19] anticonvulsant[20–22] and antitubercular.[23–27] Furthermore, they are used as a versatile reagent in the synthesis of heterocyclic compounds and/or as a raw material in drug synthesis[28a–e] (Fig. 1 shows some examples of clinical drugs in practice containing 1,2,4-triazole ring system). To expand and further explore the clinical significance of triazoles, we have undertaken to synthesize new Schiff base derivatives of triazole ($\mathbf{L^1}$–$\mathbf{L^3}$; Scheme 1). These triazole-derived Schiff bases have potential sites for metal complexation, especially with the zinc atom to form their Zn(II) complexes (**1**–**3**; Scheme 1). We hope that these newly synthesized Zn(II) complexes will have a tendency to reduce the resistivity of bacterial strains.[29] The newly synthesized Schiff bases and their metal complexes were characterized by their IR, NMR, molar conductance, magnetic moments, electronic and elemental analyses data. The synthesized ligands and their Zn(II) chelates were evaluated for *in vitro* antibacterial activity against *Escherichia coli, Salmonella sonnei, Pseudomonas aeruginosa, Salmonella enterica serovar typhi, Staphylococcus aureus* and *Bacillus subtilis*, and for antifungal activity against *Trichophyton longifusus, Candida albican, Aspergillus flavus, Microsporum canis, Fusarium solani* and *Candida glabrata* strains. *In vitro* brine shrimp bioassay has also been carried out to study the cytotoxic properties of these compounds.

Experimental

Materials and Methods

All chemicals used were of reagent grade. All metal salts were used as chlorides. 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 ^{1}H and ^{13}C-NMR spectra were recorded 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 a 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 of Karachi, Pakistan and Department of Chemistry, The Islamia University of Bahawalpur, Pakistan.

General Procedure for the Synthesis of Schiff Bases (L^1–L^3)

To a hot magnetically stirred solution of 3-amino-1,2,4-triazole (0.84 g, 10 mmol) in methanol (15 ml) was added a solution of 5-chlorosalicylaldehydes (1.56 g, 10 mmol) in methanol (25 ml).The

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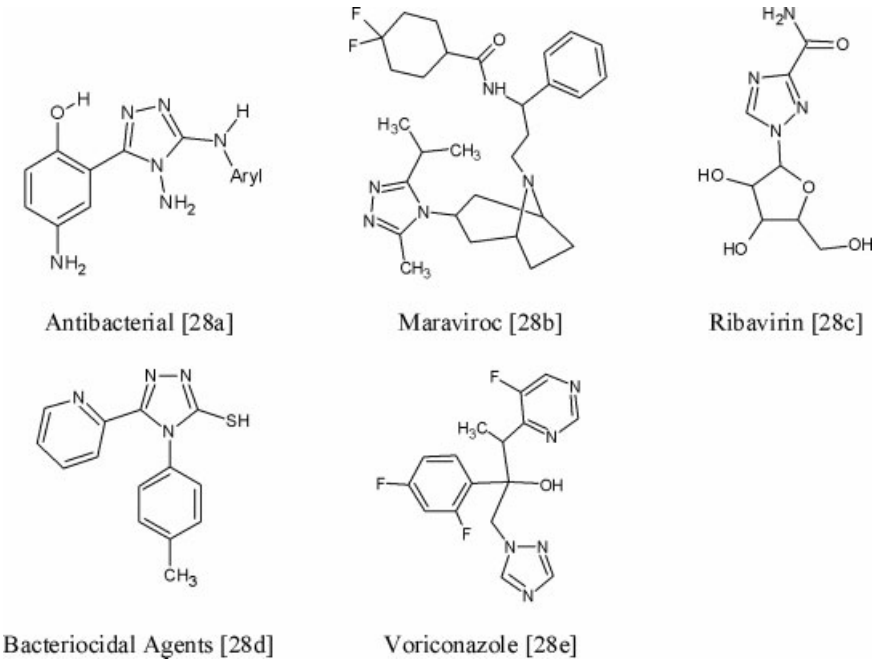
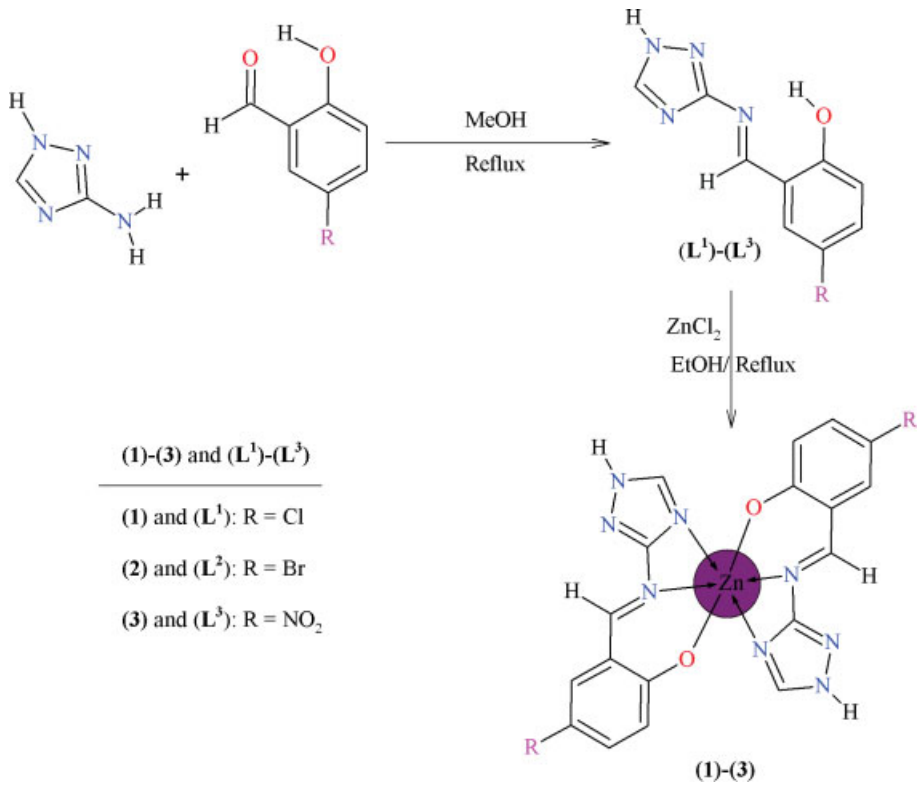


