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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. CHOHAN[†,1], ALI U. SHAIKH[2], & CLAUDIU T. SUPURAN[3,⋆]

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

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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 flexeneri,* 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.* 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 ($\mathbf{L_1}$)–($\mathbf{L_4}$) (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,Universita 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

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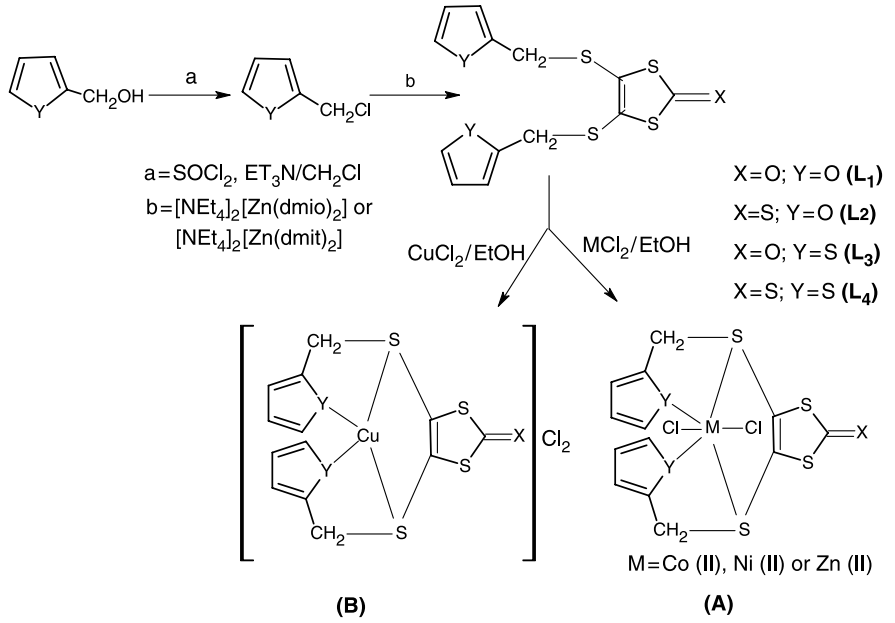

Scheme 1. Preparation of ligands ($\mathbf{L_1}$–$\mathbf{L_4}$) and their metal complexes.

Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and *Shigella flexeneri,* and two Gram-positive; *Bacillus subtilis* and *Staphylococus 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

^{1}H-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, $[Net_4]_2$[-$Zn(dmio)_2$] and $[Net_4]_2[Zn(dmit)_2]$ 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. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 4.0 [s, 4H, -CH_2S], 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. $C_{13}H_{10}O_3S_4$ 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, 0.17 mol), triethylamine (1.20 g, 0.17 mol) and dichloromethane (20 cm^3) were cooled in an

ice-bath following rest and then the same method as described for **(L_1)** except $[NEt_4]_2[Zn(dmit)_2]$ (0.18 g, 0.05 mmol in dry dichloromethane, 30 cm^3) was used instead of $[NEt_4]_2[Zn(dmio)_2]$. 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. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 4.0 [s, 4H, -CH_2S], 6.4–7.2 [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], 211.6 [C = S]. Found: C, 43.9; H, 2.6. $C_{13}H_{10}O_2S_5$ calcd: C, 43.6; H 2.8%.

4,5-Bis(2-thienylmethylthio)-1,3-dithiole-2-one (tmdo) (L_3). The same procedure was adopted as for the preparation of L_1 except 2-hydroxymethylthiophene (1.14 g, 0.95 cm^3, 0.01 mol) was used. Yield 0.68 g (21%). IR (KBr,cm^{-1}) 3030, 2959, 1765, 1660, 1410, 1215, 995. ^{1}H-NMR ($CDCl_3$) δ 3.8 [s, 4H, -CH_2S], 6.5–7.1 [m, 6H, thienyl]. ^{13}C-NMR ($CDCl_3$, 63 MHz) δ 33.5 [CH_2], 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. $C_{13}H_{10}OS_6$ calcd.: C, 41.7; H, 2.7%.

