Metal based drugs: design, synthesis and in-vitro antimicrobial screening of Co(II), Ni(II), Cu(II) and Zn(II) complexes with some new carboxamide derived compounds: crystal structures of N-[ethyl(propan-2-yl)carbamothioyl]thiophene-2-carboxamide and its copper(II) complex

Sajjad H. Sumrera1, Muhammad Hanif2, Zahid H. Chohan3, Muhammad Safwan Akram4, Javeed Akhtar5, and Saad M. Al-Shehri6

1Department of Chemistry, Institute of Natural Sciences, University of Gujrat, Gujrat, Pakistan, 2Department of Chemistry, University of Sargodha, Bhakkar, Pakistan, 3Institute of Chemical Sciences, Bahauddin Zakariya University, Multan, Pakistan, 4Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore, Pakistan, 5Department of Physics, COMSATS CIIT, Islamabad, Pakistan, and 6Chemistry Department, College of Science, King Saud University, Riyadh, Saudi Arabia

Abstract
A new series of compounds derived from thiophene-2-carboxamide were synthesized and characterized by IR, 1H-NMR and 13C-NMR, mass spectrometry and elemental analysis. These compounds were further used to prepare their Co(II), Ni(II), Cu(II) and Zn(II) metal complexes. All metal(II) complexes were air and moisture stable. Physical, spectral and analytical data have shown the Ni(II) and Cu(II) complexes to exhibit distorted square-planar and Co(II) and Zn(II) complexes tetrahedral geometries. The ligand (L1) and its Cu(II) complex were characterized by the single-crystal X-ray diffraction method. All the ligands and their metal(II) complexes were screened for their in-vitro antimicrobial activity. The antibacterial and antifungal bioactivity data showed that the metal(II) complexes were found to be more potent than the parent ligands against one or more bacterial and fungal strains.

Materials and methods

Experimental
All chemicals used were of analytical grade. All metallic salts were used as acetate. Melting points were recorded on Fisher Johns melting point apparatus. Infrared spectra were recorded on Shimadzu FT-IR spectrometer. The C, H and N analysis was carried out using a Perkin Elmer, USA, model. The 1H- and 13C-NMR spectra were recorded in DMSO-d6 using TMS as internal standard on a Bruker Spectrospin Avance DPX-500 spectrometer. Electron impact mass spectra (EIMS) were recorded
on JEOL MS Route Instrument. In vitro antibacterial and antifungal 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, Bahawalpur, Pakistan.

General procedure for the synthesis of ligands (L1-L3)

N-{[Ethyl(propan-2-yl)carbamothioyl]thiophene-2-carboxamide (L1)}

The ligand, N-[ethyl(propan-2-yl)carbamothioyl]thiophene-2-carboxamide (L1) was synthesized by following a reported method19, in which a solution of thiophenyl carbonyl chloride (20 mmol, 2.14 mL) in dry acetone (30 mL) was added dropwise to a solution of KSCN (20 mmol, 1.94 g) in acetone (30 mL). The reaction mixture was refluxed for 1 h and then cooled to room temperature. A solution of N-ethylisopropyl amine (20 mmol, 2.4 mL) in acetone (20 mL) was added dropwise to the mixture in 20 min and refluxing continued for 2 h. Then KCl was removed by filtration and light yellow filtrate was placed into water (250 mL) and added few drops of conc. HCl under constant agitation. The wax-like material was formed which was separated by filtration, washed several times with cold water and then with diethyl ether. The crude product was purified by recrystallization from a solution mixture of ethanol-dichloromethane (1:1). As a result of recrystallization, fine shiny colorless crystals of (L1) were obtained, which were suitable for X-ray analysis. The same procedure was used for the preparation of other ligands.

Yield: 81%. Color (white crystalline solid); m.p. 133–135 °C. IR (KBr, cm⁻¹): 3265 (NH), 3070–3030 (CH), 1680 (C=O), 1240 (C–O), 1235 (C–S), 1160 (C-O), 690 (C–Br). ¹H-NMR (DMSO–d6, δ, ppm): 7.85 (d, 2H, furanyl C3–H), 7.52 (d, 2H, C11–H), 2.63 (m, 4H, C11–H), 2.45 (s, 3H, thienyl-CH3), 2.04 (s, 2H, C8–H), 1.20 (t, 3H, CH3), 1.07 (d, 3H, C9–H), 1.06 (d, 3H, C10–H), 0.88 (t, 3H, C12–H). ³¹C-NMR (DMSO–d6, δ, ppm): 178.90 (C=O), 172.00 (C=O), 137.93 (C2), 136.73 (C3), 134.33 (C4), 131.10 (C5), 52.45 (C11), 20 (C9, C10), 15.0 (C12). EIMS (70 eV) m/z (%): 256.39. Anal. Calcd. for C11H15BrN2O2S: C: 41.39; H: 4.74; N: 8.78; S: 10.04; Found: C: 41.30; H: 4.79; N: 8.69; S: 10.11%.

