Journal of Enzyme Inhibition and Medicinal Chemistry, April 2006; 21(2): 193–201

Taylor & Francis
Taylor & Francis Group

Antibacterial cobalt (II), copper (II), nickel (II) and zinc (II) complexes of mercaptothiadiazole—derived furanyl, thienyl, pyrrolyl, salicylyl and pyridinyl Schiff bases

ZAHID H. CHOHAN[1,†], HUMAYUN PERVEZ[1], ABDUL RAUF[2], KHALID M. KHAN[3], & CLAUDIU T. SUPURAN[4]

[1]*Department of Chemistry, Bahauddin Zakariya University, Multan 60800, Pakistan,* [2]*Department of Chemistry, Islamia University, Bahawalpur, Pakistan,* [3]*HEJ Research Institute of Chemistry, International Centre for Chemical Sciences, University of Karachi, Karachi 75270, Pakistan, and* [4]*Dipartimento di Chimica, University of Florence, Laboratorio di Chimica Bioinorganica, Via della Lastruccia, Rm. 188, Polo Scientifico, Sesto Fiorentino, Firenze 50019, Italy*

(Received 15 July 2005; in final form 7 September 2005)

Abstract

A series of Co (II), Cu (II), Ni (II) and Zn (II) complexes of mercaptothiadiazole—derived furanyl, thienyl, pyrrorlyl, salicylyl and pyridinyl Schiff bases were 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 aureous* bacterial strains. The results of these studies show the metal complexes to be more antibacterial as compared to the prepared un-complexed Schiff bases.

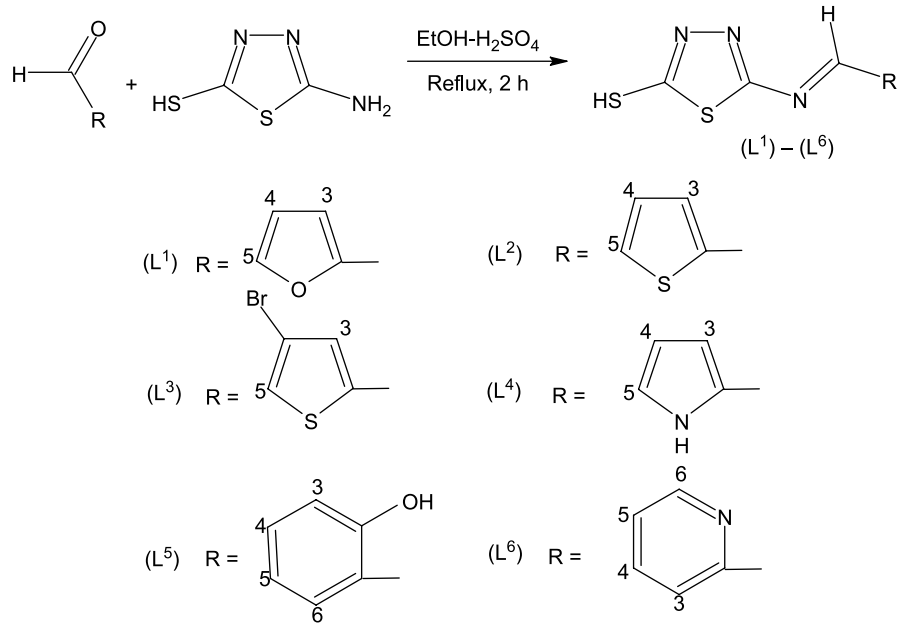
Keywords: *Mercaptothiadiazoles, metal complexes, antibacterial, organometallic compounds*

Introduction

DNA is a main target for the therapeutic treatment of various disorders and diseases. It can bind/interact with many biomolecules [1] and synthetic compounds, including proteins, polyamines, metal complexes, organometallic compounds and antibacterial/antifungal/antiviral agents [2]. Several medicinally important compounds are also excellent binding agents [3,4]. Therefore, investigations of the interaction of some antibacterial compounds with transition metals can further provide and/or improve our understanding about rational metal-based drug design chemistry. This demand is driven by an emerging medical problem of bacterial drug resistance to presently available antibiotics associated with an accelerating rate at which bacteria develop resistance [5]. This resistance is spreading alarmingly among Gram-positive as well as Gram-negative organisms [6,7]. In order to address this problem, we have commenced a program [8–15] for synthesizing classes of bactericidal and fungicidal complexes of transition metals, which could potentially reduce (interfere with) the mechanism of bacterial resistance via coordination of the metal ion (s) [16–20]. Mercaptothiadiazoles are amongst such classes of compounds already known to be associated with diverse pharmacological activities [21–23]. In continuation of this approach the present paper describes the synthesis, characterization and *in vitro* evaluation of the antibacterial activity of newly synthesized Co (II), Cu (II), Ni (II) and Zn (II) complexes (**1**)–(**24**) with the Schiff

Correspondence: C. T. Supuran, Dipartmento di Chimica, University of Florence, Laboratorio di Chimica Bioinorganica, Via della Lastruccia, Room 188, Polo Scientifico, Sesto Fiorentino, Firenze 50019, Italy. E-mail: claudiu.supuran@unifi.it
†E-mail: zchohan@ mul.paknet.com.pk

ISSN 1475-6366 print/ISSN 1475-6374 online © 2006 Taylor & Francis
DOI: 10.1080/14756360500397505



Scheme 1. Synthesis and structures of ($\mathbf{L}^1$)–($\mathbf{L}^6$).

bases ($\mathbf{L}^1$)–($\mathbf{L}^6$) obtained from the reaction of 5-amino-1,3,4-thiadiazole-2-thiol with furan-2-carboxaldehyde, thiophene-2-carboxaldehyde, 4-bromothiophene-2-carboxaldehyde, pyrrole-2-carboxaldehyde, salicylaldehyde and pyridine-2-carboxaldehyde, respectively. The structures of all the synthesized compounds were confirmed by elemental analysis, IR, NMR, UV–Visible, magnetic moment and conductance measurements. All the compounds were screened for bactericidal 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. Most compounds possessing significant activity against one or more bacterial strains.

Material and methods

Solvents used were analar grade. All metal (II) salts were used as chlorides. Thin-layer chromatography was performed using aluminium sheets (Merck) coated with silica gel 60 F_{254}. IR spectra (KBr pellets) were recorded on a Hitachi Model 200–50 IR spectrophotometer. NMR were recorded in d_6-DMSO on Bruker AM 300 and AM 400 spectrometers (Rheinstetten—Forchheim, Germany) operating at 300 and 400 MHz, respectively. CHN analyses were carried out using an Elemental Analyzer Flash EA 1112. Conductance of the metal complexes was measured on conductivity meter 4071, Jenway (USA). Magnetic susceptibility measurements of the metal complexes in the solid state were determined by a Gouy's balance at room temperature. Melting points were recorded on a Gallenkamp apparatus and are uncorrected.

