Synthesis, characterization, in-vitro antibacterial and anticancer studies on some metal(II) complexes of (methylsulfanyl)chromenol Schiff base

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ABSTRACT
Mn(II), Co(II), Ni(II), Cu(II) and Pd(II) complexes of the Schiff base, 6-methyl-3-[(4-methylsulfanyl)phenyl]imino)methyl]-3,4-dihydro-2H-chromen-4-ol are synthesized and characterized by microanalysis, conductance, $^1$H NMR, infrared and electronic spectral measurements. The ligand coordinates through the chromenol $O$ and imine $N$ atoms to the metal ions, and the Co(II) and Ni(II) complexes are in the trans-isomeric form as shown by IR measurements. All the complexes form as $[ML_2]$xH$_2$O with the exception of the Mn(II) complex which analyzed as $[MLNO]$.$\cdot$H$_2$O. Electronic measurements are indicative of a four coordinate, tetrahedral/square-planar geometry for the complexes and none is an electrolyte in nitromethane. The antibacterial studies reveal that the Schiff base and its Cu(II) complex exhibit broad-spectrum antibacterial activity against Proteus mirabilis, Escherichia coli and Staphylococcus aureus with inhibitory zones range of 11.0-13.0 mm and 10.0-16.0 mm respectively. The cytotoxic study shows that the Cu(II) complex has the best in-vitro anticancer activity against both HT-29 (colon carcinoma) and MCF-7 (human breast adenocarcinoma) cells with activities of about a half (17.02 $\mu$M) and a fifth (9.78 $\mu$M) that of Cis-platin respectively.

Introduction
Chromenyl Schiff base and chelates are associated with lots of bioactivities such as antibacterial, antifungal and anticancer e.g. Cu(II), Ni(II) and Cu(II) chelates of the Schiff base derived from aminooctaphenylene and 3-formyl chromone exhibited good in-vitro antibacterial activity against S. aureus, E. coli and P. aeruginosa [1], and various (thiomethyl)anilines are used as fungicide in agriculture against Venturia inaequalis infections in apple seedlings [2]. In addition, Schiff bases derived from 3-formyl chromone/6-methyl-3-formyl chromone and various sulfonanides(homo sulfanilamide and 4-aminoethylbenzene sulfonamide) and their Zn(II) complexes exhibited inhibitory activities against tumor-associated carbonic anhydrase isozymes [3], while those derived from 4-anilo-5H-pyridazino(4,5-b) indoles and 2-furanmethanethiol are potent antitumor agent against Bel-7402 (human liver cancer, hepatoma) and HT-1080 (human fibrosarcoma) [4]. Furthermore, the mixed Schiff base derived from mercaptobenzothiazole, formalin and phenylamine are corrosion inhibitors [5]. Extensive literature search reveals that no information is available on the Schiff base, 6-methyl-3-[(4-(methylsulphanyl) phenyl]imino)methyl]-3,4-dihydro-2H-chromen-4-ol.

Experimental
Reagent grade 3-formyl-6-methyl chromone and 4-methylthioaniline (Aldrich), hydrated cobalt(II) nitrate, nickel(II) nitrate, copper(II) nitrate and manganese(II) nitrate, and palladium(II) chloride (BDH chemicals) are used as received and the solvents are purified by distillation.

Synthesis of the ligand
The ligand, 6-methyl-3-[(4-(methylsulphanyl)phenyl]imino)methyl]-3,4-dihydro-2H-chromen-4-ol, is prepared by adding 7.97 mmol (1.10 g) of 4-methylthioaniline in 20 mL of absolute ethanol drop wise to a stirring hot 30 mL ethanolic solution of 7.97 mmol (1.50 g) of 3-formyl-6-methylchromone at 60°C. The resulting homogeneous yellow solution is then refluxed for 6 h after the addition of 6 drops of acetic acid. The yellow product, formed on cooling in ice, is filtered and recrystallized from ethanol and dried in vacuo over anhydrous calcium chloride. The yield of the resulting Schiff base (Figure 1) is 1.73 g (70 %). $^1$H Nmr (ppm) $\delta$ 10.5(s, 1H, C=$^4$ OH), 8.83(s, 1H, C$^1$), 8.07(m, 3H, C$^2$, C$^3$, C$^6$), 7.75(s, 1H, HCN), 7.45 (m, 4H, C$^4$ C$^{3'}$, C$^{5'}$), 2.48 (s, 3H(CH$_3$S), C$^{4'}$), 2.35 (s, 3H(CH$_3$), C$^{5'}$), 1.24(s, 2H, C$^{2'}$).

