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Metal based new triazoles: Their synthesis, characterization and antibacterial/antifungal activities

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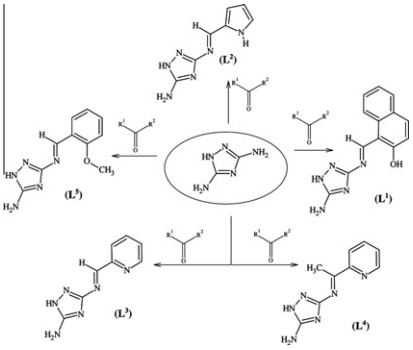
HIGHLIGHTS

- Novel series of triazole and its vanadium metal complexes are studied.
- Characterization is made on the basis of their physical, spectral and analytical data.
- Antibacterial and antifungal correlation with vanadium metal is established.
- Bioactivity is enhanced upon coordination with the vanadium metal.

GRAPHICAL ABSTRACT



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ABSTRACT

A series of new triazoles and their oxovanadium(IV) complexes have been synthesized, characterized and evaluated for antibacterial/antifungal properties. The new Schiff bases ligands (L^1)–(L^5) were prepared by the condensation reaction of 3,5-diamino-1,2,4-triazole with 2-hydroxy-1-naphthaldehyde, pyrrole-2-carboxaldehyde, pyridine-2-carboxaldehyde, 2-acetyl pyridine and 2-methoxy benzaldehyde. The structures of the ligands have been established on the basis of their physical, spectral (IR, ^{1}H and ^{13}C NMR and mass spectrometry) and elemental analytical data. The prepared ligands were used to synthesize their oxovanadium(IV) complexes **(1)**–**(5)** which were also characterized by their physical, spectral and analytical data and proposed to have a square pyramidal geometry. The ligands and their complexes were screened for *in vitro* antibacterial activity against six bacterial species such as, *Escherichia coli*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, and *Bacillus subtilis* and for *in vitro* antifungal activity against six fungal strains, *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani*, and *Candida glabrata*. Cytotoxic nature of the compounds was also reported using brine shrimp bioassay method against *Artemia salina*.

Introduction

Triazoles occupy a unique position [1] amongst the potentially used bioactive compounds due to different potential activities such as antibacterial [2], antifungal [3], anticonvulsant [4], antiproliferative [5], antitumor [6], antitubercular [7], anticancer [8], anti HIV [9] and antiviral [10]. The coordination behavior [11] of vanadium is of great interest since its discovery of a role in biotic and abiotic systems [12]. Another perspective application of vanadium compounds is their ability in promoting the biological properties like antimicrobial [13], antitumor [14], antileukemic [15], spermicidal [16], antiamoebic activity [17] and recently most significant insulin mimetic activity [18]. Keeping in view the significant bioactive nature of triazoles as well as biological functioning of vanadium metal, it was thought valuable to merge the chemistry of triazoles with the vanadium metal to form a novel class of vanadium metal based triazoles that could serve as potential antibacterial and

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antifungal agent against resistant bacterial/fungal strains. For accomplishment of this task, a series of oxovanadium(IV) complexes with different new triazole Schiff base compounds have been synthesized (Scheme 1) and characterized. These compounds were then subjected to *in vitro* antibacterial activity against six bacterial species such as, *Escherichia coli*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Bacillus subtilis* and for *in vitro* antifungal activity against six fungal strains, *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *Candida glabrata*. Cytotoxic nature of the compounds was also reported using brine shrimp bioassay method against *Artemia salina*.