Synthesis

General procedure for the preparation of ligands ($\boldsymbol{L}^1$)–($\boldsymbol{L}^6$). To a hot stirred solution of 5-amino-1,3,4-thiadiazole-2-thiol (0.01 mol) in ethanol (30–40 ml) was added the appropriate aldehyde (0.01 mol) and 2–3 drops of concentrated sulphuric acid (see Scheme 1) and the resultant mixture heated under reflux for 2 h. The reaction mixture was reduced to 10–15 ml and kept at room temperature for 0.5 h. Addition of the same to chilled water and stirring for a few minutes resulted in formation of a solid product which was collected by suction filtration. Recrystallization from aqueous ethanol furnished the required pure product. The compounds were characterized as given below:

5-[(2-Furylmethylidene) amino]-1, 3, 4-thiadiazole-2-thiol ($\boldsymbol{L}^1$). Yield 75% as yellow powder; m.p. 170–172°C; IR (KBr, cm^{-1}): 2590 (SH), 1635 (HC=N), 1620 (C=N, thiadiazole). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.87 (s,1H, SH), 6.29 (s, 1H,CH=N), 6.66 (dd,1H, $\mathcal{J} = 3.63$, 1.82 Hz, furanyl C_4-H), 6.91 (d,1H, $\mathcal{J} = 3.63$ Hz, furanyl C_3-H), 7.44 (d,1H, $\mathcal{J} = 1.82$ Hz, furanyl C_5-H). Anal. Calcd. for $C_7H_5N_3OS_2$: C, 39.8; H, 2.4; N, 19.9. Found: C, 40.0; H, 2.0; N, 19.5%.

5-[(2-Thienylmethylidene) amino]-1, 3, 4-thiadiazole-2-thiol ($\boldsymbol{L}^2$). Yield 80% as bright yellow powder; m.p. 184–186°C; IR (KBr, cm^{-1}): 2585 (SH),1635 (HC=N), 1620 (C=N, thiadiazole). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.88 (s,1H, SH), 6.33 (s, 1H,CH=N), 7.16 (dd,1H, $\mathcal{J} = 4.76$, 3.83 Hz, thienyl C_4-H),7.18 (d,1H, $\mathcal{J} = 4.76$ Hz, thienyl C_5-H), 7.25

(d,1H, $\mathcal{J} = 3.83$ Hz, thienyl C_3-H). Anal. Calcd. for $C_7H_5N_3S_3$: C, 37.0; H, 2.2; N, 18.5. Found: C, 37.3; H, 2.4; N, 18.9%.

*5-{[(4-Bromo-2-thienyl)methylidene]amino}-1,3,4-thiadiazole-2-thiol (**L**^{3})*. Yield 80% as yellow powder; m.p. 194–196°C; IR (KBr, cm^{-1}): 2585 (SH), 1640 (HC=N), 1615 (C=N, thiadiazole). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.89 (s,1H, SH), 6.31 (s, 1H,CH = N), 7.51 (s, thienyl C_5-H), 7.63 (s,1H, thienyl C_3-H). Anal. Calcd. for $C_7H_4BrN_3S_3$: C, 27.5; H, 1.3; N, 13.7. Found: C, 27.9; H, 1.0; N, 13.9%.

*5-[(1H-Pyrrol-2-yl methylidene) amino]-1, 3, 4-thiadiazole-2-thiol (**L**^{4})*. Yield 60% as yellow powder; m.p. 218–220°C; IR (KBr, cm^{-1}): 3215 (NH, pyrrole), 2585 (SH),1635 (HC=N), 1620 (C=N, thiadiazole). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.86 (s,1H, SH), 5.92 (dd,1H, $\mathcal{J} = 3.96$, 2.89 Hz, pyrrolyl, C_4-H), 6.26 (s, 1H,CH=N), 6.61 (d,1H, $\mathcal{J} = 2.89$ Hz, pyrrolyl C_5-H), 6.94 (d,1H, $\mathcal{J} = 3.96$ Hz, pyrrolyl C_3-H), 9.77 (s, 1H, NH). Anal. Calcd. for $C_7H_6N_4S_2$: C, 40.0; H, 2.9; N, 26.6. Found. C, 40.4; H, 2.5; N, 26.9%.

*5-{[(2-Hydroxyphenyl) methylidene]amino}-1,3,4-thiadiazole-2-thiol (**L**^{5})*. Yield 80% as bright yellow powder; m.p. 248–250°C; IR (KBr, cm^{-1}): 3455(OH), 2580 (SH), 1640 (HC=N), 1615 (C=N, thiadiazole). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.88 (s,1H, SH), 6.88 (ddd, 1H, $\mathcal{J} = 7.72$, 7.57, 2.12 Hz, phenol C_5-H), 6.98 (dd, 1H, $\mathcal{J} = 7.42$, 2.12 Hz, phenol C_3-H), 7.18 (ddd, 1H, $\mathcal{J} = 7.72$, 7.42, 2.14 Hz, phenol C_4-H), 7.55 (dd, 1H, $\mathcal{J} = 7.57$, 2.14 Hz, phenol C_6-H), 7.75 (s, 1H, CH=N), 9.55 (s, 1H, OH). Anal. Calcd. for $C_9H_7N_3OS_2$: C, 45.6; H, 3.0; N,17.7. Found: C, 45.9; H, 2.8; N, 17.4%.

*5-[(2-Pyridinylmethylidene) amino]-1, 3, 4-thiadiazole-2-thiol (**L**^{6})*. Yield 63% as yellow powder; m.p. 146–148°C; IR (KBr, cm^{-1}): 2590 (SH), 1635 (HC=N), 1615 (C=N mercaptothiadiazole), 1610 (C=N, pyridine). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.93 (s,1H, SH), 7.29 (ddd, 1H, $\mathcal{J} = 7.71$, 5.18, 1.21 Hz, pyridinyl C_5-H), 7.75 (dd, 1H, $\mathcal{J} = 7.88$, 1.21 Hz, pyridinyl C_3-H), 7.92 (ddd, 1H, $\mathcal{J} = 7.88$, 7.71, 1.92 Hz, pyridinyl C_4-H), 8.35 (dd, 1H, $\mathcal{J} = 5.18$, 1.92 Hz, pyridinyl C_6-H), 8.87 (s, 1H, CH=N). Anal. Calcd. for $C_8H_6N_4S_2$: C, 43.2; H, 2.7; N, 25.2. Found: C, 43.6; H, 2.3; N, 24.9%.

*General procedure for the preparation of metal complexes of ligands (**L**^{1})–(**L**^{6})*. To a hot stirred solution of an appropriate ligand (0.02 mol) in ethanol (15–20 ml) was added the solution of metal (II) chloride (0.01 mol) made in warm ethanol (10 ml) and the resultant mixture refluxed for 1 h. The solid formed during refluxing or upon cooling was collected by suction filtration. Thorough washing with hot ethanol followed by ether or recrystallization from aqueous ethanol gave purified products.

