Research Article

Synthesis, Spectroscopic, Anticancer, and Antimicrobial Properties of Some Metal(II) Complexes of (Substituted) Nitrophenol Schiff Base

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The Schiff base, 2-[(2,3-dihydro-1H-inden-4-ylimino)methyl]-5-nitrophenol coordinates to Mn(II), Cu(II), Zn(II), and Pd(II) ions through the phenolic O and imine N atoms. The complexes are characterized by physicochemical and spectroscopic methods. The metal complexes formed as [ML2]xH2O with exception of the Cu(II) complex which is anhydrous. Spectroscopic data corroborate the adoption of a four-coordinate, tetrahedral geometry for the Mn(II) and Zn(II) complexes, and a four-coordinate, square planar geometry for the Cu(II) and Pd(II) complexes. None is an electrolyte in DMSO. The in vitro anticancer activities of the metal free ligand, Cu(II), Zn(II), and Pd(II) complexes against MCF-7 (human breast adenocarcinoma) and HT-29 (colon carcinoma) cells reveal that the Pd(II) complex has the best cytotoxic activity against MCF-7 cells with an IC50 of 5.94 μM, which is within the same order of activity as cisplatin. Furthermore, the ligand and the Zn(II) complex exhibit broad-spectrum activity against two gram-positive bacteria, three gram-negative bacteria, and a fungus with inhibitory zones range of 10.0–20.0 and 10.0–17.0 mm, respectively.

1. Introduction

Tridentate aminoindane Schiff base Cr(III) compounds have been used as catalysts in enantioselective inverse-electron-demand hetero-Diels-Alder reactions of α,β-un-saturated aldehydes and ring opening of meso-aziridines [1, 2]. Fur- thermore, some amino-1-indanols possess valuable bronchodilator properties. For example, 6-methoxy-2-isopropylamino-1-indanol [3], while N-propargylamine-1(R)-aminoindane exhibits antiapoptotic properties against dopaminergic SH-SY5Y cells. Additionally, Schiff bases derived from indane-1, 3-dione-2-imine-N-acetic acid, 2-imino-N-2-propionic acid and ninyhdrin, glycine/L-alanine, and their metal(II) com-plexes exhibit unique geometries, and good antimicrobial activities against E. coli, P. mirabilis, S. aureus, and P. faecalis [4, 5], while those derived from 4-amino-1,3-dimethyl-2,6-pyrindinedione and various hydroxy benzaldehyde are potent antimicrobials. Tricyclic pyrimidine and aminobenzene sulfonamido Schiff bases showed anti-HIV activity and high antitumor activity with low therapeutic index against murine S-180 carcinoma [6–8], and N-substituted-3-chloro-2-azetidinones Schiff bases have good anthelminthic activity against earthworms [9]. Extensive literature search shows that no work is reported on the Schiff base, 2-[(2,3-dihydro-1H-inden-4-ylimino)methyl]-5-nitrophenol (derived from condensation of 4-aminoindane and 2-hydroxy-5-nitrobenzaldehyde) and its Mn(II), Cu(II), Zn(II), and Pd(II) complexes [10–14]. Thus, our aim is to synthesize and characterize the above named Schiff base and its metal(II) complexes in order to investigate their antimicrobial and anticancer properties for further studies in drug development for infectious diseases and cancer. The choice of Cu(II) and Zn(II) for cytotoxic studies is based on their importance in humans as
antioxidant, growth, and fertility promoter [15], while Pd, a rare metal with no known biological function is chosen for its renowned antitumor activity [16]. The ligand used in this study, HL and its metal(II) complexes are new and are being reported for the first time by us as a continuation of our studies on the synthesis, characterization, and bioactivities of some metal(II) complexes of various Schiff bases [17–20].

2. Experimental

Reagent grade 4-aminoidane, 2-hydroxy-5-nitrobenzaldehyde, hydrated manganese(II) nitrate, copper(II) nitrate, zinc(II) nitrate, and palladium(II) chloride were purchased from Aldrich and BDH chemicals and were used as received. Solvents were purified by distillation.

