

***In-vitro* Antibacterial, Antifungal and cytotoxic activity of cobalt (II), copper (II), nickel (II) and zinc (II) complexes with furanylmethyl- and thienylmethyl-dithiolenes: [1, 3-dithiole- 2-one and 1,3-dithiole-2-thione]**

ZAHID H. CHOCHAN^{†,1}, ALI U. SHAIKH², & CLAUDIU T. SUPURAN^{3,*}

¹Department of Chemistry, Bahauddin Zakariya University, Multan (Pakistan), ²Department of Chemistry, University of Arkansas at Little Rock, Little Rock 72204(U.S.A.), and ³University of Florence, Dipartimento di Chimica, Laboratorio di Chimica Bioinorganica, Via della Lastruccia, Rm. 188, Polo Scientifico, 50019, Sesto Fiorentino, Firenze (Italy)

(Received 30 March 2006; in final form 24 April 2006)

Abstract

Some antibacterial and antifungal furanylmethyl- and thienylmethyl dithiolenes and their Co(II), Cu(II), Ni(II) and Zn(II) complexes have been synthesized, characterized and screened for their *in vitro* antibacterial activity against four Gram-negative; *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Shigella flexneri*, and two Gram-positive; *Bacillus subtilis* and *Staphylococcus aureus* bacterial strains, and for *in-vitro* antifungal activity against *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporium canis*, *Fusarium solani* and *Candida glaberata*. All compounds showed significant antibacterial and antifungal activity. The metal complexes, however, were shown to possess better activity as compared to the simple ligands. The brine shrimp bioassay was also carried out to study their *in-vitro* cytotoxic properties.

Keywords: *Furanyl/thienyl, dithiolenes, metal complexes, antibacterial, antifungal, cytotoxicity*

Introduction

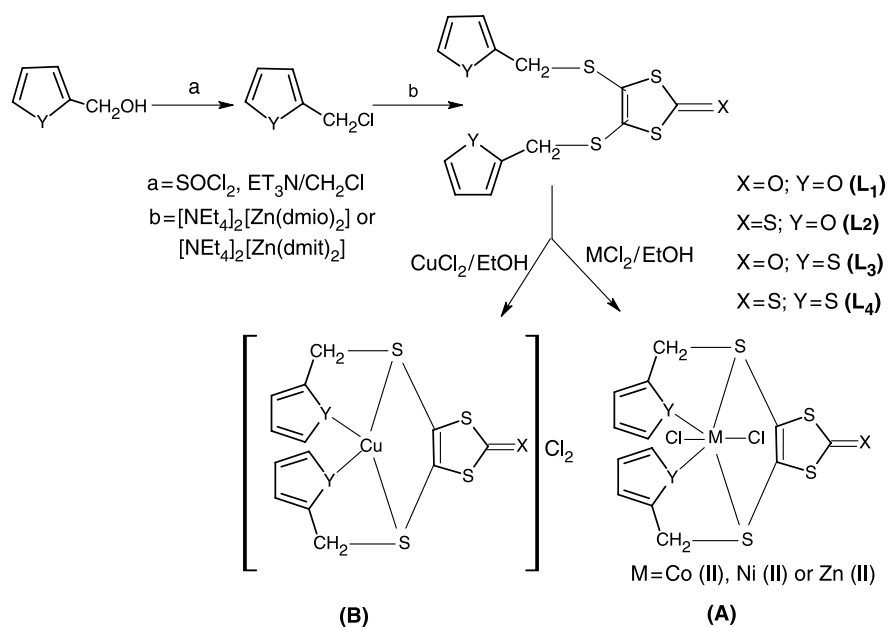
Not only are many metalloelements essential nutrients, but many are also becoming increasingly prevalent components as therapeutic agents [1] to treat a wide variety of diseases and metabolic disorders. DNA is one such target which acts as a binding agent for clinically used drugs [2]. The binding between DNA and the synthetic compounds/drugs provide better clues for rational DNA-specific drug design. This demand is driven by the emerging clinical problem of drug resistance which is alarmingly spreading at an accelerating rate.

Sulphur donor ligands mainly derived from dithiolene are known [3] to have potential as biocides. In

order to explore novel drug design systems which could combat more aggressively drug resistance, these considerations attracted the attention of the authors to combine the chemistry of furanylmethyl and thienylmethyl heterocyclic moieties with dithiolenes such as 1,3-dithiole-2-one and 1,3-dithiole-2-thione. These prepared ligands (**L**₁)–(**L**₄) (Scheme 1) were further used to react with different metalloelements such as, cobalt (II), copper (II), nickel (II) and zinc (II) to form metal chelates (**1**)–(**16**) (Scheme 1) with the hope that these compounds would potentially reduce the mechanism of bacterial resistance. These compounds have been characterized and screened *in vitro* for antibacterial activity against four Gram-negative;

Correspondence: C. Supuran, Università degli Studi di Firenze, Polo Scientifico, Laboratorio di Chimica Bioinorganica, Rm. 188, Via della Lastruccia 3, Sesto Fiorentino (Florence) 50019, Italy. Tel.: 39 055 4573005, Fax: 39 055 4573385. E-mail: claudiu.supuran@unifi.it

[†]Present address: Department of Chemistry, University of Arkansas at Little Rock, Little Rock 72204, U.S.A.
E-mail: zchohan@mul.paknet.com.pk; claudiu.supuran@unifi.it

Scheme 1. Preparation of ligands (L_1 – L_4) and their metal complexes.

