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Synthesis and spectral characterization of Zn(II) microsphere series for antimicrobial application

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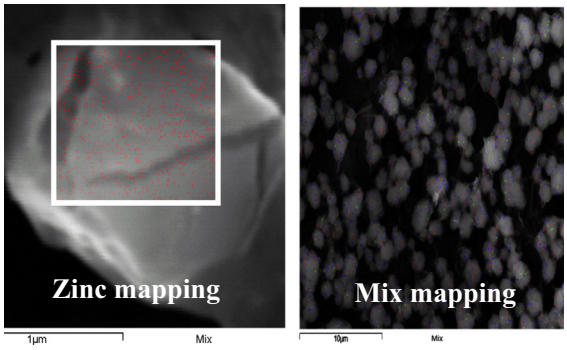
HIGHLIGHTS

- Nano-sized zinc(II) complexes were synthesized and well characterized.
- Schiff base and Zn(II) complexes showed significant antimicrobial activities.
- Zn(II) complexes look like a microsphere and their sizes are 1–2 μm.

GRAPHICAL ABSTRACT



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ABSTRACT

Microsphere series have been synthesized by reacting zinc(II) acetate dihydrate with Schiff bases derived from 2-hydrazino-5-[substituted phenyl]-1,3,4-thiadiazole/oxadiazole/triazole with salicylaldehyde. Elemental analysis suggests that the complexes have 1:2 and 1:1 stoichiometry of the type $[Zn(L)_2(H_2O)_2]$ and $[Zn(L')(H_2O)_2]$; LH = Schiff bases derived from 2-hydrazino-5-[substituted phenyl]-1,3,4-thia/oxadiazole with salicylaldehyde; $L'H_2$ = Schiff bases derived from 3-(substituted phenyl)-4-amino-5-hydrazino-1,2,4-triazole and salicylaldehyde and were characterized by elemental analyses, IR, ^1H NMR and ^{13}C NMR spectral data. Scanning electron microscopy (SEM) showed that synthesized materials have microsphere like structure and there EDX analysis comparably matches with elemental analysis. For the antimicrobial application Schiff bases and their zinc(II) complexes were screened for four bacteria e.g. *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Streptococcus pyogenes* and four fungi e.g. *Cyrtomium falcatum*, *Aspergillus niger*, *Fusarium oxysporium* and *Curvularia pallescence* by the reported method. Schiff base and Zn(II) compounds showed significant antimicrobial activities. However, activities increase upon chelation. Thermal analysis (TGA) data of compound (10) showed its stability up to 300 °C.

Introduction

Zinc has been known to be an essential trace element in eukaryotes for more than a century [1]. There is 2–3 g of zinc in adult humans, making it one of the most prevalent "trace" elements. In particular, zinc salicylate has been approved by the US Food and Drug Administration for use as an anti-oxidant, carbonless copying paper and stabiliser for polymers [2]. Metal zinc complexes became attractive for their interesting properties such as antimicrobial [3], enzymatic activity [4], personal care [5], anti-HIV drug [6], and photoluminescence as well as electroluminescence, etc. [7]. In other hand 1,3,4-thiadiazoles and their Schiff bases have received significant importance because of their diverse

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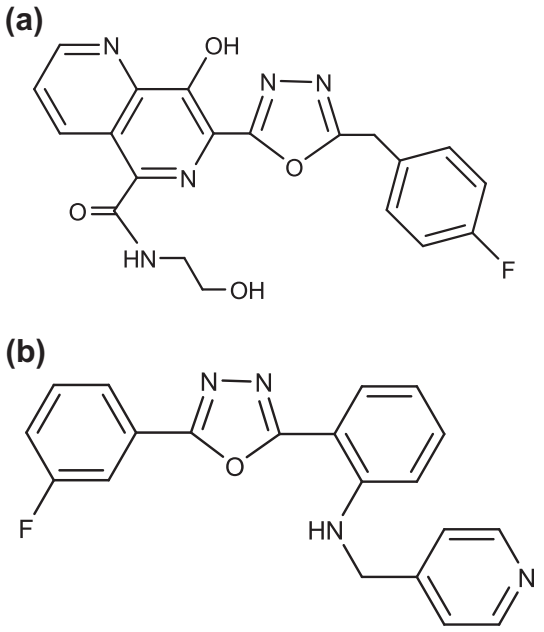


Fig. 1. Oxadiazole derived compound patented for; (a) anti-HIV drugs; (b) anti-cancer drugs.

biochemical, antifungal and antibacterial application [8,9]. Oxadiazole derivatives have been found to possess tuberculostatic [10], antiviral [11], antibacterial [12,13], antimalarial [14], fungicidal [15], anticonvulsant [16], anti-inflammatory [17], analgesic [18,19] and insecticidal [20] activities. 7-(-5-{4-fluorobenzyl}-1,3,4-oxadiazole-2-yl)-8-hydroxy-N-(2-hydroxym ethyl)-1,6-naphthyri-dine-5-caboxamide (Fig. 1a) have been patented for the HIV integrase inhibition [21] and 2-{5-(3-florophenyl)-1,3,4-oxadiazole-2-yl}-N-(pyridin-4-ylmethyl) aniline (Fig. 1) patented as anticancer agent [22]. Triazoles and their derivatives are found to be associated with various biological activities, such as anticonvulsant, antifungal, anticancer, anti-inflammatory and antibacterial properties [23–27]. Several compounds containing 1,2,4-triazole ring are well known for drugs and there amino derived Schiff bases well known for antimicrobial properties [28–30]. Salicylaldehyde isonicotinoyl hydrazone was patented for protection against retinal disease [31].

In this paper, we report the synthesis, spectroscopic, microscopic, thermal study and antimicrobial activities of zinc(II) complexes with Schiff bases derived from substituted heterocycles (thiadiazole, oxadiazole and triazole) with salicylaldehyde.

Experimental

Reagents

All reagents were purchased from Aldrich, solvents used were purified by proper methods and Milli-Q water was used for all experiments.