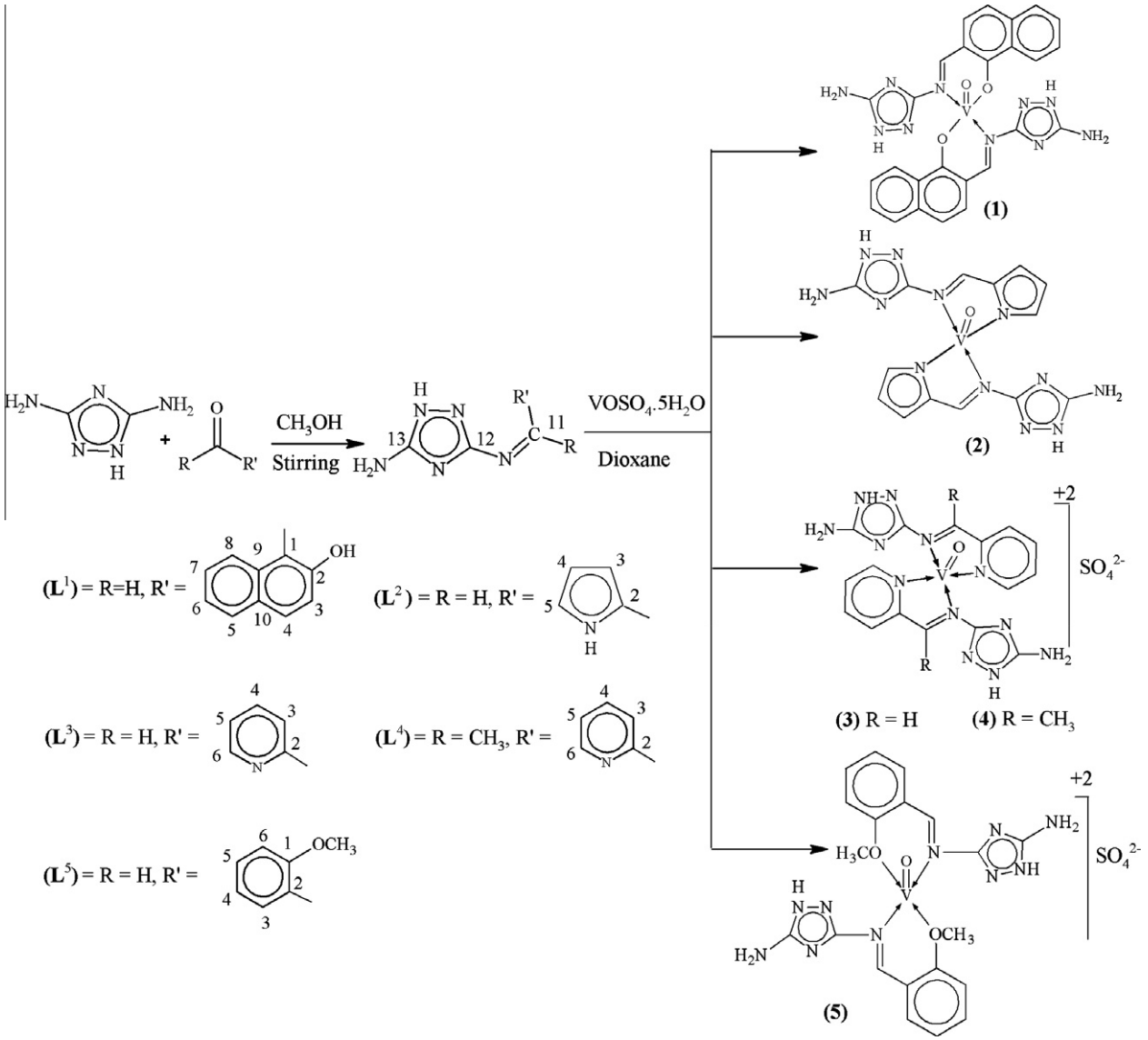
Experimental

Materials and methods

All the chemicals used were of analytical grades. 3,5-Diamino-1,2,4-triazole was purchased from Sigma Aldrich. Fisher Johns melting point apparatus was used for recording melting points. IR spectra were recorded on SHIMADZU FT-IR spectrophotometer. Elemental analysis was carried out on Perkin Elmer (USA model). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Spectrospin Avance DPX-400 spectrometer using TMS as internal standard and d_6-DMSO as a solvent. Electron impact mass spectra (EIMS) were recorded on JEOL MS route instrument. *In vitro* antibacterial, antifungal and cytotoxic properties were studied at HEJ Research Institute of Chemistry, International Centre for Chemical Sciences, University of Karachi, Pakistan.

Synthesis

An equimolar solution of 3,5-diamino-1,2,4-triazole (0.99 g, 0.01 mol) and 2-hydroxynaphthaldehyde (1.72 g, 0.01 mol) in dry methanol (50 mL) was refluxed for 3 h, a precipitated product was formed during refluxing. It was then cooled to room temperature, filtered, washed with methanol (35 mL), then with diethyl ether (2 × 5 mL) and dried under vacuum. Recrystallization in a mixture of methanol:dioxane (1:4) gave TLC pure product **(L^1)** in 83% yield. The same method was applied for the preparation of all other ligands **(L^2)–(L^5)**.



Scheme 1. Synthesis of the triazole ligands and their oxovanadium(IV) complexes.

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

Yield: 83% (2.1 g); colour (yellow); m.p. 229–231 °C; IR (KBr, cm^{-1}): 3436 (OH), 3348 (NH_2), 3203 (NH, triazole), 1637 (HC=N), 1612 (C=N, triazole), 1028 (N–N); ^{1}H NMR (DMSO–d_6): δ 6.02 (s, 2H, NH_2), 7.11 (d, 1H, J = 9.1 Hz, C_3–H), 7.39 (t, 1H, J = 7.5 Hz, C_6–H), 7.59 (t, 1H, J = 7.5 Hz, C_7–H), 7.85 (d, 1H, J = 7.9 Hz, C_4–H), 7.98 (d, 1H, J = 9.1 Hz, C_5–H), 8.19 (d, 1H, J = 8.4 Hz, C_8–H), 8.29 (s, 1H, C_{11}–H), 10.22 (s, 1H, OH), 12.10 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 115.5 (C_1), 123.2 (C_6), 124.1 (C_3), 125.8 (C_8), 126.3 (C_{10}), 127.6 (C_7), 128.9 (C_5), 133.5 (C_9), 136.1 (C_4), 156.3 (C_{13}), 158.3 (C_{11}), 159.2 (C_2), 163.8 (C_{12}); EIMS (70 eV) m/z (%): 253 ([M]$^+$, 14), 236 (100), 222 (16), 197 (11), 182 (17), 169 (68), 154 (11), 141 (12), 127 (43), 77 (9); Anal. Calcd. for $C_{13}H_{11}N_5O$ (253.26): C, 61.60; H, 4.38; N, 27.65; O: 6.32; Found: C, 61.63; H, 4.35; N, 27.63; O: 6.30%.

N^3-[(E)-1H-Pyrrol-2-ylmethylidene]-1H-1,2,4-triazole-3,5-diamine (L^2)

Yield: 78% (1.37 g); colour (light brown); m.p. 185–187 °C; IR (KBr, cm^{-1}): 3343 (NH_2), 3204 (NH, triazole), 3175 (NH, pyrrole), 1636 (HC=N), 1613 (C=N), 1030 (N–N); ^{1}H NMR (DMSO-d_6, δ, ppm): δ 5.95 (dd, 1H, J = 3.9, 2.8 Hz, C_4–H), 6.02 (s, 1H, NH_2), 6.71 (d, 1H, J = 2.8 Hz, C_5–H), 7.01 (d, 1H, J = 3.9 Hz, C_3–H), 8.72 (s, 1H, C_{11}–H), 9.46 (s, 1H, NH), 12.07 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, δ, ppm): δ 115.4 (C_3), 121.4 (C_4), 125.6 (C_5), 132.1 (C_2), 156.2 (C_{13}), 157.9 (C_{11}), 162.7 (C_{12}); EIMS (70 eV) m/z (%): 176 ([M]$^+$, 100), 160 (19), 146 (18), 134 (9), 120 (21), 105 (19), 93 (13), 79 (27), 66 (6), 52 (7); Anal. Calcd. for $C_7H_8N_6$ (176.18): C, 47.72; H, 4.59; N, 47.70; Found: C, 47.68; H, 4.56; N, 47.66%.