The microbes, Bacillus subtilis ATCC 33932, Salmonella typhi, Proteus mirabilis ATCC 21784, Salmonella paratyphi, Pseudomonas aeruginosa ATCC 27856, and Bacillus cereus ATCC 14579, were obtained from the Organic Chemistry Unit, Department of Chemistry, University of Ibadan, Ibadan, Nigeria, while MCF-7 (human breast adenocarcinoma) and HT-29 (colon carcinoma) cells were cultured at 5% CO₂ and 37°C, respectively. 4-Aminoindane, 2-hydroxy-5-nitrobenzaldehyde, hydrated manganese(II) nitrate, copper(II) nitrate, and recrystallized from ethanol and dried in vacuo over anhydrous calcium chloride. The same procedure was used to prepare the Pd(II) complexes from its chloride salt, respectively. The analytical data were as follows.

\[ \text{[MnL₂]H₂O: % yield 70 (0.14 g); Color (brown); IR (KBr, cm}^{-1}\): νOH (3427s), νC=C (1650s 1421s); UV λ \text{max} (kK): 14.79, 22.37, 36.33, 45.84; 1H NMR (300 MHz, CD₃Cl₂, \( δ \) in ppm): 8.70–8.41 (m, 3H, C 3), 7.70–7.95 (m, 3H, C 5, C 6, C 7), Indane ring: 7.0–7.14 (m, 3H, C 1, C 2, C 3), 3.0 (t, 2H, C 4), 2.16 (q, 2H, C 4), 3.06 (t, 2H, C 5), M.pt (°C), 262–264; formula mass (645.95); CHN Anal. calcd(found) for Zn(C₃₂H₂₈N₄O₇)C, 59.5 (59.6); H, 4.4 (3.8); N, 8.7 (8.0); %Zn calcd(found) 10.1 (10.0); \( Λ_m \) 10.0, 30.0.

\[ \text{[CuL₂]H₂O: % yield 70 (0.14 g); Color (green); IR (KBr, cm}^{-1}\): νOH (3427s), νC=C (1650s 1421s); UV λ \text{max} (kK): 13.89, 22.52, 31.71, 41.70; M.pt (°C), 288–300; formula mass (626.11); CHN Anal. calcd(found) for Cu₃(C₃₃H₃₂N₄O₆) C, 61.4 (61.2); H, 4.2 (4.2); N, 9.0 (8.3); %Cu calcd(found)10.2 (10.1); \( Λ_m \) 21.50, 33.60, 42.0; 1H NMR (300 MHz, CD₃Cl₂, \( δ \) in ppm): 8.76 (s, 1H, H₂C₇N), 8.19–8.41 (m, 3H, C 3, C 4, C 5), Indane ring: 7.0–7.14 (m, 3H, C 1, C 2, C 3), 3.0 (t, 2H, C 4), 2.16 (q, 2H, C 4), 3.06 (t, 2H, C 5), M.pt (°C), 262–264; formula mass (645.95); CHN Anal. calcd(found) for Zn(C₃₂H₂₈N₄O₇)C, 59.5 (59.6); H, 4.4 (3.8); N, 8.7 (8.0); %Zn calcd(found) 10.1 (10.0); \( Λ_m \) 12.58.

\[ \text{[PdL₂]O.25H₂O: % yield 50 (0.10 g); Color (brown); IR (KBr, cm}^{-1}\): νOH (3427s), νC=C (1650s 1421s); UV λ \text{max} (kK): 14.79, 22.37, 36.33, 45.84; 1H NMR (300 MHz, CD₃Cl₂, \( δ \) in ppm): 8.70 (s, 1H, H₂C₇N), 7.70–7.95 (m, 3H, C 5, C 6, C 7), Indane ring: 6.95–7.32 (m, 3H, C 1, C 2, C 3), 3.03 (t, 2H, C 4), 2.06 (q, 2H, C 4), 3.08 (t, 2H, C 5), M.pt (°C), 304–306; formula mass (673.47); CHN Anal. calcd(found) for C₆₈H₅₄N₄O₆Cl₂, 57.0 (57.0); H, 4.0 (4.4); N, 8.3 (7.2); %Pd calcd(found)15.4 (15.4); \( Λ_m \) 6.51.