Instruments

Melting points were determined on a Buchi 530 apparatus in open capillary tubes. IR spectra were recorded on a Shimadzu 8201 PC model FT IR spectrophotometer as KBr disks. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-300 spectrometer using DMSO-d_6 as solvent. Chemical shifts (δ) are reported in parts per million (ppm) relative to an internal standard of Me_4Si. Elemental analyses were recorded by Elementar Vario EL III Carlo Erba 1108 models. The thermal degradation processes and stabilities of the zinc complexes was investigated using a thermo-gravimetric analyzer (Mettler Toledo TGA/SDTA851 with *Star* software) under a nitrogen atmosphere with a heating rate of 10 °C/min from 30 to 500 °C. For scanning electron microscopy (SEM), gold sputter coating were carried out on desired zinc(II) complexes samples at pressure ranging in between 1 and 0.1 Pa. Sample was loaded in the machine, which was operated at 10^{-2} to 10^{-3} Pa with EHT 15.00 kV with 300 V collector bias using Leo microscope SEMs were recorded. Transmission electron microscope (TEM) analysis for zinc complexes was performed by a JEOL 1200EX. The JEOL 1200EX TEM with tungsten, operated at an accelerating voltage of up to 120 kV. The optical densities of microbial solutions were evaluated by using a VARIAN 50 bio UV–Vis spectrophotometer instruments. Zinc was estimated gravimetrically as its dioxide ZnO_2 [32]. A known weight of the compound was decomposed by concentrated nitric acid and the mass was extracted with distilled water, sodium carbonate solution was added. The precipitate obtained was filtered by Whatmann filter paper No. 41, and finally ignited in silica crucible to zinc oxide. Elemental analysis (C, H, N, Zn) indicates that the found and calculated values were within acceptable limits (±0.5). Molar conductance of 10^{-3} M solutions of the complexes in DMSO was recorded on a Hanna EC 215 conductivity meter by using 0.01 M KCl water solution as calibrant. The purity of compounds was checked by thin layer chromatography on silica gel plate using ether and ethyl acetate as a solvent system. Iodine chamber was used as a developing chamber.

General procedure for the synthesis of zinc(II) complexes

The route for the preparation of zinc(II) complexes is shown in Fig. 2.

Synthesis of Schiff base hydrazone derived from 5-(substituted aryl)-2-hydrazino-1,3,4-(thiadiazole, oxadiazole) and salicylaldehyde (L_1H–L_8H)

A mixture of 5-[substituted aryl]-2-hydrazino-1,3,4-(thia/oxadiazole) and salicylaldehyde in 1:1 M ratio respectively was refluxed in ethanol (30 cm^3) containing few drops of concentrated sulphuric acid for 5–6 h [33]. The product separated on evaporation of the ethanol was recrystallized from ethanol–ether mixture (1:1).

Synthesis of Schiff base hydrazone derived from 5-(substituted aryl)-3-hydrazino-1,2,4-(triazole) and salicylaldehyde (L'_9H_2–$L'_{12}H_2$)

A mixture of 3-(substituted phenyl)-4-amino-5-hydrazino-1,2,4-triazole and salicylaldehyde in 1:2 M ratio in an ethanol (30 cm^3) containing a few drops of concentrated sulphuric acid was refluxed for ca. 4–7 h [34]. On concentrating the solution up to ~10 cm^3, crystals appeared which were filtered and recrystallized from ethanol–ether mixture (1:1).

Synthesis of zinc(II) complexes (1–8)

An ethanolic solution (30 cm^3) of zinc(II) acetate dihydrate (0.01 mol) was added to a refluxing solution of appropriate Schiff base (L_1H–L_8H) (0.02 mol) in ethanol (30 cm^3) containing alcoholic solution of sodium acetate (0.02 mol). The reaction mixture was refluxed for about 8–13 h. The coloured complex was obtained. The complex was filtered off, washed thoroughly with ethanol and dried under *vacuo*.

Complex (1). Yield 70%; colour: cream; decomp. temp. 210–212 °C; IR (KBr) cm^{-1}: 3490 (—OH), 3165 (—NH), 2950 (Ar—H), 1620 (C=N azomethine), 1580 (C=N thiadiazole), 1545 (C=C), 1348 (C—O), 1155 (N—N), 1050 (C—S—C), 805, 745 (—OH wagging and rocking), 485 (Zn—O), 435 (Zn—N); ^{1}H NMR (DMSO-d_6): δ 12.45 (s, 2H,