Cobalt (II) 5-[(2-furylmethylidene) amino]-1,3,4-thiadiazole-2-thiol (1). Yield 60% as bluish-purple powder; m.p. (decomp.) 215–217°C; IR (KBr): ν_{max} cm^{-1} 2585 (SH), 1625 (HC=N), 1615 (C=N, thiadiazole), 525 (M–N), 455 (M–O); UV (DMSO): λ_{max} (cm^{-1}) 30210, 17590 and 8620; B.M. (μ_{eff}) 4.2; Molar conductance (90 $ohm^{-1}\,cm^{-2}\,mol^{-1}$). Anal. Calcd. for $C_{14}H_{10}CoCl_2N_6O_2S_4$: C, 30.4; H, 1.8; N, 15.2. Found: C, 30.8; H, 1.5; N, 15.7%.

Copper (II) 5-[(2-furylmethylidene) amino]-1,3,4-thiadiazole-2-thiol (2). Yield 58% as dirty green powder; m.p. (decomp.) 223–225°C; IR (KBr): ν_{max} cm^{-1} 2585 (SH), 1625 (HC=N), 1610 (C=N, thiadiazole), 520 (M–N), 460 (M–O); UV (DMSO): λ_{max} (cm^{-1}) 30515 and 22280; B.M. (μ_{eff}) 1.4; Molar conductance (92 $ohm^{-1}\,cm^{-2}\,mol^{-1}$). Anal. Calcd. for $C_{14}H_{10}CuCl_2N_6O_2S_4$: C, 30.2; H, 1.8; N, 15.1. Found: C, 30.5; H, 1.5; N, 15.5%.

Nickel (II) 5-[(2-furylmethylidene) amino]-1,3,4-thiadiazole-2-thiol (3). Yield 60% as green powder; m.p. (decomp.) 218–220°C; IR (KBr): ν_{max} cm^{-1} 2580 (SH), 1620 (HC=N), 1610 (C=N, mercapto-thiadiazole), 525 (M–N), 455 (M–O); UV (DMSO): λ_{max} (cm^{-1}) 29130, 15820 and 9740; B.M. (μ_{eff}) 3.3; Molar conductance (97 $ohm^{-1}\,cm^{-2}\,mol^{-1}$). Anal. Calcd. for $C_{14}H_{10}NiCl_2N_6O_2S_4$: C, 30.5; H, 1.8; N, 15.2. Found: C, 30.2; H, 1.7; N, 15.4%.

Zinc (II) 5-[(2-furylmethylidene) amino]-1,3,4-thiadiazole-2-thiol (4). Yield 58% as light yellow powder; m.p. (decomp.) 213–215°C; IR (KBr): ν_{max} cm^{-1} 2585 (SH), 1625 (HC=N), 1610 (C=N, thiadiazole), 525 (M–N), 455 (M–O); UV (DMSO):λ_{max} (cm^{-1}) 28590; B.M. (μ_{eff}) Diamagnetic; Molar conductance (97 $ohm^{-1}\,cm^{-2}\,mol^{-1}$). Anal. Calcd. for $C_{14}H_{10}ZnCl_2N_6O_2S_4$: C, 30.1; H, 1.8; N, 15.0. Found: C, 30.5; H, 1.6; N, 14.7%.

Cobalt (II) 5-[(2-thienylmethylidene) amino]-1,3,4-thiadiazole-2-thiol (5). Yield 58% as light pink powder; m.p. (decomp.) 216–218°C; IR (KBr): ν_{max} cm^{-1} 2580 (SH), 1625 (HC=N), 1610 (C=N, thiadiazole), 525 (M–N), 360 (M–S); UV (DMSO):λ_{max} (cm^{-1}) 30480, 18225 and 8760; B.M. (μ_{eff}) 4.6; Molar conductance (92 $ohm^{-1}\,cm^{-2}\,mol^{-1}$). Anal. Calcd. for $C_{14}H_{10}CoCl_2N_6S_6$: C, 28.8; H, 1.7; N, 14.4. Found: C, 28.4; H, 1.4; N, 14.8%.

Copper (II) 5-[(2-thienylmethylidene) amino]-1,3,4-thiadiazole-2-thiol (6). Yield 57% as greenish powder; m.p. (decomp.) 212–214°C; IR (KBr): ν_{max} cm^{-1} 2580 (SH), 1620 (HC=N), 1605 (C=N, thiadiazole), 525 (M–N), 365 (M–S); UV (DMSO): λ_{max} (cm^{-1})

30620 and 22370; B.M. (μ_{eff}) 1.5; Molar conductance (88 $ohm^{-1}\,cm^{-2}\,mol^{-1}$). Anal. Calcd. for $C_{14}H_{10}CuCl_2N_6S_6$: C, 28.5; H, 1.7; N, 14.3. Found: C, 28.8; H, 1.6; N, 14.8%.

Nickel (II) 5-[(2-thienylmethylidene) amino]-1,3,4-thiadiazole-2-thiol (7). Yield 58% as light green powder; m.p. (decomp.) 220–222°C; IR (KBr): ν_{max} cm^{-1} 2585 (SH), 1625 (HC=N), 1610 (C=N, thiadiazole), 530 (M–N), 365 (M–S); UV (DMSO): λ_{max} (cm^{-1}) 28420, 16225 and 10130; B.M. (μ_{eff}) 3.2; Molar conductance (96 $ohm^{-1}\,cm^{-2}\,mol^{-1}$). Anal. Calcd. for $C_{14}H_{10}NiCl_2N_6S_6$: C, 28.8; H, 1.7; N, 14.4. Found: C, 28.6; H, 2.1; N, 14.7%.

Zinc (II) 5-[(2-thienylmethylidene) amino]-1,3,4-thiadiazole-2-thiol (8). Yield 60% as light yellow powder; m.p. (decomp.) 214–216°C; IR (KBr): ν_{max} cm^{-1} 2580 (SH), 1620 (HC=N), 1610 (C=N, mercaptothiadiazole), 525 (M–N), 360 (M–S); UV (DMSO): λ_{max} (cm^{-1}) 28570; B.M. (μ_{eff}) Diamagnetic; Molar conductance (95 $ohm^{-1}\,cm^{-2}\,mol^{-1}$). Anal. Calcd. for $C_{14}H_{10}ZnCl_2N_6S_6$: C, 28.5; H, 1.7; N, 14.2. Found: C, 28.4; H, 1.9; N, 14.5%.

Cobalt (II) 5-{[(4-bromo-2-thienyl)methylidene] amino}-1,3,4-thiadiazole-2-thiol (9). Yield 58% as light pink powder; m.p. (decomp.) 220–222°C; IR (KBr): ν_{max} cm^{-1} 2580 (SH), 1625 (HC=N), 1605 (C=N, thiadiazole), 525 (M–N), 360 (M–S); UV (DMSO): λ_{max} (cm^{-1}) 30420, 18190 and 8755; B.M. (μ_{eff}) 4.5; Molar conductance (94 $ohm^{-1}\,cm^{-2}\,mol^{-1}$). Anal. Calcd. for $C_{14}H_8Co\,Br_2Cl_2N_6S_6$: C, 22.6; H, 1.1; N, 11.3. Found: C, 22.9; H, 1.5; N, 11.0%.