Escherichia coli, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Shigella flexneri*, and two Gram-positive; *Bacillus subtilis* and *Staphylococcus aureus* bacterial strains, and for *in-vitro* antifungal activity against *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *Candida glaberata*. The results of these studies show the metal complexes to be more antibacterial and antifungal as compared to the uncomplexed ligands.

Materials and methods

Instrumentation

^1H -NMR spectra were obtained on a Bruker 250 MHz instrument and ^{13}C -NMR spectra on a Bruker 250 MHz instrument with 10s pulse delay. IR spectra were recorded on a Phillips analytical PU900 and Nicolet 205 Fourier-transform instruments. UV-Visible spectra were obtained in DMF on a Hitachi U-2000 double-beam spectrophotometer. Conductances of the metal complexes were determined in DMF on a Hitachi YSI-32 model conductometer. Magnetic measurements were done on solid complexes using Gouy's method. Melting points were recorded on a Gallenkamp apparatus and are not corrected. Zincate dithiolene salts, $[\text{Net}_4]_2[\text{Zn}(\text{dmio})_2]$ and $[\text{Net}_4]_2[\text{Zn}(\text{dmit})_2]$ were prepared by the published procedures [4,5] and had physical properties in agreement with the published values.

Preparation of Ligands

4,5-Bis(2-furanylmethylthio)-1,3-dithiole-2-one (*fmdo*) (L_1). A mixture of 2-hydroxymethylfuran (0.98 g,

0.01 mol) in dry dichloromethane (20 cm^3) and triethylamine (1.0 g, 0.01 mol) was cooled in an ice-bath. Thionyl chloride (1.65 g, 0.12 cm^3 , 0.21 mol) in dry dichloromethane (10 cm^3) was added under N_2 , at such a rate to keep the temperature between 15–20°C. After complete addition the reaction mixture was stirred at 20°C for 30 min and heated to 40°C for 30 min. Ice was added and mixture stirred for another 5 min. A small amount of NaHCO_3 was added to obtain pH 6.0. The organic layer was separated and dried over CaCl_2 . Filtration and evaporation of the solvent gave 2-chloromethylfuran to which was added the respective zincate salt (0.34 g, 0.05 mmol) in dry dichloromethane (30 cm^3). The mixture was refluxed overnight under a slow stream of N_2 . After cooling to room temperature, the solvent was evaporated to have a thick orange oil which was filtered through silica gel by washing several times with dichloromethane. After removal of solvent, the oil thus obtained was chromatographed on silica gel column using petroleum ether (b.p 40–60°C): dichloromethane (70:30) as eluent. After removing the solvent, an orange-red oil was obtained. Yield (58%). IR (KBr, cm^{-1}) 2953, 1660, 1211, 1410, 1065, 995. ^1H -NMR (CDCl_3 , 250 MHz) δ 4.0 [s, 4H, $-\text{CH}_2\text{S}$], 6.6–7.1 [m, 6H, furanyl]. ^{13}C -NMR (CDCl_3 , 63 MHz) δ 33.7 [CH_2], 109.7, 109.9, 110.9 [furanyl], 137.9 [C = C], 143.2 [C_i], 189.7 [C = O]. Found: C, 45.9; H, 2.7. $\text{C}_{13}\text{H}_{10}\text{O}_3\text{S}_4$ calcd: C, 45.6; H, 2.9%.

4,5-Bis(2-furanylmethylthio)-1,3-dithiole-2-thione (*fmdt*) (L_2). A mixture of 2-hydroxymethylfuran (1.20 g, 0.017 mol), triethylamine (1.20 g, 0.017 mol) and dichloromethane (20 cm^3) were cooled in an

ice-bath following rest and then the same method as described for (**L₁**) except [NEt₄]₂[Zn(dmit)₂] (0.18 g, 0.05 mmol in dry dichloromethane, 30 cm³) was used instead of [NEt₄]₂[Zn(dmio)₂]. After column chromatography and removal of the solvent an orange-red oil was obtained. Yield (60%). IR (KBr, cm⁻¹) 2959, 2811, 1410, 1065, 890, 995. ¹H-NMR (CDCl₃, 250 MHz) δ 4.0 [s, 4H, -CH₂S], 6.4–7.2 [m, 6H, furanyl]. ¹³C-NMR (CDCl₃, 63 MHz) δ 33.7 [CH₂], 109.7, 109.9, 110.9 [furanyl], 137.9 [C = C], 143.2 [C_i], 211.6 [C = S]. Found: C, 43.9; H, 2.6. C₁₃H₁₀O₂S₅ calcd: C, 43.6; H 2.8%.

4,5-Bis(2-thienylmethylthio)-1,3-dithiole-2-one (tmdo) (L₃). The same procedure was adopted as for the preparation of L₁ except 2-hydroxymethylthiophene (1.14 g, 0.95 cm³, 0.01 mol) was used. Yield 0.68 g (21%). IR (KBr, cm⁻¹) 3030, 2959, 1765, 1660, 1410, 1215, 995. ¹H-NMR (CDCl₃) δ 3.8 [s, 4H, -CH₂S], 6.5–7.1 [m, 6H, thienyl]. ¹³C-NMR (CDCl₃, 63 MHz) δ 33.5 [CH₂], 109.2, 109.9, 110.6 [thienyl], 137.5 [C = C], 143.0 [C_i], 189.5 [C = O]. Found: C, 41.4; H, 2.5. C₁₃H₁₀OS₆ calcd.: C, 41.7; H, 2.7%.