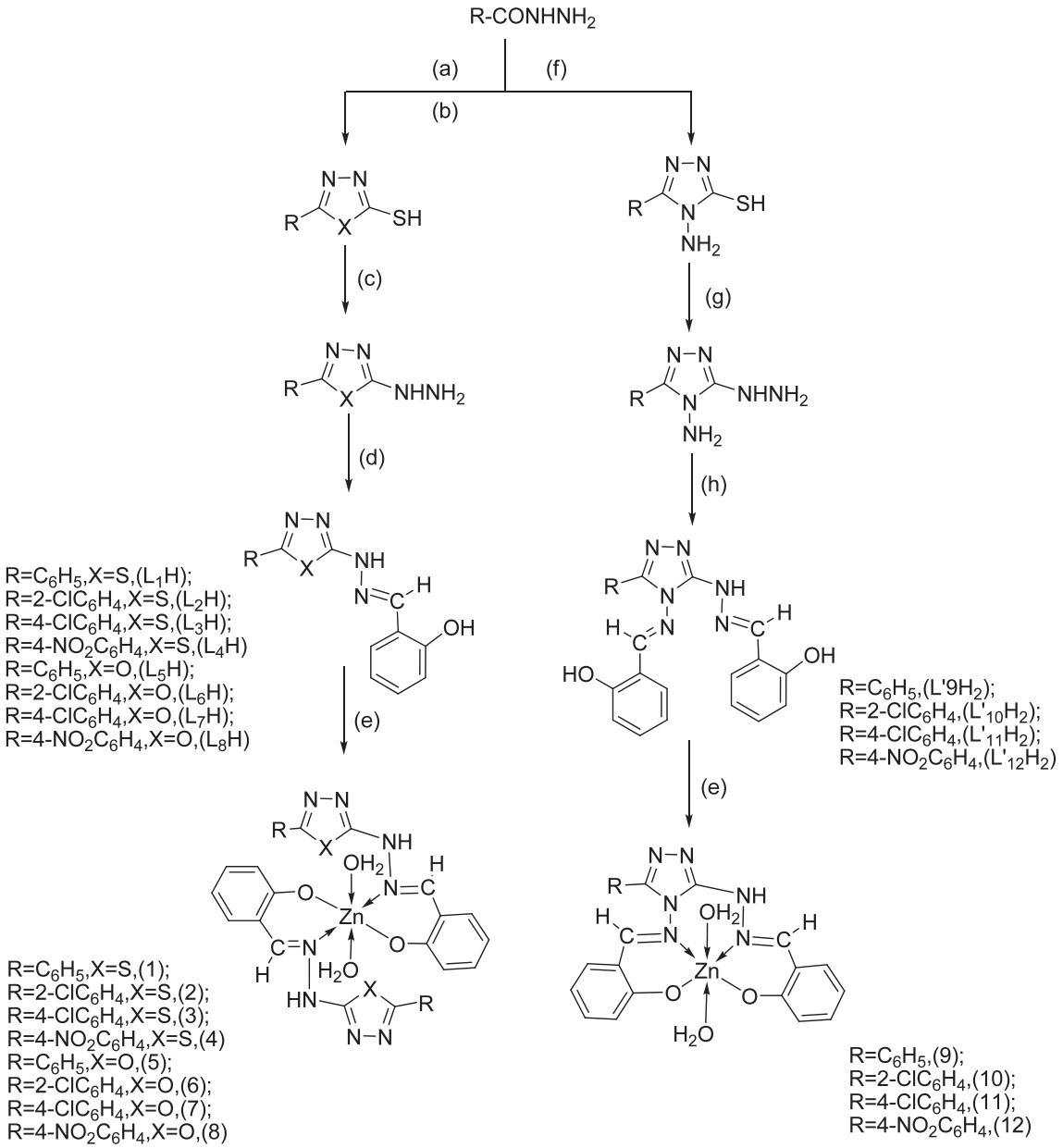


Fig. 2. Reagents and conditions (a) CS_2 + KOH, ice cold stirring/conc. H_2SO_4; for the preparation of thiadiazole (b) CS_2 + KOH/refluxed; for the preparation of oxadiazole; (c) $NH_2NH_2{\cdot}2H_2O$, refluxed ca. 5–7 h (d) salicylaldehyde, refluxed ca. 5–6 h (e) zinc acetate dihydrate/CH_3COONa (f) CS_2 + KOH/$NH_2NH_2{\cdot}2H_2O$, refluxed ca. 5–8 h (g) $NH_2NH_2{\cdot}2H_2O$, refluxed ca. 5–6 h (h) salicylaldehyde, refluxed ca. 4–7 h.

—NH), 6.95–7.40 (m, 18H, Ar—H), 5.55 (s, 4H, H_2O), 8.25 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 175.0, 174.7, 166.8, 158.6, 134.3, 132.8, 131.8, 130.7, 130.3, 129.3, 121.9, 116.9, 115.0; % Anal. Cal. for $C_{30}H_{26}N_8O_4S_2Zn$: C, 52.60; H, 3.79; N, 16.19; S, 9.27; Zn, 9.45; Found: C, 52.51; H, 3.79; N, 16.25; S, 9.10; Zn, 9.33.

Complex (2). Yield 63%; colour: light yellow; decomp. temp. 228–232 °C; IR (KBr) cm^{-1}: 3475 (—OH), 3182 (—NH), 2980 (Ar—H), 1605 (C=N azomethine), 1570 (C=N thiadiazole), 1542 (C=C), 1350 (C—O), 1142 (N—N), 1060 (C—S—C), 815, 736 (—OH wagging and rocking), 770 (C—Cl), 490 (Zn—O), 445 (Zn—N); ^{1}H NMR (DMSO-d_6): δ 12.46 (s, 2H, —NH), 7.00–7.55 (m, 16H, Ar—H), 5.45 (s, 4H, H_2O), 8.17 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 175.5, 174.6, 166.2, 158.0, 138.2, 133.7, 131.9, 131.6, 130.7, 130.0, 129.5, 128.8, 121.1, 116.0, 115.7; % Anal. Cal. for $C_{30}H_{24}N_8O_4S_2Cl_2Zn$: C, 47.35; H, 3.18; N, 14.72; S, 8.41; Zn, 8.60; Found: C, 47.23; H, 3.13; N, 14.67; S, 8.43; Zn, 8.53.

Complex (3). Yield 70%; colour: dark yellow; decomp. temp. 244–246 °C; IR (KBr) cm^{-1}: 3450 (—OH), 3200 (—NH), 3050 (Ar—H), 1605 (C=N azomethine), 1560 (C=N thiadiazole), 1540 (C=C), 1345 (C—O), 1140 (N—N), 1046 (C—S—C), 822, 748 (—OH wagging and rocking), 770 (C—Cl), 488 (Zn—O), 435 (Zn—N); ^{1}H NMR (DMSO-d_6): δ 12.52 (s, 2H, —NH), 7.06–7.65 (m, 16H, Ar—H), 5.45 (s, 4H, H_2O), 8.31 (s, 2H); ^{13}C NMR DMSO-d_6): δ 175.8, 174.0, 166.5, 158.3, 135.1, 132.2, 131.8, 131.3, 130.7, 129.6, 122.1, 116.6, 115.5; $C_{30}H_{24}N_8O_4S_2Cl_2Zn$: C, 47.35; H, 3.18; N, 14.72; S, 8.41; Zn, 8.60; Found: C, 47.27; H, 3.14; N, 14.61; S, 8.40; Zn, 8.52.