Figure 1. Examples of some bioactive compounds containing 1,2,4-triazole moiety.



Scheme 1. Preparation of triazole Schiff bases $\mathbf{L^1}$–$\mathbf{L^3}$ and their Zn(II) complexes **1**–**3**.

resultant mixture was refluxed for 3 h. During refluxing the solid product was precipitated. The solid product was separated by filtration, washed with hot methanol, then with ether and dried. It was recrystallized in a hot solution of ethanol–methanol (1 : 1) to obtain thin layer chromatography (TLC)-checked well-crystallized product. The same method was applied for the preparation of all other ligands.

*4-Chloro-2-[(E)-(1H-1,2,4-triazol-3-ylimino)methyl]phenol (**L¹**)*

Yield: 81% (1.80 g); dark yellow; m.p. 222 °C; IR (KBr, cm^{-1}): 3185 (NH), 2775 (H-bonded –OH), 1631 (HC═N), 1608 (C═N, triazole), 1036 (N–N), 815 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 7.00 (d, J = 8.8 Hz, C_3–H), 7.45 (dd, J = 8.8, 2.6 Hz, C_4–H), 7.88 (d, J = 2.6 Hz, C_6–H), 8.44 (s, C_7–H), 9.40 (s, C_9–H), 12.35 (s, 1H, OH), 14.10 (s, 1H, NH);

^{13}C NMR (DMSO-d_6): δ 118.09 (C_3), 120.45 (C_1), 129.27 (C_5), 132.72 (C_6), 134.13 (C_4), 152.43 (C_9), 157.87 (C_8), 159.93 (C_2), 162.65 (C_7); EIMS (70 eV) *m/z* (%): 224 ([M]$^+$, 32), 222 (94), 207 (72), 205 (100), 187 (9), 154 (13), 153 (17), 75 (17), 70 (26) 69 (32); Anal. calcd for $C_9H_7ClN_4O$ (222.63): C, 48.55; H, 3.17; N, 25.17; Found: C, 48.32; H, 3.21; N, 25.08%.

*4-Bromo-2-[(E)-(1H-1,2,4-triazol-3-ylimino)methyl]phenol (**L^2**)*

Yield: 80% (2.13 g); dark yellow; m.p. 239 °C; IR (KBr, cm^{-1}): 3175 (NH), 3268 (H-bonded –OH), 1632 (HC═N), 1610 (C═N, triazole),1038 (N–N); ^{1}H NMR (DMSO-d_6): δ 6.96 (d, J = 8.7 Hz,C_3–H), 7.56 (dd, J = 8.7, 2.5 Hz, C_4–H), 8.00 (d, J = 2.5 Hz, C_6–H), 8.45 (s, C_7–H), 9.39 (s, C_9–H), 12.3 (s, 1H, OH), 14.05 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 116.57 (C_5),119.24 (C_3), 121.35 (C_1), 133.86 (C_6), 136.12 (C_4), 151.89 (C_9), 158.26 (C_8), 160.09 (C_2), 163.15 (C_7); EIMS (70 eV) *m/z* (%): 268 ([M]$^+$, 44), 266 (50), 251 (100), 249 (95), 199 (9), 76 (10), 69 (12); Anal. calcd for $C_9H_7BrN_4O$ (267.08): C, 40.47; H, 2.64; N, 20.98; Found: C, 40.29; H, 2.56; N, 21.07%.