4,5-Bis(2-thienylmethylthio)-1,3-dithiole-2-thione (tmdt) (L₄). The same procedure was adopted as for the preparation of L₂ except 2-hydroxymethylthiophene (1.14 g, 0.95 cm³, 0.01 mol) was used. Yield 0.94 g (61%). IR (KBr, cm⁻¹) 2918, 1531, 1458, 1410, 1230, 1184, 1065, 995. ¹H-NMR (CDCl₃) δ 3.7 [s, 4H, CH₂S], 6.7–7.0 [m, 6H, thienyl]. ¹³C-NMR (CDCl₃) δ 35.5 [CH₂], 127.0, 127.4, 127.5 [thienyl], 137.6 [C = C], 138.3 [C_i], 211.5 [C = S]. Found: C, 39.5; H, 2.9. C₁₃H₁₀S₇ calcd: C, 39.9; H, 2.6%.

Preparation of Metal (II) Complexes

The metal (II) complexes were prepared by mixing the respective ligand (1 mmol) in methanol (10 cm³) with the respective metal (II) as chloride (1 mmol) in methanol (50 cm³). The pH of the solution was adjusted to 8.0 with 5.0 M NaOH and then the mixture was refluxed for 1 h and then cooled to room temperature. On cooling, a solid product was formed which was filtered off, washed with methanol, then with ether and dried. Crystallization from hot ethanol gave the desired metal complex. All complexes (**1**)–(**16**) were prepared following this method.

Antibacterial bioassay (in-vitro)

The synthesized compounds were screened *in vitro* for their antibacterial activity against four Gram-negative; *E. coli*, *P. aeruginosa*, *S. typhi* and *S. flexneri*, and two Gram-positive; *B. subtilis* and *S. aureus* bacterial strains by the agar-well diffusion method [6]. The wells

(6 mm in diameter) were dug in the media with the help of a sterile metallic borer with centers at least 24 mm apart. Two to eight hours old bacterial inocula containing approximately 10⁴–10⁶ 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 were supplemented with DMSO and the reference antibacterial drug, imipenem, served as a standard drug. The plates were incubated immediately at 37°C for 24 h. Activity was determined by measuring the diameter of the zones showing complete inhibition (mm). In order to clarify any participating role of DMSO in the biological screening, separate studies were carried out with solutions alone of DMSO and they showed no activity against any bacterial strains.

Antifungal Activity (in-vitro)

Antifungal activities of all compounds were studied against six fungal cultures, *T. longifusus*, *C. albicans*, *A. flavus*, *M. canis*, *F. solani* and *C. glabrata*. Sabouraud dextrose agar (Oxoid, Hampshire, England) was seeded with 10⁵ (cfu) ml⁻¹ fungal spore suspensions and transferred to petri plates. Discs soaked in 20 cm³ (200 µg/cm³ in DMSO) of all compounds were placed at different positions on the agar surface. The plates were incubated at 32°C for seven days. The results were recorded as % of inhibition of growth and compared with standard drugs miconazole and Amphotericin B.

Minimum Inhibitory Concentration (MIC)

Compounds containing high antibacterial activity (over 80%) were selected for minimum inhibitory concentration (MIC) studies. The minimum inhibitory concentration was determined using the disc diffusion technique [6] by preparing discs containing 10, 25, 50 and 100 µg/cm³ of the compounds and applying the protocol.

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 other compartment was open to ordinary light. After two days nauplii were collected by a pipette from the lighted side. A sample of the test compound was prepared by dissolving 20 mg of each compound in 2 cm³ of DMF. From this

stock solutions 500, 50 and 5 $\mu\text{g}/\text{cm}^3$ were transferred to 9 vials (three for each dilutions were used for each test sample and LD₅₀ is the mean of three values) and one vial was kept as control having 2 cm^3 of DMF only. The solvent was allowed to evaporate overnight. After two days, when shrimp larvae were ready, 1 mL of sea water and 10 shrimps were added to each vial (30 shrimps/dilution) and the volume was adjusted with sea water to 5 cm^3 per vial. After 24 h the number of survivors was counted. Data were analyzed by a Finney computer program to determine the LD₅₀ values [7].

Result and discussion

Chemistry

The ligands (**L**₁–**L**₄) are oily liquids, which are soluble in ethanol, methanol, chloroform, acetone and dichloromethane. They are stable in air and light and can be stored at room temperature for indefinite period. The structure of these air stable compounds have been characterised by their IR, ¹H-NMR, ¹³C-NMR spectral data and elemental analysis. The interaction of metal ions with these ligands in a 1:1 molar ratio resulted in the formation of the complexes, [M(**L**₁–**L**₄)Cl₂] where M = cobalt (II), nickel (II) and zinc (II) and [Cu(**L**₁–**L**₄)]Cl₂ (Scheme 1) (Table I). The molar conductance values (in DMSO) fell within the range 13–20 $\Omega^{-1} \text{cm}^2 \text{mol}^{-1}$ for the cobalt (II), nickel (II) and zinc (II) complexes, showing their non-electrolytic nature [8] and for the copper (II) complexes in a higher range (114–120 $\Omega^{-1} \text{cm}^2 \text{mol}^{-1}$) showing their electrolytic nature. This in turn, suggests that the chloride ions are coordinated with the metal ions in cobalt (II), nickel (II) and zinc (II) complexes and uncoordinated in the copper (II) complexes. All complexes decomposed rather than melted above 200°C.

IR Spectra. Dithiolenes generally display intense electronic transitions in the near the IR region. These intense absorptions [9] arise from a transition between the highest occupied (HOMO) and lowest unoccupied molecular orbital (LUMO) states. The infrared spectra of the metal complexes are depicted in Table II with some tentative assignments for the important characteristic bands. The IR spectra of the ligands showed the absence of a band at 3225 cm^{-1} assigned to OH stretching providing evidence for the conversion of the methanol groups into chloromethyl on reacting with thionyl chloride. In situ reaction with [Zn(dmio)₂][Net₄]₂ and [Zn(dmit)₂][Net₄]₂ salts respectively indicated the presence of carbonyl stretching (C = O) at 1660 cm^{-1} and of (C = S) at 1065 cm^{-1} , thus confirming [10] that the starting chloro derivatives had been converted into the expected ligands (**L**₁–**L**₄).