General procedure for the synthesis of metal(II) complexes

The solution of Cu(CH3COO)2·H2O (0.5 mmol) in methanol (15 mL) was added dropwise to a stirred solution of ligand N-[ethyl(propan-2-yl)carbamothioyl]thiophene-2-carboxamide (L1) (1 mmol) in methanol (20 mL). The reaction mixture was refluxed for 2 h. The precipitates were obtained by cooling the reaction mixture and filtered, washed with methanol and dried under vacuum. The precipitates were dissolved in a mixture of ethanol and dichloromethane (1:1) and a clear solution was kept in a refrigerator for one week. Suitable dark green color crystals for X-ray studies were obtained for Cu(L1)2 complex. The same method was used for the preparation of all other complexes.

¹H- and ¹³C-NMR data of Zn(II) complexes

[Zn (L1-H)2] (4)

1H-NMR (DMSO–d6, δ, ppm): 8.30 (d, 2H, thienyl C2–H), 8.20 (d, 2H, thienyl C3–H), 7.57 (dd, 2H, thienyl C1–H), 2.60 (m, 4H, C11–H), 2.35 (m, 2H, C8–H), 1.16 (d, 12H, C9–H, C10–H), 0.98 (t, 3H, C12–H). ¹³C-NMR (DMSO–d6, δ, ppm): 179.80 (C=O), 172.00 (C=O), 137.93 (C2), 136.73 (C3), 134.33 (C4), 131.10 (C5), 52.45 (C11), 20 (C9, C10), 15.0 (C12). EIMS (70 eV) m/z (%): 256.39. Anal. Calcd. for C11H15N2O2S2: C: 46.41; H: 4.10; N: 8.78; S: 10.04; Found: C: 46.35; H: 4.08; N: 8.71; S: 10.04%.

[Zn (L2-H)2] (8)

1H-NMR (DMSO–d6, δ, ppm): 7.95 (d, 2H, thienyl C3–H), 7.20 (d, 2H, thienyl C2–H), 2.63 (m, 4H, C11–H), 2.50 (s, 2H, thienyl-CH2), 2.35 (m, 2H, C8–H), 1.16 (d, 12H, C9–H, C10–H), 0.98 (t, 3H, C12–H). ¹³C-NMR (DMSO–d6, δ, ppm): 180.50 (C=O), 173.42 (C=O), 141.48 (C3), 137.30 (C2), 133.15 (C5), 52.72 (C11), 15.15 (C12).

[Zn (L3-H)2] (12)

1H-NMR (DMSO–d6, δ, ppm): 7.85 (d, 2H, furanyl C1–H), 7.52 (d, 2H, furanyl C2–H), 2.67 (m, 4H, C11–H), 2.38 (m, 2H, C8–H), 1.11 (d, 12H, C9–H, C10–H), 1.03 (t, 3H, C12–H). ¹³C-NMR (DMSO–d6, δ, ppm): 180.60 (C=O), 173.5 (C=O), 140.23 (C2), 134.13 (C3), 130.39 (C4), 127.47 (C5), 52.17 (C11), 46.15 (C12), 21.2 (C9, C10), 15.65 (C12).

Biological activity

Antibacterial studies (in-vitro)

The synthesized ligands (L1-L3) and their respective metal(II) complexes were tested against four Gram-negative (E. coli, S. sonnei, P. aeruginosa and S. typhi) and two Gram-positive (St. aureus and B. subtilis) bacterial strains by the disc diffusion method20,21. The test compounds (ligand/complex) were dissolved (10 mg/mL) in DMSO. A known volume (10 µL) of the solution was applied with the help of a micropipette onto the sterilized filter paper discs. The discs were dried at room temperature overnight and stored in sterilized dry containers. Discs soaked with 10 µL of DMSO and dried in air at room temperature were used as the negative control. The standard antibiotic discus used as positive control were prepared as mention above in the laboratory.

DOI: 10.3109/14756366.2015.1050011

Metal based drugs 591
by applying a known concentration of the standard antibiotic solution. Ampicillin was used as a standard antibiotic. Bacterial culture was grown in nutrient broth medium at 37 °C overnight and spread on to solidified nutrient agar medium in Petri plates using sterilized cotton swabs. Test and control disks were then applied to the medium surface with the help of sterilized forceps. The plates were incubated at 37 °C for 24–48 h. The results were recorded by measuring the zone of inhibition in mm against each compound. The experiments were carried out in triplicate and the values obtained were statistically analyzed.

Antifungal activity (in-vitro)

Antifungal activity of all the compounds was studied against six fungal strains (T. longifusus, C. albicans, A. flavus, M. canis, F. solani and C. glabrata) according to literature protocol. Sabouraud dextrose agar (Oxoid, Hampshire, England) was seeded with 10⁵ (cfu) mL⁻¹ fungal spore suspensions and transferred to petri dishes. Discs soaked in 20 mL (200 μg/mL in DMSO) of test compounds were placed at different positions on the agar surface. The plates were incubated at 32° C for 7 days. The results were recorded as percentage of inhibition and compared with the standard drugs miconazole and amphotericin B.

