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Design, synthesis, characterization and antibacterial properties of copper(II) complexes with chromone-derived compounds

Zahid H. Chohan[a]*, Mohammad S. Iqbal[b] and Syed K. Aftab[c]

A new series of six chromone-derived compounds and their Cu(II) complexes was synthesized and characterized by their physical, spectral and analytical data The elemental analysis data of the complexes agree well with the proposed composition of the compounds, which were found to be dimeric in nature with two hydrazine molecules bridging the two copper atoms through coordination. The ligands and their Cu(II) complexes were screened for their *in-vitro* antibacterial activity against four Gram-negative (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Shigella flexneri*) and two Gram-positive (*Bacillus subtilis, Staphylococcus aureus*) bacterial strains by the agar-well diffusion method. The ligands were found to exhibit either no or low to moderate activities against one or more of the bacterial species, whereas all the metal complexes exhibited moderate to high activities against different bacterial species. The ligands which were inactive before complexation turned active and less active ones became more active upon coordination with copper ions. Overall, the complexes 7–12 showed comparatively much higher activities than the ligands. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: chromone derivatives; Cu(II) complexes; antibacterial

Introduction

Chromones are one of the most widespread[1] classes of naturally occurring oxygen-containing heterocyclic compounds. The pharmaceutical and chemical significance[2–8] of these compounds offer interesting possibilities in exploring their more pharmacological and biocidal potentials.[9] 3-Formylchromone has been found to have plastid effects[10] on the system of *Euglena gracillis* and antimycobacterial activity similar to isonicotinic acid hydrazide (INH). Such compounds are also attractive synthons in synthetic chemistry due to comparable behavior with α-,β-unsaturated aldehyde.[11–13] Natural chromones of the abundant flavonoid family contain one or several hydroxyl groups as free or protected. Keeping in view the biological significance and remarkable structural conduct of these compounds, we wish to report in this paper some of our findings in the preparation of a novel class of chromone derived compounds which were obtained by the condensation of the respective formylchromone with different aromatic/heteroaromatic hydrazides. The new ligands thus obtained were studied for their complexation behavior with the copper(II) ion. The synthesized compounds were subjected to *in vitro* antibacterial screening against four Gram-negative (*E. coli*, *S. flexneri*, *P. aeruginosa* and *S. typhi*) and two Gram-positive (*B. subtilis* and *S. aureus*) bacterial strains. The compounds reported in this paper constitute a new class of metal based antibacterial agents.

Material and Methods

All reagents and solvents were used as obtained from the supplier or recrystallized/redistilled as necessary. Thin-layer chromatography was performed using aluminum sheets (Merck) coated with silica gel 60 F_{254}. IR (KBr disk) was recorded with a Hitachi Model 200-50 FTIR spectrophotometer. ^{1}HNMR spectra were recorded in DMSO-d_6 with Bruker AM 300 and AM 400 spectrometers (Rheinstetten–Forchheim, Germany) operating at 300 and 400 MHz, respectively. Tetramethylsilane was used as an internal standard. Mass spectra of the ligands were obtained using a Jeol MS Route spectrometer using EI ionization mode. CHN analyses were carried out using an Elemental Analyzer Flash EA 1112. Conductance of the metal complexes was measured on a 4071 conductivity meter, Jenway (USA). Magnetic susceptibility measurements of the metal complexes in the solid state were performed on Gouy's Balance at room temperature. Melting points were recorded on a Gallenkamp apparatus and reported as uncorrected.

General Procedure for the Preparation of Ligands 1–6

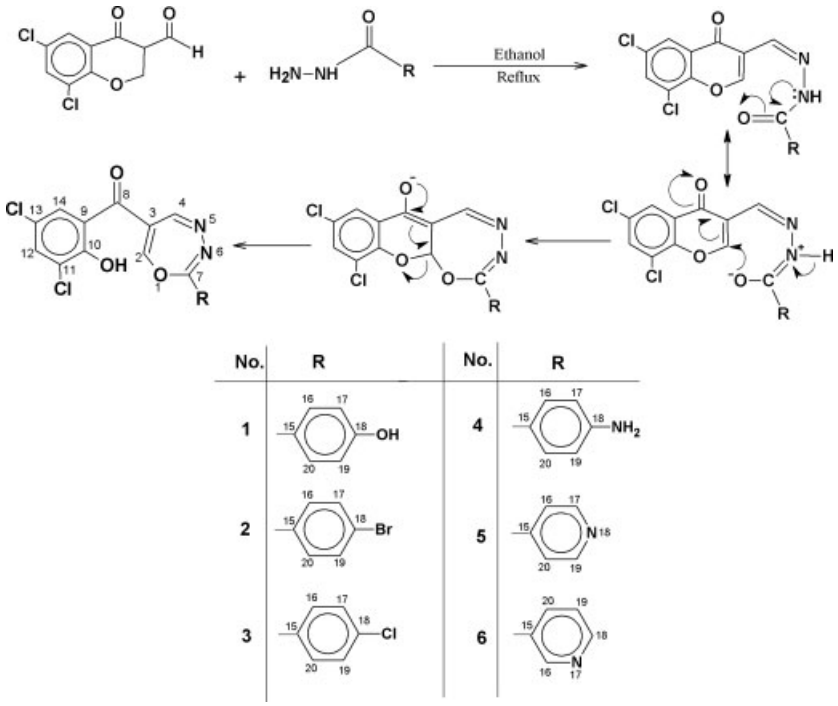
To a stirred solution of 3,5-dichloroformylchromone (0.01 mol) in ethanol (30 ml) was added the appropriate hydrazide (0.01 mol) in ethanol (20 ml) (Scheme 1). The resultant mixture in each case was heated under reflux for 3–4 h. The solid formed during refluxing was collected by suction filtration. Thorough washing with hot ethanol followed by ether furnished the required product in pure form as a single spot on TLC. All compounds were prepared similarly and characterized as below.

* *Correspondence to: Zahid H. Chohan, Department of Chemistry, Bahauddin Zakariya University, Multan-60800, Pakistan. E-mail: dr.zahidchohan@gmail.com*

a *Department of Chemistry, Bahauddin Zakariya University, Multan-60800, Pakistan*

b *Department of Chemistry, G. C. University, Lahore, Pakistan*

c *Department of Chemistry, University of Sargodha, Sargodha, Pakistan*



Scheme 1. Proposed scheme for the preparation of ligands **1**–**6**.