Copper (II) 5-{[(4-bromo-2-thienyl)methylidene] amino}-1,3,4-thiadiazole-2-thiol (10). Yield 63% as dark brown powder; m.p. (decomp.) 222–224°C; IR (KBr): ν_{max} cm^{-1} 2585 (SH), 1625 (HC=N), 1610 (C=N, thiadiazole), 530 (M–N), 365 (M–S); UV (DMSO): λ_{max} (cm^{-1}) 30610 and 22360; B.M. (μ_{eff}) 1.4; Molar conductance (96 $ohm^{-1}\,cm^{-2}\,mol^{-1}$). Anal. Calcd. for $C_{14}H_8Cu\,Br_2Cl_2N_6S_6$: C, 22.5; H, 1.1; N, 11.3. Found: C, 22.9; H, 1.4; N, 11.0%.

Nickel (II) 5-{[(4-bromo-2-thienyl)methylidene] amino}-1,3,4-thiadiazole-2-thiol (11). Yield 58% as light green powder; m.p. (decomp.) 217–219°C; IR (KBr): ν_{max} cm^{-1} 2580 (SH), 1625 (HC=N), 1605 (C=N, mercaptothiadiazole), 525 (M–N), 360 (M–S); UV (DMSO): λ_{max} (cm^{-1}) 28430, 16235 and 10155; B.M. (μ_{eff}) 3.4; Molar conductance (90 $ohm^{-1}\,cm^{-2}\,mol^{-1}$). Anal. Calcd. for $C_{14}H_8Ni\,Br_2Cl_2N_6S_6$: C, 22.7; H, 1.1; N, 11.3. Found: C, 22.3; H, 1.5; N, 11.1%.

Zinc (II) 5-{[(4-bromo-2-thienyl)methylidene]amino}-1,3,4-thiadiazole-2-thiol (12). Yield 60% as off white powder m.p. (decomp.) 218–220°C; IR (KBr): ν_{max} cm^{-1} 2585 (SH), 1620 (HC=N), 1605 (C=N, thiadiazole), 530 (M–N), 360 (M–S); UV (DMSO): λ_{max} (cm^{-1}) 28580; B.M. (μ_{eff}) Diamagnetic; Molar conductance (98 $ohm^{-1}\,cm^{-2}\,mol^{-1}$). Anal. Calcd. for $C_{14}H_8Zn\,Br_2Cl_2N_6S_6$: C, 22.5; H, 1.1; N, 11.2. Found: C, 22.7; H, 1.0; N, 11.0%.

Cobalt (II) 5-[(1H-pyrrol-2-yl methylidene) amino]-1,3,4-thiadiazole-2-thiol (13). Yield 59% as orange powder m.p. (decomp.) 232–234°C; IR (KBr): ν_{max} cm^{-1} 2580 (SH), 1620 (HC=N), 1605 (C=N, thiadiazole), 525 (M–N), 540 (M–N); UV (DMSO): λ_{max} (cm^{-1}) 30280, 18175 and 8865; B.M. (μ_{eff}) 4.5; non-electrolyte. Anal. Calcd. for $C_{14}H_{10}CoN_8S_4$: C, 35.2; H, 2.1; N, 23.5. Found: C, 35.0; H, 2.5; N, 23.1%.

Copper (II) 5-[(1H-pyrrol-2-yl methylidene) amino]-1,3,4-thiadiazole-2-thiol (14). Yield 65% as dark green powder; m.p. (decomp.) 258–260°C; IR (KBr): ν_{max} cm^{-1} 2585 (SH), 1625 (HC=N), 1610 (C=N, mercaptothiadiazole), 520 (M–N), 540 (M–N), UV (DMSO): λ_{max} (cm^{-1}) 30630 and 22340; B.M. (μ_{eff}) 1.3; non-electrolyte. Anal. Calcd. for $C_{14}H_{10}CuN_8S_4$: C, 34.8; H, 2.1; N, 23.2. Found: C, 35.3; H, 2.0; N, 23.6%.

Nickle (II) 5-[(1H-pyrrol-2-yl methylidene) amino]-1,3,4-thiadiazole-2-thiol (15). Yield 60% as light green powder; m.p. (decomp.) 228–230°C; IR (KBr): ν_{max} cm^{-1} 2580 (SH), 1620 (HC=N), 1605 (C=N, thiadiazole), 525 (M–N), 535 (M–N); UV (DMSO): λ_{max} (cm^{-1}) 28585 and, 16180 and 9860; B.M. (μ_{eff}) 3.4; non-electrolyte. Anal. Calcd. for $C_{14}H_{10}NiN_8S_4$: C, 35.2; H, 2.1; N, 23.5. Found: C, 35.6; H, 2.0; N, 23.8%.

Zinc (II) 5-[(1H-pyrrol-2-yl methylidene) amino]-1,3,4-thiadiazole-2-thiol (16). Yield 61% as off white powder; m.p. (decomp.) 240–242°C; IR (KBr): ν_{max} cm^{-1} 2585 (SH), 1620 (HC=N), 1610 (C=N, thiadiazole), 525 (M–N), 535 (M–N), UV (DMSO): λ_{max} (cm^{-1}) 28535; B.M. (μ_{eff}) Diamagnetic; non-electrolyte. Anal. Calcd. for $C_{14}H_{10}ZnN_8S_4$: C, 34.7; H, 2.1; N, 23.1. Found: C, 34.5; H, 2.6; N, 23.4%.

Cobalt (II) 5-{[(2-Hydroxyphenyl)methylidene] amino}-1,3,4-thiadiazole-2-thiol (17). Yield 59% as orange powder; m.p. (decomp.) 268–270°C; IR (KBr): ν_{max} cm^{-1} 2575 (SH), 1630 (HC=N), 1605 (C=N, thiadiazole), 1380 (C–O), 520 (M–N), 445 (M–O); UV (DMSO): λ_{max} (cm^{-1}) 30355, 17890 and 8895; B.M. (μ_{eff}) 4.4; non-electrolyte. Anal. Calcd. for $C_{18}H_{12}CoN_6\,O_2\,S_4$: C, 40.7; H, 2.3; N, 15.8. Found: C, 40.3; H, 2.5; N, 16.1%.

Copper (II) 5-{[(2-Hydroxyphenyl)methylidene] amino}-1,3,4-thiadiazole-2-thiol (18). Yield 61% as dirty green powder; m.p. (decomp.) 265–267°C; IR (KBr): ν_{max} cm^{-1} 2575 (SH), 1625 (HC=N), 1610 (C=N, thiadiazole), 1380 (C–O), 520 (M–N),

440 (M–O); UV (DMSO): λ_{max} (cm^{-1}) 30580 and 22325; B.M. (μ_{eff}) 1.4; non-electrolyte. Anal. Calcd. for $C_{18}H_{12}CuN_6O_2S_4$: C, 40.3; H, 2.2; N, 15.7. Found: C, 40.6; H, 2.5; N, 15.4%.

Nickle (II) 5-{[(2-Hydroxyphenyl)methylidene] amino}-1,3,4-thiadiazole-2-thiol (19). Yield 60% as light green powder; m.p. (decomp.) 266–268°C; IR (KBr): ν_{max} cm^{-1} 2580 (SH),1620 (HC=N), 1610 (C=N, thiadiazole), 1380 (C–O), 525 (M–N), 450 (M–O); UV (DMSO): λ_{max} (cm^{-1}) 28570, 16220 and 9870; B.M. (μ_{eff}) 3.3; non-electrolyte. Anal. Calcd. for $C_{18}H_{12}NiN_6O_2S_4$: C, 40.7; H, 2.3; N, 15.8. Found: C, 41.0; H, 2.2; N, 15.4%.