Results and discussion

Chemistry

The ligands (L¹–L³) were synthesized by the reaction of potassium thiocyanate with thiophene-2-carbonyl chloride, 5-methyl-thiophene-2-carbonyl chloride and 5-bromo-furan-2-carbonyl chloride, respectively, in dry acetone followed by condensation of the resulting product with N-ethyl-isopropyl amine (Scheme 1). The ligands (L¹–L³) thus formed were soluble in ethanol, ethyl acetate, DMF and DMSO; however, slightly soluble in tetrahydrofuran, diethyl ether and insoluble in aliphatic and aromatic hydrocarbons. The metal(II) complexes were obtained by stoichiometric reaction of the corresponding ligands with metals [Co(II), Ni(II), Cu(II) and Zn(II)] as acetate in a molar ratio of metal:ligand (M:L) as 1:2 (Scheme 1). All metal(II) complexes were air and moisture stable. They were soluble in mixture of ethanol and dichloromethane (1:1), DMF and DMSO. The ligands and their metal(II) complexes were characterized by their physical, spectral and analytical data. The structure of ligand, N-(ethyl(propan-2-yl)carbamothioyl)thiophene-2-carboxamide (L¹) and its Cu(L¹)₂ complex was determined from single crystal X-ray diffraction data. Physical measurements and analytical data of the metal(II) complexes are given in Supplementary Tables 1 and 2.

Spectral characterization of ligands (L¹–L³) and their metal(II) complexes

IR spectra

The main IR vibrational bands of the synthesized ligands (L¹–L³) and their metal(II) complexes were found in their expected region. The characteristic IR bands, are given in “Experimental” section and Table 1. The IR spectra of newly synthesized ligands showed the strong peak at 3265–3280 cm⁻¹ due to N–H vibrations and medium peaks at 1675–1680 and 1235–1250 cm⁻¹, respectively, due to carbonyl (C=O) and thiocarbonyl (C=S) vibrations, which strongly support the preparation of desired compounds. The ligands (L¹) and (L²) showed bands at 880–885 due to (C–S) vibrations assigned to thiophene and (L³) displayed band at 1160 cm⁻¹ due to (C–O) vibrations assigned to furane moiety. A weaker peak at 690 cm⁻¹ was also observed by ligand (L³), which was due to v(C-Br) vibrations. The IR spectra of all the metal(II) complexes exhibited major changes in comparison to the spectra of the subsequent ligands. The most prominent change observed was, the carbonyl (C=O) and thiocarbonyl (C=S) bands originally appearing at 1675–1680 and 1235–1250 cm⁻¹ in the spectra of the ligand, shifted to lower frequency by 13–15 cm⁻¹ at 1660–1670 and 1220–1237 cm⁻¹, respectively, in the spectra of metal(II) complexes indicating the involvement in coordination with the metal(II) ions. The decrease in frequency is due to delocalization of electrons. The N–H vibrations appearing in the ligands at 3265–3280 cm⁻¹ were also disappeared in the metal complexes giving a clue of deprotonation, which may undergo through tautomerism. However, keto form was identified as a stable product. Coordination of carbonyl-O and thiocarbonyl-S is further justified.
by the appearance of new bands at 450–460 and 525–536 cm\(^{-1}\) corresponding to M–S and M–O linkages, respectively. This linkage is also supported by X-ray structure of the Cu(L^1)_2 complex as shown in Figures 3 and 4.

\(^1\)H-NMR spectra

The spectra of all the ligands (L^1–L^3) showed a broad singlet peak at 11.53–11.62 ppm due to N–H protons\(^{1,28}\) and the protons of ethyl and isopropyl groups (C\(_2\)H\(_2\), C\(_3\)H\(_5\), C\(_{10}\)H\(_{18}\)). C\(_{12}\)H\(_{19}\) and C\(_{12}\)H\(_{17}\) were observed as doublet to multiplet at 0.93–2.60 ppm. The methyl (CH\(_3\)) protons of (L^1) and C\(_{2}\)H\(_2\) to C\(_{5}\)H\(_{11}\) protons of all ligands were observed at 2.45 and 7.10–8.19 ppm as a singlet and doublet, respectively. On comparison of the spectra of ligand with those of Zn(II) complexes, the N–H protons disappeared which is also supported by the IR spectra and X-rays technique and all other remaining protons underwent downfield shift by 0.05–0.15 ppm due to coordination and increased conjugation\(^{30}\).