N^3-[(E)-Pyridin-2-ylmethylidene]-1H-1,2,4-triazole-3,5-diamine (L^3)

Yield: 74% (1.39 g); colour (grey); m.p. 172–174 °C; IR (KBr, cm^{-1}): 3347 (NH_2), 3201 (NH, triazole), 1633 (HC=N), 1609 (C=N), 1030 (N–N); ^{1}H NMR (DMSO-d_6, δ, ppm): δ 6.01 (s, 2H, NH_2), 7.33 (ddd, 1H, J = 7.8, 5.2, 1.3 Hz, C_5–H), 7.71 (dd, 1H, J = 7.9, 1.3 Hz, C_3–H), 8.04 (ddd, 1H, J = 7.9, 7.8, 1.9 Hz, C_4–H), 8.68 (dd, 1H, 5.2, 1.9 Hz, C_6–H), 8.95 (s, 1H, C_{11}–H), 12.11 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, δ, ppm): δ 121.2 (C_3), 126.8 (C_5), 136.6 (C_4), 149.6 (C_6), 153.9 (C_2), 156.1 (C_{13}), 158.6 (C_{11}), 162.3 (C_{12}); EIMS (70 eV) m/z (%): 188 ([M]$^+$, 15), 172 (100), 161 (11), 133 (36), 118 (74), 105 (13), 92 (15), 78 (22), 51 (8); Anal. Calcd. for $C_8H_8N_6$ (188.19): C, 51.06; H, 4.28; N, 44.66; Found: C, 51.02; H, 4.26; N, 44.61%.

N^3-[(1E)-1-(Pyridin-2-yl)ethylidene]-1H-1,2,4-triazole-3,5-diamine (L^4)

Yield: 70% (1.42 g); colour (grey); m.p. 126–128 °C; IR (KBr, cm^{-1}): 3349 (NH_2), 3199 (NH), 1634 (HC=N), 1612 (C=N), 1033 (N–N); ^{1}H NMR (DMSO-d_6, δ, ppm): δ 2.36 (s, 3H, CH_3), 6.04 (s, 2H, NH_2), 7.33 (ddd, 1H, J = 7.8, 5.2, 1.3 Hz, C_5–H), 7.71 (dd, 1H, J = 7.9, 1.3 Hz, C_3–H), 8.04 (ddd, 1H, J = 7.9, 7.8, 1.9 Hz, C_4–H), 8.68 (dd, 1H, 5.2, 1.9 Hz, C_6–H), 8.95 (s, 1H, C_{11}–H), 12.11 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, δ, ppm): δ 16.8 (CH_3), 121.2 (C_3), 126.8 (C_5), 136.6 (C_4), 149.6 (C_6), 153.9 (C_2), 156.1 (C_{13}), 158.6 (C_{11}), 162.3 (C_{12}); EIMS (70 eV) m/z (%): 202 ([M]$^+$, 18), 186 (24), 172 (100), 161 (9), 146 (22), 133 (16), 118 (11), 105 (18), 92 (20), 78 (17), 51 (13); Anal. Calcd. for $C_9H_{10}N_6$ (202.22): C, 53.46; H, 4.98; N, 41.96; Found: C, 53.43; H, 4.96; N, 41.91%.

N^3-[(E)-(2-Methoxyphenyl)methylidene]-1H-1,2,4-triazole-3,5-diamine (L^5)

Yield: 73% (2.59 g); colour (reddish brown); m.p. 238–240 °C; IR (KBr, cm^{-1}): 3346 (NH_2), 3199 (NH), 2910 (OCH_3), 1632 (HC=N), 1603 (C=N, triazole), 1027 (N–N); ^{1}H NMR (DMSO-d_6): δ 3.86 (s, 3H, OCH_3), 6.02 (s, 2H, NH_2), 6.87 (t, 1H, J = 8.5 Hz, C_5–H), 6.95 (d, 1H, J = 7.9 Hz, C_3–H), 7.32 (t, 1H, J = 7.7 Hz, C_4–H), 7.59 (d, 1H, J = 8.1 Hz, C_6–H), 8.36 (s, C_{11}–H), 11.89 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 56.1 (OCH_3), 113.7 (C_6), 120.9 (C_4), 122.5 (C_2), 128.6 (C_3), 133.8 (C_5), 156.1 (C_{13}), 159.6 (C_1), 160.2 (C_{11}), 163.1 (C_{12}); EIMS (70 eV) m/z (%): 217 ([M]$^+$, 28), 201 (100), 186 (19), 171 (12), 161 (32), 146 (9), 134 (23), 107 (9), 104 (43), 77 (13); Anal. Calcd. for $C_{10}H_{11}N_5O$ (217.23): C, 55.29; H, 5.10; N, 32.24; O: 7.37; Found: C, 55.26; H, 5.09; N, 32.22; O: 7.34%.

Synthesis of oxovanadium(IV) complexes (1)–(5)

Vanadyl sulfate (0.163 g, 0.001 mol) in a dry methanol solution (20 mL) was treated with Schiff base ligand 1-{(E)-[(5-amino-1H-1,2,4-triazol-3-yl)imino]methyl}naphthalen-2-ol **(L^1)** (0.51 g, 0.002 mol) in dry dioxane (40 mL). The reaction mixture was refluxed for 3 h during which a precipitated product was formed. The reaction mixture was then cooled to room temperature. The precipitates thus formed were filtered, washed with methanol, dioxane and then with diethyl ether and dried under vacuum. The precipitated product was recrystallized in a mixture of water:dioxane (1:3) to obtain TLC pure vanadium complex **(1)**. All other complexes **(2)–(5)** were prepared following the same method (Scheme 1).

Estimation of vanadium metal

The vanadium content present in complex **(1)** was determined volumetrically by using 0.1 N $KMnO_4$ solution as an oxidizing agent in the presence of dil. H_2SO_4. For this purpose, sample of the oxovanadium(IV) complex **(1)** (100 mg) was placed in a silica crucible, decomposed by gentle heating and then by adding 1–2 mL of conc. HNO_3 two to three times. An orange colored mass (V_2O_5) was obtained after decomposition and complete drying. It was dissolved in miminum amount of dil. H_2SO_4 and solution so obtained was diluted with distilled water to 100 mL in a measuring flask. The amount of vanadium in the tested sample was calculated [19] by using the standard relationship given below. All other oxovanadium(IV) complexes **(2)–(5)**, were estimated following the same method.

1 mL of 0.1 N $KMnO_4$ = 0.005094 g vanadium.