Zinc (II) 5-{[(2-Hydroxyphenyl)methylidene]amino}-1,3,4-thiadiazole-2-thiol (20). Yield 61% as light yellow powder; m.p. (decomp.) 263–265°C; IR (KBr): ν_{max} cm^{-1} 2580 (SH), 1630 (HC=N), 1605 (C=N, thiadiazole), 1380 (C–O), 525 (M–N), 445 (M–O); UV (DMSO): λ_{max} (cm^{-1}) 28565; B.M. (μ_{eff}) Diamagnetic; non-electrolyte. Anal. Calcd. for $C_{18}H_{12}ZnN_6O_2S_4$: C, 40.2; H, 2.2; N, 15.6. Found: C, 39.9; H, 2.5; N, 15.9%.

Cobalt (II) 5-[(2-pyridinylmethylidene) amino]-1, 3,4-thiadiazole-2-thiol (21). Yield 59% as orange red powder; m.p. (decomp.) 202–204°C; IR (KBr): ν_{max} cm^{-1} 2585 (SH), 1620 (HC=N), 1610 (C=N, thiadiazole), 1605 (C=N, pyridine), 525 (M–N), 515 (M–N); UV (DMSO): λ_{max} (cm^{-1}) 30380, 17825 and 8875; B.M. (μ_{eff}) 4.4; Molar conductance (97 $ohm^{-1}cm^{-2}mol^{-1}$). Anal. Calcd. for $C_{16}H_{12}CoCl_2N_8S_4$: C, 33.5; H, 2.1; N, 19.5. Found: C, 33.8; H, 2.6; N, 19.7%.

Copper (II) 5-[(2-pyridinylmethylidene) amino]-1, 3,4-thiadiazole-2-thiol (22). Yield 60% as dark green powder; m.p. (decomp.) 206–208°C; IR (KBr): ν_{max} cm^{-1} 2585 (SH), 1615 (HC=N), 1610 (C=N, thiadiazole), 1600 (C=N, pyridine), 520 (M–N), 510 (M–N); UV (DMSO): λ_{max} (cm^{-1}) 30595 and, 22350; B.M. (μ_{eff}) 1.4; Molar conductance (94 $ohm^{-1}cm^{-2}mol^{-1}$). Anal. Calcd. for $C_{16}H_{12}CuCl_2N_8S_4$: C, 33.2; H, 2.1; N, 19.4. Found: C, 33.6; H, 2.4; N, 19.8%.

Nickle (II) 5-[(2-pyridinylmethylidene) amino]-1, 3,4-thiadiazole-2-thiol (23). Yield 60% as light green powder; m.p. (decomp.) 201–203°C; IR (KBr): ν_{max} cm^{-1} 2585 (SH), 1620 (HC=N), 1610 (C=N, thiadiazole), 1605 (C=N, pyridine), 525 (M–N), 510 (M–N); UV (DMSO): λ_{max} (cm^{-1}) 28555, 16195 and 9865; B.M. (μ_{eff}) 3.3; Molar conductance (97 $ohm^{-1}cm^{-2}mol^{-1}$). Anal. Calcd. for $C_{16}H_{12}NiCl_2N_8S_4$: C, 33.5; H, 2.1; N, 19.5. Found: C, 33.1; H, 2.5; N, 19.1%.

Zinc (II) 5-[(2-pyridinylmethylidene) amino]-1, 3,4-thiadiazole-2-thiol (24). Yield 61% as pale yellow powder; m.p. (decomp.) 198–200°C; IR (KBr): ν_{max} cm^{-1} 2585 (SH), 1620 (HC=N), 1610 (C=N, thiadiazole), 1605 (C=N, pyridine), 525 (M–N), 515 (M–N); UV (DMSO): λ_{max} (cm^{-1}) 28575; B.M. (μ_{eff}) Diamagnetic; Molar conductance (95 $ohm^{-1}cm^{-2}mol^{-1}$). Anal. Calcd. for $C_{16}H_{12}ZnCl_2N_8S_4$: C, 33.1; H, 2.1; N, 19.3. Found: C, 33.3; H, 2.5; N, 19.0%.

Antibacterial bioassay

Preliminary screening. The synthesized compounds ($\mathbf{L^1}$)–($\mathbf{L^6}$) and their corresponding metal (II) complexes **(1)–(24)** 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 (Bacillus *subtilis* and *S. aureus*) bacterial strains by the agar-well diffusion method [24]. The wells (6 mm in diameter) were dug in the media with the help of a sterile metallic borer with centres 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 using a sterile cotton swab. The recommended concentration of the test sample (1 mM/ml in DMSO) was introduced in the respective wells. Other wells supplemented with DMSO and reference antibacterial drug, imipenum, served as negative and positive controls, respectively. The plates were incubated immediately at 37°C for 24 h. Activity was determined by measuring the diameter (mm) of zones showing complete inhibition. Growth inhibition was calculated with reference to a positive control.

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 by preparing discs containing 10, 25, 50 and 100 mM/ml of the compounds and applying the reported protocol [25].

Results and discussion

Chemistry

The Schiff bases ($\mathbf{L^1}$)–($\mathbf{L^6}$), were prepared by refluxing equimolar (0.01 mol) quantities of 5-amino-1,3,4-thiadiazole-2-thiol and the respective aldehydes for 2 hours in ethanol (30–40 ml) containing a few drops of conc. H_2SO_4 (Scheme 1). All the synthesized Schiff bases were characterized by spectroscopic techniques (IR and ^{1}H-NMR) and their elemental analyses.

The metal complexes (**1**)–(**24**) of the Schiff bases ($\mathbf{L^1}$)–($\mathbf{L^6}$) were air stable and prepared by the

stoichiometric reaction of the corresponding metal (II) chlorides with the Schiff base ligands in a molar ratio (M:L) of 1:2. These complexes are intensely coloured amorphous solids except those of zinc (which are light yellow in colour) and decompose without melting. They are insoluble in common organic solvents such as ethanol, methanol, chloroform or acetone but are soluble in DMSO and DMF. Molar conductance values ($88-98\ ohm^{-1}\ cm^{-2}\ mol^{-1}$) of the complexes (**1**)–(**12**) and (**21**)–(**24**) of cobalt (II), copper (II), nickel (II) and zinc (II), soluble in DMF (10^{-3} M solution at 25°C), indicated that they are electrolytic in nature; however, the metal complexes (**13**)–(**20**), are found to be non electrolytic in nature [26]. The elemental analysis data agree well with the proposed formulae for the ligands and the $[M(L)_2]Cl_2$ and $M(L)_2$ composition of the metal (II) complexes.