*4-Nitro-2-[(E)-(1H-1,2,4-triazol-3-ylimino)methyl]phenol (**L^3**)*

Yield: 78% (1.82 g); yellow; m.p. 210 °C; IR (KBr, cm^{-1}): 3196 (NH), 3285 (H-bonded –OH), 1635 (HC═N), 1612 (C═N, triazole), 1370 (NO_2), 1029 (N–N); ^{1}H NMR (DMSO-d_6): δ 7.17 (d, J = 9.0 Hz, C_3–H), 8.28 (dd, J = 9.0, 2.3 Hz, C_4–H), 8.6 (s, C_7–H), 8.81 (d, J = 2.3 Hz, C_6–H), 9.53 (s, C_9–H), 13.05 (s, 1H, OH), 14.10 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 117.09 (C_3), 120.05 (C_1), 125.13 (C_4), 127.72 (C_6),142.21 (C_5), 152.38 (C_9), 158.17 (C_8), 161.73 (C_2), 163.85 (C_7); EIMS (70 eV) *m/z* (%): 397 ([M]$^+$, 48), 380 (100), 335 (36), 259 (18), 245 (26), 191 (11), 164 (13), 150 (10), 138 (9), 121 (21), 76 (14); Anal. calcd for $C_9H_7N_5O_3$ (233.18): C, 46.36; H, 3.03; N, 30.03; Found: C, 46.28; H, 2.96; N, 30.15%.

Procedure for Preparation of Zinc(II) Complexes (1–3)

*Zn(II) complex with 4-chloro-2-[(E)-(1H-1,2,4-triazol-3-ylimino) methyl]phenol (**L^1**)*

To a hot magnetically stirred solution of (**L^1**; 0.445 g, 2 mmol) in ethanol (20 ml) was added dropwise a solution of Zn(II) $Cl_2{\cdot}6H_2O$ (0.238 g, 1 mmol) in ethanol (15 ml). The resultant mixture was refluxed for 2 h. The precipitated product formed during refluxing was collected by filtration, thorough washing with hot ethanol followed by ether and dried. It was then recrystallized in dioxane. The same method was used for the preparation of all other complexes. The physical and analytical data for each metal complex are given below.

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

Yield: 64% (0.325 g); dirty yellow; m.p. 282–284 °C (decomp); IR (KBr, cm^{-1}): 3185 (NH); 1618 (HC═N); 1608/1592 (C═N); 1388 (C–O); 1035 (N–N); 815 (C–Cl); 522 (Zn–N); 450 (Zn–O). Conductivity, 25.6 Ω^{-1} cm^2 mol^{-1}. Diamagnetic. λ_{max} 28 856 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 7.15 (d, J = 8.2 Hz, C_3–H), 7.60 (dd, C_4–H), 8.02 (d, C_6–H), 8.78 (s, C_7–H), 9.65 (s, C_9–H), 14.20 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 118.2 (C_3), 120.58 (C_1), 129.4 (C_5), 132.8 (C_6), 134.25 (C_4), 152.65 (C_9), 158.0 (C_8), 160.0 (C_2), 162.89 (C_7). Anal. calcd for $C_{18}H_{12}N_8O_2Cl_2Zn$ (508.63): C, 42.50; H, 2.38; N, 13.94; Found: C, 42.83; H, 2.24; N, 13.69%.

*[Zn(L^1-H)$_2$] (**2**)*

Yield: 61% (0.365 g); dirty yellow; m.p. 285–287 °C (decomp); IR (KBr, cm^{-1}): 3175 (NH); 1616 (HC═N); 1610/1595 (C═N); 1384 (C–O); 1040 (N–N); 532 (Zn–N); 445 (Zn–O). Conductivity, 22.7 Ω^{-1} cm^2 mol^{-1}. Diamagnetic. λ_{max} 28670 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 7.13 (d, J = 8.2 Hz, C_3–H), 7.71 (dd, C_4–H), 8.13 (d, C_6–H), 8.79 (s, C_7–H), 9.68 (s, C_9–H), 14.18 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 116.7 (C_5), 119.35 (C_3), 121.46 (C_1), 133.95 (C_6), 136.2 (C_4), 152.05 (C_9), 158.4 (C_8), 160.23 (C_2), 163.45 (C_7). Anal. calcd for $C_{18}H_{12}N_8O_2Br_2$ Zn (597.56): C, 36.18; H, 2.02; N, 18.75; Found: C, 36.71; H, 1.91; N, 18.98%.

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

Yield: 67% (0.355 g); lght yellow; m.p. 258–260 °C (decomp); IR (KBr, cm^{-1}): 3196 (NH); 1619 (HC═N); 1612/1596 (C═N); 1384 (C–O); 1030 (N–N); 1370 (NO_2); 528 (Zn–N); 455 (Zn–O). Conductivity, 24.9 Ω^{-1} cm^2 mol^{-1}. Diamagnetic. λ_{max} 28 738 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 7.30 (d, J = 8.3 Hz, C_3–H), 8.40 (dd, C_4–H), 8.88 (s, C_7–H), 8.90 (d, C_6–H), 9.84 (s, C_9–H), 14.20 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 117.23 (C_3), 120.2 (C_1), 125.25 (C_4), 127.85 (C_6),142.35 (C_5), 152.55 (C_9), 158.3 (C_8), 161.9 (C_2),164.15 (C_7). Anal. calcd for $C_{18}H_{12}N_{10}O_6Zn$ (527.79): C, 41.81; H, 2.28; N, 26.44; Found: C, 42.09; H, 2.44; N, 26.69%.

