

SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL PROPERTIES OF SOME HETEROLEPTIC METAL(II) COMPLEXES OF PARACETAMOL AND VANILLIN

ADEROJU A. OSOWOLE *¹, OLUWATOOSIN B. A. AGBAJE ² AND BABATOPE O. OJO ¹

¹Inorganic Chemistry Unit, Department of Chemistry, University of Ibadan, Ibadan, Nigeria, ²Organic Chemistry Unit, Department of Chemistry, University of Ibadan, Ibadan, Nigeria
 Email: aderoju30@gmail.com

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ABSTRACT

Objective: This study was aimed at investigating the suitability of Paracetamol and Vanillin in forming low spin, novel heteroleptic, octahedral metal(II) complexes and the potentials of the metal(II) complexes as broad-spectrum antibacterial agents *in-vitro*.

Methods: Heteroleptic metal(II) complexes of Paracetamol (HL) and Vanillin(HL¹) were synthesized and characterized by percentage metal analysis, infrared and electronic spectroscopies, room temperature magnetic moments, melting points, conductance measurements. The antibacterial activity of the synthesized complexes as well as their metal-free ligands was studied using the agar diffusion technique.

Results: Infrared spectra data confirmed that coordination was via the Paracetamol and Vanillin phenol and carbonyl oxygen atoms. The room temperature magnetic moment and electronic spectra data indicated that all the metal(II) complexes assumed octahedral geometry. Furthermore, the Ni(II) complex showed high spin \rightleftharpoons low spin octahedral equilibrium, and the Cu(II) complex was dimeric and antiferromagnetic. The molar conductances of the metal(II) complexes in DMSO showed that the complexes were covalent. Interestingly, the *in-vitro* antibacterial studies on these metal(II) complexes, Paracetamol and Vanillin against *Bacillus cereus*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca* and *Staphylococcus aureus* showed that the Cobalt(II) complex had the best antibacterial activity, being active against all the bacteria used with inhibitory zones range of 19.0-26.0 mm proving it's potentials as a broad spectrum antibacterial agent.

Conclusion: Infrared and electronic spectroscopies, with room temperature magnetic moments indicated a monomeric, 6-coordinate octahedral geometry for all the complexes with the exception of the Cu(II) complex, which was dimeric.

Keywords: antibacterial, covalent, equilibrium, paracetamol, vanillin

INTRODUCTION

The advancement in the field of medicinal / bioinorganic chemistry has led to the use of metal complexes and metal nano-particles due to their imperative applications for the development of advanced novel technologies which includes chemotherapeutic and diagnostic devices [1] e. g. Platinum compounds, cisplatin (cis-[Pt(NH₃)₂Cl₂]), carboplatin and oxaliplatin are among the most widely used for cancer treatment; myocrisin and auranofin which contain gold complexes are used as drugs in treating rheumatoid arthritis. Furthermore, many metal complexes have been found to have potentials as antibacterial, anticancer and anti-diabetic agents [2-9]. Paracetamol (Acetaminophen) is a mild analgesic with weak anti-inflammatory activity, commonly used for the relief of aches and pains [10]. However, overdose of Paracetamol may cause liver damage [11]. Vanillin (4-hydroxy-3-methoxyphenol) is the largest use flavouring agent in foods and medicines. In addition, vanillin has very good antibacterial activity, which is exploited in perfumes and cleaning products [12-13]. Detailed literature search shows that mixed drug metal complexes of o-vanillin and 4-methylthiosemicarbazone; Aspirin and Paracetamol; Paracetamol and Ibuprofen; Aspirin, Paracetamol and Naproxen have been reported [3, 5, 14]. However, no information is available on the heteroleptic metal(II) complexes of Paracetamol and Vanillin. Thus, we present the synthesis, characterization and antibacterial activities of some novel heteroleptic metal(II) complexes of Paracetamol and Vanillin, with the aims of investigating the suitability of these natural ligands in forming low spin octahedral complexes, and the potentials of their metal(II) complexes as broad-spectrum antibacterial agents *in-vitro*. This is a continuation of the research activities of our group on the

search for biologically active metal(II) complexes that could serve as lead compounds in drug research for pain management and antiseptics; and as flavorants in the food and perfume industries [6-8].