*(3,5-Dichloro-2-hydroxyphenyl)[2-(4-hydroxyphenyl)-1,3,4-oxadiazepin-6-yl]methanone (**1**)*

Yield 68% as dark yellow powder; m.p. 264–266 °C; IR (KBr, cm^{-1}): 3450 (dichlorohydroxyphenyl, OH), 3354 (hydroxyphenyl, OH), 1670 (C═O), 1655 (oxadiazepin, $C_7H{=}N_6$), 1630 (oxadiazepin, $C_4H{=}N_5$), 1240 (oxadiazepin, $C_2{-}O_1$), 1035 (N–N, oxadiazepin), 615 (dichlorohydroxyphenyl, C–Cl); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.79 (*d*, 2H, J = 8.22 Hz, hydroxyphenyl $C_{17,19}$–H), 7.81 (d, 2 H, J = 8.21 Hz, hydroxyphenyl $C_{16,20}$–H), 8.02 (s, 1 H, oxadiazepin C_2–H), 8.22 (s, 1 H, dichlorohydroxyphenyl, C_{12}–H), 8.23 (s, 1 H, dichlorohydroxyphenyl, C_{14}–H), 8.54 (s, 1 H, OH, hydroxyphenyl). 8.86 (s, 1 H, CH═N), 12.02 (s, 1 H, OH, dichlorohydroxyphenyl); ^{13}C NMR (DMSO-d_6, δ, ppm): 99.3 (C_3-oxadiazepin), 116.0 ($C_{17,19}$-hydroxyphenyl), 122.5 (C_{15}-hydroxyphenyl), 124.2 (125.9 (C_9-dichlorohydroxyphenyl), 126.8 (C_{11}-dichlorohydroxyphenyl), 129.8 (C_{14}-dichlorohydroxyphenyl), 130.6 ($C_{16,20}$-hydroxyphenyl), 137.6 (C_{12}-dichlorohydroxyphenyl), 159.6 (C_{10}-dichlorohydroxyphenyl), 160.8 (C_{18}-hydroxyphenyl), 163.2 (C_4-oxadiazepin), 164.8 (C_7-oxadiazepin), 183.6 (C_2-oxadiazepin), 192.3 (C_8-carbonyl): EIMS (70 eV) *m/z* (%): 377.17 (M^+, 68%); anal. calcd for $C_{17}H_{10}Cl_2N_2O_4$ (377.20): C, 54.28; H, 2.95; N, 7.42; Cl, 18.82. Found: C, 54.45; H, 2.72; N, 7.52, Cl, 18.73%.

*[2-(4-Bromophenyl)-1,3,4-oxadiazepin-6-yl](3,5-dichloro-2-hydroxyphenyl)methanone (**2**)*

Yield 75% as light yellow powder; m.p. 165–167 °C; IR (KBr, cm^{-1}): 3452 (dichlorohydroxyphenyl, OH), 1675 (C═O), 1655 (oxadiazepin, $C_7H{=}N_6$), 1625 (oxadiazepin, $C_4H{=}N_5$), 1239 (oxadiazepin, $C_2{-}O_1$), 1035 (N–N, oxadiazepin), 615 (dichlorohydroxyphenyl, C–Cl), 585 (bromophenyl, C–Br); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.75 (*d*, 2H, J = 8.22 Hz, bromophenyl $C_{17,19}$–H), 7.76 (d, 2 H, J = 8.21 Hz, bromophenyl $C_{16,20}$–H), 8.03 (s, 1 H, oxadiazepin C_2–H), 8.24 (s, 1 H, dichlorohydroxyphenyl C_{12}–H), 8.25 (s, 1 H, dichlorohydroxyphenyl C_{14}–H), 8.91 (s, 1 H, CH═N), 12.03 (s, 1 H, OH, dichlorohydroxyphenyl); ^{13}C NMR (DMSO-d_6, δ, ppm): 99.3 (C_3-oxadiazepin), 125.4 (C_{18}-bromophenyl), 125.9 (C_9-dichlorohydroxyphenyl), 126.8 (C_{11}-dichlorohydroxyphenyl), 128.9 (C_{15}-bromophenyl),129.8 (C_{14}-dichlorohydroxyphenyl), 131.4 ($C_{16,20}$-bromophenyl), 131.8 ($C_{17,19}$-bromophenyl), 137.6 (C_{12}-dichlorohydroxyphenyl), 159.6 (C_{10}-dichlorohydroxyphenyl), 163.2 (C_4-oxadiazepin), 164.8 (C_7-oxadiazepin),183.6 (C_2-oxadiazepin), 192.3 (C_8-carbonyl): EIMS (70 eV) *m/z* (%): 440.07 (M^+, 88%); anal. calcd for $C_{17}H_9BrCl_2N_2O_3$ (440.07): C, 46.40; H, 2.06; N, 6.37; Cl, 16.13. Found.: C, 46.26; H, 2.15; N, 6.45; Cl, 16.05%.

*[2-(4-Chlorophenyl)-1,3,4-oxadiazepin-6-yl](3,5-dichloro-2-hydroxyphenyl)methanone (**3**)*

Yield 72% as yellow powder; m.p. 163–165 °C; IR (KBr, cm^{-1}): 3455 (dichlorohydroxyphenyl, OH), 1665 (C═O), 1655 (oxadiazepin, $C_7H{=}N_6$), 1622 (oxadiazepin, $C_4H{=}N_5$), 1241 (oxadiazepin, $C_2{-}O_1$), 1035 (N–N, oxadiazepin), 615 (dichlorohydroxyphenyl, C–Cl), 605 (chlorophenyl, C–Cl); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.91 (*d*, 2H, J = 8.22 Hz, chlorophenyl $C_{17,19}$–H), 8.02 (s, 1 H, oxadiazepin C_2–H), 7.93 (d, 2 H, J = 8.21 Hz, chlorophenyl $C_{16,20}$–H), 8.23 (s, 1 H, dichlorohydroxyphenyl C_{12}–H), 8.24 (s, 1 H, dichlorohydroxyphenyl C_{14}–H), 8.91 (s, 1 H, CH═N), 12.11 (s, 1 H, OH, dichlorohydroxyphenyl); ^{13}C NMR (DMSO-d_6, δ, ppm): 99.3 (C_3-oxadiazepin), 125.9 (C_9-dichlorohydroxyphenyl), 126.8 (C_{11}-dichlorohydroxyphenyl), 128.0 (C_{15}-chlorophenyl), 129.0 ($C_{17,19}$-chlorophenyl), 129.8 (C_{14}-dichlorohydroxyphenyl), 130.6 ($C_{16,20}$-chlorophenyl), 136.6 (C_{18}-chlorophenyl), 137.6 (C_{12}-dichlorohydroxyphenyl), 159.6 (C_{10}-dichlorohydroxyphenyl), 163.2 (C_4-oxadiazepin), 164.8 (C_7-oxadiazepin),183.6 (C_2-oxadiazepin), 192.3 (C_8-carbonyl); EIMS (70 eV) *m/z* (%): 395.62 (M^+, 79%); anal. calcd for $C_{17}H_9Cl_3N_2O_3$ (395.62): C, 51.61; H, 2.29; N, 7.08; Cl, 26.91. Found: C, 51.40; H, 2.16; N, 7.25; Cl, 26.86%.