\(^1\)C-NMR spectra

The thiophene, furane, carbonyl and thiocarbonyl carbons of all the ligands were observed\(^{30}\) at 131.1–141.3, 127.3–139.8, 172.0–172.12 and 179.90–179.20 ppm, respectively. The comparison of the spectra of Zn(II) complexes showed downfield shifting of carbonyl-C and thiocarbonyl-S from 172.0–172.1 and 179.9–179.2 ppm in free ligands to 173.42–173.5 and 180.3–180.6 ppm in the zinc(II) complexes, respectively, revealing the coordination of carbonyl-O and thiocarbonyl-S with the Zn(II) metal ion. Furthermore, all other carbons in the spectra of the Zn(II) complexes underwent downfield shifting by 0.15–0.4 ppm due to increased conjugation and coordination.

Electronic spectra

The electronic spectral values of Co(II), Ni(II), Cu(II) and Zn(II) complexes in DMF are recorded in Table 1. The spectra of Co(II) complexes showed only one absorption band at 17995–18380 cm\(^{-1}\) assigned to the transition \(4A_2 (F) \rightarrow 4T_1 (F)\)\(^{31}\). This in turn, propose tetrahedral geometry for the Co(II) complexes. This is also supported by magnetic moment values (3.78–3.94 B.M) of Co(II) complexes\(^{31}\). The Ni(II) complexes exhibited two absorption bands at 13 390–13 515 cm\(^{-1}\) and at 18 770–19 230 cm\(^{-1}\) assigned to the transitions \(1A_{1g} \rightarrow 1B_{2g}\) and \(1A_{1g} \rightarrow 1A_{2g}\), respectively, which give clue for square-planar geometry\(^{32}\). The electronic spectra of Cu(II) complexes showed low-energy absorption bands at 15 190–15 660 cm\(^{-1}\) assigned to the transitions, \(2B_{1g} \rightarrow 2E_{1g}\). The high-energy bands at 21 160–21 640 cm\(^{-1}\) was assigned to the transition, \(2B_{1g} \rightarrow 2A_{1g}\). These transitions as well as the measured magnetic moment values (1.83–190 B.M) propose a square-planar geometry for Cu(II) complex\(^{33}\). The diamagnetic Zn(II) complexes did not show any d–d transitions and their spectra were dominated\(^{32}\) only by the charge transfer band at 27 190–27 360 cm\(^{-1}\) proposing a tetrahedral geometry for the Zn(II) complexes.

Molar conductivity and magnetic properties of the metal(II) complexes

Molar conductance was carried out in DMF solution and the results reported in Table 1 indicate the values in a lower range (10.9–18.2 Ω\(^{-1}\) cm\(^2\) mol\(^{-1}\)), thus showing their non-electrolytic nature\(^{34}\). The magnetic moment values of Co(II) complexes were found in the range of 3.78–3.94 B.M, expected for three unpaired electrons and are in agreement with their tetrahedral geometry\(^{35}\). The Cu(II) and Ni(II) complexes showed \(\mu_{\text{eff}}\) values compatible for their square-planar geometry. The Zn(II) complexes exhibited diamagnetic nature\(^{36}\).
X-Ray crystallographic studies of ligand (L1) and Cu(L1)2 complex

Single crystal X-ray structure of ligand (L1)

The molecular structure of N-[ethyl(propan-2-yl)carbamothioyl]thiophene-2-carboxamide (L1) (Figure 1) showed their expected bond lengths and angles\textsuperscript{37,38}. Data collection and refinement of (L1) are listed in Supplementary Table 3. The bond lengths of the carbonyl C5–O1 and thiocarbonyl C6–S2 groups were, C5–O1 = 1.221(2) Å, C6–S2 = 1.6792(16) Å, respectively, for double bonds. All C–N bonds, C7–N2 = 1.475(2) Å and C9–N2 = 1.493(2) Å represented single bond and C6–N2 = 1.323(2) Å, C6–N1 = 1.417(2) Å and C5–N1 = 1.374(2) Å showing a partial double bond character (Supplementary Table 4). This information indicated partial electron delocalization within the C5–N1–C6–N2 fragment. The C6–N2 bond, adjacent to the alkyl group, was slightly shorter than C6–N1. These bond distances were in good agreement with those observed in literature, as reported in the Cambridge Structural Database\textsuperscript{37,38}.