Pharmacology

Antibacterial studies (in vitro)

All newly synthesized triazole Schiff bases **(L^1)–(L^5)** and their oxovanadium(IV) complexes **(1)–(5)** were screened *in vitro* for their antibacterial activity against four Gram-negative (*E. coli*, *S. flexneri*, *P. aeruginosa*, *S. typhi*) and two Gram-positive (*S. aureus*, *B. subtilis*) bacterial strains by the agar-well diffusion method [20]. The wells (6 mm in diameter) were dug in the media with the help of a sterile metallic borer. Bacterial inocula (2–8 h old) containing approximately 10^4–10^6 colony-forming units (CFU/mL) were spread on the surface of the nutrient agar with the help of a sterile cotton swab. The recommended concentration of the test sample (1 mg/mL in DMSO) was introduced in the respective wells. Other wells supplemented with DMSO and reference antibacterial drug, imipenum served as negative and positive controls, respectively. The plates were incubated at 37 °C for 24 h. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). In order to evaluate the interfering effect of DMSO on the biological screening, alternate studies on DMSO solution showed no activity against any bacterial strains.

Antifungal activity (in vitro)

Antifungal activities of all compounds were studied [20] against six fungal strains (*T. longifusus*, *C. albican*, *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. Discs soaked in 20 mL (200 μg/mL 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 of inhibition and compared with standard drugs miconazole and amphotericin B.

Minimum inhibitory concentration (MIC)

Compounds containing promising antibacterial (above 80%) activity were selected for minimum inhibitory concentration (MIC) studies. The minimum inhibitory concentration was determined using the disc diffusion technique [21] by preparing discs containing 10, 25, 50 and 100 g mL^{-1} concentrations of the compounds along with standards at the same concentrations.

Cytotoxicity (in vitro)

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 two days a pipette collected nauplii from the lighted side. A sample of the test compound was prepared by dissolving 20 mg of each compound in 2 mL of DMF. From this stock solutions 500, 50 and 5 μg/mL were transferred to 9 vials (three for each dilutions were used for each test sample and LD_{50} is the mean of three values) and one vial was kept as control having 2 mL of DMF only. The solvent was allowed to evaporate overnight. After two days, when shrimp larvae were ready, 1 mL of seawater and 10 shrimps were added to each vial (30 shrimps/dilution) and the volume was adjusted with seawater to 5 mL per vial [22]. After 24 h the number of survivors were counted and analyzed by Finney computer program to determine the LD_{50} values [23].

Results and discussion

Chemistry

The triazole Schiff base ligands **(L^1)–(L^5)** were prepared by the condensation reaction of 3,5-diamino-1,2,4-triazole with 2-hydroxy-1-naphthaldehyde, pyrrole-2-carboxaldehyde, pyridine-2-carboxaldehyde, 2-acetyl pyridine and 2-methoxybenzaldehyde respectively, under stirring. The prepared ligands were air, moisture stable, different colored compounds and only soluble in dioxane, DMF and DMSO. All were amorphous solids which melted at 126–240 °C. The oxovanadium(IV) complexes **(1)–(5)** of the synthesized ligands were prepared in a molar ratio 1:2 (metal:ligand). All the oxovanadium(IV) complexes were green colored amorphous solid. All the complexes decomposed on heating rather than melting. They were all only soluble in DMSO and DMF. The elemental analysis and solubility data were consistent with monomers and showed 1:2 stoichiometry of type ML_2 where M is metal and L is ligand. The analytical data given in (Table 1), also agreed well with the proposed structure of the complexes. The spectral data of the vanadyl(IV) complexes is reported in Tables 1 and 2.

IR spectra

IR spectral data of the ligands is reported in experimental section and oxovanadium(IV) complexes in Table 2. All the triazole ligands have active donor sites such as, azomethine (–C=N) linkage, hydroxyl (–OH), methoxy ($-OCH_3$), pyrrole (N–H), pyridine nitrogen (–C–N) and triazole-N, for coordination with the vanadium metalloelement. All the ligands showed bands at 3180–3204, 1594–1616 and 1016–1033 cm^{-1} respectively due to N–H, C=N and N–N vibrations of triazole [24] moiety. Originally, 3,5-diamino-1,2,4-triazole had two bands [25] at 3310 and 3350 cm^{-1} due to two amino groups present in the molecule. All the ligands showed an absence of a band at 3310 cm^{-1} emerging into a new band of azomethine linkage [26] at 1626–1636 cm^{-1} however, a band at 3350 cm^{-1} was remained unchanged giving thus an evidence for condensation of only one amino group of the triazole moiety [27]. The hydroxyl and methoxy, [υ(–OH) and $\upsilon(OCH_3)$] groups of ligands **(L^1)** and **(L^5)** appeared at 3436 and 2910 cm^{-1}.

On comparison of the IR spectral data of all the oxovanadium(IV) complexes with the data of triazole Schiff base ligands, the absorption modes indicated that the triazole Schiff base ligands were principally coordinated to the vanadium(IV) metal ion bidentately. The coordination modes of bonding are discussed as under:

(1) All the ligands showed azomethine ν(HC=N) bands at 1632–1637 cm^{-1} which shifted [28] to lower frequency (12–15 cm^{-1}) at 1618–1624 cm^{-1} indicating the coordination of the azomethine-N with the vanadium(IV) metal atom.
(2) The appearance of a new band at 1387–1388 cm^{-1} was assigned [29] to ν(C–O) in the spectra of vanadyl(IV) complex **(1)** and **(5)**, confirming deprotonation and coordination of hydroxyl-OH to the vanadium(IV) metal.
(3) A new lower frequency bands at 518–522 were assigned to ν(V–O) of complexes **(1)** and **(5)**, and all complexes showed bands at 423–428 cm^{-1} due to ν(V–N) [30] respectively indicating the coordination of these ligands with the vanadyl(IV) metal ion.
(4) A new band at 960–970 cm^{-1} which was only observed in all the spectra of the complexes was assigned to the presence of (V=O) [31]. The oxovanadium complexes **(2)–(4),** showed bands at 1080–1086 due to the presence of (SO_4) [32].
(5) The bands at 3343–3349, 3199–3204, 1603–1613 and 1027–1033 cm^{-1} respectively assigned to the vibrations, $\upsilon(NH_2)$, υ(N–H), υ(C=N) and υ(N–N) of triazole moiety remained unchanged in all the ligands indicating their non-involvement in the coordination.

All the above mentioned evidences supported the coordination of the ligands with the vanadium(IV) metal though azomethine-N, pyridine-N, deprotonation of hyroxyl-OH and pyrrol-N.