*[2-(4-Aminophenyl)-1,3,4-oxadiazepin-6-yl](3,5-dichloro-2-hydroxyphenyl)methanone (**4**)*

Yield 75% as yellow powder; m.p. 225–227 °C; IR (KBr, cm^{-1}): 3450 (dichlorohydroxyphenyl, OH), 3380, 3320 (aminophenyl, NH_2), 1660 (C═O), 1655 (oxadiazepin, $C_7H{=}N_6$), 1620 (oxadiazepin, $C_4H{=}N_5$), 1240 (oxadiazepin, C_2-O_1), 1035 (N–N, oxadiazepin), 615 (dichlorohydroxyphenyl, C–Cl); ^{1}H NMR (DMSO-d_6, δ, ppm): 6.14 (*d*, J = 8.10 Hz 2H, Ph-NH_2), 7.79 (*d*, 2H, J = 8.22 Hz, aminophenyl $C_{17,19}$–H), 7.80 (d, 2 H, J = 8.21 Hz, aminophenyl $C_{16,20}$–H), 8.15 (s, 1 H, oxadiazepin C_2–H), 8.30 (s, 1 H, dichlorohydroxyphenyl C_{12}–H), 8.34 (s, 1 H, dichlorohydroxyphenyl C_{14}–H), 8.97 (s, 1 H, CH═N), 12.04 (s, 1 H, OH, dichlorohydroxyphenyl); ^{13}C NMR (DMSO-d_6, δ, ppm): 99.3 (C_3-oxadiazepin), 116.4 ($C_{17,19}$-aminophenyl), 119.9 (C_{15}-aminophenyl), 125.9 (C_9-dichlorohydroxyphenyl), 126.8 (C_{11}-dichlorohydroxyphenyl), 129.8 (C_{14}-dichlorohydroxyphenyl), 130.0 ($C_{16,20}$-aminophenyl), 137.6 (C_{12}-dichlorohydroxyphenyl), 150.7 (C_{18}-aminophenyl), 159.6 (C_{10}-dichlorohydroxyphenyl), 163.2 (C_4-oxadiazepin), 164.8 (C_7-oxadiazepin),183.6 (C_2-oxadiazepin), 192.3 (C_8-carbonyl); EIMS (70 eV) *m/z* (%): 376.117 (M^+, 62%); anal. calcd for $C_{17}H_{11}Cl_2N_3O_3$ (376.19): C, 54.13; H, 2.67; N, 11.17; Cl, 18.87. Found: C, 53.92; H, 2.78; N, 11.25; Cl, 18.92%.

*(3,5-Dichloro-2-hydroxyphenyl)(2-pyridin-4-yl-1,3,4-oxadiazepin-6-yl)methanone (**5**)*

Yield 72% as yellow powder; m.p. 171–173 °C; IR (KBr, cm^{-1}): 3452 (dichlorohydroxyphenyl, OH), 1680 (C═O), 1655 (oxadiazepin, $C_7H{=}N_6$),1632 (oxadiazepin, $C_4H{=}N_5$), 1238 (oxadiazepin, C_2-O_1), 1505 (pyridine, C═N), 1035 (N–N, oxadiazepin), 615 (dichlorohydroxyphenyl, C–Cl); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.14 (d, 2H, J = 5.72 Hz, pyridine $C_{17,19}$–H), 8.15 (s, 1H, oxadiazepin C_2–H), 8.18 (s, 1 H, dichlorohydroxyphenyl C_{12}–H), 8.36 (s, 1 H, dichlorohydroxyphenyl C_{14}–H), 8.74 (s, 1 H, CH═N), 8.87 (d, 2 H, J = 5.72 Hz, pyridine $C_{16,20}$–H), 12.04 (s, 1 H, OH, dichlorohydroxyphenyl); ^{13}C NMR (DMSO-d_6, δ, ppm): 99.3 (C_3-oxadiazepin), 124.1 ($C_{16,20}$-pyridine), 125.9 (C_9-dichlorohydroxyphenyl), 126.8 (C_{11}-dichlorohydroxyphenyl), 129.8 (C_{14}-dichlorohydroxyphenyl), 137.6 (C_{12}-dichlorohydroxyphenyl), 138.4 (C_{15}-pyridine),149.5 ($C_{17,19}$-pyridine), 159.6 (C_{10}-dichlorohydroxyphenyl), 163.2 (C_4-oxadiazepin), 164.8 (C_7-oxadiazepin), 183.6 (C_2-oxadiazepin), 192.3 (C_8-carbonyl); EIMS (70 eV) *m/z* (%): 362.16 (M^+, 72%); anal. calcd for $C_{16}H_9Cl_2N_3O_3$ (362.17): C, 53.06; H, 2.50; N, 11.60; Cl, 19.6. Found: C, 53.48; H, 2.69; N, 11.46; Cl, 19.72%.

*(3,5-Dichloro-2-hydroxyphenyl)(2-pyridin-3-yl-1,3,4-oxadiazepin-6-yl)methanone (**6**)*

Yield 70% as creamish yellow powder; m.p. 160–163 °C; IR (KBr, cm^{-1}): 3450 (dichlorohydroxyphenyl, OH), 1685 (C═O), 1655 (oxadiazepin, $C_7H{=}N_6$), 1632 (oxadiazepin, $C_4H{=}N_5$), 1510 (pyridine, -C═N), 1239 (oxadiazepin, C_2-O_1), 1035 (N–N, oxadiazepin), 615 (dichlorohydroxyphenyl, C–Cl); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.14 (dd, 1 H, J = 7.10, 5.36 Hz, pyridine C_{19}–H), 8.19 (s, 1 H, dichlorohydroxyphenyl C_{12}–H), 8.38 (s, 1 H, dichlorohydroxyphenyl C_{14}–H), 8.62 (dd, 1 H, J = 5.30, 2.10 Hz, pyridine C_{20}–H), 8.39 (s, 1 H, oxadiazepin C_2–H), 8.70 (dd, 1 H, J = 5.32, 2.10 Hz, pyridine C_{18}–H), 8.77 (s, 1 H, CH═N), 8.90 (s, 1 H, pyridine C_{16}–H), 12.30 (s, 1 H, OH, dichlorohydroxyphenyl); ^{13}C NMR (DMSO-d_6, δ, ppm): 99.3 (C_3-oxadiazepin), 123.9 (C_{19}-pyridine), 125.9 (C_9-dichlorohydroxyphenyl), 126.3 (C_{15}-pyridine), 126.8 (C_{11}-dichlorohydroxyphenyl), 129.8 (C_{14}-dichlorohydroxyphenyl), 136.8 (C_{20}-pyridine), 137.6 (C_{12}-dichlorohydroxyphenyl), 151.5 (C_{16}-pyridine), 152.6 (C_{18}-pyridine), 159.6 (C_{10}-dichlorohydroxyphenyl), 163.2 (C_4-oxadiazepin), 164.8 (C_7-oxadiazepin), 183.6 (C_2-oxadiazepin), 192.3 (C_8-carbonyl); EIMS (70 eV) *m/z* (%): 362.17 (M^+, 78%); anal. calcd for $C_{16}H_9Cl_2N_3O_3$ (362.17): C, 53.06; H, 2.50; N, 11.60; Cl, 19.6. Found: C, 52.88; H, 2.60; N, 11.40; Cl, 19.65%.