Complex (4). Yield 66%; colour: brown; decomp. temp. 248–250 °C; IR (KBr) cm^{-1}: 3425 (—OH), 3190 (—NH), 2982 (Ar—H), 1610 (C=N azomethine), 1565 (C=N thiadiazole), 1540 (C=C), 1515 (C—NO_2), 1345 (C—O), 1150 (N—N), 1055 (C—S—C), 805, 750 (—OH wagging and rocking), 490 (Zn—O), 445 (Zn—N); ^{1}H NMR (DMSO-d_6): δ 12.44 (s, 2H, —NH), 6.90–7.40 (m, 16H, Ar—H), 5.46 (s, 4H, H_2O),

8.22 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 175.0, 174.6, 166.8, 158.0, 150.1, 140.2, 131.2, 131.0, 129.7, 124.6, 122.1, 116.8, 115.3; Anal. Cal. for $C_{30}H_{24}N_{10}$ $O_{10}S_2Zn$: C, 46.07; H, 3.09; N, 17.91; S, 8.20; Zn, 8.36; Found: C, 46.00; H, 3.11; N, 17.81; S, 8.04; Zn, 8.12.

Complex (5). Yield 78%; colour: light orange; decomp. temp. 218–222 °C; IR (KBr) cm^{-1}: 3420 (—OH), 3180 (—NH), 2955 (Ar—H), 1615 (C=N azomethine), 1585 (C=N oxadiazole), 1545 (C=C), 1340 (C—O), 1170 (N—N), 1080 (C—O—C), 810, 740 (—OH wagging and rocking), 492 (Zn—O), 435 (Zn—N); ^{1}H NMR (DMSO-d_6): δ 12.50 (s, 2H, —NH), 7.00–7.60 (m, 18H, Ar—H), 5.45 (s, 4H, H_2O), 8.30 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 170.2, 168.6, 165.5, 158.6, 131.6, 131.4, 131.9, 130.7, 129.4, 127.8, 122.6, 115.9, 114.0; Anal. Cal. for $C_{30}H_{26}N_8O_6Zn$: C, 54.60; H, 3.97; N, 16.98; Zn, 9.91; Found: C, 54.38; H, 3.83; N, 16.90; Zn, 9.77.

Complex (6). Yield 71%; colour: orange; decomp. temp. 200–202 °C; IR (KBr) cm^{-1}: 3485 (—OH), 3205 (—NH), 2970 (Ar—H), 1605 (C=N azomethine), 1560 (C=N oxadiazole), 1542 (C=C), 1355 (C—O), 1140 (N—N), 1080 (C—O—C), 825, 758 (—OH wagging and rocking), 772 (C—Cl), 485 (Zn—O), 440 (Zn—N); ^{1}H NMR (DMSO-d_6): δ 12.48 (s, 2H, —NH), 7.00–7.55 (m, 16H, Ar—H), 5.45 (s, 4H, H_2O), 8.26 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 170.9, 168.1, 165.0, 158.3, 137.8, 133.4, 130.8, 130.4, 130.0, 129.1, 128.6, 127.8, 122.6, 116.7, 115.8; Anal. Cal. for $C_{30}H_{24}N_8O_6Cl_2$ Zn: C, 49.44; H, 3.32; N, 15.37; Zn, 8.97; Found: C, 49.31; H, 3.38; N, 15.25; Zn, 8.90.

Complex (7). Yield 65%; colour: light brown; decomp. temp. 216–218 °C; IR (KBr) cm^{-1}: 3455 (—OH), 3195 (—NH), 3040 (Ar—H), 1612 (C=N azomethine), 1570 (C=N oxadiazole), 1530 (C=C), 1340 (C—O), 1145 (N—N), 1075 (C—O—C), 820, 755 (—OH wagging and rocking), 770 (C—Cl), 495 (Zn—O), 448 (Zn—N); ^{1}H NMR (DMSO-d_6): δ 12.52 (s, 2H, —NH), 7.04–7.55 (m, 16H, Ar—H), 5.50 (s, 4H, H_2O), 8.41 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 170.6, 168.2, 165.6, 158.4, 135.6, 130.8, 130.6, 129.7, 129.0, 125.3, 123.4, 115.7, 114.0; Anal. Cal. for $C_{30}H_{24}N_8O_6Cl_2$ Zn: C, 49.44; H, 3.32; N, 15.37; Zn, 8.97; Found: C, 49.28; H, 3.34; N, 15.20; Zn, 8.80.

Complex (8). Yield 66%; colour: brown; decomp. temp. 214–260 °C; IR (KBr) cm^{-1}: 3430 (—OH), 3210 (—NH), 2950 (Ar—H), 1605 (C=N azomethine), 1565 (C=N oxadiazole), 1555 (C=C), 1510 (C—NO_2), 1360 (C—O), 1155 (N—N), 1087 (C—O—C), 830, 720 (—OH wagging and rocking), 480 (Zn—O), 435 (Zn—N); ^{1}H NMR (DMSO-d_6): δ 12.54 (s, 2H, —NH), 7.04–7.60 (m, 16H, Ar—H), 5.51 (s, 4H, H_2O), 8.32 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 170.9, 168.1, 165.1, 158.4, 148.6, 133.4, 131.9, 131.5, 130.1, 129.4, 122.6, 115.7, 114.3; Anal. Cal. for $C_{30}H_{24}N_{10}O_{10}Zn$: C, 48.04; H, 3.23; N, 18.68; Zn, 8.72; Found: C, 48.00; H, 3.01; N, 18.47; Zn, 8.55.

Synthesis of zinc(II) complexes (9–12)

An ethanolic solution (30 cm^3) of zinc(II) acetate dihydrate (0.01 mol) was added to a refluxing solution of appropriate Schiff base (L′$_9$H$_2$–L′$_{12}$H$_2$) (0.01 mol) in ethanol (30 cm^3) containing alcoholic solution of sodium acetate (0.02 mol). The reaction mixture was refluxed for about 8–13 h. The coloured complex was obtained. The complex was filtered off, washed thoroughly with ethanol and dried under *vacuo*.