^{1}H NMR spectra

^{1}H NMR spectral data of the triazole Schiff bases recorded in DMSO-d_6 along with its possible assignments is reported in the experimental part. All the protons due to aromatic and heteroaromatic were found in their expected region [33]. All the ligands **(L^1)–(L^5)** possessed a singlet at 12.07–12.11 and 8.29–8.95 ppm due to N***H*** proton of triazole moiety and azomethine protons (C^{11}–***H***). Also, all ligands demonstrated [34] characteristic amino (N$\boldsymbol{H_2}$) protons at 5.98–6.08 ppm as a singlet which provided an evidence for condensation of one mono group of triazole moiety. The ligand **(L^1)** possessed hydroxyl (O***H***) proton at 10.22 ppm as a singlet. Other C^3–***H***, C^4–***H***, C^5–***H*** and C^8–***H*** protons were found as a double doublet at 7.11, 7.85, 7.98 and 8.19 ppm, respectively, but C^6–***H*** and C^7–***H*** protons appeared as a triplet at 7.39 and

Table 1
Physicaland microanalytical data of the oxovanadium(IV) complexes.

No.	Structure	MW (g/mol) Formula	M.P (dec.) (°C)	Yield (%) [colour]	Found (calculated)%			
					C	H	N	V
(1)	$[VO(\mathbf{L^1}–H)_2]$	[571.44]	240–241	85	54.69	3.51	24.48	8.81
		$C_{26}H_{20}N_{10}O_3V$		[Green]	(54.65)	(3.53)	(24.51)	(8.85)
(2)	$[VO(\mathbf{L^2}–H)_2]$	[417.28]	248–250	74	40.33	3.36	40.25	12.17
		$C_{14}H_{14}N_{12}OV$		[Sea-green]	(40.30)	(3.38)	(40.28)	(12.21)
(3)	$[VO(\mathbf{L^3})_2]$	SO_4 [539.31]	263–265	72	35.59	2.99	31.13	9.48
		$C_{16}H_{16}N_{12}O_5SV$		[Light green]	(35.63)	(2.97)	(31.21)	(9.44)
(4)	$[VO(\mathbf{L^4})_2]$	SO_4 [567.37]		74	38.14	3.51	29.57	8.94
		$C_{18}H_{20}N_{12}O_5SV$	254–257	[Light green]	(38.10)	(3.53)	(29.61)	(8.98)
(5)	$[VO(\mathbf{L^5})_2]$	SO_4 [597.46]		81	40.17	3.65	23.41	8.55
		$C_{20}H_{22}N_{10}O_7SV$	229–232	[Sea-green]	(40.21)	(3.68)	(23.44)	(8.53)

Table 2
Physical, spectral and IR data of oxovanadium(IV) complexes.

No.	M [cm^{-1} (nm)] (Ω^{-1} cm^2 mol^{-1})	B.M ($_{eff}$)	λ_{max} [cm^{-1} (nm)]	IR (cm^{-1})
(1)	41.8	1.75	12412 (806), 17541 (570), 24912 (401), 37256 (268)	1622 (C=N), 1388 (C–O), 960 (V=O), 518 (V-O), 428 (V-N)
(2)	39.5	1.73	12576 (795), 17534 (570), 24978 (400), 37364 (268)	1624 (C=N), 970 (V=O), 425 (V–N)
(3)	88.9	1.78	12536(798), 17418(574), 25013 (400), 36751 (272)	1623 (C=N), 1085 (SO_4), 968 (V=O), 425 (V–N)
(4)	85.8	1.73	12642 (791), 17639 (567), 25057 (399), 36632 (273)	1622 (C=N), 1088 (SO_4), 965 (V=O), 427 (V–N)
(5)	87.5	1.68	12475 (801), 17498 (572), 24783 (404), 36712 (272)	1618 (C=N), 1378 (C–O), 1085 (SO_4), 965 (V=O), 522 (V–O), 422 (V–N)

7.59 ppm, respectively. The N***H*** proton of pyrrolyl ring found in the ligand **($\mathbf{L^2}$)** exhibited a singlet at 9.46 ppm. Also, C^5–***H*** and C^3–***H*** protons were found in the region at 6.71 and 7.01 ppm as a doublet, and C^4–***H*** proton at 5.95 ppm as double of doublet. The ligands **($\mathbf{L^3}$)** and **($\mathbf{L^4}$)** showed C^3–***H*** and C^6–***H*** protons at 7.71 and 8.68 ppm as double of doublet, and C^4–***H*** and C^5–***H*** protons appeared as double of double doublet at 7.33 and 8.04 ppm, respectively. The ligand **($\mathbf{L^5}$)** showed (OC***H***$_3$) proton at 2.86 ppm as a singlet. Other protons, C^4–***H*** and C^5–***H*** appeared as a triplet at 6.87–7.32 ppm. The C^3–***H*** and C^6–***H*** protons were observed at 6.95–7.59 ppm as a doublet. The conclusions drawn from this study provided further support to the modes of bonding discussed earlier in IR spectra.

^{13}C NMR spectra

The spectra of ligands **($\mathbf{L^1}$)**–**($\mathbf{L^5}$)** possessed the triazole carbons ***C***^{12} and ***C***^{13} in the region at 156.12–163.78 ppm. In addition to this, the azomethine carbons ***C***^{11} of all ligands appeared in the region at 157.9–160.23 ppm. Moreover, the aromatic and heteroaromatic carbons of these two series were found at 115.35–159.17 ppm. The peak for ***C***^{2} of ligand **($\mathbf{L^1}$)** was observed at 159.17 ppm due to higher inductive effect of hydroxyl (OH) group. However, the methoxy-***C*** in the ligand **($\mathbf{L^5}$)** appeared in the region at 55.39 ppm. The methyl-***C*** in ligand **($\mathbf{L^4}$)** was observed at 16.83 ppm. The results of these studies were found to be in agreement with the results of IR and ^{1}H NMR spectra of these compounds.