IR spectra. The IR spectra of all the Schiff bases ($\mathbf{L}^1$)–($\mathbf{L}^6$) showed the absence of bands at 1735 and 3420 cm^{-1} due to carbonyl ν(C=O) and ν (NH_2) stretching vibrations, respectively (present in the starting materials) and instead, a strong new band at $\sim 1635-1640^{-1}$ assigned to azomethine ν(HC=N) vibrations appeared indicating condensation of the starting carbonyl moieties with the amino group containing heteroaromatic systems [27]. The IR spectra of all the ligands exhibited the azomethine (HC=N) and thiadiazole stretchings in the regions 1635–1640 and 1615–1620 cm^{-1}, respectively. In addition, the spectrum of ($\mathbf{L}^4$) showed a band resulting from the NH stretching of the pyrrole moiety at 3215 cm^{-1}. The SH stretchings appeared in the 2580–2590 cm^{-1} region. The IR spectrum of the Schiff base ($\mathbf{L}^5$) showed bands resulting from the SH and OH stretchings of the thiol and phenol functions at 2580 and 3455 cm^{-1}, respectively. The azomethine (HC=N) and the thiadiazole (C=N) stretchings were observed respectively at 1640 and 1615 cm^{-1}. In the case of the Schiff base ($\mathbf{L}^6$), the SH stretching appeared at 2590 cm^{-1}, whereas the azomethine (HC=N) and thiadiazole (C=N) stretchings were observed at 1635 and 1615 cm^{-1}, respectively [28]. The pyridine (C=N) stretching appeared at 1610 cm^{-1}.

The comparison of the infrared spectra of the Schiff bases ($\mathbf{L}^1$)–($\mathbf{L}^6$) with their metal complexes (**1**)–(**24**) principally revealed that the ligands are tridentately coordinated to the metal ions. The band appearing at $\sim 1635-1640\ cm^{-1}$ due to the azomethine stretching vibration is shifted to lower frequency by $\sim 10-15\ cm^{-1}$ indicating participation of the azomethine nitrogen in complexation. The band at $\sim 1615-1620\ cm^{-1}$ assigned to the thiadiazole ring ν(C=N) vibrations is also shifted to lower frequency by $\sim 10-15\ cm^{-1}$, which is indicative of the involvement of the thiadiazole ring in chelation. Also, a band at $\sim 3215\ cm^{-1}$ attributed to νNH in the ligand ($\mathbf{L}^4$) disappeared in its metal complexes (**13**)–(**16**) indicating deprotonation of the NH moiety during coordination [29]. Also, a band at $\sim 3455\ cm^{-1}$ attributed to νOH in the ligand ($\mathbf{L}^5$) disappeared in its metal complexes (**17**)–(**20**) and instead appearance of a (C–O) band at $\sim 1380\ cm^{-1}$ indicated deprotonation of the OH moiety during coordination. The band at $\sim 1610\ cm^{-1}$ assigned to the pyridine ring ν(C=N) vibrations in the ligand ($\mathbf{L}^6$) is also shifted to lower frequency by $\sim 5\ cm^{-1}$ in its metal complexes (**21**)–(**24**), which is indicative of the involvement of the N atom of the pyridine ring in chelation. However, further conclusive evidence of the coordination of the ligands with the metal atoms was established by the far IR spectra in which new bands at 360–365, 440–450, 455–460, 510–515, 520–530 and 535–540 cm^{-1} assigned to (M-S (thienyl), (M-O (deprotonated salicylyl), (M-O (furanyl), M–N (pyridyl), (M–N (thiadiazole) and (M–N (pyrrolyl) in the spectra of the metal complexes were observed, which are not present in the spectra of their corresponding ligands [29].

Magnetic moment. The room temperature magnetic moments of the solid cobalt (II) complexes were found to lie in the range 4.2–4.6 B.M indicative of three unpaired electrons per Co (II) ion in an octahedral environment. The Cu(II) complexes showed μ_{eff} values in the range 1.3–1.5 B.M indicative of one unpaired electron per Cu(II) ion, suggesting [30] that these complexes had structures within the range consistent to spin-free distorted octahedral geometry. Similarly, the Ni (II) complexes showed μ_{eff} values in the range 3.2–3.4 B.M corresponding to two unpaired electrons per Ni (II) ion for their ideal six-coordinated configuration. The Zn (II) complexes were all found to be diamagnetic.

Electronic spectra. The electronic spectra of the Co(II) complexes (**1**), (**5**), (**9**), (**13**), (**17**) and (**21**) showed three bands at 8620–8895, 17590–18225 and 30210–30480 cm^{-1}, which may be assigned to $^4T_{1g} \rightarrow {}^4T_{2g}(F)$, $^4T_{1g} \rightarrow {}^4T_{2g}(P)$ and $^4T_{1g} \rightarrow {}^3A_{2g}(F)$ transactions, respectively, and are suggestive of their octahedral geometry around the cobalt ions [31]. The Cu (II) complexes (**2**), (**6**), (**10**), (**14**), (**18**) and (**22**) showed absorption bands between 10Dq band for a distorted octahedral geometry corresponding to the transitions $^2E_g \rightarrow {}^2T_{2g}$ [32]. The Ni (II) complexes (**3**), (**7**), (**11**), (**15**), (**19**) and (**23**) exhibited three spin-allowed bands at 9740–10155, 15820–16235 and 28420–29130 cm^{-1} assignable, respectively to the transitions $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)(\nu_1)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)(\nu_2)$ and $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)(\nu_3)$, which are characteristic of Ni(II) in octahedral geometry [33].

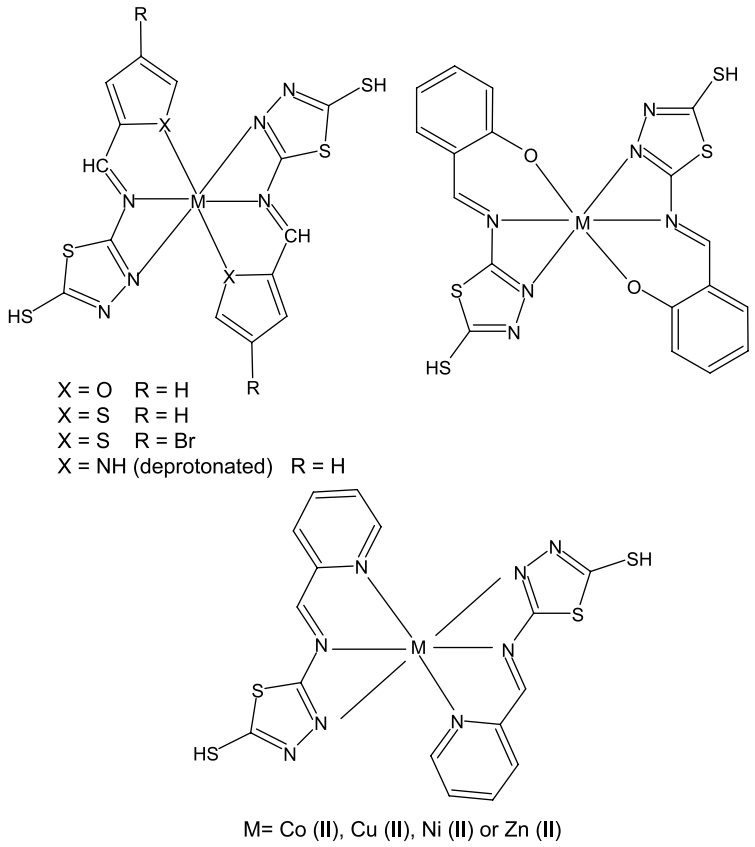


Figure 1. Proposed structural formulae of the metal (II) complexes.