Fig 1 Structural formula for the ligand
Preparation of the Metal(II) Complexes

The various complexes are prepared by refluxing a homogeneous solution of 0.30 mmol (0.053-0.089 g) of hydrated M(II) nitrates (M = Co, Ni, Cu, Zn) and 0.60 mmol (0.19 g) of the ligand to which 0.06 mmol (0.061 g) of triethylamine is added in 30 mL of ethanol for 6 h. The products formed on cooling in ice are filtered, washed with ethanol, and dried in vacuo over anhydrous calcium chloride. Similar procedure is used to isolate the Pd(II) complex from its chloride.

Biological Studies

The antibacterial tests are done at the Department of Microbiology, University of Ibadan, Ibadan, Nigeria while the anticancer tests are carried out at the Institute of Medicinal and Pharmaceutical Chemistry, Technical University Braunschweig, Beethovenstrasse, 55, 38106 Braunschweig, Germany.

Antimicrobial assay

The assay is carried out on the ligand and its metal(II) complexes using the Agar diffusion technique. The surface of Antimicrobial assay Beethovenstrasse, 55, 38106 Braunschweig, Germany. Pharmaceutical Chemistry, Technical University Braunschweig, Beethovenstrasse, 55, 38106 Braunschweig, Germany.

Cytotoxicity assay

The MCF-7 (human breast adenocarcinoma) and HT-29 (colon carcinoma) cells are maintained in minimum essential medium (MEM) supplemented with 10% of fetal calf serum (FCS), and 25 mg of gentamycin at 37°C in a humidified atmosphere with 5% CO₂. In 96 well plates, 100 mL of a cell suspension in culture medium at 7500 cells/mL (MCF-7) and 2500 cells/ml (HT-29) are plated into each well and incubated for three days under culture conditions. After the addition of various concentrations of the test compounds, cells are incubated for another 96 h and 24 h respectively. The medium is removed and the cells are fixed with glutardialdehyde solution 1% and stored under phosphate buffered saline (PBS) at 4°C. Cell biomass is determined by a crystal violet staining, followed by extracting of the bound dye with ethanol and a photometric measurement at 590 nm. Mean values are calculated and the effects of the compounds are expressed as % Treated/Controlcorr values according to the following equations:

\[ \text{T/C}_{\text{corr}}[^\%] = \left( \frac{\text{T} - \text{C}_{\text{corr}}}{\text{C}_{\text{corr}}} \right) \times 100 \]

(\text{C}_{\text{corr}} is the biomass of control cells at the time of compound addition; \text{C} is the biomass of control cells at the time of the test end; \text{T} is the biomass of probes/samples at the time of the test end). The test compounds are prepared fresh as stock solutions in DMF and diluted with the cell culture medium to the final assay concentrations (0.1% v/v DMF) and Cis-platin is used as the reference drug. The IC_{50} value is determined as the concentration causing 50% inhibition of cell proliferation [16].

Physical Measurements

The electronic spectra are recorded on a Perkin-Elmer A25 spectrophotometer while infrared spectra are done with Thermo Nicolet FTIR 200 spectrophotometer in the range 4000-400 cm⁻¹ as KBr discs. The ¹H nmr spectrum is recorded on a 300 MHz Bruker DRX-400 NMR instrument in CDCl₃ at 295K, and ¹H chemical shifts are referenced to the residual signals of the protons of CDCl₃ and are quoted in ppm. The elemental analyses for C, H and N are recorded on Thermo Quest CE Instruments flash EA1112 analyser, while cobalt, nickel, copper, zinc and palladium are determined titrimetrically [17]. Electrolytic conductivities in nitromethane are determined using a HANNA HI 991300 conductivity meter and melting points (uncorrected) are done with Mel-Temp electro thermal machine.

Results and Discussion

The formation of ligand is confirmed by microanalysis and 1HNMR measurements. All the complexes adopt [ML₂]xH₂O stoichiometry with the exception of the Mn(II) complex which analyzed as [MLNO₃]xH₂O, and the Co(II) complex is hygroscopic. The generalized equation for the formation of the complexes is:

\[ \text{M(NO₃)₃·6H₂O + 2HL} \rightarrow [\text{ML₂}]\text{aH₂O + 2HNO₃ + bH₂O} \]

(When \text{M} = \text{Ni(II)}/\text{Cu(II)} (a = 3, b = 3))

The analytical data, colors, percentage yields, melting points, molar conductivities, and room temperature magnetic moments of the complexes are presented in Table 1. Attempts to isolate suitable crystals for single X-ray structural determination are not successful.