Pharmacology

In vitro antibacterial bioassay

All the newly synthesized compounds (**L^1**–**L^3**) and their respective metal (II) chelates (**1**–**3**) were tested against four Gram-negative (*E. coli*, *S. sonnei*, *P. aeruginosa* and *S. typhi*) and two Gram-positive (*S. aureus* and *B. subtilis*) bacterial strains by the disk diffusion method.[30,31] The tested compounds (ligand and complex) were dissolved in DMSO to get 10 mg ml^{-1} solution. A known volume (10 µl) of the solution was applied with the help of a micropipette onto the 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 positive control were either purchased from manufacturer or prepared as above in the laboratory by applying a known concentration of the standard antibiotic solution. Bacterial cultures were grown in nutrient broth medium at 37 °C overnight and spread on to solidified nutrient agar medium in Petri plates using sterilized cotton swabs in standard microbiological working environment. 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. As reference compound, ampicillin was used and the experiments were carried out in triplicate and the values obtained were statistically analyzed.

In vitro antifungal bioassay

Antifungal activities of all compounds were studied[31] against six fungal strains (*T. longifusus*, *C. albican*, *A. flavus*, *M. canis*, *F. solani* and *C. glabrata*) using the disk diffusion method. Sabouraud dextrose agar (Oxoid, Hampshire, UK) was seeded with 10^5 (cfu) ml^{-1} fungal spore suspensions and transferred to Petri plates. Disks soaked in 20 ml (200 µg ml^{-1} in DMSO) of the 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 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^{-1} of the compounds and applying the literature protocol.[32]

In Vitro Cytotoxic Bioassay

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. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened while the matter compartment was opened to ordinary light. After 2 days nauplii were collected by a pipette from the lighted side. A sample of the test compound was prepared by dissolving 20 mg of each compound in 2 ml of DMSO. From this stock solution 500, 50 and 5 μg ml^{-1} were transferred to nine vials (three for each dilutions were used for each test sample and LD_{50} is the mean of three values) and one vial was kept as control having 2 ml of DMSO only. The solvent was allowed to evaporate overnight. After 2 days, when shrimp larvae were ready, 1 ml of sea water and 10 shrimps were added to each vial (30 shrimps per 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 using the Finney computer program to determine the LD_{50} values.[33,34]

Results and Discussion

Chemistry

The triazole-derived Schiff bases ($\mathbf{L^1}$–$\mathbf{L^3}$) were prepared by the reaction of 3-amino-1,2,4 triazole with a series of chloro-, bromo- and nitro- substituted salicylaldehydes under reflux as shown in Scheme 1. All the Schiff base derivatives ($\mathbf{L^1}$–$\mathbf{L^3}$) were soluble in ethanol, dioxane, dimethylformamide and dimethyl sulfoxide. The composition of which is consistent with their micro-analytical and mass spectral data. The Zn(II) complexes (**1**–**3**) were obtained by stoichiometric reaction of the corresponding ligand with the zinc metal as its chloride salt in a molar ratio M : L of 1 : 2. All the complexes were stable against air and moisture at room temperature. They were insoluble in common organic solvents and only soluble in dimethylformamide (DMF) and DMSO. Physical measurements and analytical data of the Zn(II) complexes (**1**–**3**) are given in the Experimental section.

Spectroscopic Characterization of Ligands ($\mathbf{L^1}$–$\mathbf{L^3}$) and their Zn(II) Complexes (1–3)