Mass spectra

The electron impact mass spectra (EIMS) of the synthesized triazole Schiff bases is recorded in experimental part. The molecular mass of ligand **($\mathbf{L^1}$)** with molecular formula $[C_{13}H_{11}N_5O]^+$, was observed at *m/z* 253 (calcd. 253.26) and its base peak stable fragment $[C_{13}H_{10}N_5]^+$ was found at *m/z* 236. Similarly, the mass of ligand **($\mathbf{L^2}$)** with molecular formula $[C_7H_8N_6]^+$ was found at *m/z* 176 (calcd. 176.18) as a stable fragment. The ligand **($\mathbf{L^3}$)** showed molecular mass at *m/z* 188 (calcd. 188.19) with molecular formula $[C_8H_8N_6]^+$ and its most stable fragment $[C_8H_6N_5]^{\cdot+}$ was appeared at *m/z* 172. In addition, the mass of ligand **($\mathbf{L^4}$)** with molecular formula $[C_9H_{10}N_6]^+$, was observed at *m/z* 202 (calcd. 202.22) and its most stable fragment $[C_8H_6N_5]^+$ was shown at *m/z* 172. Moreover, the mass of ligand **($\mathbf{L^5}$)** with molecular formula $[C_{10}H_{11}N_5O]$ was observed at *m/z* 217 (calcd. 217.22) and the most stable fragment $[C_{10}H_9N_4O]^+$ was observed at *m/z* 201. All ligands fragmentation pattern followed the cleavage of C=N (exocyclic as well as endocyclic), C–N, C–C, C=C and C–O bonds. The proposed mass fragmentation pattern of **($\mathbf{L^1}$)** has been shown in Fig. 1.

Conductance and magnetic susceptibility measurements

The molar conductance studies of the oxovanadium(IV) complexes **(1)**–**(5)** were carried out in DMF solution and the results reported in Table 2. The molar conductance values of compounds **(1)** and **(2)** fall in the range 39.5–41.8 Ω^{-1} cm^2 mol^{-1} showing their non-electrolytic nature [35]. Conductance values of compounds **(3)**–**(5),** however, fall in the range 85.8–88.9 Ω^{-1} cm^2 mol^{-1} indicating their electrolytic [36] nature (Table 2). The effective magnetic moments of the oxovanadium(IV) complexes at room temperature were found in the range 1.68–1.78 B.M. which are indicative of the presence of a single electron consistent with half-spin ($S = 1/2$) orbital contribution. These results gave a clue for vanadium metal to possess +4 oxidation state which is indicative of having square-pyramidal geometry of all these oxovanadium(IV) complexes. These values were also compatible to the reported values for the compounds having square-pyramidal geometry.

Electronic spectra

The UV/Visible spectra of all the oxovanadium(IV) complexes in DMF displayed three (Table 2) distinctive low to high intensity bands (v_1, v_2 and v_3) which were assigned to $b_2(d_{xy}) \rightarrow e_\pi(d_{xz}, d_{yz}), b_2(d_{xy}) \rightarrow b_1(d_x^2 - y^2)$ and $b_2(d_{xy}) \rightarrow a_1(d_z^2)$ transitions, respectively. The first band observed at 12412–12642 cm^{-1} was assigned to $b_2 \rightarrow e_\pi$ d–d transitions. The second band was observed at 17418–17639 cm^{-1} which can be attributed to $b_2 \rightarrow b_1$ and the presence of third band at 24783–25057 cm^{-1} can be assigned to the transitions $b_2 \rightarrow a_1$. The fourth band of high intensity observed at 36632–37251 cm^{-1} was due to metal → ligand charge transfer

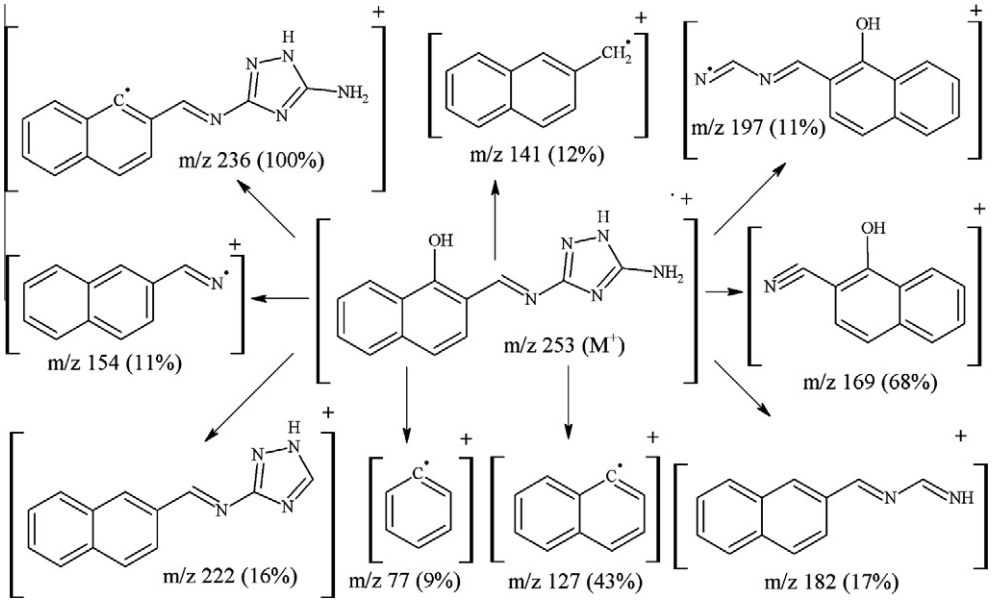


Fig. 1. Proposed mass fragmentation pattern of ($\mathbf{L^1}$).

(MLCT). All these observations provided a strong evidence for the complexes to show a square-pyramidal geometry [37,38].

Biological activity

Antibacterial Bioassay

The results obtained from *in vitro* antibacterial studies are summarized in (Table 3) and (Fig. 2). All the compounds were tested against four Gram-negative (*E. coli*, *S. flexneri*, *P. aeruginosa*, *S. typhi*) and two Gram-positive (*S. aureus*, *B. subtilis*) bacterial strains. The results obtained were compared with that of the standard drug imipenum. The percentage of activity was compared with the activity of the standard drug considering its activity as 100%. All ligands and their oxovanadium(IV) complexes possessed wide-ranging biological activity against all Gram-negative and Gram-positive bacterial strains. The synthesized compounds showed varying degree of inhibitory effects: low (up to 33%), moderate (up to 53%) and significant (above 53%). The studies of results showed that the ligand **($\mathbf{L^1}$)** exhibited significant (55–59%) activity against ***(a)***, ***(c)*** and ***(d)*** bacterial and moderate (45–50%) activity against ***(b)***, ***(e)*** and ***(f)***. Also, the ligands **($\mathbf{L^2}$)–($\mathbf{L^4}$)** exhibited a significant (54–72%) activity against all the strains except ***(b)*** of the ligand **($\mathbf{L^3}$)** which displayed moderate (50%) activity. Likewise, significant (55%) activity against ***(d)*** strain was observed by the ligands **($\mathbf{L^5}$)** and remaining strains demonstrated moderate (42–50%) activity. All the oxovanadium(IV) complex **(1)–(5)** showed significant (54–88%) activity against all bacterial strains. The antibacterial studies revealed that all the Schiff base ligands and their oxovanadium(IV) complexes contributed significantly towards enhancing the biological activity.