General procedure for the preparation of Cu(II) complexes 7–12

To a hot magnetically stirred solution of an appropriate ligand (0.02 mol) in methanol (20 ml) was added a solution of copper(II) chloride (0.01 mol) in warm methanol (10 ml) and resultant mixture refluxed for 1 h. The solid thus formed during reflux was cooled and collected by suction filtration. Thorough washing with hot methanol followed by ether furnished a desired product. It was recrystallized from aqueous–methanol (30 : 70) by standing at room temperature for 24 h, gave purified product.

*Copper (II) complex of (3,5-dichloro-2-hydroxyphenyl)[2-(4-hydroxyphenyl)-1,3,4-oxadiazepin-6-yl]methanone (**7**)*

Yield 63% as bluish-green powder; m.p. (decomp.) 310–312 °C; IR (KBr, cm^{-1}): 3455 (OH), 3422 (-NH), 1665 (C═O), 1648 (dichlorohydroxyphenyl, C═O), 1635 (CH═N), 610 (C–Cl), 455 (M–O, carbonyl), 425 (M–O, dichlorohydroxyphenyl); UV (DMSO): λ_{max} (cm^{-1}); 14 080; BM (μ_{eff}) 1.97; molar conductance (36 Ohm^{-1} cm^2 mol^{-1}); water contents, 0.76%; anal. calcd for $C_{68}H_{44}Cu_2Cl_8N_{12}O_{16}$ (1695.86): C, 48.16; H, 2.62; N, 9.9; Cl, 16.74. Found: C, 48.25; H, 2.50; N, 9.84; Cl, 16.69%.

*Copper (II) complex of [2-(4-bromophenyl)-1,3,4-oxadiazepin-6-yl](3,5-dichloro-2-hydroxy-phenyl)methanone (**8**)*

Yield 59% as green powder; m.p. (decomp.) 216–218 °C; IR (KBr, cm^{-1}): 3433 (-NH), 1662 (C═O), 1641 (dichlorohydroxyphenyl, C═O), 1628 (CH═N)), 610 (C–Cl), 470 (M–O, carbonyl), 430 (M–O, dichlorohydroxyphenyl); UV (DMSO): λ_{max} (cm^{-1}); 13 680; B.M.(μ_{eff}) 2.35; molar conductance (38 Ohm^{-1} cm^2 mol^{-1}); Water contents, 3.16%; anal. calcd for $C_{68}H_{40}Cu_2Br_4Cl_8N_{12}O_{12}$ (1947.45): C, 41.98; H, 2.07; N, 8.63; Cl, 14.58. Found.: C, 41.81; H, 2.22; N, 8.71; Cl, 14.65%.

*Copper (II) complex of [2-(4-chlorophenyl)-1,3,4-oxadiazepin-6-yl](3,5-dichloro-2-hydroxy-phenyl)methanone (**9**)*

Yield 62% as brown powder; m.p. (decomp.) 200–202 °C; IR (KBr, cm^{-1}): 3427 (-NH), 1655 (C═O), 1625 (CH═N); 465 (M–O, carbonyl), 610 (C–Cl), 430 (M–O, dichlorohydroxyphenyl); UV (DMSO): λ_{max} (cm^{-1}); 14 735; BM (μ_{eff}) 2.25; molar conductance (34 Ohm^{-1} cm^2 mol^{-1}); water contents, 0.97%; anal. calcd for $C_{68}H_{40}Cu_2$ $Cl_{12}N_{12}O_{12}$ (1769.64): C, 46.15; H, 2.28; N, 9.50; Cl, 24.07. Found: C, 46.22; H, 2.36; N, 9.58; Cl, 24.07%.

*Copper (II) complex of [2-(4-aminophenyl)-1,3,4-oxadiazepin-6-yl](3,5-dichloro-2-hydroxy-phenyl)methanone (**10**)*

Yield 64% as brown powder; m.p. (decomp.) 264–266 °C; IR (KBr, cm^{-1}): 3419 (-NH), 3386, 3326 (amino, NH/NH_2), 1651 (C═O), 1638 (dichlorohydroxyphenyl, C═O), 1627 (CH═N), 610 (C–Cl), 480 (M–O, carbonyl), 455 (M–O, dichlorohydroxypheny); UV

(DMSO): λ_{max} (cm^{-1}); 16 670; BM (μ_{eff}) 2.18; molar conductance (36 Ohm^{-1} cm^2 mol^{-1}); water contents, 4.26%; anal. calcd for $C_{68}H_{48}Cu_2Cl_{18}N_{16}O_{12}$ (1691.92): C, 48.27; H, 2.86; N, 13.25; Cl, 37.76. Found: C, 48.35; H, 2.66; N, 13.34; Cl, 37.85%.

*Copper (II) complex of (3,5-dichloro-2-hydroxyphenyl)(2-pyridin-4-yl-1,3,4-oxadiazepin-6-yl)methanone (**11**)*

Yield 62% as dull-green powder; m.p. (decomp.) 260–262 °C; IR (KBr, cm^{-1}): 3443 (-NH), 1678 (C═O), 1655 (dichlorohydroxyphenyl, C═O), 1636 (CH═N), 1512 (pyridine, -C═N), 610 (C–Cl), 460 (M–O, carbonyl), 435 (M–O, dichlorohydroxyphenyl); UV (DMSO): λ_{max} (cm^{-1}); 12 230; BM (μ_{eff}) 2.50; molar conductance (35 Ohm^{-1} cm^2 mol^{-1}); water contents, 3.92%; anal. calcd for $C_{64}H_{40}Cu_2Cl_8N_{16}O_{12}$ (1635.82): C, 46.99; H, 2.46; N, 13.70; Cl, 17.36. Found: C, 46.82; H, 2.37; N, 13.53; Cl, 17.43%.

*Copper (II) complex of (3,5-dichloro-2-hydroxyphenyl)(2-pyridin-3-yl-1,3,4-oxadiazepin-6-yl)methanone (**12**)*

Yield 60% as dark brown powder; m.p. (decomp.) 230–232 °C; IR (KBr, cm^{-1}): 3444 (-NH), 1674 (C═O), 1652 (dichlorohydroxyphenyl, C═O), 1638 (CH═N), 1516 (pyridine, -C═N), 610 (C–Cl), 455 (M–O, carbonyl), 440 (M–O, dichlorohydroxyphenyl); UV (DMSO): λ_{max} (cm^{-1}); 14 810; BM (μ_{eff}) 2.08; molar conductance (38 Ohm^{-1} cm^2 mol^{-1}); water contents, 3.54%; anal. calcd for $C_{64}H_{40}Cu_2Cl_8N_{16}O_{12}$ (1635.82): C, 46.99; H, 2.46; N, 13.70; Cl, 17.36. Found: C, 46.78; H, 2.24; N, 12.59; Cl, 17.42%.