Complex (9). Yield 70%; colour: cream; decomp. temp. 260–262 °C; IR (KBr) cm^{-1}: 3450 (—OH), 3165 (—NH), 2940 (Ar—H), 1612 (C=N azomethine), 1555 (C=N triazole), 1545 (C=C), 1330 (C—O), 1155 (N—N), 800, 732 (—OH wagging and rocking), 490 (Zn—O), 420 (Zn—N); ^{1}H NMR (DMSO-d_6): δ 12.45 (s, 1H, —NH), 7.05–7.60 (m, 13H, Ar—H), 5.45 (s, 4H, H_2O), 8.25 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 166.0, 159.4, 158.7, 157.4, 151.6, 132.3, 130.8, 130.4, 129.3, 129.0, 127.5, 121.7, 116.9, 114.9; Anal. Cal. for $C_{22}H_{20}N_6O_4Zn$: C, 53.08; H, 4.05; N, 16.88; Zn, 13.14; Found: C, 53.12; H, 4.01; N, 16.73; Zn, 13.03.

Complex (10). Yield 75%; colour: light yellow; decomp. temp. 255–258 °C; IR (KBr) cm^{-1}: 3460 (—OH), 3180 (—NH), 3040 (Ar—H), 1612 (C=N azomethine), 1545 (C=N triazole), 1540 (C=C), 1145 (N—N), 815, 720 (—OH wagging and rocking), 762 (C—Cl), 485 (Zn—O), 440 (Zn—N); ^{1}H NMR (DMSO-d_6): δ 12.56 (s, 1H, —NH), 6.90–7.55 (m, 12H, Ar—H), 5.45 (s, 4H, H_2O), 8.27 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 166.4, 159.0, 158.1, 157.2, 152.7, 139.7, 133.4, 130.8, 130.5, 130.0, 129.6, 128.7, 122.6, 115.7, 114.8; Anal. Cal. for $C_{22}H_{19}N_6O_4ClZn$: C, 49.64; H, 3.60; N, 15.79; Zn, 12.29; Found: C, 49.55; H, 3.53; N, 15.70; Zn, 12.31.

Complex (11). Yield 72%; colour: light yellow; decomp. temp. 256–260 °C; IR (KBr) cm^{-1}: 3465 (—OH), 3195 (—NH), 3030 (Ar—H), 1608 (C=N azomethine), 1575 (C=N triazole), 1540 (C=C), 1365 (C—O), 1160 (N—N), 815, 748 (—OH wagging and rocking), 777 (C—Cl), 495 (Zn—O), 435 (Zn—N); ^{1}H NMR (DMSO-d_6): δ 12.55 (s, ^{1}H, —NH), 6.95–7.45 (m, 12H, Ar—H), 5.48 (s, 4H, H_2O), 8.31 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 166.7, 159.8, 158.0, 157.5, 152.0, 135.6, 131.7, 131.4, 130.8, 129.6, 129.4, 128.3, 122.4, 115.7, 114.7; Anal. Cal. for $C_{22}H_{19}N_6O_4ClZn$: C, 49.64; H, 3.60; N, 15.79; Zn, 12.29; Found: C, 49.58; H, 3.51; N, 15.85; Zn, 12.22.

Complex (12). Yield 66%; colour: yellow; decomp. temp. 248–250 °C; IR (KBr) cm^{-1}: 3425 (—OH), 3210 (—NH), 2950 (Ar—H), 1615 (C=N azomethine), 1515 (C=N triazole), 1550 (C=C), 1520 (C—NO_2), 1350 (C—O), 1140 (N—N), 825, 760 (—OH wagging and rocking), 480 (Zn—O), 435 (Zn—N); ^{1}H NMR (DMSO-d_6): δ 12.44 (s, 2H, —NH), 7.04–7.44 (m, 12H, Ar—H), 5.46 (s, 4H, H_2O), 8.30 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 166.5, 159.3, 158.8, 157.3, 152.6, 148.6, 137.4, 131.7, 130.5, 128.1, 124.4, 122.6, 116.7, 115.3; Anal. Cal. for $C_{22}H_{24}N_{10}O_6Zn$: C, 48.68; H, 3.53; N, 18.06; Zn, 12.05; Found: C, 48.59; H, 3.53; N, 18.16; Zn, 12.00.

Biological activity

The antimicrobial properties of the Schiff bases (L_1H–L_8H and L′$_9$H$_2$–L′$_{12}$H$_2$) and there Zn(II) complexes were tested against four fungal strains *Cyrtomium falcatum*, *Aspergillus niger*, *Fusarium oxysporium* and *Curvularia pallescence* and four bacteria namely *Bacillus subtilis*, *Pseudomonas Aeruginosa*, *Salmonella typhi* and *Streptococcus pyogenes*. Bacteria/fungi are potentially hazardous and care has been taken while working with them. Standard bio safety lab techniques were followed while handling bacteria/fungi and various media. Gloves were used during all experimentation, and any accidental spills were immediately sterilized using 70% isopropanol/water followed by bleach. The work area was also sterilized with 70% isopropanol/water after completion of work. Unused media and bacterial suspensions were first deactivated with commercial bleach for 1 h before being disposed in bio safety bags. All material that had come in contact with bacteria (e.g., pipette tips, tubes, agar plates, etc.) was also thrown in bio safety bags in tightly closed bins. Bio safety bags were autoclaved for 2 h before final disposal.

All newly prepared Schiff bases and Zn(II) complexes were screened for their antimicrobial activity against four fungi and four bacteria by reported method [35,36]. Microbial activity of each compound was evaluated at three different concentrations, i.e., 10, 100 and 200 μg/ml. Petridishes of equal diameter were sterilised at 180 °C. Solution of 1 ml of each concentration was poured in presterilised petridishes and 9 ml of LB agar was added immediately. Each dish was rotated on the table top in order to achieve thorough mixing of medium with the compound. After this,

bacterial strains were inoculated in the dishes (diameter 5 mm). These set were then inoculated at appropriate temperature. The colony diameter of the test organism was measured with mm scale after 6 days. The percentage inhibition of the growth of the test organism was calculated by following formula