MATERIALS AND METHODS

Reagent grade Vanillin, Copper(II)chloride tetrahydrate, Nickel(II) chloride hexahydrate, Cobalt(II)chloride hexahydrate, Manganese(II) chloride tetrahydrate, Zinc(II) nitrate hexahydrate, and Zinc(II) acetate dihydrate were obtained from Aldrich chemicals, and Paracetamol was a gift from Bentos Pharmaceutical products limited, New Garage Ibadan, Nigeria and were used as received. Solvents were purified by distillation.

Preparation of [Mn(L)(HL¹)(H₂O)Cl]

0.5g (3.29 mmole) of Vanillin and 0.5g (3.29 mmole) Paracetamol were dissolved in 30 mL of methanol. To the resulting homogenous solution, 0.3g (3.29 mmole) of the Mn(II) Chloride tetrahydrate was added while stirring and heating at 60°C. The resulting homogenous solution was then refluxed for 3 h, during which the product formed. This was filtered, washed with methanol and dried over silica gel. The same procedure was used for the preparation of Co(II), Ni(II), Cu(II), and Zn(II) complexes from their chloride, acetate and nitrate salts respectively.

[Mn(L)(HL¹)(H₂O)Cl]: 60% yield (0.81g); Color (Lt yellow); IR (KBr, cm⁻¹): νOH (3525s, 3116s), νC=O + νC=C (1652s 1590s 1560s 1432s), νM-N (586m), νM-O (427 m); νM-Cl (388s) VIS/UV λ_{max}(kK): 16.67, 23.81, 31.25, 37.04; D.T (°C), 233; Formula mass (410.75);%Mn. calcd(found)13.38 (13.19); Λ_m: 37.90; μ_{eff}:2.45

[Co(L)(HL¹)(H₂O)Cl]: 60% yield (0.82g); Color (Ash); IR (KBr, cm⁻¹): νOH (3217s), νC=O + νC=C (1647 s 1583 s 1553s 1448s 1467s), νM-N (503 m), νM-O (440 m); νM-Cl (388s) VIS/UV λ_{max}(kK): 15.39, 20.00, 23.53, 31.75, 35.71, 40.0; D.T (° C), 206; Formula mass (414.76); %Co. calcd(found)14.21 (14.14); Λ_m: 15.06; μ_{eff}:2.0

[Ni(L)(HL¹)(H₂O)Cl]: 60% yield (0.87g); Color (Green); IR (KBr, cm⁻¹): νOH (3247b), νC=O + νC=C (1647 s 1583 s 1447s), νM-N (574m 516m), νM-O (433m); νM-Cl (360s) VIS/UV λ_{max}(kK): 11.77, 23.26, 31.75, 35.71; D.T (° C), 194; Formula mass (441.59); %Ni. calcd(found)14.16 (13.65); Λ_m: 11.72; μ_{eff}:1.59

[Cu(L)(HL¹)₂Cl₂]: 60% yield (0.79g); Color (Green); IR (KBr, cm⁻¹): νOH (3500b, 3434s), νC=O + νC=C (1759s 1651s 1587s 1422s), νM-N (511m), νM-O (427s); νM-Cl (356s) VIS/UV λ_{max}(kK): 11.11, 21.98, 37.04, 40.82, 43.48; D.T (° C), 208; Formula mass (802.70); %Cu. calcd(found)15.83 (15.65); Λ_m: 4.14; μ_{eff}:3.32

[Zn(L)(HL¹)(H₂O)NO₃]: 40% yield (0.60g); Color (Lt yellow); IR (KBr, cm⁻¹): νOH (3164s), νC=O + νC=C (1648s 1554s 1466s), νM-N (583m), νM-O (425m); VIS/UV λ_{max}(kK): 23.81, 31.25, 35.71; D.T (° C), 235; Formula mass (447.69); %Zn. calcd(found)14.61 (14.71); Λ_m: 5.60; μ_{eff}:0.86