2.2. Preparation of the Metal(II) Complexes. The various complexes were prepared by refuxing a homogeneous solution of 0.30 mmol (0.053–0.089 g) of hydrated M(II) nitrates (M = Mn, Cu, Zn) and 0.60 mmol (0.17 g) of the ligand, to which 0.06 mmol (0.061 g) of triethylamine was added in 30 mL ethanol for 3 h. The products formed were filtered, washed with ethanol, and dried in vacuo over anhydrous calcium chloride. The resulting homogeneous brown solution was then refluxed for 3 h after addition of 4 drops of acetic acid. The orange product, formed on cooling in ice, was filtered and recrystallized from ethanol and dried in vacuo over anhydrous calcium chloride. The resulting homogeneous brown solution was then refluxed for 3 h after addition of 4 drops of acetic acid. The orange product, formed on cooling in ice, was filtered and recrystallized from ethanol and dried in vacuo over anhydrous calcium chloride.
Table 1: IC50 values of the ligand and its Cu(II), Zn(II), Pd(II) complexes against MCF-7 and HT-29 cells.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>MCF-7 (μM)</th>
<th>HT-29 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL</td>
<td>33.3 ± 0.0</td>
<td>&gt;100</td>
</tr>
<tr>
<td>[CuL₂]</td>
<td>78.0 ± 0.1</td>
<td>&gt;100</td>
</tr>
<tr>
<td>[ZnL₂]H₂O</td>
<td>19.9 ± 0.0</td>
<td>51.6 ± 0.0</td>
</tr>
<tr>
<td>[Pd(L)₂]0.25H₂O</td>
<td>5.9 ± 0.0</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

1Results are expressed as means (± error) of at least 2 independent experiments.

to the final assay concentrations (0.1% V/V DMF) and cisplatin was used as the reference drug. The IC50 value was taken as the concentration causing 50% inhibition of cell proliferation and calculated as mean of at least two independent experiments [22].

2.4. Antimicrobial Assay. The assay was carried out on the ligand and its metal(II) complexes using Agar diffusion technique. The surface of the agar in a Petri dish was uniformly inoculated with 0.3 mL of 18 hours old test bacteria/fungus culture. Using a sterile cork borer, 6 mm wells were bored into agar. Then 0.06 mL of 10 mg/mL concentration of each metal complex in DMSO was introduced into the wells and the plates are allowed to stand on bench for 30 min before incubation at 37°C for 24 h after which inhibitory zones (in mm) were taken as a measure of antibacterial activity. The experiments were conducted in duplicates and gentamycin was used as the reference drug.

3. Results and Discussion

The Schiff base and its complexes are obtained in good yields of 70% with the exception of Pd(II) complex with a yield of 50%. All complexes isolated adopt [ML₂]xH₂O stoichiometry, with exception of the Cu(II) complexes which is anhydrous. Evidence for the formation of HL (Figure 1) in pure form is from microanalyses and 1H NMR. The generalized equation for the formation of the complexes is

\[
M(NO₃)₂·2H₂O+2HL \rightarrow [ML₂]xH₂O+2HNO₃ + bH₂O
\]

(when M = Mn(II), x = 2, b = 0; Zn(II), x = 1, b = 1; Cu(II), x =0, b = 2).

Attempts to isolate suitable crystals for single X-ray structural determination are not successful so far.

The molar conductivities of the complexes in DMSO are in the range 1.05–30.0 ohm⁻¹ cm² mol⁻¹, showing that they are covalent in the solvent. A value of 94–105 ohm⁻¹ cm² mol⁻¹ is expected for a 1:1 electrolyte [23].

The infrared bands are assigned by comparing the spectra of the compounds with reported literature on similar systems [5, 13, 14]. The band at 3427 cm⁻¹ in the hydrated ligand is assigned as νOH and its absence in the complexes indicates the involvement of the phenolic O atom in bonding to the metal atoms. The broad band at 3500 cm⁻¹ in the hydrated complexes is assigned to ν(OH) of crystallization water. The uncoordinated C=N and C=C stretching vibrations in the ligand are expectedly coupled in the range 1650–1421 cm⁻¹ [16] and are observed in the range 1675–1404 cm⁻¹ in the metal complexes, due to coordination via the imine N atom. Further evidence of coordination is the presence of the bands due to ν(M=O) and ν(M–N) in the complexes at 481–410 and 580–502 cm⁻¹, respectively; these bands are absent in the ligand.