$$\% \text{ Inhibition} = 100(\text{Cd} - \text{Td})/\text{Cd}$$

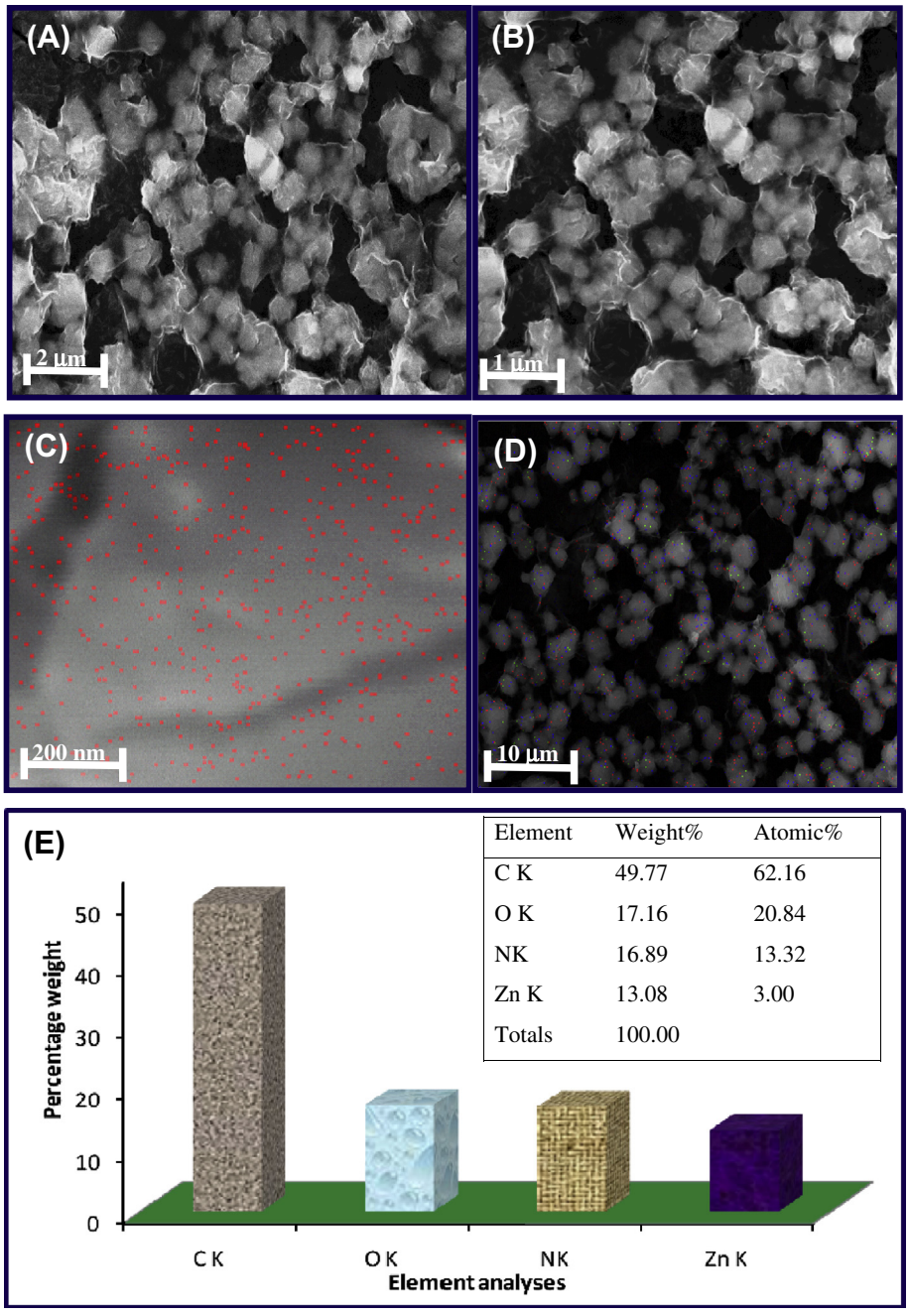
where Cd is the colony diameter of control set; and Td is the colony diameter of treated set. To ensure that solvent had no effect on fungal and bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment.

Results and discussion

All the final compounds (1–12) were obtained in good yields. The physical properties of these compounds are mentioned in experimental section and the synthetic route is given in Fig. 2.

Infrared spectra

The IR spectra provide valuable information regarding the nature of the functional groups attached to the metal atom. The spectra of Schiff bases of type (L_1H–L_8H) show a medium band at ca. 3210–3165 cm^{-1} due to ν(N—H) which remains almost at the same position in the complexes indicating the non-involvement



Element	Weight%	Atomic%
C K	49.77	62.16
O K	17.16	20.84
NK	16.89	13.32
Zn K	13.08	3.00
Totals	100.00	

Fig. 3. SEM image of zinc(II) compound 10 and EDX performance; (A) low magnification SEM image; (B) high magnification image; (C) zinc mapping shown by red colour dot; (D) carbon, oxygen, nitrogen mix mapping represent by blue, red, and green colour dot; (E) EDX performed results shown in table and also present in bar diagram. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of (N—H) group in bond formation [29]. The ligands show one medium intensity band at ca. 1635 cm^{-1} assignable to ν(C=N) which shifts to lower frequency (ca. 20–15 cm^{-1}) in the complexes [36]. This shift indicates the coordination of azomethine nitrogen to metal ion [37–39]; the band at ca. 448–420 cm^{-1} is assigned to ν(Zn—N). Schiff bases show a broad band at ca. 2660 cm^{-1} due to intramolecular H-bonded OH group This band disappears in their corresponding Zn(II) complexes indicating the coordination of phenolic oxygen to metal ion through deprotonation. This is further supported by shift in phenolic C—O band from ca. 1290 cm^{-1} (in the free ligands) to ca. 1350 cm^{-1} in the complexes. The coordination through phenolic oxygen is further confirmed by the appearance of band at ca. 480 cm^{-1} assignable to ν(Zn—O) phenolic [40]. The Schiff bases type (L_1H–L_4H) shows strong band at ca. 1050 cm^{-1} due to the ν(C—S—C) vibration; this position remains unchanged in their corresponding Zn(II) complexes type (1–4), indicating non-coordination of thiadiazole ring sulphur to metal atom. The ν(C—O—C) vibration appears as a strong band at ca. 1080 cm^{-1} in the free ligands type (L_5H–L_8H) [41]; the position of which also remains the same in their corresponding complexes type (5–8), indicating non-coordination of oxadiazole ring oxygen to metal atom. The presence of coordinated water molecules in these complexes is indicated by a broad band in the region 3490–3420 cm^{-1} and two weaker bands in the region ca. 830–805 and 760–720 cm^{-1} due to ν(—OH) rocking and wagging modes of vibrations, respectively [42].