IR spectra

The characteristic infrared spectral assignment of ligands ($\mathbf{L^1}$–$\mathbf{L^3}$) and their Zn(II) complexes are reported in experimental section. The presence of a broad band in the region at 2768–2790 cm^{-1} in the spectra of Schiff bases exhibited the intramolecular H-bonded –OH. The medium intensity peak in the spectra of ligands at 3175–3196 cm^{-1} was attributed to the *v*(NH) of triazole. The appearance of a strong new band at 1631–1635 cm^{-1} in the spectra of ligands was assigned[35] to azomethine (HC═N) linkage, which resulted from condensation of amino (NH_2) group of triazole with carbonyl *v*(C═O) group of salicylaldehydes. The azomethine (HC═N) linkage is confirmed by the disappearance of bands at 1722 and 3330 cm^{-1} owing to carbonyl *v*(C═O) and amine *v*(NH_2) stretching, respectively. The other bands appearing in the spectra of ligands (newly synthesized) at 1608–1612 and 1029–1038 cm^{-1} were assigned to the vibrations of C═N and N–N of triazole. The medium intensity band at 3175–3196 cm^{-1} present in all the Zn(II) complexes was attributed[36] to the *v*(NH) vibration. The strong new band appearing at 1631–1635 cm^{-1} owing to azomethine *v*(HC═N), exhibited a lower shift of 10–20 cm^{-1} (1613–1621 cm^{-1}) in all the metal complexes. This shift indicates that the azomethine *v*(HC═N) is coordinated with the zinc metal ion via its nitrogen atom. Similarly, the band at 1608–1612 cm^{-1} already assigned to triazole *v* (C═N) is also shifted to a lower frequency at 1592–1599 cm^{-1} by 10–20 cm^{-1}, indicating the coordination of *v*(C═N) of triazole with the Zn(II) metal atom. Moreover, the disappearance of the broad band at 3268–3290 cm^{-1} in the spectra of ligands and the appearance of new band at 1384–1388 cm^{-1} owing to *v*(C–O) was evidence[37] of deprotonation and coordination of the zinc metal atom with the ligand through oxygen of salicylidene. All the above evidence was further supported by the emergence of new bands at 520–532 and 445–460 cm^{-1} owing to *v*(Zn–N) and *v*(Zn–O) vibrations.[38] These new bands were only observed in the spectra of the zinc complexes and not in its ligands. As a conclusion, comparison of the spectra of the ligands ($\mathbf{L^1}$–$\mathbf{L^3}$) and their Zn(II) complexes (**1**–**3**) confirmed the coordination of ligands with the Zn(II) metal tridentately through oxygen of salicylidene, nitrogen of azomethine (HC═N) and nitrogen of triazole (C═ N).

^{1}H NMR spectra

^{1}H NMR spectral data of the triazole-derived Schiff bases ($\mathbf{L^1}$–$\mathbf{L^3}$) and their diamagnetic Zn(II) complexes (**1**–**3**) are provided in the Experimentalal section. The displayed signals of all the protons of free ligands owing to heteroaromatic/aromatic groups were found to be in their expected regions.[39] The ^{1}H NMR spectrum of Schiff bases ($\mathbf{L^1}$) and ($\mathbf{L^2}$) displayed the OH proton of the phenol moiety at δ12.3–12.35 and of ($\mathbf{L^3}$) at δ13.0 as singlets. The compounds ($\mathbf{L^1}$–$\mathbf{L^3}$) showed characteristic azomethine C_7–H and triazole C_9–H protons at δ 8.44–8.60 and δ 9.39–9.53, respectively, as a singlet. The ^{1}H NMR spectra of $\mathbf{L^1}$ and $\mathbf{L^2}$ exhibited phenyl C_3–H and C_6–H as doublet at 6.96–7.00 and 7.88–8.00 ppm, respectively. However, the phenyl C_4–H of ($\mathbf{L^1}$) and ($\mathbf{L^2}$) appeared as a double doublet at 7.45–7.56 ppm. The ^{1}H NMR spectrum of $\mathbf{L^3}$ showed the phenyl C_3–H and C_6–H protons as multiplets at 7.17 and 8.81 ppm, respectively. The proton, C_4–H appearing at 8.28 ppm was assigned to the phenyl as double doublet. A broad singlet at 14.05–14.10 ppm displayed the NH proton of triazole in all the ligands that disappeared on exchangement with D_2O. The coordination of the azomethine (CH═N) nitrogen and nitrogen of triazole (C═N) ring were assigned by the downfield shifting of the azomethine C_7–H proton and triazole C_9–H proton signal from 8.44–8.60 and 9.39–9.53 ppm in free ligand to 8.78–8.88 and 9.65–9.84 ppm in its Zn(II) complexes, respectively. The downfield shifting of azomethine proton in Zn(II) complexes was attributed

to the discharging of electronic cloud towards the Zn(II) ion. The hydroxyl (OH) proton at 12.30–13.0 ppm in the ligands **L^1**–**L^3** disappeared in the spectra of its Zn(II) complexes, indicating deprotonation and coordination of the oxygen with the metal ion. All other protons underwent downfield shift by 0.15–0.30 ppm owing to the increased conjugation[40] on complexation with the zinc metal atom.