[Zn(L)(HL¹)CH₃CO₂]: 40% yield (0.60g); Color (Lt yellow); IR (KBr, cm⁻¹): νOH (3159s), νC=O + νC=C (1648s 1570s 1448s), νM-N (528m), νM-O (425 m); VIS/UV λ_{max}(kK): 23.53, 30.30, 37.74; D.T (° C), 196; Formula mass (444.74); %Zn. calcd(found)15.32 (15.20); Λ_m: 4.98; μ_{eff}:0.27

NB: D.T: Decomposition Temperature

Physical measurement

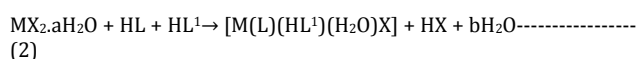
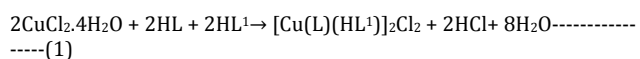
The solid reflectance spectra of the complexes were recorded on a Perkin-Elmer λ25 spectrophotometer and infrared spectra were recorded as KBr disc on a Perkin-Elmer FT-IR spectrum BX spectrometer in the range 4000-400 cm⁻¹. The room temperature magnetic susceptibilities at 303K were measured on Sherwood Susceptibility Balance MSB Mark 1, melting points were determined with Mel-Temp electrothermal machine, and molar conductivity measurements of 1 x 10⁻³ M solutions in DMSO were obtained using electrochemical analyzer Consort C933.

Antibacterial assay

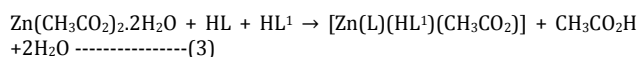
The antibacterial activity of the synthesized complexes as well as their metal-free ligands was studied using the agar diffusion technique. The bacterial used were identified laboratory strains of *Bacillus cereus*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca* and *Staphylococcus aureus*. The surface of the agar in a Petri dish was uniformly inoculated with 0.2 mL of 18 hour old test bacterial culture. Using a sterile cork borer, 9 mm wells were bored into the agar. Then 0.06 mL of 10 mg/mL concentration of each metal complex in DMSO was introduced into the wells and the plates were allowed to stand on the bench for 30 minutes before incubation at 37°C for 24 hours, after which inhibitory zones (in mm) were taken as a measure of antibacterial activity. The experiments were conducted in duplicates and Streptomycin was used as the reference drug.

RESULTS AND DISCUSSION

The reaction of the Paracetamol (HL), Vanillin(HL¹) with the metal(II) chlorides (Mn, Ni, Cu and Co) and metal(II) acetate and nitrate (Zn) gave coloured complexes in good yields according to equations 1- 3.



(When M = Mn, a=4, b=3; Co, Ni; a =6, b=5, X = Cl; M = Zn, a=6, b=5, X=NO₃)



The formation of the metal complexes was confirmed by percentage metal, distinct decomposition temperature, infrared and electronic spectroscopies. The ligands, Paracetamol (HL) and Vanillin (HL¹) melt at 168-170°C and 84°C respectively, whereas their metal complexes decomposed in the range 190-235 °C, confirming coordination. In the absence of suitable crystal for single x-ray diffraction measurement, percentage metal, magnetic and spectroscopic data were used to propose possible structures (Figure 1a).

Solubility and Conductance measurements

The complexes were slightly soluble in water, methanol, ethanol, nitromethane, and chloroform but were soluble in DMSO. Consequently, their molar conductances were measured in DMSO with values obtained in the range 4.14– 37.9 Ω⁻¹cm²mol⁻¹ indicating their covalent nature [15].

Electronic Spectra and Magnetic moments

The ultraviolet spectra of the HL (Paracetamol) and HL¹ (Vanillin) were characterized by strong absorption maxima in the range 33.3-35.0 kK and 40.0-42.5 kK respectively, assigned to π→π* and charge transfer transitions. In the metal complexes, these bands shifted to the range 30.0-37.74 kK and 40.0-43.48 kK respectively due to coordination.