Antibacterial Bioassay *(in Vitro)*

Preliminary screening

The synthesized ligands **1**–**6** and their corresponding copper(II) complexes **7**–**12** 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.[14] 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-hour-old bacterial inocula containing approximately 10^4–10^6 colony-forming units (CFU ml^{-1}) were spread on the surface of the nutrient agar using a sterile cotton swab. The appropriate amount of the test sample (1 mg ml^{-1} in DMSO) was introduced into 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. In order to clarify any participating role of DMSO in the biological screening, separate studies were carried out with pure DMSO and they showed no activity against any bacterial strains.

Minimum Inhibitory Concentration

Compounds exhibiting most significant antibacterial activity (>16 mm) were selected for minimum inhibitory concentration (MIC) determinations by use of disc diffusion technique by employing discs containing 5, 10, 25, 50 and 100 µg ml^{-1} of the compound under investigation and applying the reported protocol.[15]

Results and Discussion

Chemistry

The ligands **1**–**6** were prepared by refluxing the equimolar (0.01 mol) quantities of 3,5-dichloroformylchromone and the respective aryl hydrazide for 3–4 h in ethanol (30–40 ml) (Fig. 1). All the synthesized ligands were characterized by their physical, spectroscopic (IR and ^{1}H-NMR), mass spectral and elemental analyses data. The metal complexes **7**–**12** of these ligands **1**–**6**, were all air-stable and prepared by the stoichiometric reaction of the corresponding copper (II) chloride with the prepared compounds in a metal to ligand molar ratio (M : L) of 1 : 2. The complexes were intensely colored and amorphous solids which decomposed without melting. They were insoluble in common organic solvents such as ethanol, methanol, chloroform and acetone and soluble in aqueous methanol, DMSO and DMF. Molar conductance (34–38 ohm^{-1} cm^2 mol^{-1}) of the complexes **7**–**12** in DMF (10^{-3} M solution at 25 °C), indicated that they are non-electrolytic in nature.[16] The elemental analysis data of the complexes agree well with the proposed composition of the compounds, which were found to be dimeric in nature with two hydrazine molecules bridging the two copper atoms through coordination. Efforts to grow good crystals of the metal complexes for X-ray diffraction studies were unsuccessful due to their poor solubility in common organic solvents.

IR Spectra

In the IR spectra of the ligands, a strong new band at ~1620–1640 cm^{-1} assigned to ν (CH═N) vibrations appeared, indicating condensation of the starting carbonyl moiety with amino group.[17] The IR spectrum of all the ligands displayed ν (C═O) and ν(OH) stretching at 1650–1685 and 3450 cm^{-1}. In the case of **4** the amino NH_2 asymmetric and symmetric stretching appeared at 3380 and 3320 cm^{-1}, respectively. The IR spectra of ligands **5** and **6** exhibited the 4-pyridine and 3-pyridine C═N stretching in the region 1505 and 1510 cm^{-1}, respectively.

The comparison of the IR spectra of the ligands **1**–**6** with their metal complexes **7**–**12** principally revealed that the compounds are bidentately coordinated to the metal ions. In all the complexes the band appearing at 1650–1670 cm^{-1} due to the ν C═O vibrations is shifted to higher frequency by 17–25 cm^{-1}, which is indicative of the involvement of the C═O in chelation. In addition to this band at 1680–1690 cm^{-1} attributed to ν (C═O) stretching in the ligands **1**–**6** shifted to lower frequency by 15–25 cm^{-1} in its metal complexes **7**–**12**, indicating the involvement of the oxygen atom (O) of the carbonyl group. These new bands were not present in the spectra of their corresponding ligands. Further conclusive evidence of the coordination of the ligands **1**–**6** with the metal ions was established by the far-IR spectra in which new bands at 455–470 and 420–440 cm^{-1} assigned to M–O (carbonyl)[18] and M–O (dichlorohydroxyphenyl) in the spectra of the metal complexes were observed. Moreover, bands at 615 cm^{-1} were assigned to C–Cl in the spectra of all compounds.

^{1}H-NMR Spectra

The ^{1}H-NMR spectral data of the ligands listed in experimental showed that all the ligands **1**–**6** displayed the CH═N and dichlorohydroxyphenyl OH chemical shifts at δ 8.62–8.99 and δ 12.00–12.70 respectively as singlets. The C_2–H of the oxadiazepin moiety of the ligands also appeared as a singlet at δ 8.02–8.39.

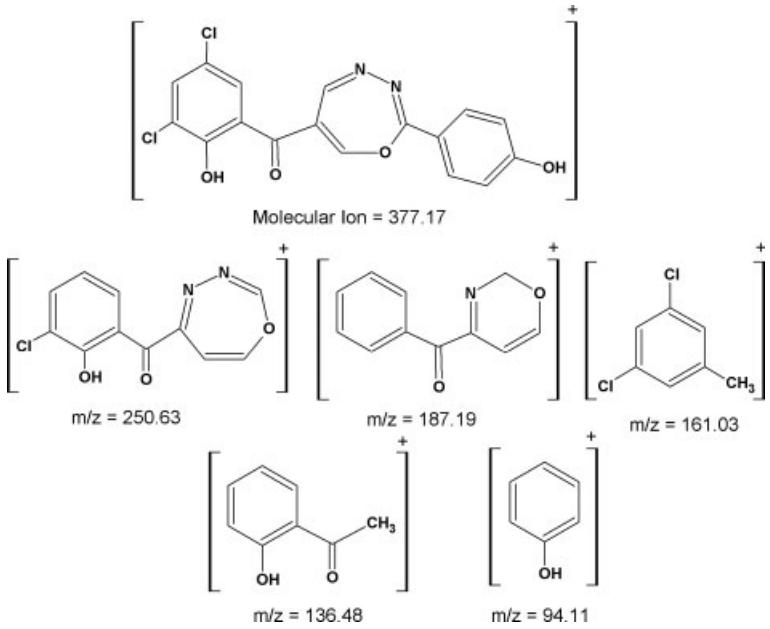


Figure 1. Fragmentation pattern of ligand **1**.

The 1H-NMR spectra of the ligands **1**–**4** demonstrated the phenyl $C_{17,19}$–H and $C_{16,20}$–H as doublets at δ 7.75–7.91 and δ 7.76–7.93 while the ligand **5** showed spectra of the pyridine $C_{17,19}$–H and $C_{16,20}$–H protons as doublets at δ 8.14 and δ 8.87, respectively. In addition, in the spectra of all the ligands, the protons of the hydroxyl group (at dichlorohydroxyphenyl moiety) appeared as a singlet at δ 12.02–12.30. The peak due to NH_2 appeared at δ 6.14 in case of ligand **4**. In case of ligand **6**, the pyridine C_{18}–H, C_{19}–H and C_{20}–H protons appeared as a doublet of doublet at δ 8.70, δ 8.14 and δ 8.62, and C_{16}–H appeared as a singlet at δ 8.90.