^{13}C NMR spectra

The ^{13}C NMR spectra of the free ligands and their Zn(II) chelates were determined 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.[39] The conclusion obtained from these studies provides further support to the mode of bonding explained in their IR and ^1H NMR spectral data. The azomethine carbon (CH═N) of the ligands appeared in the region δ 162.65–163.85 ppm. The aromatic carbons of all the free ligands were found in the region δ 116.57–161.73 ppm. All the Schiff bases exhibited triazole carbons in the region δ 151.89–158.26 ppm. Downfield shifting of the azomethine carbon from δ 162.65–163.85 ppm in the free ligands to δ 162.89–164.15 ppm in its zinc(II) complexes was due to shifting of electronic density towards the Zn(II) ion. The downfield shifting also confirmed the coordination of the azomethine to the zinc metal atom. Furthermore, the presence of the number of carbons agreed well with their expected values.[40]

Mass spectra

The electron impact mass spectral (EIMS) data of the ligands was found to show[41,42] the compositions as: **L^1**, $C_9H_7N_4ClO$, 222.2 (calcd 222.63); **L^2**, $C_9H_7N_4BrO$, 267.0 (calcd 267.08); and **L^3**, $C_9H_7N_5O_3$ 233.08 (calcd 233.18). The **L^1** showed a base peak at 205.0 for the fragment $[C_9H_6N_4Cl]^+$. Similarly, **L^2** showed its base peak for the fragment $[C_9H_6N_4Br]^+$ at 249 and **L^3** at 216.05 for fragment $[C_9H_6N_5O_2]^+$. These were the most expected stable fragments. The most fragmented pattern was followed by the cleavage of C═N (exocyclic as well as endocyclic), C═C, C–C, C–N, C–X (X = Cl, Br) and C–O bonds.

Conductance, magnetic susceptibility and electronic spectra of Zn(II) complexes

The molar conductance values of the Zn(II) complexes **1**–**3** in DMF fell in the range 21.2–29.5 Ω^{-1} cm^2 mol^{-1}, showing a nonelectrolytic nature.[43] The room temperature magnetic moment values of Zn(II) complexes were found to be diamagnetic, as expected. The electronic spectral values of Zn(II) complexes **1**–**3** are recorded in the Experimental section. The diamagnetic Zn(II) chelates did not show any d–d transition and their spectra were dominated only by the charge transfer band at 28 670–28 856 cm^{-1}.

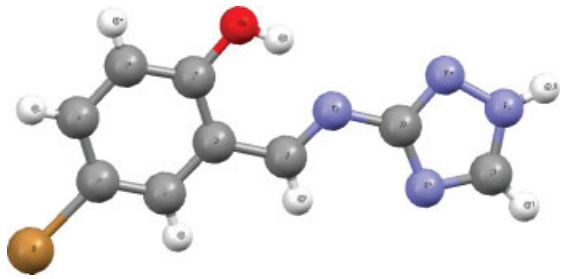


Figure 2. Crystal structure of ligand **L^2**.

Crystallographic structure of ligand *L^2*

The molecular structure of ligand **L^2** (Fig. 2) shows the expected bond lengths and angles. The triazol ring and the benzene ring of the triazole-imino-phenol unit are not perfectly planar, making a dihedral angle of 9.16°(3). The functionalized arm at C(1) atom adopts a *cisoidal*-conformation, with O–H···N(1) distance of 1.837(6) Å and C1–C2 and N1–C7 bonds in a common plane of central system and atoms N1–C8 bond deviating from this plane by 3.03°(2).

The molecular structure data with the atom-numbering scheme and 30% probability displacement ellipsoids are shown in Tables 1–3. H atoms have been saved for O–H···N interaction elucidation. Intermolecular interactions in the crystal structure, $C_9H_7BrN_4O$, has spatial group: P 4_3 2_1 2; a = 5.8778(3) Å; b = 5.8778(3) Å; c = 55.458(6) Å; α = 90°; β = 90°; γ = 90°; V = 1915.99(6) Å^3; Z = 8.

The hydroxyl-C2 group makes a dihedral angle of 4.48°(3) with the plane of the 1,2,4-triazole ring system. The hydroxyl group adopts a *cisoidal* conformation with N3 atom. Within the molecule, there are O–H···N intramolecular contacts, as detailed in Table 3.

Table 1. Selected geometric parameters (Å)

O1–H1	0.839(4)	C1–C6	1.406(7)	N1–C8	1.395(7)
O1–C2	1.348(6)	Br1–C5	1.895(5)	N2–C8	1.355(7)
C1–C2	1.413(7)	C1–C7	1.457(7)	N3–N4	1.371(7)
C1–C6	1.406(7)	N1–C7	1.295(7)	N4–C9	1.333(7)
O1–C2–C1	121.6(4)	N1–C8–N3	117.5(5)	C2–C1–C6	119.2(5)
O1–C2–C3	118.6(5)	C8–N3–N4	101.2(4)	C2–O1–H1	109.4(4)
C1–C2–C3	119.8(5)	C8–N3–N4	101.2(4)	C2–C3–H3	120.0(5)
Br1–C5–C4	118.6(4)	N3–N4–C9	109.9(4)	C1–C6–H6	119.9(5)
Br1–C5–C6	120.7(4)	C8–N2–C9	101.6(4)	C1–C7–H7	120.0(5)
C4–C5–C6	120.7(5)	N2–C9–N4	110.9(5)	N1–C7–H7	120.0(5)
C1–C6–C5	120.2(5)	N2–C8–N3	116.3(5)	N3–N4–H4A	125.1(5)
C1–C7–N1	120.0(5)	C2–C3–C4	120.1(5)	N4–C9–C9	124.5(5)
C7–N1–C8	119.2(4)	C3–C4–C5	119.9(5)	N2–C9–H9	124.6(5)