Antifungal bioassay

The triazole ligands **($\mathbf{L^1}$)–($\mathbf{L^5}$)** and their oxovanadium(IV) complexes **(1)–(5)** were subjected to screen against six fungal strains (Table 4 and Fig. 3). It was indicated from the obtained antifungal results that ligand **($\mathbf{L^1}$)** possessed significant (61%) activity against ***(c)*** and moderate (39–48%) against ***(b)***, ***(d)–(f)*** but inactive against fungal strain ***(a)***. However, the ligand **($\mathbf{L^2}$)** demonstrated overall significant (55–63%) activity against all fungal strains except ***(c)*** which showed moderate (49%) activity. Similarly, the ligand **($\mathbf{L^3}$)** exhibited significant (54–62%) activity against ***(d)–(f)*** and moderate (47–52%) against ***(a)–(c)*** fungal strains. Furthermore, significant (58–63%) activity was observed by the ligand **($\mathbf{L^4}$)** against ***(a)***, ***(d)–(f)*** and moderate (42–44%) against ***(b)***, ***(c)*** fungal strains.

Table 3
Antibacterial activity (concentration used 1 mg/mL of DMSO) of triazole Schiff base ligands and their oxovanadium(IV) complexes.

Bacteria	Compounds [Zone of Inhibition (mm)]											
	(L^1)	(L^2)	(L^3)	(L^4)	(L^5)	(1)	(2)	(3)	(4)	(5)	(A)	SD
Gram-negative												
(a)	16	16	17	18	12	17	19	24	25	20	10	29
(b)	14	17	19	17	14	18	22	25	22	16	12	31
(c)	16	16	17	20	13	21	18	20	24	17	11	30
(d)	17	20	19	21	16	19	27	24	25	18	10	29
Gram-positive												
(e)	12	17	16	18	11	16	23	21	22	16	08	26
(f)	14	18	14	17	14	17	24	20	23	16	09	28
SA =	1.83	1.51	1.90	1.64	1.75	1.79	3.31	2.25	1.38	1.60	1.41	1.72
Average of bacterial strains												
	13.6	16.8	17.5	18.5	12.2	17	22.2	22.3	23.5	16.2	10	28.8

(a) *E. coli*, (b) *S. flexneri*, (c) *P. aeruginosa*, (d) *S. typhi*, (e) *S. aureus*, (f) *B. Subtilis*; <10 = weak; 11–15 = moderate; 16 and above = significant; (A) = simple triazole; SD = standard drug = imipenum; SA = statistical analysis.

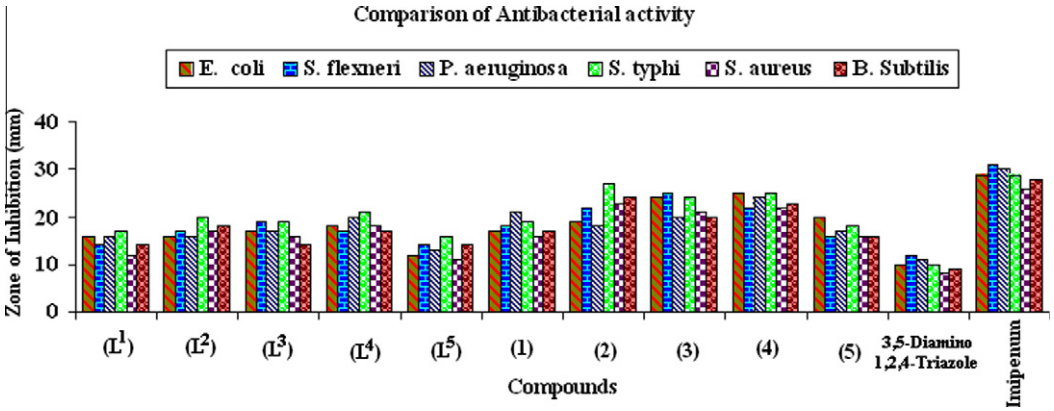


Fig. 2. Comparison of antibacterial activity.

Table 4
Antifungal activity (concentration used 200 μg/mL) of triazole Schiff base ligands and oxovanadium(IV) complexes.

Organism	Compounds										
	(L^1)	(L^2)	(L^3)	(L^4)	(L^5)	(1)	(2)	(3)	(4)	(5)	SD
(a)	00	55	47	60	45	40	72	59	71	57	A
(b)	48	63	52	42	48	61	77	66	55	46	B
(c)	61	49	45	44	56	70	65	54	63	62	C
(d)	43	59	54	58	37	57	69	74	73	54	D
(e)	43	56	64	63	49	35	70	80	79	58	E
(f)	39	60	62	58	00	55	75	78	75	39	F
	SA20.58	4.86	7.72	8.86	20.15	13.16	4.32	10.58	8.80	7.37	
Average of fungal strains											
	39	57	54	54.2	39.2	53	71.3	68.5	69.3	51.5	

(a) *T. longifusus*, (b) *C. albicans*, (c) *A. flavu*, (d) *M. canis*, (e) *F. Solani*, (f) *C. glabrata*, SD = standard drugs MIC μg/mL; a = miconazole (70 μg/mL:1.6822 × 10^{-7} M/mL), b = miconazole (110.8 μg/mL:2.6626 × 10^{-7} M/mL), c = amphotericin B (20 μg/mL:2.1642 × 10^{-8} M/mL), d = miconazole (98.4 μg/mL:2.3647 × 10^{-7} M/mL), e = miconazole (73.25 μg/mL: 1.7603 × 10^{-7} M/mL), f = miconazole (110.8 μg/mL: 2.66266 × 10^{-7} M/mL), SA = statistical analysis.