^{13}C NMR Spectra

The ^{13}C NMR spectral data of ligands **1**–**6** along with possible assignments is reported in the Experimental and all the carbons were found in their expected region. These studies are well supported by their IR and ^{1}H NMR spectra. Five out of six spectra of dichlorohydroxyphenyl moiety ($C_{9,11-14}$) of all ligands appeared in the region of 125.5–137.8 ppm while the sixth carbon spectra (C_{10}) appeared at 158.6 ppm due to the attachment of hydroxyl group at this carbon. The spectra of $C_{2,4,7}$ of oxadiazepin moiety of all the ligands appeared in the range 163.2–183.6 ppm while C_3 of this moiety appeared at 99.3 ppm. ^{13}C NMR spectra of carbonyl (C_8) moiety of all ligands appeared at 192.3 ppm. Five ^{13}C spectra of hydroxylphenyl moiety ($C_{15-17,19,20}$) of ligand **1** appeared at 116.0–130.6 ppm while C_{18} appeared at 160.8 ppm due to the electron-withdrawing effect of hydroxyl group at this position. In case of ligands **2** and **3**, the spectra of all six carbons of bromophenyl and chlorophenyl moieties showed at 125.4–136.6 ppm. Spectra of C_{18} in chlorophenyl showed an upfield value of 136.8 ppm as compared with bromophenyl 125.4 ppm due to the greater electronegativity of the chloro group. ^{13}C NMR spectra of all carbons of aminophenyl and pyridine moieties of ligands **4** and **5** appeared at 116.4–150.7 ppm. In the case of ligand **6**, the spectra of C_{18} and C_{16} appeared in an upfield direction as compared with other carbon atoms in pyridine moiety due to the effect of nitrogen atom.

Mass Spectra

Mass spectral data along with the fragments of the ligand **1** under study is given in Fig. 1. The molecular ion peaks (M^+) were visible in this spectra. The data clearly indicate the formation of the ligand having the proposed structures. The fragmentation pattern of ligand **1** is reproduced as Fig. 1.

Magnetic Susceptibility Measurements

The room temperature magnetic moment of the solid Cu(II) complexes was found to lie in the range 1.97–2.50 BM. The values of the magnetic moments of these complexes **7**–**12** were higher than the normal values (~1.73 BM) for Cu(II) ion, suggesting[19] that these complexes are not mononuclear. Thus the magnetic moment values support the proposed molecular structure of the complexes as binuclear.[20]

Conductivity Measurements

The conductance values in DMF at the concentration 10^{-3} mol dm^{-3} fall in the range 34–38 Ohm^{-1} cm^2 mol^{-1}. The conductance values indicate the non-electrolytic nature of the complexes as there are no anions present in the lattice.[16] However, slightly higher conductivity values are due to the binuclear nature of the complexes.

Water Content

Water contents of the Cu (II) complexes were determined by the Karl–Fischer titration method. The values do not equate with any whole number of water molecules, suggesting that the water content is just the lattice water and the complexes are not completely dry.

Thermal Analysis

The TG, DSC and DTA thermograms of one respective copper (II) complex is shown in Fig. 2. The relevant data obtained from the

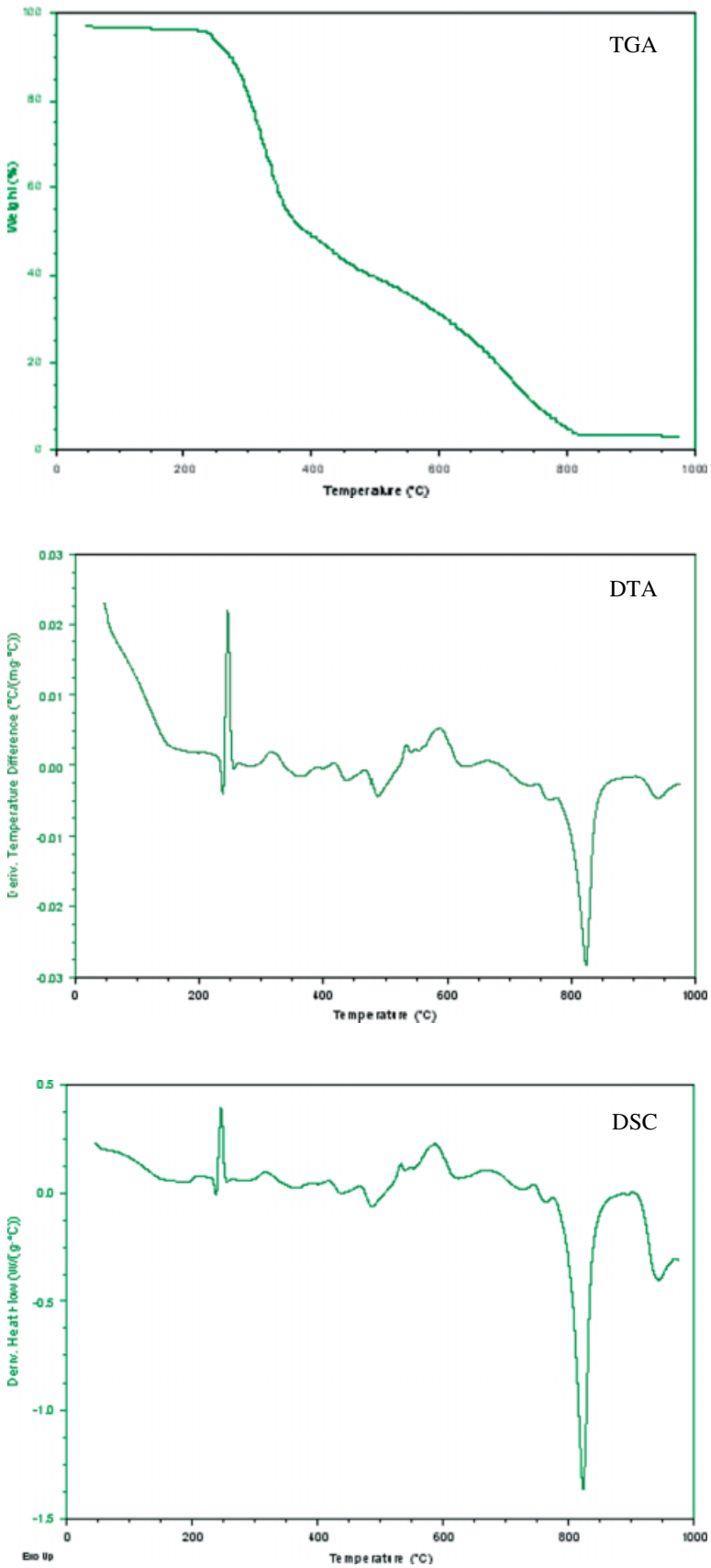


Figure 2. TGA, DTA and DSC curves of the complex **7**.

thermograms of all the metal complexes is given in Table 1. The TG–DTA curves of all the complexes showed three to five steps in their decomposition. In the first step of each complex, one out of two hydrazine molecules was lost between 100 and 156 °C.[20,21] The complexes began to lose weight around 200–500 °C; a sharp decrease in weight shows the loss of one of the ligands from the complexes. The DTA curves show different peaks in the range of 210–390 °C. The endothermic peaks in these complexes in the range of 210–375 °C were assigned to the loss of the ligands.