The spectra of the metal complexes reported in Table II showed that in the far-infrared region, new bands at 375 cm^{-1} and 460 cm^{-1} appeared which were attributed [11] to $\nu(\text{M-S})$ and $\nu(\text{M-O})$ which were not present in the spectra of ligands. It clearly indicated that the ligands are coordinated to the respective metals via the sulphur atoms of the thiolene moiety and oxygens or sulphurs of the furanyl or thienyl groups. Also, a weak band at 310 cm^{-1} due to the $\nu(\text{M-Cl})$ mode was observed in the spectra of the Co (II), Ni (II) and Zn (II) complexes strongly suggesting [10,12] coordination of the respective metal atom with the chloride ions in their octahedral environment (Fig. A of Scheme 1). This band however, was not found in the spectra of the Cu (II) complexes thus suggesting a four coordinated square-planar geometry for the Cu (II) complexes (Fig. B of Scheme 1).

¹H NMR and ¹³C NMR Spectra. The ¹H NMR and ¹³C NMR spectra of the free ligands and their Zn (II) complexes were recorded in DMSO-d₆ with TMS as

Table I. Physical and Analytical Data of the Metal (II) Complexes

| No | | M.P (°C) | Yield (%) | B.M (μ_{eff}) | Calcd. C | (Found) % II |
|-----|---|----------|-----------|----------------------------|-------------|--------------|
| 1. | [Co(fmdo)Cl ₂] [472.03] C ₁₃ H ₁₀ CoCl ₂ O ₃ S ₄ | 203–205 | 65 | 4.0 | 33.0 (33.4) | 2.1 (2.5) |
| 2. | [Cu(fmdo)Cl ₂] [476.65] C ₁₃ H ₁₀ CuCl ₂ O ₃ S ₄ | 208–210 | 67 | 1.5 | 32.7 (32.9) | 2.1 (2.2) |
| 3. | [Ni(fmdo)Cl ₂] [471.79] C ₁₃ H ₁₀ NiCl ₂ O ₃ S ₄ | 214–216 | 65 | 3.5 | 33.1 (32.8) | 2.1 (2.5) |
| 4. | [Zn(fmdo)Cl ₂] [478.49] C ₁₃ H ₁₀ ZnCl ₂ O ₃ S ₄ | 205–207 | 67 | Dia | 32.6 (32.2) | 2.1 (1.8) |
| 5. | [Co(fmdt)Cl ₂] [488.15] C ₁₃ H ₁₀ CoCl ₂ O ₂ S ₅ | 210–212 | 66 | 3.9 | 31.9 (32.3) | 2.0 (2.5) |
| 6. | [Cu(fmdt)Cl ₂] [492.8] C ₁₃ H ₁₀ CuCl ₂ O ₂ S ₅ | 214–216 | 65 | 1.4 | 31.7 (31.9) | 2.0 (2.3) |
| 7. | [Ni(fmdt)Cl ₂] [487.94] C ₁₃ H ₁₀ NiCl ₂ O ₂ S ₅ | 205–207 | 66 | 3.4 | 31.9 (31.6) | 2.0 (1.7) |
| 8. | [Zn(fmdt)Cl ₂] [494.64] C ₁₃ H ₁₀ ZnCl ₂ O ₂ S ₅ | 208–210 | 65 | Dia | 31.5 (31.9) | 2.0 (2.3) |
| 9. | [Co(tmdo)Cl ₂] [504.25] C ₁₃ H ₁₀ CoCl ₂ OS ₆ | 208–210 | 65 | 4.1 | 30.9 (31.3) | 2.0 (2.4) |
| 10. | [Cu(tmdo)Cl ₂] [508.87] C ₁₃ H ₁₀ CuCl ₂ OS ₆ | 211–213 | 67 | 1.4 | 30.7 (30.4) | 2.0 (1.7) |
| 11. | [Ni(tmdo)Cl ₂] [504.01] C ₁₃ H ₁₀ NiCl ₂ OS ₆ | 208–210 | 65 | 3.4 | 30.9 (31.3) | 2.0 (2.4) |
| 12. | [Zn(tmdo)Cl ₂] [510.71] C ₁₃ H ₁₀ ZnCl ₂ OS ₆ | 212–21 | 67 | Dia | 30.5 (30.2) | 2.0 (2.3) |
| 13. | [Co(tmdt)Cl ₂] [520.32] C ₁₃ H ₁₀ CoCl ₂ S ₇ | 205–207 | 66 | 4.0 | 29.9 (29.5) | 1.9 (1.6) |
| 14. | [Cu(tmdt)Cl ₂] [524.94] C ₁₃ H ₁₀ CuCl ₂ S ₇ | 210–212 | 65 | 1.7 | 29.7 (29.9) | 1.9 (2.2) |
| 15. | [Ni(tmdt)Cl ₂] [520.08] C ₁₃ H ₁₀ NiCl ₂ S ₇ | 202–204 | 66 | 3.2 | 30.1 (30.5) | 1.9 (1.5) |
| 16. | [Zn(tmdt)Cl ₂] [526.78] C ₁₃ H ₁₀ ZnCl ₂ S ₇ | 208–210 | 65 | Dia | 29.6 (29.9) | 1.9 (2.3) |