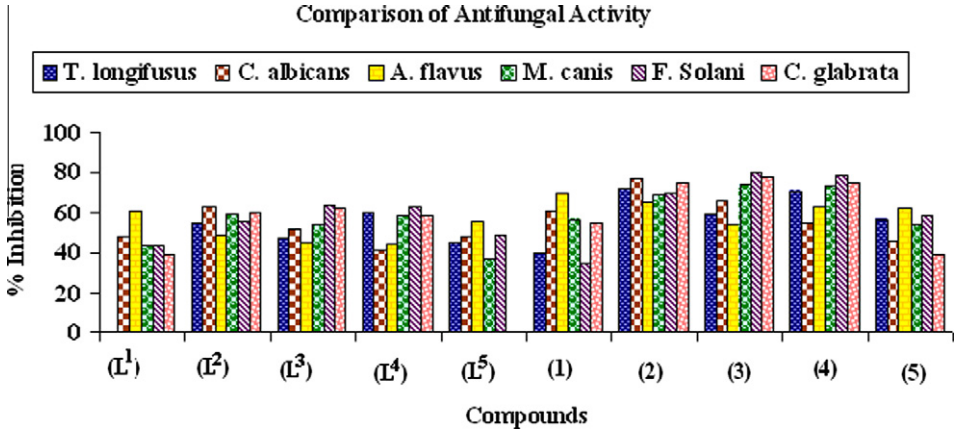


Fig. 3. Comparison of antifungal activity.

Beside this, **(L^5)** showed significant (56%) activity against ***(c)***, and moderate (37–49%) against ***(a)***, ***(b)***, ***(d)*** and ***(e)*** and showed no activity against ***(f)*** fungal strains. The vanadyl complex **(1)** showed moderate (35–40%) activity against ***(a)*** and ***(e)*** and rest of all strains possessed significant (55–70%) activity. In addition to this, the complexes **(2)–(5)** possessed significant (54–81%) activity against all strains except ***(f)*** of complex **(5)** which displayed weaker (38%) activity. The comparison of the average antifungal activity data revealed that the oxovanadium(IV) metal complexes displayed average value (60.4%) greater than the average value of the ligands (49.2%). It was also concluded that the heterocyclic aldehyde ring system and imine (–CH=N) group play a key role in enhancing the activity. On the other hand, the comparison of average values of the complexes versus ligands, a conclusion can be drawn that the antifungal activity is overall enhanced upon chelation with the oxovanadium(IV) metal atom.

Minimum inhibitory concentration (MIC)

The synthesized ligands and their oxovanadium(IV) complexes possessing promising antibacterial activity (above 80%) were selected for MIC studies and obtained results are reported in Table 5.

Table 5
Minimum inhibitory concentration (mol/mL) of the selected compounds (2–4) against selected bacteria.

Microorganisms	MIC (mol/mL)[a]		
	(2)	(3)	(4)
Gram-negative			
E. coli	ND	1.435×10^{-6}	4.751×10^{-8}
S. flexneri	ND	6.378×10^{-3}	ND
P. aeruginosa	ND	ND	4.117×10^{-7}
S. typhi	5.223×10^{-4}	2.383×10^{-2}	3.832×10^{-3}
Gram-positive			
S. aureus	3.132×10^{-7}	1.391×10^{-4}	4.237×10^{-5}
B. subtilis	1.879×10^{-6}	ND	5.688×10^{-7}

[a] Each compound was measured three times, with a standard deviation (SD) less than 10% in all the cases. ND is not determined at tested concentrations.

Table 6
Brine shrimp bioassay of triazole Schiff bases (L^1–L^5) and oxovanadium(IV) complexes (1–5).

Ligands/ complexes	LD_{50} (mol/mL)
(L^1)	$>4.623 \times 10^{-2}$
(L^2)	$>3.439 \times 10^{-3}$
(L^3)	$>3.956 \times 10^{-4}$
(L^4)	$>2.428 \times 10^{-3}$
(L^5)	$>1.996 \times 10^{-4}$
(1)	$>3.234 \times 10^{-3}$
(2)	$>2.532 \times 10^{-2}$
(3)	5.637×10^{-4}
(4)	6.192×10^{-4}
(5)	$>4.145 \times 10^{-3}$

The antibacterial results indicated that the oxovanadium(IV) complexes **(2)–(4)** were found to display activity greater than 80%, therefore these complexes were selected for MIC screening. The complex **(2)** showed activity against *S. typhi*, *S. aureus*, *B. subtilis* but unable to possess activity against *E. coli*, *S. flexneri* and *P. aeruginosa*. Moreover, the complex **(3)** demonstrated activity against *E. coli*, *S. flexneri*, *S. typhi* and *S. aureus*. Similarly, the complex **(4)** possessed activity against all bacterial strains except *S. flexneri*. Therefore, the MIC values of these complexes **(2)–(4)** were found at 4.751×10^{-8} and 2.383×10^{-2} mol, respectively.

Cytotoxic bioassay (in vitro)

The data recorded in Table 6 showed that only complexes **(3)** and **(4)** had potent cytotoxic activity against *Artemia salina*, while all other were inactive for this bioassay. The complexes **(3)** and **(4)** exhibited activity as LD_{50} values in the range of 5.637×10^{-4} to 6.192×10^{-4} mol/mL. The recorded data clearly indicated that only the oxovanadium complexes showed potent cytotoxicity rather than their non-coordinated ligands. However, some of the oxovanadium(IV) complexes possessed weaker cytotoxic activity while others showed more toxicity against *A. salina*.

Conclusions

The triazole Schiff bases were prepared by an equimolar ratio (triazole:aldehydes) which act as bidentate ligands for coordination with the vanadium metal atom. Physical, spectral and analytical data indicated the triazole Schiff base ligands to coordinate with the vanadium metal atom via azomethine-N, deprotonated-O and deprotonated-N forming a square-pyramidal geometry. The data obtained from the results of antibacterial and antifungal studies revealed that all the oxovanadium(IV) complexes showed more biological activity against one or more bacterial and/or fungal strains as compared to the parent ligands, hence showing better results of bioactivities rather than their uncomplexed parent ligands [39]. Generally, it is claimed that functional groups present in the compounds are responsible for enhancing antibacterial and antifungal activities.

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