Table 2. Torsion angle (degree) of ligand L^2

O1–C2–C1–C7	1.9(8)	N2–C8–N1–C7	−7.8(8)	N1–C7–C1–C6	−178.0(5)
N1–C7–C1–C2	3.0(8)	N3–C8–N1–C7	170.8(5)	N1–C8–N2–C9	178.6(5)

Table 3. Hydrogen-bond parameters (Å)

D–H	H···A	D···A	D–H···A
O1–H1	1.837	2.583	1477.31
N4–H4A	2.190	2.963	146.43

Pharmacology

In vitro antibacterial bioassay

The antibacterial activity of newly synthesized Schiff bases and their corresponding Zn(II) complexes was determined against four Gram-negative (*E. coli*, *S. sonnei*, *P. aeruginosa* and *S. typhi*) and two Gram-positive (*S. aureus* and *B. subtilis*) bacterial strains (Table 4) according to the literature protocol.[30,31] The obtained results of all the compounds were compared with those of the standard drug ampicillin (Table 4). The percentage of activity was compared with the activity of the standard drug considering its activity as 100%. All the ligands (L^1–L^3) and their Zn(II) complexes (**1**–**3**) showed moderate to significant activity against all the bacterial strains; however, weak activity was observed for L^1 against *f* and L^2 against *b*. The activity of ligand L^1 was found to be significant (59–61%) against strains *a* and *e*, moderate (46–50%) against *b*–*d* and weaker (31–33%) against *f*. The compound L^2 showed moderate activity (43–51%) against *a* and *c*–*f* and weaker (33%) against *b*. Significant activity (57–75%) was observed by ligand L^3 against *b*–*e* and moderate (50–51%) against *a* and *f*. Compounds **1**–**3** showed overall a significant activity (53–85%) against strains *a*–*f*. The results given in Table 4 more precisely show that compounds **1**–**3** possessed activity, 61–84% against *a*, 54–87% against *b*, 56–82% against *d*, 56–85% against *c* and *e* and 55–77% against *f*. These results evidently show that the activity of the Schiff base compounds enhanced on coordination with the zinc metal. Enhancement in activity upon coordination can be explained on the basis of our previous studies according to the chelation theory.[44–46] Chelation reduces the polarity of metal ion to a significant extent owing to overlapping with the donor groups. Further, the delocalization of π-electrons over the whole chelate ring is increased, which in turn increases the lipophilicity of the complexes. The increased lipophilicity then enhances the penetration of the complexes into lipid membranes of the bacterial strains, thus killing more of them.[47,48]

In Vitro Antifungal Bioassay

The antifungal screening of all the synthesized compounds was carried out against *T. longifusus*, *C. albican*, *A. flavus*, *M. canis*, *F. solani* and *C. glabrata* fungal strains (Table 5) according to the literature protocol.[31] The obtained results were compared with the standard drugs miconazole and amphotericin B. The activity of standard drug is considered as 100%. The Schiff base derivatives of triazole and their Zn(II) complexes exhibited a moderate to significant degree of inhibitory effects on the growth of testing fungal strains. However, only a few compounds showed low degree of inhibitory effect on the growth of testing strains. The antifungal results illustrated in Table 5 indicate that ligand L^1 showed significant activity (57–65%) against *c* and *e*, and moderate activity (42–45%) against *a* and *d*. Schiff base ligand L^2 possessed significant activity (53–66%) against *a*, *d* and *f* and moderate activity (43%) against *d*. L^3 exhibited significant activity (64–68%) against *b* and *d* and moderate activity (38–51%) against *a*, *c*, *e* and *f*. L^1 showed low activity (18–25%) against *b* and *f* and L^2 possessed weak activity (27–28%) against *c* and *e*. Compound **1** showed good antifungal activity (54–69%) against *a*, *c* and *e* and moderate (37–48%) against *b*, *d* and *f*. Compound **2** was found to show a significant activity (57–82%) against *a*, *d* and *f*, moderate (39–51%) against *b* and weaker (29–36%) against *c* and *e*. Significant activity (53–81%) was observed by compounds **3** against *b*–*d*, and moderate (45–52%) against *a*, *e* and *f*. It was evident that the overall potency of the uncoordinated compounds/ligands was enhanced on coordination with the metal ion.