Conductance measurements

The molar conductivities of the complexes in nitromethane are in the range 10.0-18.0 ohm⁻¹ cm² mol⁻¹, showing that they are non-electrolytes in the solvent. A value of 94.0-105.0 ohm⁻¹ cm² mol⁻¹ is expected for a 1:1 electrolyte [18].

Infrared and Electronic Spectra

The relevant infrared data are presented in Table 2. The band at 3417 cm⁻¹ in the ligand is assigned as ν(OH) of crystallization water. The uncoordinated C=N vibrations in the ligand are observed as four bands in the range 1650-1556 cm⁻¹. These bands are observed as three to four bands in the complexes with the exceptions of the Co(II) and Ni(II) complexes which have two bands and are bathochromic shifted to 1644-1501 cm⁻¹, confirming the involvement of the imine N atom in coordination to metal(II) ion. Moreover, it has been documented that some metal(II) Schiff base complexes do exhibit geometric isomerism [20], with the trans-isomer having δH vibrations of the ligand is observed at 981 cm⁻¹ and it suffers a bathochromic shift to 826-775 cm⁻¹ due to the pseudo-aromatic nature of the complexes [21]. Further evidence of coordination is the appearance of the C=C →π* and CT transitions. These bands are observed as three to four bands in the complexes due to the pseudo-aromatic nature of the complexes [21]. Further evidence of coordination is the appearance of the bands due to ν(M-O) and ν(M-N) in the complexes at 498-514 and 572-534 cm⁻¹ respectively, these bands are absent in the ligand spectrum.

The electronic spectra are presented in Table 2. The band at 253-260, 3680 and 40.98 kK, and are assigned to n→π*, π→π* and CT transitions. These bands are bathochromic / hypsochromic shifted in the complexes to 25.5-26.02, 30.12-33.78 and 36.90-40.68 kK due to coordination [20]. The Mn(II) complex, shows two absorption bands at 14.01 and 25.20, 36.80 and 40.98 kK, and it is assigned to n→π* bands due to the pseudo-aromatic nature of the complexes [21]. Further evidence of coordination is the appearance of the bands due to ν(M-O) and ν(M-N) in the complexes at 498-514 and 572-534 cm⁻¹ respectively, these bands are absent in the ligand spectrum.

The electronic spectra are presented in Table 2. The ligand bands are observed at 25.20, 36.80 and 40.98 kK, and are assigned to n→π*, π→π* and CT transitions. These bands are bathochromic / hypsochromic shifted in the complexes to 25.51-26.02, 30.12-33.78 and 36.90-40.68 kK due to coordination [20]. The Mn(II) complex, shows two absorption bands at 14.01 and 25.32 kK respectively, consistent with a four-coordinate, tetrahedral geometry and are assigned to 2E₂ (v₁) and A₁ → E₂ (v₂) transitions [22]. The Co(II) complex shows two absorption bands at 15.30 and 20.94 kK typical of a 4-
bands at 15.39 and 25.0 kK assigned to Pd(II) complex is expectedly square-planar, with absorption single bands below and above 10.0 kK respectively [12]. The coordinate, tetrahedral geometry and are assigned to $T_1(S) \rightarrow T_1(P), (v_4) \rightarrow \pi$ transitions [23]. The Ni(II) complex has absorptions typical of a tetrahedral geometry at 14.71 and 20.04 kK assigned to $T_1(F) \rightarrow T_2, (v_2)$ and $T_1(F) \rightarrow \pi$, $\nu_2$ transitions [23]. The observance of two bands at 15.0 and 24.07 kK in the Cu(II) complex is indicative of square planar geometry with the assignment $B_{1g} \rightarrow A_{1g}$ and $B_{2g} \rightarrow E_{2g}$, since tetrahedral and octahedral Cu(II) complexes have single bands and above 10.0 kK respectively [12]. The Pd(II) complex is expectedly square-planar, with absorption bands at 15.39 and 25.0 kK, assigned to $A_{1g} \rightarrow B_{1g}$ and $A_{1g} \rightarrow E_{2g}$ transitions [21].