Table II. Selected IR, NMR and UV-Visible Spectral Data of the Metal Complexes

| No. | IR (cm ⁻¹) | ¹ HNMR (DMSO-d ₆)(ppm) | ¹³ CNMR(DMSO-d ₆)(ppm) | λ _{max} (cm ⁻¹) |
|-----|--|--|--|--------------------------------------|
| 1. | 1660 (C = O), 460 (M-O), 375 (M-S), 310 (M-Cl) | - | - | 8620, 17310, 30210 |
| 2. | 1660 (C = O), 460 (M-O), 375 (M-S), | - | - | 15105, 19360, 30315 |
| 3. | 1660 (C = O), 460 (M-O), 375 (M-S), 310 (M-Cl) | - | - | 10325, 16280, 29485 |
| 4. | 1660 (C = O), 460 (M-O), 375 (M-S), 310 (M-Cl) | 4.2 [s, 4H, -CH ₂ S], 6.4 [m, 2H, furanyl], 6.5 [m, 2H, furanyl], 7.6 [d, 2H, furanyl] | 33.8 [CH ₂], 109.7, 110.1, 111.2 [furanyl], 137.9 [C = C], 143.4 [C _i], 189.9 [C = O] | 28335 |
| 5. | 1065 (C = S), 460 (M-O), 375 (M-S), 310 (M-Cl) | - | - | 8795, 17655, 30115 |
| 6. | 1065 (C = S), 460 (M-O), 375 (M-S) | - | - | 15330, 19595, 30285 |
| 7. | 1065 (C = S), 460 (M-O), 375 (M-S), 310 (M-Cl) | - | - | 10495, 16225, 29690 |
| 8. | 1065 (C = S), 460 (M-O), 375 (M-S), 310 (M-Cl) | 4.3 [s, 4H, -CH ₂ S], 6.5 [m, 2H, furanyl], 6.6 [m, 2H, furanyl], 7.5 [d, 2H, furanyl] | 33.8 [CH ₂], 109.9, 110.2, 110.9 [furanyl], 138.2 [C = C], 143.4 [C _i], 211.9 [C = S] | 28375 |
| 9. | 1660 (C = O), 460 (M-O), 375 (M-S), 310 (M-Cl) | - | - | 8685, 17565, 30155 |
| 10. | 1660 (C = O), 460 (M-O), 375 (M-S) | - | - | 15235, 19415, 30355 |
| 11. | 1660 (C = O), 460 (M-O), 375 (M-S), 310 (M-Cl) | - | - | 10385, 16300, 29575 |
| 12. | 1660 (C = O), 460 (M-O), 375 (M-S), 310 (M-Cl) | 4.0 [s, 4H, -CH ₂ S], 6.2 [m, 2H, thienyl], 5.9 [m, 2H, thienyl], 7.4 [m, 2H, thienyl] | 35.7 [CH ₂], 109.5, 110.3, 110.8 [thienyl], 137.7 [C = C], 143.2 [C _i], 189.7 [C = O] | 28480 |
| 13. | 1660 (C = S), 460 (M-O), 375 (M-S), 310 (M-Cl) | - | - | 8780, 17615, 29995 |
| 14. | 1660 (C = S), 460 (M-O), 375 (M-S) | - | - | 15325, 19510, 30245 |
| 15. | 1660 (C = S), 460 (M-O), 375 (M-S), 310 (M-Cl) | - | - | 10455, 16175, 29615 |
| 16. | 1660 (C = O), 460 (M-O), 375 (M-S), 310 (M-Cl) | 3.8 [s, 4H, -CH ₂ S], 6.2 [m, 2H, thienyl], 6.4 [m, 2H, thienyl], 7.4 [d, 2H, thienyl] | 33.7 [CH ₂], 109.7, 109.9, 110.9 [thienyl], 137.9 [C = C], 143.3 [C _i], 211.7 [C = S] | 28410 |

internal reference and are summarized in Table II with some tentative assignments [13]. The conclusions drawn from these studies lend further support to the mode of bonding discussed above in the IR spectra. In the spectra of the diamagnetic Zn (II) complexes these protons shifted downfield as expected [14] due to the increased conjugation during coordination to the metal atoms. The number of protons calculated from the integration curves, and those obtained from the values of the expected C and H analyses are in agreement. It was observed that DMSO did not have any coordinating effect on the spectra of the zinc (II) complexes.

Electronic Spectra and Magnetic Moments. The UV-Visible spectral bands of the ligands and their complexes in DMSO are recorded in Table II. The Co (II) complexes showed three bands at 8,620–8,795, 17,310–17,655 and 29,995–30,210 cm⁻¹

which may be assigned to ${}^4T_{1g} \rightarrow {}^4T_{2g}(F)$, ${}^4T_{1g} \rightarrow {}^3A_{2g}(F)$ and ${}^4T_{1g} \rightarrow {}^4T_{1g}(P)$ transitions respectively and are suggestive [15] of an octahedral geometry around the cobalt ions. The electronic spectra of the Cu (II) complexes showed two low-energy weak bands at 15,105–15,330 and 19,360–19,595 cm⁻¹ and a strong high-energy band at 30,245–30,355 cm⁻¹. The low-energy bands in this region are typically expected [16] for a square-planar configuration and may be assigned to ${}^2B_{1g} \rightarrow {}^2A_{1g}$ and ${}^2B_{1g} \rightarrow {}^2E_g$ transitions, respectively. The strong high-energy band, in turn is assigned to a metal \rightarrow ligand charge transfer. The Ni (II) complexes exhibited three spin-allowed bands at 10,325–10,495, 16,175–16,300, and 29,485–29,690 cm⁻¹ assignable [17] respectively, to the transitions ${}^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)(\nu_1)$, ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)(\nu_2)$ and ${}^3A_{2g}(F) \rightarrow {}^3T_{2g}(P)(\nu_3)$ which were characteristic of an octahedral geometry. The electronic spectra of the Zn (II) complexes showed only

a high intensity band at 28,335–28,480 cm^{-1} due to ligand \rightarrow metal charge transfer in a distorted octahedral environment [18].