MIC for Antibacterial Activity

The preliminary screening results of all the compounds showed that the Zn(II) complexes **1** and **3** were found to be the most (above

Table 4. Antibacterial bioassay (concentration used 1 mg ml^{-1} of DMSO) of ligands L^1–L^3 and Zn(II)-diaquo complexes **1**–**3**; zone of inhibition (mm)

	Gram-negative				Gram-positive		
Compound	*Escherichia coli*	*Salmonella sonnei*	*Pseudomonas aeruginosa*	*Salmonella enterica serovar typhi*	*Staphylococcus aureus*	*Bacillus subtilis*	SA
L^1	16	12	15	14	16	09	2.73
L^2	12	08	14	13	14	14	2.34
L^3	13	18	21	16	17	15	2.73
1	21	19	26	18	23	17	3.39
2	19	13	24	14	20	16	4.13
3	20	21	27	22	23	19	2.83
SD	26	24	32	28	27	29	2.73

>10, Weak; >10, moderate; >16, Significant. SD, standard drug (ampicillin). SA, statistical analysis.

Table 5. Antifungal bioassay (concentration used, 200 μg ml^{-1}) of ligands **L^1**–**L^3** and their Zn(II) complexes **1**–**3**

	Antifungal activity (*in vitro*)					
Compound	*Trichophyton longifusus*	*Candida albican*	*Aspergillus flavus*	*Microsporum canis*	*Fusarium solani*	*Candida glabrata*
L^1	42	18	57	45	65	25
L^2	58	43	28	66	27	53
L^3	49	68	51	64	38	41
1	54	37	69	48	68	37
2	70	51	36	80	29	66
3	51	81	53	67	50	45
SD	A	B	C	D	E	F

SD, standard drugs. MIC (μg ml^{-1}); A, Miconazole (70 μg ml^{-1}; 1.6822×10^{-7} M ml^{-1}); B, miconazole (110.8 μg ml^{-1}; 2.663×10^{-7} M ml^{-1}); C, amphotericin B (20 μg ml^{-1}; 2.164×10^{-8} M ml^{-1}); D, miconazole (98.4 μg ml^{-1}; 2.365×10^{-7} M ml^{-1}); E, miconazole (73.25 μg ml^{-1}; 1.760×10^{-7} M ml^{-1}); F, miconazole (110.8 μg ml^{-1}; 2.663×10^{-7} M ml^{-1}).

Table 6. Minimum inhibitory concentration (M ml^{-1}) of the selected compounds **1** and **3** against selected bacteria and *in vitro* cytotoxic bioassay of ligands, **L^1**–**L^3**, and their Zn(II) complexes, **1**–**3**

	In vitro antibacterial bioassay. Minimum inhibitory concentration (M ml^{-1})				Cytotoxic bioassay
No.	*Escherichia coli*	*Salmonella sonnei*	*Psudomonas aeruginosa*	*Staphylococcus aureus*	LD_{50} (M ml^{-1})
L^1	–	–	–	–	$>2.88 \times 10^{-4}$
L^2	–	–	–	–	$>2.77 \times 10^{-4}$
L^3	–	–	–	–	$>2.92 \times 10^{-4}$
1	2.15×10^{-7}	–	2.13×10^{-7}	1.34×10^{-7}	$>5.76 \times 10^{-4}$
2	–	–	–	–	$>1.05 \times 10^{-4}$
3	–	0.65×10^{-7}	1.29×10^{-7}	1.25×10^{-7}	$>4.23 \times 10^{-4}$

80%) active compounds. Therefore, these two compounds were selected for MIC studies (Table 6). The MIC values of compounds **1** and **3** fell in the range 6.475×10^{-8} to 2.252×10^{-7} M. The MIC results showed that compound **3** is the most active, showing a maximum inhibition of 6.475×10^{-8} M against bacterial strain *S. sonnei*.

In Vitro Cytotoxic Bioassay

The synthesized ligands **L^1**–**L^3** and their Zn(II) complexes (**1**–**3**) were screened for their cytotoxicity (brine shrimp bioassay) using the protocol of Meyer *et al*.[33] The cytotoxic data recorded in Table 6 revealed that only compound **2** displayed potent cytotoxic activity ($LD_{50} = 1.676 \times 10^{-4}$ M) against *Artemia salina*, while all other compounds were considered as almost inactive in this assay. It was interesting to note that the zinc(II) complexes showed potent cytotoxicity as compared with the ligands. This activity relationship may serve as a basis for future development of certain cytotoxic agents in clinical practices.

Conclusions

The improvement of antibacterial and antifungal activity in ligands **L^1**–**L^3** increased upon chelation/coordination. It has been proposed that the chelation process reduces the polarity of the metal ion by coordinating with ligands, which in turn increases the lipophilic nature of the zinc metal. This lipophilic nature of metal thus enhances its penetration through the lipoid layer of cell membrane of the microorganism. Further, it has been suggested that some functional groups such as azomethine (HC═N) or hetero-aromatics present in these compounds also play an important role in antibacterial and antifungal activity that may be responsible for the enhancement of the hydrophobic character and liposolubility of the molecules.

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Supporting Information

Crystallographic data for the structural analysis has been deposited with the Cambridge Crystallographic Data Centre, CCDC no. 774 652 for ligand **L^2**. A copy of this information can be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-1223–336 033; email: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk). Supporting information can be found in the online version of this article.

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