4,5-Bis(2-thienylmethylthio)-1,3-dithiole-2-thione (tmdt) (L_4). The same procedure was adopted as for the preparation of L_2 except 2-hydroxymethylthiophene (1.14 g, 0.95 cm^3, 0.01 mol) was used. Yield 0.94 g (61%). IR (KBr, cm^{-1}) 2918, 1531, 1458, 1410, 1230, 1184, 1065, 995. ^{1}H-NMR ($CDCl_3$) δ 3.7 [s, 4H, CH_2S], 6.7–7.0 [m, 6H, thienyl]. ^{13}C-NMR ($CDCl_3$) δ 35.5 [CH_2], 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. $C_{13}H_{10}S_7$ 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 cm^3) with the respective metal (II) as chloride (1 mmol) in methanol (50 cm^3). 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. flexeneri,* 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 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 were supplemented with DMSO and the reference antibacterial drug, imipenum, 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. glaberata*. Sabouraud dextrose agar (Oxoid, Hampshire, England) was seeded with 10^5 (cfu) ml^{-1} fungal spore suspensions and transferred to petri plates. Discs soaked in 20 cm^3 (200 μg/cm^3 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/cm^3 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 cm^3 of DMF. From this

stock solutions 500, 50 and 5 μg/cm^3 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 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 LD_{50} 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, ^{1}H-NMR, ^{13}C-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, $[M(L_1–L_4)Cl_2]$ where M = cobalt (II), nickel (II) and zinc (II) and $[Cu(L_1–L_4)]Cl_2$ (Scheme 1) (Table I). The molar conductance values (in DMSO) fell within the range 13–20 Ω^{-1} cm^2 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 Ω^{-1} cm^2 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 $[Zn(dmio)_2][Net_4]_2$ and $[Zn(dmit)_2][Net_4]_2$ 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 ν(M-S) and ν(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 ν (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).