The Mn(II) complex showed two absorption bands at 16.67 kK and 23.81 kK, consistent with a low spin octahedral geometry and were assigned to ²T_{2g} → ²T_{1g} and ²T_{2g} → ²A_{1g} transitions. This complex had an effective magnetic moment of 2.45 B.M. which was complementary of low spin octahedral Mn(II) [16].

Similarly, the Co(II) complex exhibited two absorption bands at 15.39 and 20.0 kK, typical of a low spin octahedral geometry and were assigned to ²E_g → ²T_{1g} and ²E_g → ²T_{2g} transition. An observed moment of 2.0 B. M was indicative of low spin octahedral geometry since moments in the range 1.7-2.2 B.M were reported for low spin octahedral Co(II) complexes [17-18].

The Ni(II) complex showed two absorption bands at 11.77 and 23.26 kK typical of six coordinate high spin and low spin octahedral geometry, and were assigned to ³A_{2g} → ³T_{1g} and ¹A_{1g} → ¹B_{2g} transitions. Room temperature magnetic moments in the range 2.8-3.3 B.M was expected for high spin octahedral complexes Ni(II) while low spin octahedral Ni(II) complexes were expected to be diamagnetic. Thus, an observed moment of 1.59 B.M for this complex corroborated equilibrium between high spin ⇌ low spin octahedral geometry [19].

The Zn(II) complexes, [Zn(CH₃CO₂)(L)(HL¹)] and [Zn(NO₃)(L)(HL¹)(H₂O)] expectedly showed M→L CT transitions around 23.67 kK, as no d-d transition was expected. The former complex was diamagnetic expectedly while the latter complex had a moment of 0.86 B. M. due to polarisation paramagnetism and they assumed a 6-coordinate octahedral geometry [20].

The Cu(II) complex exhibited two bands at 11.11 kK and 21.98 kK typical of a 6-coordinate, tetragonal (octahedral) geometry and were assigned to ²B_{1g} → ²B_{2g} and ²B_{1g} → ²E_g transitions [21]. A moment in the range 1.9–2.2 B.M is usually observed for mononuclear copper(II) complexes, regardless of stereochemistry, expectedly higher than the spin only moment due to orbital contribution and spin-orbit coupling. The Cu(II) complex in this study, had a moment of 3.32 B. M, which was higher than expected due to antiferromagnetism, operating through a dimeric structure in which

the chlorine atoms bridged two copper centers, and each copper centre reinforcing the overall magnetic susceptibility of the complex [22]. However, we are unable to confirm this due to the absence of viable crystals for single crystal X-ray structural investigation (Figure 1b).

Infrared spectra

The strong and medium bands at 3328 cm^{-1} and 3184 cm^{-1} in Paracetamol and Vanillin were assigned as νOH band [3, 14]. This band in Paracetamol was absent in the metal complexes, due to the coordination of the phenol oxygen atom. On the contrary, the νOH band in Vanillin still remained but shifted to $3247\text{-}3116\text{ cm}^{-1}$, due to the coordination of the phenol oxygen atom without deprotonation [4]. The broad band at 3500 cm^{-1} in the metal complexes were assigned to $\nu(\text{OH})$ of coordinated water. The sharp bands in the range $1666\text{-}1503\text{ cm}^{-1}$ in Paracetamol and Vanillin were assigned to $\nu\text{C}=\text{O}$ stretching vibrations. These bands shifted to $1759\text{-}1553\text{ cm}^{-1}$ in the metal complexes, confirming coordination through their carbonyl oxygen atoms [23]. Furthermore, the new bands in the range $586\text{ - }503\text{ cm}^{-1}$, $440\text{-}425\text{ cm}^{-1}$ and $388\text{-}356\text{ cm}^{-1}$ in the spectra of the metal complexes were assigned to $\nu(\text{M-N})$, $\nu(\text{M-O})$ and $\nu(\text{M-Cl})$ respectively. These bands were absent in the spectra of Paracetamol and Vanillin, a further evidence of coordination [2, 23].