Molecular structure of Cu(L1)\textsubscript{2} complex

The molecular structure of Cu(L1)\textsubscript{2} complex (Figure 2) was obtained from X-ray single crystal studies and, the data collection and crystal refinement parameters are given in Supplementary Table 3 and selected bond lengths and bond angles are given in Supplementary Tables 7 and 8. The structure of Cu(L1)\textsubscript{2} complex showed the bond lengths and angles\textsuperscript{15} as expected. The Cu(L1)\textsubscript{2} complex existed as a monomer unit in which each copper Cu1 atom was coordinated with each oxygen and each sulfur of two ligands (Figure 2) in a \textit{cis}-fashion, with slightly distorted square planar geometry\textsuperscript{15}. In both chelate rings, the distances of N1–C6 (1.333(6) Å) and N3–C17 (1.337(6) Å) in the thiourea fragment were almost same, but the distances of N1–C5 (1.310(6) Å) and N3–C16 (1.325(6) Å) (Supplementary Table 8) were slightly different, which supported the distorted square planar geometry of the complex. The bond lengths of the carbonyl O1–C5 = 1.273(5) Å; O2–C16 = 1.267(5) Å and thiocarbonyl S1–C6 = 1.746(4) Å; S3–C17 = 1.716(5) Å groups were in between those for double and single bonds (Supplementary Table 7). The investigated
complex observed same behavior for C–N–C–N bond lengths, which is shorter than the average single C–N bond length of 1.48 Å and greater than average double C–N bond length 1.16 Å, being C5–N1 = 1.310(6) Å, C6–N1 = 1.333(6) Å, C17–N3 = 1.337(6) Å, C16–N3 = 1.344(6) Å, C6–N2 = 1.336(5) Å, thus showing variable degree of double and single bond character.

Impact of metal/ligand coordination on bioactivity

In vitro antibacterial bioassay

Antibacterial activity of newly synthesized ligands (L1–L3) and their metal(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 and obtained data are recorded in Table 2. The antibacterial activity of prepared compounds was compared with the activity of standard drug (ampicillin). The synthesized compounds showed varying degree of inhibitory effects: low (up to 10 mm), moderate (up to 11–15 mm) and significant (above 15 mm). The obtained data indicated that all the ligands (L1–L3) showed moderate (11–15 mm) activity against all the tested bacterial strains except, (b) and (e) which are weakly inhibited (05–10 mm) by (L1) and (L3), respectively. All Co(L1)2 complexes possessed significant (17 mm) activity against (c) and (d), and moderate (11–14 mm) against (a), (b), (e) and (f) strains. Similarly, the Ni(L1)2 complexes displayed significant (16–19 mm) activity against (c) and (e), and moderate (12–14 mm) against (a), (b), (d) and (f) bacterial strains. The Cu(L1)2 complexes also showed significant (17 mm) activity against (e) and (f) and moderate (11–15 mm) against (a)–(d) strains. The metal(II) complexes, Co(L2)2 and Cu(L2)2 possessed significant (16–19 mm) activity against (c) and (f), and moderate (11–15 mm) against (a), (b), (d) and (e) bacterial strains. In the same way, the Co(L3)2, Ni(L3)2, Cu(L3)2 and Zn(L3)2 complexes also showed significant (16–20 mm) activity against (c), (e) and (f), and moderate (11–15 mm) against...
Table 3. Antifungal bioassay (concentration used 200 μg/mL) of ligands (L₁–L₃) and metal(II) complexes.

| Compounds | (a) | (b) | (c) | (d) | (e) | (f) | (SA) | Average |
|-----------|-----|-----|-----|-----|-----|-----|------|---------|
| (L₁)      | 37  | 53  | 30  | 46  | 30  | 40  | 9.5  | 39.3    |
| (L₂)      | 44  | 15  | 57  | 42  | 36  | 48  | 14.2 | 40.3    |
| (L₃)      | 44  | 13  | 50  | 48  | 39  | 62  | 16.4 | 42.7    |
| Co(L₁)₂   | 42  | 54  | 31  | 48  | 33  | 43  | 8.8  | 41.8    |
| Ni(L₁)₂   | 41  | 57  | 36  | 49  | 36  | 41  | 8.2  | 43.3    |
| Cu(L₁)₂   | 40  | 56  | 35  | 47  | 34  | 46  | 9.9  | 44.7    |
| Zn(L₁)₂   | 36  | 59  | 34  | 50  | 30  | 44  | 10.9 | 42.1    |
| Co(L₂)₂   | 40  | 18  | 62  | 44  | 41  | 53  | 14.8 | 43.0    |
| Ni(L₂)₂   | 45  | 14  | 63  | 45  | 39  | 50  | 16.2 | 42.7    |
| Cu(L₂)₂   | 46  | 20  | 59  | 40  | 42  | 45  | 12.7 | 42.0    |
| Zn(L₂)₂   | 47  | 17  | 60  | 44  | 37  | 52  | 14.8 | 42.8    |
| Co(L₃)₂   | 50  | 10  | 54  | 52  | 39  | 67  | 19.5 | 45.3    |
| Ni(L₃)₂   | 47  | 15  | 55  | 46  | 44  | 59  | 15.5 | 44.3    |
| Cu(L₃)₂   | 49  | 17  | 56  | 47  | 42  | 65  | 16.3 | 46.0    |
| Zn(L₃)₂   | 40  | 20  | 57  | 50  | 40  | 64  | 15.5 | 45.1    |