^{1}H NMR and ^{13}C NMR Spectra. The ^{1}H NMR and ^{13}C NMR spectra of the free ligands and their Zn (II) complexes were recorded in DMSO-d_6 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) % H
1.	$[Co(fmdo)Cl_2]$ [472.03] $C_{13}H_{10}CoCl_2O_3S_4$	203–205	65	4.0	33.0 (33.4)	2.1 (2.5)
2.	$[Cu(fmdo)]Cl_2$ [476.65] $C_{13}H_{10}CuCl_2O_3S_4$	208–210	67	1.5	32.7 (32.9)	2.1 (2.2)
3.	$[Ni(fmdo)Cl_2]$ [471.79] $C_{13}H_{10}NiCl_2O_3S_4$	214–216	65	3.5	33.1 (32.8)	2.1 (2.5)
4.	$[Zn(fmdo)Cl_2]$ [478.49] $C_{13}H_{10}ZnCl_2O_3S_4$	205–207	67	Dia	32.6 (32.2)	2.1 (1.8)
5.	$[Co(fmdt)Cl_2]$ [488.15] $C_{13}H_{10}CoCl_2O_2S_5$	210–212	66	3.9	31.9 (32.3)	2.0 (2.5)
6.	$[Cu(fmdt)]Cl_2$ [492.8] $C_{13}H_{10}CuCl_2O_2S_5$	214–216	65	1.4	31.7 (31.9)	2.0 (2.3)
7.	$[Ni(fmdt)Cl_2]$ [487.94] $C_{13}H_{10}NiCl_2O_2S_5$	205–207	66	3.4	31.9 (31.6)	2.0 (1.7)
8.	$[Zn(fmdt)Cl_2]$ [494.64] $C_{13}H_{10}ZnCl_2O_2S_5$	208–210	65	Dia	31.5 (31.9)	2.0 (2.3)
9.	$[Co(tmdo)Cl_2]$ [504.25] $C_{13}H_{10}CoCl_2OS_6$	208–210	65	4.1	30.9 (31.3)	2.0 (2.4)
10.	$[Cu(tmdo)]Cl_2$ [508.87] $C_{13}H_{10}CuCl_2OS_6$	211–213	67	1.4	30.7 (30.4)	2.0 (1.7)
11.	$[Ni(tmdo)Cl_2]$ [504.01] $C_{13}H_{10}NiCl_2OS_6$	208–210	65	3.4	30.9 (31.3)	2.0 (2.4)
12.	$[Zn(tmdo)Cl_2]$ [510.71] $C_{13}H_{10}ZnCl_2OS_6$	212–21	67	Dia	30.5 (30.2)	2.0 (2.3)
13.	$[Co(tmdt)Cl_2]$ [520.32] $C_{13}H_{10}CoCl_2S_7$	205–207	66	4.0	29.9 (29.5)	1.9 (1.6)
14.	$[Cu(tmdt)]Cl_2$ [524.94] $C_{13}H_{10}CuCl_2S_7$	210–212	65	1.7	29.7 (29.9)	1.9 (2.2)
15.	$[Ni(tmdt)Cl_2]$ [520.08] $C_{13}H_{10}NiCl_2S_7$	202–204	66	3.2	30.1 (30.5)	1.9 (1.5)
16.	$[Zn(tmdt)Cl_2]$ [526.78] $C_{13}H_{10}ZnCl_2S_7$	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_2S], 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_2S], 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_2S], 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_2S], 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 $^{4}T_{1g} \rightarrow {}^{4}T_{2g}(F)$, $^{4}T_{1g} \rightarrow {}^{3}A_{2g}(F)$ and $^{4}T_{1g} \rightarrow {}^{4}T_{1g}(P)$ 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 $^{2}B_{1g} \rightarrow {}^{2}A_{1g}$ and $^{2}B_{1g} \rightarrow {}^{2}E_{g}$ 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 $^{3}A_{2g}(F) \rightarrow {}^{3}T_{2g}(F)(\nu_1)$, $^{3}A_{2g}(F) \rightarrow {}^{3}T_{1g}(F)(\nu_2)$ and $^{3}A_{2g}(F) \rightarrow {}^{3}T_{2g}(P)(\nu_3)$ 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 → 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. flexenari, 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 imipenum. 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 imipenum

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. glaberata* 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 $1.9651x10^{-8}$– $1.9651x10^{-7}$ 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 (L_1-L_4) and its Metal Complexes (1-16) for Antibacterial Activity (zone of inhibition in mm).

No. Ligands/Complexes	**Microbial species**					
	E.coli	*P.aeruginosa*	*S.typhi*	*S.flexneri*	*B.subtilis*	*S.aureus*
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
Imipenum	27	27	28	27	28	30

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

Table IV. Primary Screening Data of the Ligands (L_1-L_4) and its Metal Complexes (1-16) for Antifungal Activity (% inhibition).

	Microbial species					
No. Ligands/Complexes	*T. longifusus*	*C. albicans*	*A. flavus*	*M. canis*	*F. solani*	*C. glaberata*
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)**
E. coli	2.0899×10^{-8}	2.0216×10^{-8}	1.9651×10^{-8}	4.8951×10^{-8}	4.7458×10^{-8}
S. typhi	2.0899×10^{-8}	$>2.0216 \times 10^{-7}$	$>1.9651 \times 10^{-7}$	4.8951×10^{-8}	9.4916×10^{-8}
B. subtilis	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 lipoid layer of the micro-organism [29–32] thus making the chelate compounds more bacteriostatic and fungistatic.

Table VI. Brine Shrimp Bioassay Data of Ligands (L^1-L^4) 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.

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