The geometry of the metal complexes has been further deduced from the magnetic moment data of the complexes. The room temperature magnetic moment of the solid cobalt (II) complexes was in the range (3.9–4.1 B.M), indicative [15,19] of three unpaired electrons per Co (II) ion in an octahedral environment. The magnetic moment of the Cu (II) complexes was in the range (1.4–1.7 B.M), consistent [19] for square-planar geometry. The Ni (II) complexes showed μ_{eff} values (3.2–3.5 B.M), corresponding [15] to two unpaired electrons per Ni (II) ion for their six-coordinated configuration.

On the basis of the above observations, it is suggested that the Co (II), Ni (II) and Zn (II) complexes show an octahedral geometry and distorted octahedral geometry respectively and the Cu (II) complexes, a square-planar geometry (Figs. A & B of Scheme 1).

Antibacterial bioassay

All compounds were tested against four Gram-negative; *E. coli*, *S. flexneri*, *P. aeruginosa* and *S. typhi* and two Gram-positive; *B. subtilis* and *S. aureus* bacterial strains according to literature protocol [8]. The results were compared with those of the standard drug imipenem. The synthesized compounds exhibited varying degree of inhibitory effects on the growth of different tested strains (Table III). The ligands displayed inhibitory effects

which became overall more pronounced on coordination with the metal ions but were not as potent as imipenem

Antifungal Bioassay

The antifungal screening of all compounds was carried out against *T. richophyton longifusus*, *C. albicans*, *A. flavus*, *M. canis*, *F. solani* and *C. glabrata* fungal strains according to the literature protocol [6]. The results were compared with those for the standard drugs miconazole and Amphotericin B. The results Table IV indicate that all the ligands exhibited an activity which was enhanced on complexation. The moderate or weak activity against certain species was also enhanced which in turn suggests that complexation played a significant role in enhancement of the activity although they were not as potent as the standard drugs.

Minimum Inhibitory Concentration (MIC). Preliminary screening showed that compounds **4, 8, 10, 12** and **16** were the most active ones against *E. coli* and compounds **4, 8** and **16** were the most active against *B. subtilis* and, **4** and **12** against *S. typhi*. These compounds were therefore, selected for minimum inhibitory concentration (MIC) studies (Table V). The MIC of all these compounds varied from 1.9651×10^{-8} – 1.9651×10^{-7} M.

Cytotoxic Bioassay

All the synthesized compounds were screened for their cytotoxicity (brine shrimp bioassay) using the protocol

Table III. Screening Data of the Ligands (L₁-L₄) and its Metal Complexes (1-16) for Antibacterial Activity (zone of inhibition in mm).

| No. Ligands/Complexes | Microbial species | | | | | |
|-----------------------|-------------------|--------------------|----------------|-------------------|-------------------|-----------------|
| | <i>E.coli</i> | <i>Paeruginosa</i> | <i>S.typhi</i> | <i>S.flexneri</i> | <i>B.subtilis</i> | <i>S.aureus</i> |
| L ₁ | 20 | 18 | 17 | 09 | 20 | 19 |
| L ₂ | 18 | 17 | 18 | 10 | 17 | 18 |
| L ₃ | 17 | 17 | 20 | 08 | 18 | 20 |
| L ₄ | 19 | 16 | 17 | 07 | 20 | 19 |
| 1 | 20 | 20 | 20 | 12 | 22 | 22 |
| 2 | 20 | 19 | 20 | 13 | 22 | 18 |
| 3 | 20 | 18 | 20 | 10 | 19 | 22 |
| 4 | 22 | 17 | 23 | 15 | 23 | 20 |
| 5 | 21 | 20 | 21 | 16 | 21 | 23 |
| 6 | 19 | 19 | 22 | 10 | 20 | 22 |
| 7 | 20 | 18 | 20 | 11 | 21 | 22 |
| 8 | 22 | 18 | 18 | 14 | 23 | 20 |
| 9 | 20 | 17 | 22 | 12 | 20 | 22 |
| 10 | 22 | 20 | 19 | 12 | 20 | 21 |
| 11 | 18 | 21 | 18 | 11 | 21 | 20 |
| 12 | 22 | 20 | 23 | 13 | 22 | 22 |
| 13 | 20 | 19 | 19 | 12 | 22 | 19 |
| 14 | 20 | 19 | 18 | 14 | 20 | 20 |
| 15 | 19 | 20 | 19 | 12 | 20 | 22 |
| 16 | 22 | 18 | 20 | 11 | 23 | 21 |
| Imipenem | 27 | 27 | 28 | 27 | 28 | 30 |

10 <: weak; 10-16: moderate; > 16: significant. Imipenem (standard drug).