![Fig 2 Proposed structure for the Ni(II) and Co(II) complexes](image)

The chromenol proton is observed at 10.5 ppm, while the imine proton is seen as a singlet at 7.75 ppm. The protons on $C_5$, $C_1'$ and $C_8$ in the chromenol ring resonate as a singlet and multiplet at 8.83 and 8.07 ppm respectively. In addition, the protons on $C_3$, $C_2'$, $C_3'$ and $C_4'$ in the methyl thio phenyl ring are observed as a multiplet at 7.45 ppm. The methyl group in thio phenyl ring is seen as a singlet at 7.45 ppm while the methyl in chromenol ring is observed at 2.35 ppm. Finally, the 2H at $C_2$ resonate as a singlet at 1.24 ppm.

Antibacterial activity

The results of antibacterial activities are presented in Table 3 and shown in Figure 3. The ligand is active against Staphylococcus aureus, Esherichia coli and Proteus mirabilis with inhibitory zones range of 12.0-13.0 mm but it is inactive against Klebsiella pneumoniae. The ligand is more susceptible to the gram negative bacteria due to their thin peptidoglycan layer, which makes it more permeable to the complexes [24]. Furthermore, none of the complex is active against Klebsiella pneumoniae, and all the metal complexes are active against Proteus mirabilis with inhibitory zones range of 11.0 -13.0 mm with the exception of the Pd(II) complex. The resistance of Klebsiella pneumoniae to the ligand and the metal complexes may be attributed to its ability to produce extended-spectrum beta-lactamases (ESBL) which inactivates the compounds [25]. The Mn(II) and Co(II) complexes are active against two organisms each i.e. E. coli and P. mirabilis and S. aureus and P. mirabilis with inhibitory zones of 13.0 mm and 12.0 mm, and 9.0 mm and 12.0 mm respectively. Furthermore, the Ni(II) and Pd(II) complexes are active against P. mirabilis and E. coli only with inhibitory zone range of 13.0 mm and 11.0 mm respectively.

It is obvious from this study that the ligand is mostly more effective than the complexes against the bacteria with the exceptions of Mn(II) and Ni(II) complexes whose activities of 13.0 mm are the same as the ligand, against E. coli and P. mirabilis respectively. The lower activities of the metal complexes relative the ligand may be attributed to the degree of permeability of the cells of the bacteria or the difference in the}

bacteria ribosomes [26]. In addition the Cu(II) complex activity of 16.0 mm against E. coli is greater than that of the ligand (13.0 mm) due to chelation and π-electron delocalisation which increase the lipophilic character, favouring its permeation into the bacterial membrane, causing the death of the organism [27]. Sulfamethoxazole activities (25.0-39.0 mm) against the various bacterial isolates relative to the metal complexes (10.0-16.0 mm), show that the activities of the metal complexes are much lower with the optimum activity of about half in Cu(II) complex against E. coli. Thus, the ligand and Cu(II) complex exhibit broad-spectrum antibacterial activity against Staphylococcus aureus, Esherichia coli and Proteus mirabilis with inhibitory zones range of 12.0-13.0 and 10.0-16.0 mm respectively, proving their usefulness as potential broad-spectrum antibacterial agents.

![Fig 3. The comparative antibacterial activities of the complexes with the standard Antibiotic](image)

Anticancer activity

The results of the anticancer activities are presented in Table 4, and shown in Figures 4 and 5. The metal complexes are more susceptible to the MCF-7 cells. The sensitivity of MCF-7 cells to the ligand and its Co(II), Cu(II) and Pd(II) complexes decrease as follows; CuL$_2$ (9.78 µM) > CoL$_2$ (17.48 µM) > PdF$_2$ (21.01 µM) > HL (26.54 µM). The Cu(II) complex activity is the best, being about 1/5 that of Cis-platin (Figure 4). Thus, chelation enhances the cytotoxic activities of the compounds.

The colon carcinoma cells (HT-29) are not sensitive to the Pd(II) complex but are sensitive to the ligand, Co(II) and Cu(II) complexes with IC$_{50}$ values of 46.68 µM, 43.50 µM and 17.02 µM respectively.

![Fig 4 The inhibitory effect of the ligand, Co(II), Cu(II) and Pd(II) complexes against Human Breast Adenocarcinoma cells](image)
planar geometry which avoids possible steric hindrance during physiological actions [28].