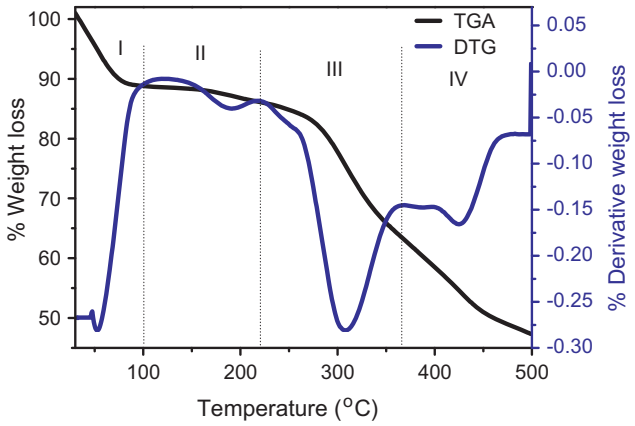


Fig. 4. TGA and DTGA curve for zinc(II) complex 10.

Proton magnetic resonance spectra

The proton magnetic resonance spectra of these complexes have been recorded in DMSO-d_6. The intensities of all the resonance lines were determined by planimetric integration. The following conclusions can be derived by comparing the spectra of ligands with their corresponding complexes. The spectra of Schiff bases exhibit signals at 12.50 and 8.05 ppm due to NH and azomethine protons, respectively. In zinc(II) complexes (1–12), the first signal remains almost at the same position but the second signal shifts to downfield. The downfield shift indicates the deshielding effect due to the co-ordination of azomethine nitrogen to central metal ion. The Schiff bases shows ca. 10.40 ppm signal due to phenolic —OH these signals disappears in their corresponding complexes (1–12), this confirms that the hydroxyl group reacted with metal ion via deprotonation. Schiff bases and their corresponding Zn(II) complexes show multiplet at 6.90–7.60 ppm due to aromatic protons. Zinc(II) complexes (1–12) show new signal at about 5.5 ppm due to the water protons.

^{13}C NMR spectra

The ^{13}C NMR spectra were recorded in DMSO-d_6. Schiff bases derived from thiadiazole and oxadiazole (L_1H–L_8H) show signals at ca. δ 164 for their azomethine carbon which shift downfield in their corresponding zinc(II) complexes (1–8) due to coordination through the azomethine nitrogen. Schiff bases of type (L_1H–L_4H) show signals at ca. δ 175, 174 due to the thiadiazole ring carbon and oxadiazole derived Schiff bases (L_5H–L_8H) show signals at δ 170, 168. These signals remain unchanged in their corresponding complexes indicating that thiadiazole and oxadiazole ring nitrogen are not participated in bond formation.

Schiff bases derived from hydrazino triazole (V–VI) show signal at δ 164 and 157 for azomethine carbon and which shift downfield in zinc metal complexes due to coordination through the azomethine nitrogen. For triazole ring carbons, two signals appear at ca. δ 158, 157 which remain unchanged in their corresponding complexes (9–12) indicating that triazole ring carbons are not participated in bond formation. For aromatic ring, a number of signals were appeared.

Microscopic characterization

The compound (**10**) was dissolved in DMSO and solution with concentration 100 μg/ml was made. One drop of sample was paste in aluminium grid and dried under IR lamp. Typical SEM image of the compound 10 was taken and result obtained is shown in Fig. 3A and B. The compound (**10**) looks like microsphere. For the

Table 1
Antifungal results of Zn(II) complexes (1–12) and standard drug at concentration (μg/ml).

Compound	% Fungicidal inhibition in μg/ml concentration											
	C. falcatum			*A. niger*			*F. oxysporum*			*C. pallescence*		
	200	100	10	200	100	10	200	100	10	200	100	10
1	72	64	44	80	64	46	77	64	43	75	62	42
2	82	73	55	86	75	55	82	72	54	84	68	51
3	77	67	49	82	69	52	80	66	48	82	63	47
4	79	69	51	84	66	54	76	67	50	83	65	44
5	74	60	41	75	65	44	70	58	45	72	57	37
6	79	68	52	83	67	50	76	68	51	79	62	47
7	77	65	45	79	64	46	75	64	47	76	60	43
8	75	56	49	78	63	47	72	69	49	75	63	44
9	74	67	46	81	66	48	81	63	44	75	62	43
10	85	75	62	89	73	59	87	72	56	86	69	54
11	78	69	55	84	70	53	83	66	57	78	65	49
12	80	70	57	83	72	55	82	69	58	77	66	51
Fluconazole	100	100	100	100	100	100	100	100	100	100	100	100

elemental visualization EDX mapping were performed and results obtained are shown in Fig. 3C and D. Zinc elemental mapping of compound (**10**) show that Zn element equivalently distributed in microsphere (Fig. 3C). For the confirmation of other element availability in the microsphere mix elemental mapping were performed and results obtained are systematized in Fig. 3D. EDX performed results are systematically arranged in Fig. 3E, and these results are closely comparable to elemental analysis results.

Thermal analysis

Thermal stability of compound (**10**) was analysed by thermogravimetric analysis (TGA) (Fig. 4) and which shows four steps weight losses. The first decomposition step ranges from 50 to 100 °C showing loss of 11.18 percentage mass involving losing bound water (moisture) and second step ranges from 100 to 220 °C with loss of 2.5 weight percent indicating that compound has coordinated water [43]. Subsequently, in stage III and IV (a range from 220 to 366 °C and 366 to 500 °C) weight losses are 22.63% and 16.13%, respectively. Third and four step weight losses indicate the decomposition of Schiff bases. At 500 °C temperature, 47.56 mass percentage remained.