Table IV. Primary Screening Data of the Ligands (L₁-L₄) and its Metal Complexes (1-16) for Antifungal Activity (% inhibition).

| No. Ligands/Complexes | Microbial species | | | | | |
|-----------------------|----------------------|--------------------|------------------|-----------------|------------------|---------------------|
| | <i>T. longifusus</i> | <i>C. albicans</i> | <i>A. flavus</i> | <i>M. canis</i> | <i>F. solani</i> | <i>C. glaberata</i> |
| L ₁ | 18 | 18 | 10 | 20 | 20 | 20 |
| L ₂ | 20 | 18 | 13 | 19 | 20 | 21 |
| L ₃ | 19 | 17 | 15 | 18 | 18 | 20 |
| L ₄ | 21 | 18 | 12 | 20 | 20 | 19 |
| 1 | 22 | 20 | 18 | 23 | 22 | 22 |
| 2 | 19 | 22 | 20 | 22 | 23 | 23 |
| 3 | 20 | 20 | 19 | 20 | 24 | 24 |
| 4 | 22 | 19 | 20 | 21 | 22 | 23 |
| 5 | 21 | 18 | 21 | 22 | 21 | 22 |
| 6 | 20 | 18 | 18 | 22 | 20 | 22 |
| 7 | 19 | 20 | 18 | 20 | 22 | 23 |
| 8 | 22 | 20 | 20 | 22 | 22 | 20 |
| 9 | 21 | 22 | 14 | 23 | 23 | 20 |
| 10 | 21 | 20 | 19 | 21 | 21 | 22 |
| 11 | 22 | 21 | 20 | 21 | 19 | 23 |
| 12 | 20 | 19 | 20 | 23 | 20 | 22 |
| 13 | 20 | 20 | 19 | 20 | 20 | 21 |
| 14 | 22 | 21 | 18 | 23 | 19 | 23 |
| 15 | 22 | 22 | 19 | 23 | 21 | 22 |
| 16 | 21 | 21 | 20 | 22 | 22 | 23 |
| Miconazole | 30 | 24 | 25 | 27 | 33 | 30 |
| AmphotericinB | 28 | 30 | 30 | 32 | 30 | 32 |

10 <: weak; 10-16: moderate; > 16: significant. Miconazole & Amphotericin B (standard drugs).

Table V. Minimum Inhibitory Concentration (M) of the Compounds (4), (8), (10), (12) and (16) against Selected Bacteria.

| No. | (4) | (8) | (10) | (12) | (16) |
|--------------------|-------------------------|---------------------------|---------------------------|---------------------------|-------------------------|
| <i>E. coli</i> | 2.0899×10^{-8} | 2.0216×10^{-8} | 1.9651×10^{-8} | 4.8951×10^{-8} | 4.7458×10^{-8} |
| <i>S. typhi</i> | 2.0899×10^{-8} | $> 2.0216 \times 10^{-7}$ | $> 1.9651 \times 10^{-7}$ | 4.8951×10^{-8} | 9.4916×10^{-8} |
| <i>B. subtilis</i> | 5.2247×10^{-8} | $> 1.0108 \times 10^{-7}$ | $> 1.9651 \times 10^{-7}$ | $> 1.9580 \times 10^{-7}$ | 4.7458×10^{-8} |

of Meyer *et. al* [20]. From the data recorded in Table VI, it is evident that four compounds, **2**, **6**, **11** and **14** displayed potent cytotoxic activity against *Artemia salina*, while the other compounds were inactive in this assay

Some of the compounds generally showed good antibacterial activity against one or more bacterial species. It was evident from the data that this activity significantly increased on coordination. This enhancement in the activity may also be rationalized [21–25] on the basis of their structures by mainly possessing additional electron donor groups. Furthermore, coordination reduces the polarity [26–28] of the metal ion mainly because of the partial sharing of its positive charge with the donor groups within the chelate ring system, which is mainly formed during chelation process. This process, in turn, increases the lipophilic nature of the central metal atom, which favours its permeation more efficiently through the lipid layer of the micro-organism [29–32] thus making the chelate compounds more bacteriostatic and fungistatic.

Table VI. Brine Shrimp Bioassay Data of Ligands (L¹-L⁴) and their Metal (II) Complexes (1-16).

| Compound | LD ₅₀ (M) |
|----------------|---------------------------|
| L ¹ | $> 2.9239 \times 10^{-6}$ |
| L ² | $> 2.7932 \times 10^{-6}$ |
| L ³ | $> 2.6737 \times 10^{-6}$ |
| L ⁴ | $> 2.5641 \times 10^{-6}$ |
| 1 | $> 2.1185 \times 10^{-6}$ |
| 2 | 7.2380×10^{-7} |
| 3 | $> 2.1195 \times 10^{-6}$ |
| 4 | $> 2.0899 \times 10^{-6}$ |
| 5 | $> 2.0485 \times 10^{-6}$ |
| 6 | 7.1428×10^{-7} |
| 7 | $> 2.0494 \times 10^{-6}$ |
| 8 | $> 2.0216 \times 10^{-6}$ |
| 9 | $> 1.9831 \times 10^{-6}$ |
| 10 | $> 1.9651 \times 10^{-6}$ |
| 11 | 5.6546×10^{-7} |
| 12 | $> 1.9580 \times 10^{-6}$ |
| 13 | $> 1.9218 \times 10^{-6}$ |
| 14 | 6.1340×10^{-7} |
| 15 | $> 1.9227 \times 10^{-6}$ |
| 16 | $> 1.8983 \times 10^{-6}$ |

Conclusion

New types of dithiolene units attached to the heteroaromatic systems, furanyl and thienyl have been prepared. The chemistry of such heteroaromatics combined with sulphur-based compounds within one molecular unit offers a novel approach in the design and development of metal-based antibacterial and antifungal compounds.

Acknowledgements

One of us (ZHC) wishes to thank United States Department of State (U.S.A) for a William Fulbright award that made this research possible.