Antibacterial activities

Paracetamol (HL), Vanillin (HL^1) and the Mn(II) complex had no activity against the bacteria used. Several metal and metal complexes reportedly shown promise as strong antimicrobial agents against a broad spectrum of microorganisms [24]. Similarly in this study, the Ni(II) complex was active against only *S. aureus* with inhibitory zone of 14.0 mm. The Cu(II) and the Zn(II) complexes were active against three organisms each namely *S. aureus*, *B. cereus*

and *P. mirabilis*; and *K. oxytoca*, *S. aureus* and *B. cereus* with inhibitory zones range of 14.0-20.0 mm and 15.0-19.0 mm respectively. Hence, the Co(II) complex had the best antibacterial activity, being active against all the bacteria used with inhibitory zones range of 19.0-26.0 mm proving it's potentials as a broad spectrum antibacterial agent (Figure 2). Generally, metal(II) complexes are expected to be more effective than the metal-free ligands due to chelation, which reduces the polarity of the metal atom mainly because of partial sharing of its positive charge with donor groups of the ligand and possible π -electron delocalization over the aromatic rings. This increases lipophilic character, favouring its permeation through lipid layers of the bacterial membrane [25]. Conversely, the inactivity of the Mn(II) and Ni(II) complexes may be attributed to their propable lipophobic character [26]. Expectedly, Streptomycin was more active than the metal complexes with inhibitory zones range of 22.0-43.0 mm.

CONCLUSION

Heteroleptic metal(II) complexes of Paracetamol (HL) and Vanillin (HL^1) mostly analyzed as $[\text{M}(\text{L})(\text{HL}^1)(\text{H}_2\text{O})\text{X}]$, where $\text{X} = \text{Cl}/\text{NO}_3$; $\text{M} = \text{Mn, Co, Ni, Cu}$ and Zn based on percentage metal and conductance measurements. Infrared and electronic spectroscopies, with room temperature magnetic moments indicated a monomeric, 6-coordinate octahedral geometry for all the complexes with the exception of the Cu(II) complex, which was dimeric. The *in-vitro* antibacterial studies on these metal(II) complexes against *B. cereus*, *E. coli*, *P. mirabilis*, *P. aeruginosa*, *K. oxytoca* and *S. aureus* showed that the Cobalt(II) complex exhibited a broad spectrum antibacterial activity against these bacteria.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

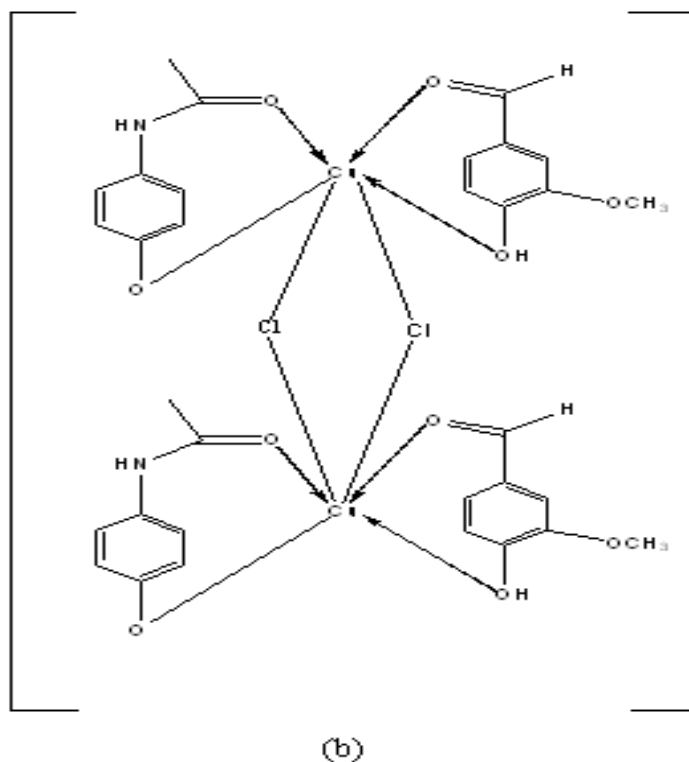
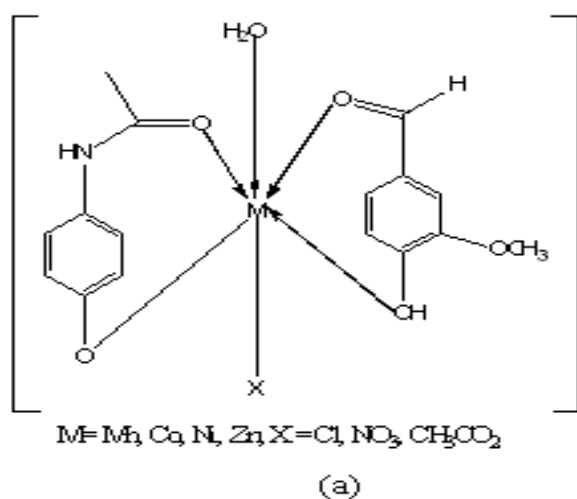