The diamagnetic zinc (II) complexes did not show any d–d bands and their spectra are dominated only by charge transfer bands. The diamagnetic Zn (II) complexes (**4**), (**8**), (**12**), (**16**), (**20**) and (**24**) did not show any d–d bands and their spectra were dominated only by the charge transfer bands 28535–28590 cm^{-1} [34] (Figure 1).

Antibacterial bioassay

Preliminary screening. Antibacterial activity of the synthesized Schiff bases ($\mathbf{L}^{1}$)–($\mathbf{L}^{6}$) and their corresponding metal (II) complexes (**1**)–(**24**) was determined against four Gram-negative (*E. coli, P. aeruginosa*, *S. typhi* and *S. flexneri*) and two Gram-positive (*B. subtilis and S. aureus*) bacterial strains. The synthesized Schiff base compounds exhibited varying degree of inhibitory effects (low to moderate) on the growth of different tested strains (Table I). It is evident from the results collected in the Table that the potency of all the ligands was enhanced upon coordination with the metal ions. These results substantiate our own findings [35–42] and the findings of some other workers [43–46] that biologically inactive compounds become active and less biologically active compounds become more active upon coordination with the metal ions. The results given in the Table show that eight of the synthesized complexes i.e. (**5**)–(**6**), (**9**)–(**10**), (**12**), (**14**), (**21**)–(**22**) have comparatively much more activity and of these, (**10**) is the most active one.

Minimum inhibitory concentration (MIC). The preliminary screening showed that compounds (**5**)–(**6**), (**9**)–(**10**), (**14**) and (**22**) were the most active ones against both Gram-negative and Gram-positive organisms. These six compounds were selected for minimum inhibitory concentration (MIC) studies (Table II). The MIC values of all the six active compounds ranges from 0.013–0.207 μM. Compound (**10**) again proved to be the most active one; it inhibited the growth of *S. typhi*, *S. flexneri* and *B. subtilis* at 0.013 μM concentration.

The present investigation suggests that of all the metal complexes, those derived from the Schiff base ($\mathbf{L}^{2}$) bearing merceptothiadiazole and thiophene moieties have comparatively more biological activity. Further, replacement of thiophene-2- carboxaldehyde with 4- bromothiophene-2-caboxaldehyde as one of the components of the Schiff base causes enhancement of the activity of the metal complexes derived from. These studies may serve as a basis for chemical modifications directed towards the development of a new class of antibacterial agents.

Table I. Preliminary screening of the schiff bases ($\mathbf{L}^1$)–($\mathbf{L}^6$) and their metal (II) complexes (**1**)–(**24**) for antibacterial activity (zone of inhibition in mm).

	Microbial species					
	Gram-negative				Gram-positive	
Schiff base/Complex	*E. coli*	*P. aeruginosa*	*S. typhi*	*S. flexneri*	*B. subtilis*	*S. aureus*
$\mathbf{L}^1$	12	11	10	11	13	12
$\mathbf{L}^2$	10	10	10	11	11	13
$\mathbf{L}^3$	11	11	09	13	12	11
$\mathbf{L}^4$	13	10	10	10	11	13
$\mathbf{L}^5$	11	11	12	13	12	12
$\mathbf{L}^6$	12	12	13	13	14	14
1	18	16	17	18	21	16
2	18	14	15	16	19	20
3	18	16	15	16	17	20
4	19	13	13	17	19	21
5	20	16	21	23	27	27
6	21	17	21	23	28	28
7	20	17	17	18	23	22
8	21	16	17	18	22	24
9	22	18	22	23	28	28
10	23	19	23	24	30	30
11	21	18	19	20	25	23
12	23	17	20	22	26	26
13	19	14	17	17	22	19
14	20	16	21	22	28	27
15	16	16	16	19	18	19
16	20	15	16	16	19	21
17	18	15	16	16	20	22
18	20	14	15	17	20	19
19	18	14	14	17	16	22
20	16	13	17	15	21	21
21	18	16	20	22	25	26
22	20	17	21	22	27	27
23	15	16	16	15	22	19
24	17	15	16	18	17	20
Imipenum	30	24	25	27	33	33

$<$ 9: weak; 9–16: moderate; $>$16: significant.

Table II. Minimum inhibitory concentration (mM/ml) of compounds (**5**)–(**6**), (**9**)–(**10**), (**14**) and (**22**) against selected bacteria.

Compound no.	**5**	**6**	**9**	**10**	**14**	**22**
Gram-negative						
S. typhi	0.017	0.042	0.013	0.013	0.021	0.017
S. flexneri	0.017	0.017	0.013	0.013	$>$0.207	$>$0.173
Gram-positive						
B. subtilis	$>$0.171	0.042	0.034	0.013	$>$0.207	$>$0.173
S. aureus	$>$0.171	$>$0.170	0.135	0.033	0.052	0.086

References

[1] Ming L.-J. Metalloantibiotics. Med Res Rev 2003;23:697.

[2] Tullius TD. Metal-DNA chemistry. ACS Symposium Series 402. New York: The American Chemical Society; 1989.

[3] Barton JK. Metal-nucleic acid interactions. London: Bio-inorganic Chemistry, University Science Books; 1994.

[4] Hinton JF, Koeppe RE. Complexing properties of gramicidins. Met Ion Biol Syst 1985;19:173.

[5] Claussen CA, Long EC. Nucleic acid recognition by metal complexes of bleomycin. Chem Rev 1999;99:2797.

[6] Bravo A, Anacona JR. Synthesis and characterization of metal complexes with ampicillin. J Coord Chem 1998;44:173.

[7] Anacona JR, Figueroa EM. Zinc (II), cadmium (II), mercury (II) and lead (II) semiquinone-type complexes of a new Schiff base ligand: Antibacterial studies. J Coord Chem 1999;48:181.

[8] Chohan ZH, Scozzafava A, Supuran CT. Synthesis of biologically active Co(II), Cu(II), Ni(II) and Zn(II) complexes of symmetrically 1,1′-disubstituted ferrocene-derived compounds. Synth React Inorg Met-Org Chem 2003;33:241.

[9] Chohan ZH, Scozzafava A, Supuran CT. Zinc complexes of benzothiazole-derived Schiff-bases with antibacterial activity. J Enz Inhib Med Chem 2003;17:261.

[10] 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 Orga-nomet Chem 2002;16:17.

[11] 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.

[12] Chohan ZH, Rauf A, Supuran CT. Antibacterial Co(II) and Ni(II) complexes of N-(2-furanylmethylene)-2-aminothisdiazole and role of SO_4^{2-}, NO_3, $C_2O_4^{2-}$ and $CH_3CO_2^-$ anions on biological properties. Metal-Based Drugs 2002;8:287.