Electronic Absorption Spectra

The electronic absorption bands of the complexes under investigation are listed in experimental data. The d–d spectra are consistent with the distorted octahedral geometry of the complexes. In the absorption spectra there is an intense broad band observed at 11 760–16 670 cm^{-1} which is assigned to a $^2E_g \rightarrow {}^2T_{2g}$ transition.[21] Although three transitions are expected in this case, they are very close in energy and often appear in the form of one broad band envelope.[22] The values of the electronic transitions for the Cu(II) complexes are specific to an axially deformed octahedral geometry.[23]

Proposed Structures of the Copper (II) Complexes

Based on the experimental evidences available through this study the complexes are proposed to have binuclear structures as shown in Fig. 3. Interestingly, the presence of hydrazine molecule (N_2H_4) is shown by thermal analysis data that acts as a bridging link between the two copper atoms. However, the origin of this moiety during the reaction is not known. Construction of the molecular model reveals that the proposed structure for the complexes is justifiable.

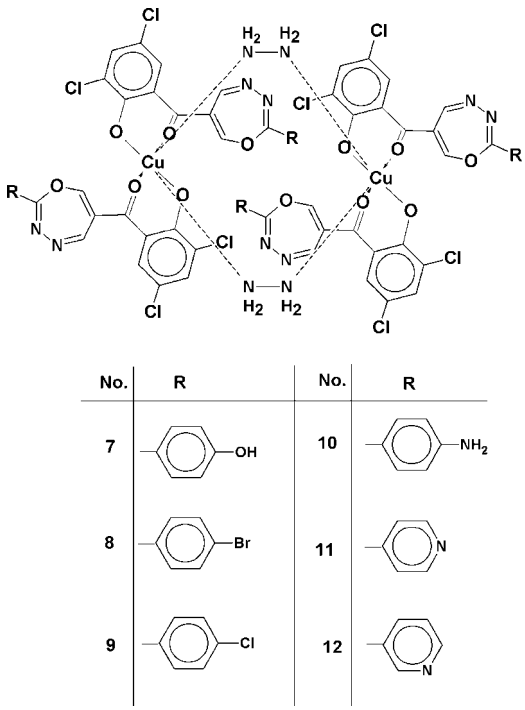


Figure 3. Proposed structure of the Cu(II) complexes **1**–**6**.

Table 1. Thermal analysis data of the Cu (II) Complexes **7**–**12**

Sample no.	Temperature (°C)	Decomposition stages	TGA weight loss (% found)	DTA temperature peak (°C/ thermal effect (exo/endo)	Evolved moiety Formula	Evolved moiety Mass calcd (%)
7	100–156	1	1.66	163.4 (endo)	N_2H_4	32.2
	156–217	2	6.13	341.1 (endo)	$C_5H_4N_2O$	108.5
	217–295	3	14.82	402.7 (exo)	$C_{10}H_9Cl_2NO_3$	262.2
	295–357	4	35.84	742.8 (endo)	$C_{26}H_9Cl_4CuN_2O_5$	634.2
8	100–145	1	1.21	88.76 (endo)	N_2H_4	32.0
	77–246	2	6.47	107.7 (exo)	$C_5H_2N_2O$	105.8
	246–385	3	68.92	180.0 (endo)	$C_{46}H_{27}Cl_3Cu_2N_{10}O_{11}$	1127.4
	385–586	4	81.11	285.4 (endo)	$C_{54}H_{31}Cl_5Cu_2N_{13}O_{10}$	1326.8
	586–670	5	89.96	296.1 (exo)	$C_{34}H_{20}Cl_3CuN_6O_6$	1471.5
9	100–150	1	0.12	249.4 (exo)	N_2H_4	32.6
	210–356	2	97.35	259.3 (endo)	$C_{64}H_{34}Cl_8Cu_2F_8N_9O_8$	1619.3
10	100–155	1	2.13	230.9 (endo)	N_2H_4	32.4
	217–357	2	57.96	247.5 (exo)	$C_{24}H_{24}Cl_5CuN_{10}O_4S_3$	841.3
	357–893	3	91.85	901.6 (endo)	$C_{41}H_{27}Cl_7Cu_2N_{13}O_8S_4$	1333.2
11	100–153	1	2.08	229.5 (endo)	N_2H_4	32.4
	223–242	2	18.12	239.4 (exo)	$C_{11}H_{13}Cl_2N_3O_3$	306.5
	242–371	3	45.99	345.6 (endo)	$C_{34}H_{20}Cl_{13}CuN_6O_6$	778.4
	371–691	4	73.16	848.2 (endo)	$C_{51}H_{38}Cl_6CuN_{13}O_8$	1237.8
12	100–154	1	2.55	198.9 (endo)	N_2H_4	32.38
	164–277	2	20.81	226.2 (exo)	$C_{10}H_7Cl_2N_3O_3$	288.74
	277–403	3	52.32	326.9 (endo)	$C_{23}H_{18}Cl_4CuN_4O_7$	725.96
	403–864	4	96.35	381.4 (endo)	$C_{41}H_{39}Cl_8Cu_2N_{16}O_{12}$	1336.9

Table 2. Antibacterial activity data of the ligands and their copper(II) complexes

Compounds	Inhibition zone (mm)					
	Gram-negative				Gram-positive	
	Escherichia coli	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Shigella flexneri*	*Bacillus subtilis*	*Staphylococcus aureus*
1	0	0	6.33	0	0	0
2	6.33	6.33	6.66	0	6.66	6
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	7.33	6.66	7.0	6.33	7.0	6.33
7	25.0	22.0	21.0	23.0	28.0	23.0
8	0	0	0	0	0	0
9	21.0	12.0	19.0	13.0	29.0	21.0
10	21.0	11.0	16.0	19.0	22.0	19.0
11	14.0	13.0	17.0	18.0	22.0	24.0
12	21.0	12.0	16.0	19.0	22.0	23.0
Standard	30.0	24.0	25.0	27.0	33.0	33.0

0, Absence of measurable inhibitory action; <9 weak; 9–16, moderate; >16, significant.
No activity observed against negative control.