Conclusion

The ligand coordinates to the Mn(II), Co(II), Ni(II), Cu(II) and Pd(II) ions using the azomethine N and Chromenol O atoms, and the Co(II) and Ni(II) complexes are in the trans-isomeric form as shown by the IR measurements. The assignment of a 4-coordinate, tetrahedral geometry to Mn(II), Co(II), Ni(II) and Zn(II) complexes, and square-planar geometry to the Cu(II) and Pd(II) complexes is corroborated by electronic spectral measurements. The ligand and the Cu(II) complex exhibit broad-spectrum antibacterial activities Staphylococcus aureus, Escherichia coli and Proteus mirabilis with inhibitory zones range of 12.0-13.0 and 10.0-16.0 mm. The cytotoxic study shows that the Cu(II) complex has the best in-vitro anticancer activity against both MCF-7(human breast adenocarcinoma) and HT-29 (colon carcinoma) cells, with IC\textsubscript{50} values of 9.78 µM and 17.02 µM, which are about a fifth as, and half as active as Cis-platin respectively.

Acknowledgments

I thank TWAS (The Academy of Sciences for The Developing World) and DFG (Deutsche Forschungsgemeinschaft) for the award of a Fellowship, and Prof Ingo Ott of Institute for Medicinal and Pharmaceutical Chemistry, Technical University, Braunschweig, Germany for the chemical analyses and provision of facilities for the cell lines study.

References

4. R. Li, P. Huang, J. Qiao, Zhongnan Yaoxue 6 (2) (2008) 144.

![Fig 5 The inhibitory effect of the ligand, Co(II) and Cu(II) complexes against Colon Carcinoma cells](image)

### Table 1: Analytical data for the compounds

<table>
<thead>
<tr>
<th>Compound (Empirical formula)</th>
<th>Formula mass</th>
<th>Color</th>
<th>% Yield</th>
<th>λ\textsubscript{max} (nm)</th>
<th>M.p. (°C)</th>
<th>Analysis</th>
<th>(Calculated)</th>
</tr>
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<tbody>
<tr>
<td>NaL</td>
<td>309.38</td>
<td>Yellow</td>
<td>70</td>
<td>94</td>
<td>69.52</td>
<td>5.54</td>
<td>4.15</td>
</tr>
<tr>
<td>(C\textsubscript{18}H\textsubscript{20}NO\textsubscript{4})</td>
<td>(69.80)</td>
<td>(5.21)</td>
<td>(4.53)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[MnL\textsubscript{2}H\textsubscript{2}O\textsubscript{4}]</td>
<td>443.34</td>
<td>Green</td>
<td>60</td>
<td>13.0</td>
<td>140</td>
<td>48.81</td>
<td>3.67</td>
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<tr>
<td>(C\textsubscript{20}H\textsubscript{22}N\textsubscript{2}O\textsubscript{6})</td>
<td>(48.77)</td>
<td>(3.87)</td>
<td>(6.32)</td>
<td>(12.39)</td>
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<td></td>
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<td>Na\textsubscript{2}[Co\textsubscript{2}L\textsubscript{2}H\textsubscript{2}O\textsubscript{4}]</td>
<td>801.83</td>
<td>Orange</td>
<td>50</td>
<td>10.0</td>
<td>300</td>
<td>53.94</td>
<td>4.40</td>
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<td>(C\textsubscript{24}H\textsubscript{26}N\textsubscript{2}O\textsubscript{4})</td>
<td>(53.93)</td>
<td>(5.53)</td>
<td>(3.49)</td>
<td>(7.35)</td>
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<tr>
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<td>729.53</td>
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<td>12.0</td>
<td>200</td>
<td>59.25</td>
<td>4.69</td>
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<td>(C\textsubscript{20}H\textsubscript{20}N\textsubscript{2}O\textsubscript{4})</td>
<td>(59.27)</td>
<td>(4.97)</td>
<td>(3.84)</td>
<td>(8.05)</td>
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<tr>
<td>Na\textsubscript{2}[Cu\textsubscript{2}L\textsubscript{2}H\textsubscript{2}O</td>
<td>734.37</td>
<td>Lemon</td>
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<td>15.0</td>
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<td>58.25</td>
<td>4.89</td>
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<td>(C\textsubscript{24}H\textsubscript{26}N\textsubscript{2}O\textsubscript{4})</td>
<td>(58.88)</td>
<td>(4.94)</td>
<td>(3.82)</td>
<td>(8.65)</td>
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<td>Na\textsubscript{2}[PdL\textsubscript{2}H\textsubscript{2}O</td>
<td>777.20</td>
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<td>176</td>
<td>55.82</td>
<td>4.74</td>
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<td>(C\textsubscript{24}H\textsubscript{26}N\textsubscript{2}O\textsubscript{4})</td>
<td>(55.64)</td>
<td>(4.67)</td>
<td>(3.60)</td>
<td>(13.69)</td>
<td></td>
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# = hygroscopic; ω = cm^{-1} mol^{-1}; + = decomposition temperature
Table 2. Relevant infrared and electronic spectral data of the complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>νOH</th>
<th>ν(C=N)</th>
<th>δC-H</th>
<th>ν(M—N)</th>
<th>ν(M—O)</th>
<th>Electronic transitions (kK)</th>
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</thead>
<tbody>
<tr>
<td>HL</td>
<td>3417b</td>
<td>1650s</td>
<td>1618s</td>
<td>1581s</td>
<td>1556s</td>
<td>981s</td>
</tr>
<tr>
<td>[MnLNO₃]H₂O</td>
<td>3500b</td>
<td>1644s</td>
<td>1526s</td>
<td>826s</td>
<td></td>
<td>537s</td>
</tr>
<tr>
<td>[CoL₂]H₂O</td>
<td>3500b</td>
<td>1595s</td>
<td>1541s</td>
<td>823s</td>
<td></td>
<td>534s</td>
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<tr>
<td>[NiL₂]H₂O</td>
<td>3500b</td>
<td>1599s</td>
<td>1506s</td>
<td>822s</td>
<td></td>
<td>542s</td>
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<tr>
<td>[CuL₂]H₂O</td>
<td>3500b</td>
<td>1598s</td>
<td>1579s</td>
<td>1509s</td>
<td></td>
<td>825s</td>
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<tr>
<td>[PdL₂]H₂O</td>
<td>3500b</td>
<td>1598s</td>
<td>1561s</td>
<td>1501s</td>
<td></td>
<td>824m</td>
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Key: s = strong, m = medium, b = broad, s = strong; 1 kK = 1000 cm⁻¹