SD  A  B  C  D  E  F  –  –

Activity of ligands (L₁–L₃) = 40.8%; Average activity of metal complexes = 43.6%; (a) = T. longifusus; (b) = C. albican; (c) = A. flavus; (d) = M. canis; (e) = F. solani; (f) = C. glaberata. SD = Standard drugs. MIC μg/mL: A = Miconazole (70 μg/mL); 1.6822 x 10⁻⁷ M/mL, B = Miconazole (110.8 μg/mL; 2.6626 x 10⁻⁷ M/mL), C = Amphotericin B (20 μg/mL; 2.1642 x 10⁻⁷ M/mL), D = Miconazole (98.4 μg/mL; 2.3647 x 10⁻⁷ M/mL), E = Miconazole (73.25 μg/mL; 1.7603 x 10⁻⁷ M/mL), F = Miconazole (110.8 μg/mL; 2.6626 x 10⁻⁷ M/mL). SA = Statistical analysis.

Figure 4. Comparison of antifungal activity of (L₁–L₃) and their metal(II) complexes.

(a), (b) and (d) strains. Moreover, the Zn(L₁)₂, Ni(L₂)₂ and Zn(L₃)₂ complexes showed significant (16–19 mm) activity against (c), (d) and (f) strains, respectively. The same complexes, showed moderate (16–20 mm) activity against all the remaining tested bacterial strains. Conclusively, the ligands (L₁–L₃) possessed smaller average (11.5 mm) activity against different strains than the average (14.3 mm) activity of the metal(II) complexes, which showed that the activity is enhanced upon coordination. The comparative and average activity data of the ligands and their metal(II) complexes is reproduced in Figure 3.

In vitro antifungal bioassay

The newly synthesized ligands (L₁–L₃) and their metal(II) complexes were subjected to screening for their antifungal activity against, T. longifusus, C. albican, A. flavus, M. canis, F. solani and C. glaberata fungal strains and results are recorded in Table 3. The obtained results were compared with the standard drugs Miconazole and Amphotericin B. The ligand (L₁) showed moderate (37–53%) activity against (a), (b), (d) and (f) and weak (30%) against (c) and (e) fungal strains. The ligand (L₂) displayed significant activity (57%) against (e), moderate (36–48%) against (a) and (d) and weak (15%) against (b) fungal strains. The activity of ligand (L₃) was found to be significant (62%) against (f), moderate (39–50%) against (a) and (c)–(f) and weak (13%) against (b) fungal strains. The results of antifungal activity exhibited that the metal(II) complexes Co(L₁)₂, Ni(L₁)₂, Cu(L₁)₂ and Zn(L₁)₂ showed significant activity (54–59%) against (b), moderate (34–50%) against (a), (c), (d) and (f) and weak activity (30–33%) against (e) fungal strains. The Co(L₂)₂, Ni(L₂)₂, Cu(L₂)₂ and Zn(L₂)₂ complexes also showed significant activity (59–63%) against (c), moderate (37–53%) against (a) and (d)–(f) and weak (14–20%) against (b) fungal strains. Similarly, the Co(L₃)₂, Ni(L₃)₂, Cu(L₃)₂ and Zn(L₃)₂ complexes showed significant activity (54–67%) against (c) and (f), moderate
that the antifungal activity of the ligands increased \(^{41,42}\) upon average activity of the ligands (40.8%). It is therefore, concluded complexes exhibited greater average activity (43.6%) than the fungal strains. The average activity data showed that the metal(II) coordination with the metal ions.

In vitro antibacterial and antifungal studies of the ligands and their metal(II) complexes against representative bacterial and fungal strains showed that the ligands (\(L_1\)) and their Co(II), Ni(II), Cu(II) and Zn(II) complexes were found to have moderate to significant activity. However, against one or more bacterial strains the bioactivity of the ligands enhanced upon coordination with the metal ions.

Conclusions

All the newly synthesized ligands (\(L_1-L_3\)) act as bidentate and coordinate through carbonyl-O and thiocarbonyl-S to the metal(II) ions. The bonding of ligands to the metal(II) ion is supported by their physical, analytical and spectral data. These observations are further supported by the X-ray crystallographic data of Cu(\(L_1\))\(_2\) complex. In vitro antibacterial and antifungal studies of the ligands and their metal(II) complexes against representative bacterial and fungal strains showed that the ligands (\(L_1-L_3\)) and their Co(II), Ni(II), Cu(II) and Zn(II) complexes were found to have moderate to significant activity. However, against one or more bacterial strains the bioactivity of the ligands enhanced upon coordination with the metal ions.

Acknowledgements

The authors are thankful to HEJ research Institute of Chemistry, University of Karachi, Pakistan, for providing their help in taking NMR, mass spectral and antibacterial/antifungal data.

Declaration of interest

This work was supported by Collaboration between University of Manchester, UK and Bahauddin Zakariya University, Multan (Pakistan) on Indigenous Fellowship program sponsored by the Higher Education Commission, Government of Pakistan to one of the authors (MH).