Antimicrobial activity

The Schiff bases are found to be biologically active and their corresponding Zn(II) complexes show significantly enhanced antifungal (Table 1) and antibacterial (Table 2) activities. As chelation increases, bacterial and fungal growth inhibition also increases. Actual mechanism of increased activity of complexes is not certain but factors like solubility, dipole moment and cell permeability mechanism and their enzymatic action may be the possible reason. According to Overtone's concept of cell permeability, the lipid membrane surrounding the cell favours the passage of lipid-soluble materials, making the solubility an important factor controlling the antimicrobial activity [44]. Tweedy's chelation theory the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity of the hetero chelates. The increased lipophilicity enhances the penetration of the hetero chelates into lipid membranes and blocks the metal binding sites in the enzymes of microorganisms. These hetero chelates also disturb the respiration process of the cell and block the synthesis of proteins, which actually restricts further growth of the organisms. Furthermore, the mode of action comprising the compounds may involve the

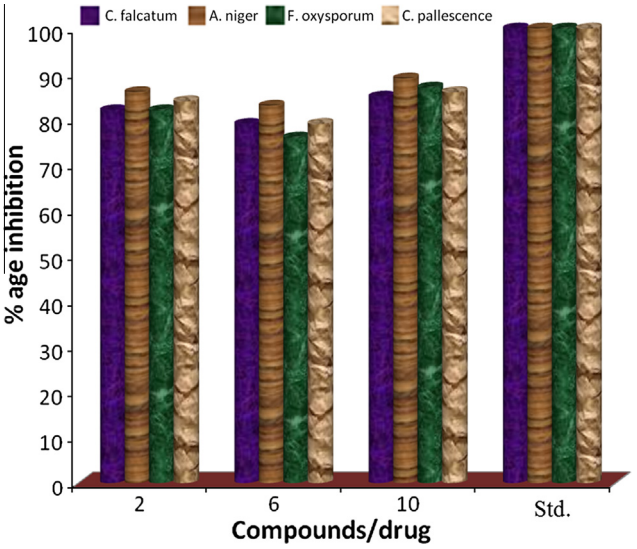


Fig. 5. *In vitro* antifungal spectrum of compounds 2, 6, 10 and fluconazole (Std.) at 200 μg/ml concentration.

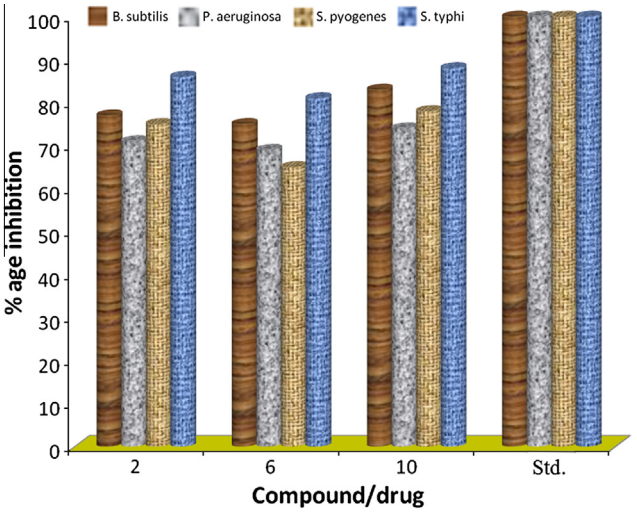


Fig. 6. *In vitro* antibacterial spectrum of compounds 2, 6, 10 and streptomycin (Std.) at 200 μg/ml concentration.

Table 2
Antibacterial results of Zn(II) complexes (1–12) and standard drug at concentration (μg/ml).

Compounds	% Antibacterial inhibition in μg/ml concentration											
	B. subtilis			*P. aeruginosa*			*S. pyogenes*			*S. typhi*		
	200	100	10	200	100	10	200	100	10	200	100	10
1	69	48	27	63	54	20	69	47	25	76	54	31
2	77	55	30	71	59	30	75	58	29	86	62	40
3	71	51	27	69	57	26	69	55	27	79	59	35
4	74	50	26	66	58	24	73	54	26	81	57	36
5	66	43	23	60	47	22	61	40	20	70	49	27
6	75	50	27	69	54	27	65	52	25	81	55	33
7	72	46	25	64	51	25	60	45	23	76	52	29
8	68	45	24	68	50	23	65	48	22	79	53	31
9	72	51	29	64	54	26	68	54	26	78	62	37
10	83	56	35	74	63	33	78	60	32	88	66	43
11	79	53	30	72	59	29	72	58	30	83	63	39
12	77	52	29	76	57	27	70	54	29	81	63	37
Ciprofloxacin	100	100	100	100	100	100	100	100	100	100	100	100

formation of hydrogen bond through the azomethine/carbonyl/amine group with the active center of cell constituents and interferences forced with the normal cell process [45].

In antifungal activity all ligands and Zn(II) complexes are found to be more active against *A. niger* (Fig. 5). It is found that substitution in the ligands increases the activity against bacteria and fungi. 2-Chloro substituted ligands/compounds are more active than the other substituted ligands/compounds. Due to the chelating properties of 2-chloro group, antibacterial and antifungal activity increases. The compound (10) is more active against all bacteria and fungi due to the chelation of ligands. In antibacterial activity, all Schiff bases and Zn(II) complexes are more active against *S. typhi* (Fig. 6).

Conclusions

Schiff bases (L_1H–L_8H) are monobasic, bidentate ligands coordinating through azomethine nitrogen and phenolate oxygen atom (NO donor). Schiff bases (L'_9H_2–$L'_{12}H_2$) are dibasic, tetradentate ligands coordinating through azomethine nitrogen atoms and phenolate oxygen atoms (N_2O_2 donor). The complexes are insoluble in all common organic solvent except DMF and DMSO. The structures of Schiff bases and complexes have been established by elemental analysis and spectral studies IR, ^{1}H NMR and ^{13}C NMR. All these data puts together leads us to propose the structure of zinc(II) complexes shown in Fig. 2. The presence of coordinated water molecules in complexes has been confirmed by TGA data. Scanning electron microscope image showed that zinc complexes look like a microsphere and their sizes are 1–2 μm. Antifungal and antibacterial activities of the ligands and corresponding complexes have also been evaluated which showed that the activities increase on chelation.

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