Table 3 Zones of inhibition (mm) of the compounds against various bacteria isolates

<table>
<thead>
<tr>
<th>Compounds</th>
<th>S.aureus</th>
<th>E. coli</th>
<th>P. mirabilis</th>
<th>K.pneumoniae</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL</td>
<td>12.0±1.4</td>
<td>13.0±0.3</td>
<td>13.0±1.7</td>
<td>R</td>
</tr>
<tr>
<td>[MnLNO₃]H₂O</td>
<td>R</td>
<td>13.0±0.2</td>
<td>11.0±0.2</td>
<td>R</td>
</tr>
<tr>
<td>[CoL₂]H₂O</td>
<td>9.0±0.0</td>
<td>R</td>
<td>12.0±1.4</td>
<td>R</td>
</tr>
<tr>
<td>[NiL₂]H₂O</td>
<td>R</td>
<td>R</td>
<td>13.0±0.7</td>
<td>R</td>
</tr>
<tr>
<td>[CuL₂]H₂O</td>
<td>10.0±1.4</td>
<td>16.0±1.4</td>
<td>11.0±0.5</td>
<td>R</td>
</tr>
<tr>
<td>[PdL₂]H₂O</td>
<td>R</td>
<td>11.0±0.0</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>25.0±2.0</td>
<td>39.0±0.0</td>
<td>34.0±2.2</td>
<td>R</td>
</tr>
</tbody>
</table>

Key: R = Resistance; + = positive standard.

Table 4. IC₅₀ values of the ligand and its Co(II), Cu(II), Pd(II) complexes against MCF-7 and HT-29 cells

<table>
<thead>
<tr>
<th>Compounds</th>
<th>MCF-7 (human breast adenocarcinoma) [µM]</th>
<th>HT-29 (colon carcinoma cells) [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDDP (Cis-platin)</td>
<td>2.0</td>
<td>7.0</td>
</tr>
<tr>
<td>HL</td>
<td>26.5±0.2</td>
<td>46.6±0.0</td>
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<tr>
<td>[CoL₂]H₂O</td>
<td>17.48±0.0</td>
<td>43.50±1.0</td>
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<tr>
<td>[CuL₂]H₂O</td>
<td>9.78 ± 0.2</td>
<td>17.02 ± 0.0</td>
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<tr>
<td>[PdL₂]H₂O</td>
<td>21.01±0.1</td>
<td>&gt;100</td>
</tr>
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</table>

Results are expressed as means (± error) of at least two independent experiments.