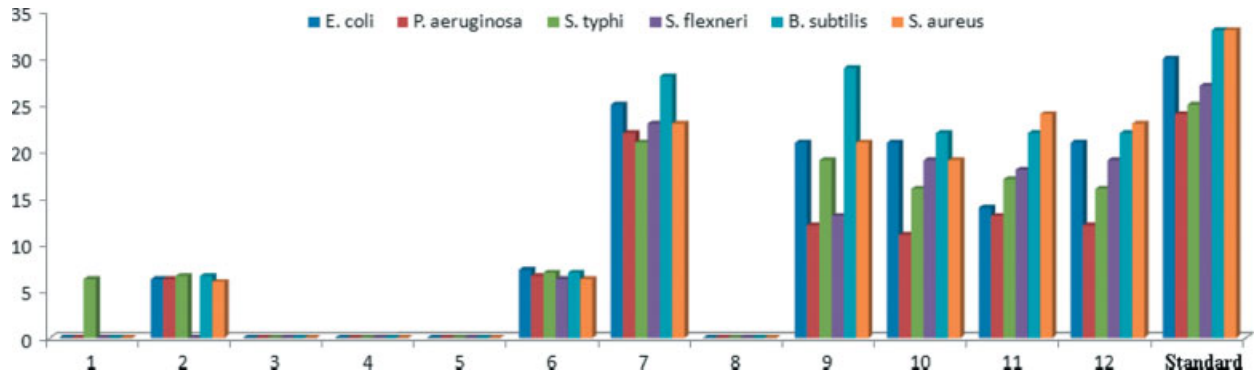


Figure 4. Comparison of antibacterial activity.

Antibacterial studies of the ligands 1–6 and their copper(II) complexes 7–12

Preliminary screening

Antibacterial activities of the synthesized ligands **1**–**6** and their corresponding copper (II) complexes **7**–**12** were determined against four Gram-negative (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Shigella flexneri*) and two Gram-positive (*Bacillus subtilis and Staphylococcus aureus*) bacterial strains. The results of all the synthesized compounds were compared with those of the standard drug (Table 2). The ligands either exhibited no activity or have varying degree of inhibitory effects (low to moderate) against different tested strains. Amongst all the tested compounds, **2, 6,7** and **9**–**12** were found to be active against Gram-negative and Gram-positive testing bacterial strains. In comparison (Figs 4 and 5), the activity against all the Gram-negative and Gram-positive species was increased by coordination of the ligands with the Cu(II) metal ion except compound **8**. Interestingly, five complexes (**7** and **9**–**12**) were found to be much more active than the ligands. These results substantiate our own findings and the findings of some other workers[24–27] that biologically inactive compounds become active and less biologically active compounds become more active upon coordination/complexation. Such induction or enhancement in activity of the metal complexes can be explained on the basis of Overtone's concept and chelation theory. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid soluble materials, due to which liposolubility is an important factor that controls antimicrobial activity. On chelation, the polarity of the metal ion is reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity of the complex. This increased lipophilicity in turn enhances the penetration of the complexes into lipid membranes and blocking of metal binding sites on the enzymes of the microorganisms.[28] The metal complex may also be a vehicle for activation of the ligand as the cytotoxic agent. Moreover, coordination may lead to significant reduction of drug resistance.[29–31] Apart from this, other factors such as solubility, conductivity and dipole moment as influenced by the presence of metal ions may also be amongst the possible reasons for enhancement of the bactericidal activity of the metal complexes as compared with the uncomplexed compounds.[32–35]

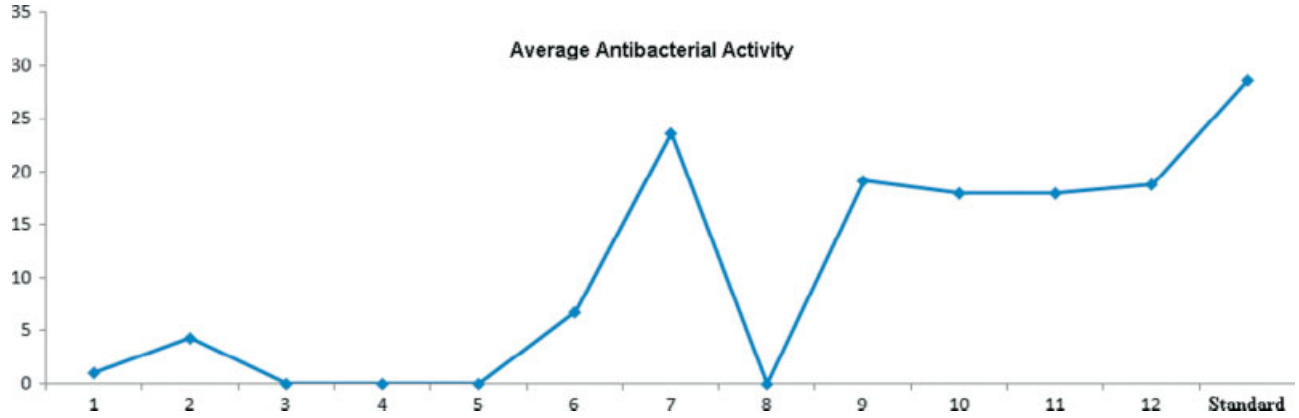


Figure 5. Average antibacterial activity.

Table 3. Minimum inhibitory concentration ($\mu g\ ml^{-1}$) of ligands/complexes (**6, 7, 9–12**) against selected bacteria

	Compound no.					
	6	**7**	**9**	**10**	**11**	**12**
Gram-negative						
Pseudomonas aeruginosa	25	10	25	25	50	25
Shigella flexneri	25	10	10	25	25	25
Gram-positive						
Bacillus subtilis	>100	10	10	>100	>100	50
Staphlococcus aureus	50	10	25	10	25	10

Minimum inhibitory concentration

The preliminary screening showed that the ligands **1–6** were found to be insignificantly active; however, the metal complexes **7** and **9–12** were significantly active against both Gram-negative and Gram-positive organisms. These compounds were selected for MIC studies. The MICs of these compounds varied from 10–100 $\mu g\ ml^{-1}$. The Cu(II) complex (**7**) showed the most significant activity. It inhibited the growth of *Pseudomonas aeruginosa, Shigella flexneri, Bacillus subtilis* and *Staphylococcus aureus* at 10 $\mu g\ ml^{-1}$ concentration (Table 3). The MIC of selected compounds was also tested up to 5 $\mu g\ ml^{-1}$; however, the activity at 10 $\mu g\ ml^{-1}$ concentration was found to be maximum.

The present investigations suggest that in most of the cases the metal complexes were found to be more active than the ligands. These studies may serve as a basis for the development and designing of a new class of metal based antibacterial agents.

Conclusion

The increase in antibacterial activity of the ligands upon coordination is rationalized on the basis of their structure activity relationship and mode of coordination/chelation. It has been suggested that chelation reduces the polarity of the metal ion on partial sharing of its positive charge with the donor groups such as O and/or N. The process of chelation thus increases the lipophilic nature of the compounds, which in turn favors its permeation through the cell membrane of the micro-organism. It has also been suggested that some electron donor groups present in the ligands display extensive biological activity that may be responsible for the increase in hydrophobic character and liposolubility of the complexes that ultimately enhances the biological activity of the compounds.

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