Fig 1: Propose structures for some of the Metal(II) complexes

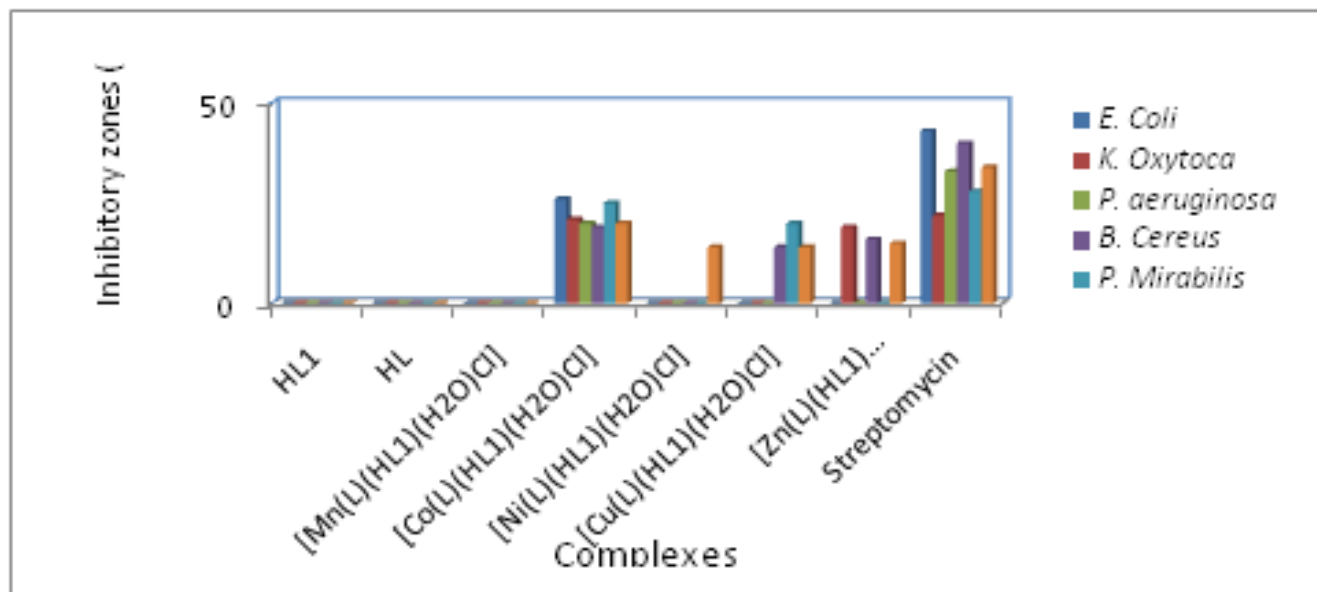


Fig 2: The activities of the metal complexes against bacteria and Streptomycin

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