References

1. Binzet G, Arslan H, Florke U, et al. Synthesis, characterization and antimicrobial activities of transition metal complexes of N,N-diaryl-N-(2-chlorobenzoyl)thiourea derivatives. J Coord Chem 2006;59:1395–406.
2. Arslan H, Duran N, Borecki G, et al. Antimicrobial activity of some thiourea derivatives and their nickel and copper complexes. Molecules 2009;14:519–27.
3. Saeed S, Rashid N, Ali M, Hussain R. Synthesis, characterization and antibacterial activity of nickel (II) and copper (II) complexes of N-alkyl(aryl)carbamothioyl-4-nitrobenzamide. Eur J Chem 2010;1:200–5.
4. Saeed S, Rashid N, Hussain R, et al. Synthesis, characterization and biological evaluation of some thiourea derivatives bearing benzothiazole moiety as potential antimicrobial and anticancer agents. Eur J Med Chem 2010;45:1323–31.
5. Campo RD, Criado JJ, Garcia E, et al. Thiourea derivatives and their nickel(II) and platinum(II) complexes: antifungal activity. J Inorg Biochem 2002;89:74–82.
6. Saeed S, Rashid N, Hussain R, et al. Synthesis, spectroscopic characterization, crystal structure and antifungal activity of thiourea derivatives containing a thiazole moiety. Central Eur J Chem 2010;8:550–8.
7. Sacht C, Datt MS, Otto S, Roodt A. Synthesis, characterization and coordination chemistry of novel chiral N,N-diaryl-N-thienyl-3-oxo-2-thioxo-2,3-dihydroindazole and related compounds. J Inorg Biochem 2003;90:461–72.
8. Abdel HMM, Gad-Elkareem MAMGE, El-Adasy ABAEAM, Othman IM. N-1-Naphthyl-3-oxobutanamide in heterocyclic synthesis: a facile synthesis of nicotinamide, thieno[2,3-b]pyridine, and bi- or tricyclic annulated pyridine derivatives containing naphthyl moiety. Phosph Sulf Silicon Relat Elem 2009;18:2263–80.
9. Khan MH, Bano Q, Nizamuddin. Pesticidal activities of some 3-aryl-4-methylpyrazol[3,4-b]1,5 benzodiazepines and 4-aryl-2-imino-5-methyl-1,3-thiazolino[4,5-b]1,5 benzodiazepines. J Agr Food Chem 1995;43:2719–21.
10. Fuks L, Anuszewska E, Kruszewska H, et al. Platinum(II) complexes with thio urea derivatives containing oxygen, sulfur or selenium in a heterocyclic ring: computational studies and cytotoxic properties. Trans Metal Chem 2010;35:639–47.
11. Ke SY, Xue SJ. Synthesis and herbicidal activity of N-(O-Fluoro phenoxycetacyl)thiourea derivatives and related fused heterocyclic compounds. Arkivoc 2006;10:63–8.
12. Zhang YM, Wei TB, Xian L, Gao LM. An efficient synthesis of polyme thylene-bis-aryl thio urea derivatives under the condition of phase-transfer catalysis. Phosphorus Sulfur Silicon Relat Elem 2004;179:2007–13.
13. Jung SH, Kim DY. Catalytic enantioselective electrophilic nitrogen zation of \(\beta\)-ketooesters using bifunctional organocatalysts. Tetrahedron Lett 2008;49:5527–30.
14. Koch KR. New chemistry with old ligands: \(N\)-alkyl- and \(N,N\)-dialkyl-N'-acylthioureas in co-ordination, analytical and process chemistry of the platinum group metals. Coord Chem Rev 2001;216:473.
15. Binzet G, Florke U, Kulcu N, Arslan H. Crystal and molecular structure of bis(4-bromo-\((\text{di-n-butylcarbamothioyl})\)benzamido) copper(II) complex. Eur J Chem 2012;3:211–13.
16. Kharodawala MJ, Rana AK. Synthesis, characterization, and biological activity of some transition metal chelates of 4-acyloxime-2-pyrAzolin-5-ones. Synth React Inorg Metal-Org Chem 2003;33:1483–504.
17. Baghiali GB, Patil SA. Synthesis, spectral characterization, in vitro biological and DNA cleavage studies of Co(II), Ni(II), Cu(II), and Zn(II) complexes with 1,2,4-triazole Schiff bases. J Coord Chem 2009;62:1690–700.
18. Banerjee P, Pandey OP, Sengupta SK. Microwave assisted synthesis, spectroscopic and antibacterial studies of titanocene chelates of Schiff bases derived from 3-substituted-4-amino-5-hydradino-1,2,4-triazoles. Trans Met Chem 2008;33:1047–52.
19. Hernandez W, Spodine E, Vega A, et al. \(\text{cis-\text{trans}}\) isomerism in copper(II) complexes with \(N\)-acyl thio urea ligands. Z Anorg Allg Chem 2004;630:1381–6.
20. Chohan ZH, Praveen M, Ghaffar A. Synthesis, characterisation and biological role of anions (nitrate, sulphate, oxalate and acetate) in Co(II), Cu(II) and Ni(II) metal chelates of some Schiff base derived amino acids. Synth React Inorg Met-Org Chem 1998;28:1673–87.
21. Rahman AU, Choudhary MI, Thomsen WJ. Bioassay techniques for drug development. The Netherlands: Harwood Academic Publishers; 2005.
22. Chohan ZH, Youssoufi MH, Jarrahpour A, Ben Hadda T. Identification of antibacterial and antifungal pharmacophore sites for potent bacteria and fungi inhibition: indol enyl sulfonamide derivatives. Eur J Med Chem 2010;45:1189–99.
23. Halli MB, Sumathi RB. Synthesis, spectroscopic, antimicrobial and DNA cleavage studies of new Co(II), Ni(II), Cu(II), Cd(II), Zn(II) and Hg(II) complexes with naphthofuran-2-carbodihydrazide Schiff base. J Mol Str 2012;1022:130–8.
24. Prashanthi Y, Raj S. Synthesis and characterization of transition metal complexes with N,O, N,N and S,N-donor Schiff base ligands. J Sci Res 2010;2:114–26.
25. Patel IB, Parmar SJ. Synthesis and studies of novel optically active Schiff’s Base derivatives and their antimicrobial activities. E-J Chem 2010;7:617–23.
26. Prakash A, Singh BK, Bhojak N, Adhikari D. Synthesis and characterization of bioactive zinc(II) and cadmium(II) complexes with new Schiff bases derived from 4-nitrobenzaldehyde and acetonaphone with ethylendiamine. Spectrochim Acta A 2010;76:356–62.
27. Mikami N, Nakagawa I, Shimanouch T. Far infra-red spectra and metal-ligand force constants of acetylenoacetates of transition metals. Spectrochim Acta A 1967;23:1037–42.
28. Freeman RA. Handbook of nuclear magnetic resonance. 2nd ed. Harlow, UK: Longman Publishing; 1997.