References

- [1] Orvig C, Abrams MJ. Medicinal Inorganic Chemistry: Introduction. Chem Rev 1999;9:15–21.
- [2] Farrell NP. Uses of Inorganic chemistry in Medicine Cambridge, U.K: Royal Society of Chemistry; 1999.
- [3] Constable AC. Helices, supramolecular chemistry and metal induced self organization. Angew Chem Int Ed Eng 1991;30:407.
- [4] Spencer GM, Wardell JL, Aupers JH. Polyhedron, Preparation of [tris(1,3-dithiole-2-thione-4,5-dithiolato)antimonate(1-)] complexes, [SbV(dmit)3]-. Crystal structure of the 1,4-dimethylpyridinium salt 1996;15:2701–2706.
- [5] Svenstrup N, Becker J. Synthesis, The organic chemistry of 1,3-dithiole-2-thione-4,5-dithiolate (DMIT). 1995;:215–235.
- [6] Atta-ur-Rahman, Choudhary MI, Thomsen WJ. Bioassay Techniques for Drug Development. The Netherlands: Harwood Academic Publishers; 2001. p 16.
- [7] Finney DJ. Probit Analysis. 3rd ed. Cambridge: Cambridge University Press; 1971.
- [8] Geary WJ. Use of conductivity measurements in organic solvents for the characterization of coordination compounds. Coord. Chem. Rev 1971;7:81.
- [9] Mueller-Westerhoff UT, Vance B. Comprehensive Coordination Chemistry. Pergamon, Oxford 1987;pp595:165.
- [10] Nakamoto K. Infrared Spectra of Inorganic and Coordination Compounds 2nd Ed New York: Wiley Interscience; 1970.
- [11] Bellamy LJ. The Infrared Spectra of Complex Molecules New York: John Wiley; 1971.
- [12] Ferrero JR. Low-frequency Vibrations of Inorganic and Coordination Compounds New York: John Wiley; 1971.
- [13] Simmons WW. The Sadtler Handbook of Proton NMR Spectra Sadtler Research Laboratories, Inc 1978.
- [14] Pasto DJ. Organic Structure Determination London: Prentice Hall International; 1969.
- [15] Estes WE, Govel DP, Hatfield WB, Hodgson DJ. Magnetic and structural characterization of dibromo and dichlorobis(thiazole) copper (II) complex. Inorg Chem 1978;17:1415.
- [16] Balhausen CJ. An Introduction to Ligand Field New York: McGraw Hill; 1962.
- [17] Lever ABP. Inorganic Electronic Spectroscopy Amsterdam: Elsevier; 1984.
- [18] Lever ABP, Lewis J. Square-planer bisethylenediamine-metal complexes. J Chem Soc 1963;:2552.
- [19] Carlin RL. Transition Metal Chemistry 2nd Ed New York: Marcel Decker; 1965.
- [20] Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica 1982;45:31.
- [21] Chohan ZH. Antibacterial copper(II) complexes of 1,1-symmetric ferrocene-derived Schiff-base ligands: studies of the effect of anions on their antibacterial properties. Appl. Organomet. Chem 2002;16:17.
- [22] Chohan ZH, Farooq MA, Scozzafava A, Supuran CT. Antibacterial Schiff bases of oxalyl-hydrazine/diamide incorporating pyrrolyl and salicylyl moieties and of their zinc(II) complexes. J. Enz. Inhib. Med. Chem 2002;17:1.
- [23] Hassan MU, Chohan ZH, Supuran CT. Antibacterial Zn(II) compounds of Schiff bases derived from some benzothiazoles. Main Group Metal Chemistry 2002;25.
- [24] Chohan ZH, Supuran CT, Scozzafava A. Zinc complexes of benzothiazole-derived Schiff-bases with antibacterial activity. J Enz Inhib Med Chem 2003;18:1.
- [25] Saeed U Rehman, 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.
- [26] Chohan ZH, Supuran CT. Organometallic compounds with biologically active molecules: *In-vitro* antibacterial and antifungal activity of some 1,1'-(dicarbohydrazono)ferrocenes and their Co (II), Cu (II), Ni (II) and Zn (II) complexes. Appl. Organomet. Chem 2005;19:1207.
- [27] Chohan ZH, Supuran CT. In-vitro antibacterial and cytotoxic activity of cobalt (II), copper (II), nickel (II) and zinc (II) complexes of the antibiotic drug cephalothin (keflin). J Enz Inhib Med Chem 2005;20:463.
- [28] Puccetti L, Fosolis G, Vullo D, Chohan ZH, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors. Inhibition of cytosolic/tumor-associated carbonic anhydrase isozymes I, II, IX, and XII with Schiff's bases incorporating chromone and aromatic sulfonamide moieties, and their zinc complexes In-vitro antibacterial, antifungal and cytotoxic properties of sulfonamide-derived Schiff's bases and their metal complexes. Bioorg Med Chem Lett 2005;15:3096.
- [29] Chohan ZH, Supuran CT, Scozzafava A. Metal binding and antibacterial activity of ciprofloxacin complexes. J Enz Inhib Med Chem 2005;20:303.
- [30] Chohan ZH. Synthesis and biological properties of Cu(II) complexes with 1,1'-disubstituted ferrocenes. Synth React Inorg Met-Org Chem 2004;34:833.
- [31] Chohan ZH, Supuran CT, Scozzafava A. Metalloantibiotics: Synthesis and antibacterial activity of cobalt(II), copper(II), nickel(II) and zinc(II) complexes of kefol. J Enz Inhib Med Chem 2004;19:79.
- [32] Hassan MU, Chohan ZH, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors: Schiff's bases of aromatic and heterocyclic sulfonamides and their metal complexes. J Enz Inhib Med Chem 2004;19:263.