[13] Chohan ZH, Iqbal MS, Iqbal HS, Scozzafava A, Supuran CT. Transition metal acetylsalicylates and their anti-inflammatory activity. J Enz Inhib Med Chem 2002;17:87.

[14] Hassan MU, Chohan ZH, Supuran CT. Antibacterial Zn(II) compounds of Schiff bases derived from some benzothiazoles. Main Group Metal Chem 2002;25:291.

[15] Chohan ZH. Biologically active Co(II) and Ni(II) complexes of N-(2-thienylmethylene)-2-aminothiadiazole. Metal Based Drugs 2002;8:323.

[16] Chohan ZH, Pervez H, Rauf A, Scozzafava A, Supuran CT. Antibacterial Co(II), Cu(II), Ni(II) and Zn(II) complexes of thiadiazole derived furanyl, thiophenyl and pyrrolyl Schiff bases. J Enz Inhib Med Chem 2002;17:117.

[17] Chohan ZH, Pervez H, Kausar S, Supuran CT. Synthesis and characterization of antibacterial metal-[Co(II), Cu(II), Ni(II) and Zn(II)] acylhydrazine derived pyrrolyl compounds. Synth React Inorg Met-Org Chem 2002;3:529.

[18] Chohan ZH, Rauf A, Noreen S, Scozzafava A, Supuran CT. Antibacterial cobalt(II), nickel(II) and zinc(II) complexes of nicotinic acid-derived Schiff-bases. J Enz Inhib Med Chem 2002;17:101.

[19] Hassan MU, Chohan ZH, Supuran CT. Antibacterial Co(II) and Ni(II) complexes of benzothiazole-derived Schiff-bases. Synth React Inorg Met-Org Chem 2002;32:1649.

[20] Chohan ZH, Pervez H, Rauf A, Supuran CT. Antibacterial role of SO_4^{2-}, NO_3^-, $C_2O_4^{2-}$ and $CH_3CO_2^-$ anions on Cu(II) and Zn(II) complexes of thidiazole derived pyrrolyl Schiff base. Metal Based Drugs 2002;8:263.

[21] Zhang L-X, Zhang A-J, Chen X-X, Lei X-X, Nan X-Y, Chen D-Y, Zhang Z-Y. Synthesis and biological activity of 3-(2-furanyl)-6-aryl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles. Molecules 2002;7:681.

[22] Abdel-Gawad SM, Elgendy MS, Abdelhamid AO. Synthesis of some new 2,3-dihydro-1,3,4-thiadiazole and triazolino[4,3-a] pyrimidines as antimicrobial agent. J Sulf Chem 2005;26:21.

[23] Bhat KS, Prasad DJ, Poojary B, Holla BS. Synthesis of some new 1,2,4-triazolo[3,4-b] thiadiazole derivatives as possible anticancer agents. Phosphorus, Sulphur, Silicon Related Elements 2004;1593:179.

[24] Carron RA, Maran JM, Montero L, Fernanddozalgo A, Dominguez A. Plants Medicinates et Phytother 1987;21:195.

[25] Atta-ur-Rahman, Choudhary MI, Thomsen WJ. Bioassay techniques for drug development. 16. The Netherlands: Harwood Academic Publishers; 2001.

[26] Geary WJ. Use of conductivity measurements in organic solvents for the characterization of coordination compounds. Coord Chem Rev 1971;7:81.

[27] Nakamoto K. Infrared spectra of inorganic and coordination compounds. 2nd ed. New York: Wiley Interscience; 1970.

[28] Bellamy LJ. The infrared spectra of complex molecules. 3rd ed. New York: John Wiley; 1971.

[29] Ferraro JR. Low frequency vibrations of inorganic and coordination compounds. 2nd ed. New York: John Wiley; 1971.

[30] Carlin RL. Transition metal chemistry. Vol 1. New York: Marcel Decker; 1965.

[31] Ballhausen CJ. An introduction to ligand field. New York: McGraw-Hill; 1962.

[32] Lever ABP. Inorganic electronic spectroscopy. Amsterdam: Elsevier; 1984.

[33] Meek DW, Drago RS, Piper TS. Spectrochemical studies of dimethyl sulfoxide, tetramethylene sulfoxide and pyridine-N-oxide as ligands with nickel (II), chromium (II) and cobalt (II). Inorg Chem 1962;1:285.

[34] Drago RS. Physical methods in inorganic chemistry. New York: Reinhold; 1965.

[35] Chohan ZH. Synthesis and biological properties of Cu(II) complexes with 1,1′-disubstituted ferrocenes. Synth React Inorg Met-Org Chem 2004;34:833.

[36] Chohan ZH, Scozzafava A, Supuran CT. Zinc complexes of benzothiazole-derived Schiff-bases with antibacterial activity. J Enz Inhib Med Chem 2003;18:259.

[37] Chohan ZH, Supuran CT, Scozzafava A. Metalloantibiotics: Synthesis and antibacterial activity of cobalt(II), copper(II), nickel(II) and zinc(II) complexes of kefzol. J Enz Inhib Med Chem 2004;19:79.

[38] Chohan ZH, Praveen M. Synthesis, characterization and coordination properties of Cu(II), Co(II), Ni(II) and Zn(II) complexes with novel asymmetric 1,1-diacetylferrocene-derived Schiff-base ligand. Syth React Inorg Met-Org Chem 2000;30:175.

[39] Chohan ZH, Praveen M. Synthesis, characterization and antibacterial properties of symmetric 1,1-ferrocene derived Schiff-base ligands and their Co(II), Cu(II), Ni(II) and Zn(II) chelates. Appl Organomet Chem 2000;14:376.

[40] Chohan ZH, Munawar A, Supuran CT. Transition metal ion complexes of Schiff bases. Synthesis, characterization and antibacterial properties. Metal Based Drugs 2001;8:137.

[41] Chohan ZH, Supuran CT. Synthesis and characterization of Zn(II) complexes with some acylhydrazine Schiff bases. Main Group Metal Chem 2001;24:399.

[42] Chohan ZH, Jaffery MF, Supuran CT. Antibacterial Co(II), Cu(II), Ni(II) and Zn(II) complexes of thiadiazoles Schiff bases. Metal Based Drugs 2001;8:95.

[43] Ming L.-J. Structure and function of metalloantibiotics. Med Resear Rev 2003;23:697.

[44] Afanaseva IB, Ostrakhovitch EA, Mikhalchik EA, Ibragimova GA, Korkina LG. Enhancement of antioxidant and anti-inflammatory activities of bioflavonoid rutin by complexation with transition metals. Biochem Pharmacol 2001;61:677.

[45] Clark MJ, Stubbs M. Interactions of metallopharmaceuticals with DNA. Met Ion Biol Syst 1996;32:727.

[46] Ilies M, Supuran CT, Scozzafava A. Carbonic anhydrase inhibitors: Part 91. Metal complexes of heterocyclic sulfonamides as potential pharmacological agents in the treatment of gastric acid secretion imbalances. Metal-Based Drugs 2000;7:57.