The spectra of Manganese(II) complexes are usually characterized by forbidden transitions from the ⁶A₁ to higher quartet states for all geometries. [MnL₂] exhibits two bands at 12.0 kK and 22.2 kK, typical of a tetrahedral geometry and are assigned to ⁶A₁ → ⁴E₁ (ν₁) and ⁶A₁ → ⁴A₁ (ν₂) transition [24]. Regular tetrahedral Copper(II) complexes have a single broad band below 10.0 kK, while square-planar complexes usually absorb in the range 10.0–20.0 kK. The observance of two bands at 13.89 kK and 22.52 kK in [CuL₂] supports the assignment of the bands to ²B₁g → ²A₁g and ²B₁g → ²E₁g transitions in a square planar environment.
[6], [ZnL_2] has a single band at 21.50 kK due to M → L CT transitions which confirms its tetrahedral geometry [14]. The spectrum of [PdL_2] expectedly shows transitions typical of square-planar geometry at 14.79 kK and 22.37 kK, which are assigned to 1A_1g → 1B_1g and 1A_2g → 1E_2g transitions [25]. The bands in the ranges 31.71–39.29 kK and 40.08–46.73 kK in the ligand and its metal complexes are assigned to π → π* and CT transitions, respectively.

The phenolic proton in HL is observed at 15.0 ppm, while the imine proton is seen as a singlet at 8.78 ppm. The protons on C^3, C^4, and C^6 resonate as a multiplet at 8.42–8.22 ppm. The protons at C^5, C^6, and C^7 in indane ring are observed as a multiplet at 7.29–7.06 ppm. The 2H at C^1 are seen as a triplet centered at 3.00 ppm while those at C^2 resonate as a quintet centered at 2.15 ppm. Finally, the 2H at C^2 resonate as a triplet centered at 3.05 ppm. [ZnL_2] spectrum shows the absence of phenolic proton at 15.0 ppm, which confirms coordination through the phenolic O atom. The imine proton is seen as a singlet at 8.76 ppm. The protons at C^3, C^4, and C^6 resonate as a multiplet at 8.41–8.19 ppm and are downshifted. Similarly, those at C^5, C^6, and C^7 in indane ring also resonate as a multiplet and are downshifted to 7.00–7.14 ppm. Likewise, the 2H at C^1 are seen as a triplet centered at 3.00 ppm and are downshifted. The 2H protons at C^2 and C^3 resonate as a quintet and triplet centered at 2.16 ppm and 3.06 ppm and are upshifted, respectively. These shifts are indicative of coordination through the imine N atom [14]. Similarly, the spectrum of [PdL_2] shows the absence of the phenolic proton at 15.0 ppm, which confirms coordination through the phenolic O atom. The imine proton is seen as a singlet at 8.70 ppm. The protons at C^3, C^4, and C^6, and those at C^5, C^6, and C^7 in indane ring resonate as multiplets at 7.70–7.95 and 6.95–7.32 ppm, respectively, and are downshifted. The 2H at C^1 are upshifted and seen as a triplet each centered at 3.03 ppm and 3.08 ppm, respectively, while 2H at C^2 are seen as a quintet at 2.06 ppm and are downshifted. These shifts are indicative of coordination through the imine N atom [25].

3.1. Antiproliferative Effects. The results of the anticancer activities of selected complexes and HL are presented in Table 1. Generally, MCF-7 cells are more sensitive towards exposure to the compounds than HT-29 cells. MCF-7 cells are also sensitive to the metal free ligand with an IC_{50} of 33.3 μM. Whereas the activity of [CuL_2] and [ZnL_2] is comparable to that of the free ligand or even decreased. [PdL_2] exhibits an enhanced activity with an IC{50} value of 5.9 μM in MCF-7 cells, which is close to that of the established metal anticancer drug cisplatin (IC{50} value of 2.0 μM in the same assay) [26]. HT-29 cells are only moderately sensitive towards [ZnL_2]. The increased activity of [PdL_2] against the growth of MCF-7 cells is of interest concerning the development of selective tumor therapeutic agents and suggests further investigations in this area.

Table 2: Zones of inhibition (in mm) of the compounds against various microorganisms.