DOI: 10.3109/14756366.2015.1050011
29. Grevy JM, Tellez F, Bernes S, et al. Coordination compounds of thiabendazole with main group and transition metal ions. Inorg Chim Acta 2002;339:532–42.
30. Pasto DJ. Organic structure determination. London: Prentice Hall International; 1969.
31. Nair MS, Josephyus RS. Synthesis and characterization of Co(II), Ni(II), Cu(II) and Zn(II) complexes of tridentate Schiff base derived from vanillin and DL-α-aminobutyric acid. Spectrochim Acta A 2008;70:749–53.
32. Lever ABP. Inorganic electronic spectroscopy. Amsterdam: Elsevier; 1984.
33. Larabi L, Hared Y, Reguig A, Mostafa M. Synthesis, structural study and electrochemical properties of copper (II) complexes derived from benzene and p-toluene sulphonyl hydrazone. J Serb Chem Soc 2003;68:85–95.
34. Badawy MA, Mohamed GG, Omar MM, et al. Synthesis, spectroscopic and thermal characterization of quinoxaline metal complexes. Eur J Chem 2010;1:282–8.
35. Cotton FA, Wilkinson G. Advanced inorganic chemistry. 5th ed. New York: Wiley Interscience Publication; 1988.
36. Chohan ZH. Metal-based antibacterial and antifungal sulfonamides: synthesis, characterization, and biological properties. Trans Met Chem 2009;34:153–61.
37. Saeed S, Rashid N, Jasinski JP, et al. N-(Diethylcarbamothioyl)-4-nitrobenzamide. Acta Cryst 2010;E66:o2589.
38. Mansuroglu DS, Arslan H, Derveer DV, Binzet G. Synthesis and characterization of N-(2,2-Diphenylacetil)-N′-substituted thiourea derivatives: the crystal structure of N-(2,2-Diphenylacetil)-N′-(4-chloro phenyl)-thiourea. Phosphorus, Sulfur and Silicon 2009;184:3221–30.
39. Rehman SU, Chohan ZH, Naz F, Supuran CT. In-vitro antibacterial, antifungal and cytotoxic activities of some coumarins and their metal complexes. J Enz Inhib Med Chem 2005;20:333–40.
40. Sumrra SH, Chohan ZH. Synthesis, characterization and biological properties of thienyl derived triazole Schiff bases and their oxovanadium(IV) complexes. J Enz Inhib Med Chem 2012;27:187–93.
41. Chohan ZH, Hanif M. Design, synthesis, and biological properties of triazole derived compounds and their transition metal complexes. J Enzyme Inhib Med Chem 2010;25:737–49.
42. Sumrra SH, Chohan ZH. In vitro antibacterial, antifungal and cytotoxic activities of some triazole Schiff bases and their vanadyl(IV) complexes. J Enz Inhib Med Chem 2013;28:1291–9.

Supplementary material available online
Supplementary Tables S1–S8.