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Pseudomonas aeruginosa</th>
<th>Proteus mirabilis</th>
<th>Bacillus subtilis</th>
<th>Bacillus cereus</th>
<th>S. thyphi</th>
<th>E. coli</th>
<th>P. mirabilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamycin</td>
<td>24.0 ± 0.1</td>
<td>20.0 ± 0.2</td>
<td>20.0 ± 0.2</td>
<td>16.0 ± 0.3</td>
<td>21.0 ± 0.1</td>
<td>18.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>PdL_2[H_2O]</td>
<td>10.0 ± 0.0</td>
<td>7.0 ± 0.1</td>
<td>7.0 ± 0.2</td>
<td>15.0 ± 0.2</td>
<td>IA</td>
<td>IA</td>
<td>16.0 ± 0.2</td>
</tr>
<tr>
<td>[PdL_2]_0.25H_2O</td>
<td>10.0 ± 0.0</td>
<td>7.0 ± 0.1</td>
<td>7.0 ± 0.2</td>
<td>15.0 ± 0.2</td>
<td>IA</td>
<td>IA</td>
<td>16.0 ± 0.2</td>
</tr>
<tr>
<td>[ZnL_2]H_2O</td>
<td>9.0 ± 0.1</td>
<td>9.0 ± 0.02</td>
<td>8.0 ± 0.0</td>
<td>IA</td>
<td>IA</td>
<td>IA</td>
<td></td>
</tr>
<tr>
<td>[MnL_2]_2H_2O</td>
<td>20.0 ± 0.1</td>
<td>16.0 ± 0.03</td>
<td>10.0 ± 0.12</td>
<td>15.0 ± 0.2</td>
<td>13.0 ± 0.1</td>
<td>20.0 ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: IA: inactive.

3.2. Antimicrobial activity. The results of antimicrobial activities are presented in Table 2 and shown in Figure 2. The ligand and the Zn(II) complex are active against all the organisms used, That is, S. thyphi, P. mirabilis, B. subtilis, B. cereus, P. aeruginosa and C. albicans with inhibitory zone ranges of 10.0–20.0 and 10.0–17.0 mm, respectively. The Pd(II) complex is active against all the organisms used with the exception of S. thyphi with inhibitory zones range of 7.0–16.0 mm, while Mn(II) complex has activity against P. aeruginosa, P. mirabilis, and B. subtilis with inhibitory zones of 8.0–9.0 mm.

Furthermore, the metal(II) complexes are mostly unexpectedly less effective than the free ligand, contrary to chelation theory (which states that chelation increases antimicrobial activity, because of partial sharing of its positive charge with donor groups of the ligand and possible π-electron delocalisation which increased the lipophilic character) with exceptions of Zn(II) and Pd(II) complexes with same activity of 10.0 mm and 15.0 mm as the ligand against Bacillus species, respectively [27]. The lower activity of the metal complexes is attributed to lower lipophilicity of the complexes, which decreases the penetration of the complexes through the lipid membrane [28].

Gentamycin activities (18.0–24.0 mm) against the various isolates relative to the metal complexes (7.0–17.0 mm) show that the activities of the latter are much lower, with the optimum activities being about the same as gentamycin in Zn(II) and Pd(II) complexes against C. albicans and B. cereus, and three quarters the activity of gentamycin in Zn(II) complex against P. mirabilis. Moreover, the ligand and Zn(II) complex exhibit broad-spectrum antimicrobial activity, like gentamycin, with inhibitory zones ranges of 10.0–20.0 and 10.0–17.0 mm. Thus, proving their usefulness as potential broad-spectrum antimicrobial agents.

4. Conclusion

The ligand coordinates to the Mn(II), Cu(II), Zn(II), and Pd(II) ions using the azomethine N and phenol O atoms.
The assignment of a 4-coordinate, square-planar/tetrahedral geometry for the Mn(II), Cu(II), Pd(II), and Zn(II) complexes is corroborated by electronic spectral measurements. The in vitro biological studies show that the Pd(II) complex has the best anticancer activity against MCF-7 cells with an IC\textsubscript{50} of 5.9 µM, which is close to the activity of cis platin. Additionally, the Zn(II) complex and the ligand have broad-spectrum activity like gentamycin, although much smaller against P. aeruginosa, P. mirabilis, B. subtilis, B. cereus, S. typhi and C. albicans with inhibitory zone ranges of 10.0–20.0 and 10.0–17.0 mm, respectively.

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