

Synthesis, Characterisation and Acetylcholinesterase Inhibition Activity of Nickel(II) and Copper(II) Complexes of 3-Hydroxybenzaldehyde-4-nitrobenzoic Acid Hydrazone

Temitope Adekunle Ajayeoba^{1,*}, Olawale Folorunso Akinyele¹, Ayowole Olaolu Ayeni¹, Idowu Julius Olawuni²

¹Department of Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria

²Department of Biochemistry, Obafemi Awolowo University, Ile-Ife, Nigeria

Email address:

topeajayeoba@yahoo.com (T. A. Ajayeoba), tajayeoba@oauife.edu.ng (T. A. Ajayeoba)

*Corresponding author

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Abstract: A new ligand namely 3-hydroxybenzaldehyde-4-nitrobenzoic acid hydrazone, its Nickel(II) and Copper(II) complexes were synthesised and characterised by spectroscopic methods: ¹H NMR, Infrared (IR), UV-Vis, magnetic susceptibility measurement and % metal analysis. ¹H NMR spectroscopy showed the diagnostic N-H signal at 12.10 ppm indicating the formation of the ligand. Infrared spectra showed that the ligand coordinated to the metal ion in keto form through the carbonyl oxygen (C=O) and the azomethine nitrogen (C=N). The UV-Vis spectrum of the ligand displayed two prominent bands at 47169 cm⁻¹ and 30303 cm⁻¹ which were ascribed to the intraligand transitions of $\pi-\pi^*$, and $n-\pi^*$ respectively. These bands had hypsochromic shifts in the metal complexes indicating coordination with the metal ion. The nickel complex had a magnetic moment of 2.92 B.M, suggesting an octahedral geometry, while the copper complex had a value of 1.65 B.M. which is close to the expected value for a d⁹ copper(II) complex with possibility antiferromagnetic interactions. The % metal calculated had a good agreement with the observed values. The acetylcholinesterase inhibition activity of the ligand measured was 190 \pm 20 μ g/mL compared to the standard Eserin that had 68 \pm 1.13 μ g/mL. The copper(II) complex had a value of 220 \pm 20 μ g/mL, while the Nickel(II) complex had the least inhibitory value at 390 \pm 80 μ g/mL. The compounds could serve as primary target in the study of acetylcholinesterase inhibitors

Keywords: 3-Hydroxybenzaldehyde-4-nitrobenzoic Acid Hydrazone, Acetylcholinesterase Inhibition, Octahedral Geometry

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia among older people and the pathogenesis of this disease is associated with oxidative stress. It is a chronic neurodegenerative and progressive disease, and is characterised by progressive impairment of memory and cognition, mood changes, and problems with communication and reasoning [1, 3]. Researches in the last four decades had correlated Alzheimer's disease with acetylcholine deficiency [4, 8]. Similarly, alterations in acetylcholinesterase (AChE) activity can affect locomotion and equilibrium in fish that are

exposed to pollutants, and may also impair their feeding, escape, and reproductive behavior [5-7].

Acetylcholinesterase inhibitors with antioxidant activities are considered as having potentials for treatments of Alzheimer disease and the last decades have seen increasingly rapid advances in the field of developing acetylcholinesterase inhibitors [8, 9]. Hydrazones have interesting biological activities and have attracted great attention in the last decades [25, 26]. There are reports that hydrazone compounds show profound acetylcholinesterase inhibition activities [10-12]. Acetylcholinesterase inhibition activity of hydrazones derived from condensation of Gallic hydrazide with some ketones were studied and it was found

that the compounds exhibited pronounced inhibitory activity [1]. This research work reports the synthesis, characterisation and acetylcholinesterase inhibition activity of nickel(II) and copper(II) complexes of 3-hydroxybenzaldehyde-4-nitrobenzoylhydrazone.

2. Experimental

2.1. Materials and Physical Measurements

All the chemicals and solvents employed were of BDH and Sigma Aldrich quality and used without further purification. The chemicals include methyl-4-nitrobenzoate, meta-hydroxybenzaldehyde, hydrazine hydrate, copper(II) chloride dihydrate, nickel(II) chloride hexahydrate. Ethanol and Methanol. The metal contents (w/w %) were determined by complexometric titration of EDTA using murexide indicator and ammonia/ammonium chloride buffer. IR spectra were recorded as nujol in the range $450\text{--}4000\text{ cm}^{-1}$ on a Perkin Elmer FT-IR Spectrophotometer. UV-Vis spectra of the samples as solid diffuse reflectance were measured in the range $200\text{--}1000\text{ cm}^{-1}$ using a Shimadzu UV-Vis 1800 Spectrophotometer. ^1H (300 MHz) NMR Spectra were recorded on a Bruker DMX avance spectrophotometer (with

internal standard) using DMSO-d_6 as solvent. The magnetic susceptibility of the samples were measured with a Sherwood Scientific Magnetic susceptibility balance, MSB Mark1. Melting Point of the compounds were determined using a Gallenkamp Melting Point Apparatus.

2.2. Preparations

The ligand: 3-hydroxybenzaldehyde-4-nitrobenzoic acid hydrazone (L) was prepared in two steps, first was the synthesis of the precursor hydrazone and the second step yielded the hydrazone ligand [13, 14]

2.2.1. 4-nitrobenzoylhydrazide

10.0 g (55.20 mmol) of methyl-4-nitrobenzoate and 4.00 ml (82.80 mmol) of hydrazine hydrate (ratio 1:1.5 moles of the esters to hydrazine) was refluxed in 75 ml of ethanol on a water bath for six hours. The resulting yellowish solution was poured into a 250 ml beaker and was concentrated by heating to reduce the volume to half the initial volume. It was kept overnight after which a brownish-yellow precipitate was formed. This was filtered under suction, washed with 40% ethanol and dried over CaCl_2 in a desiccator. (M.Pt. $218\text{--}220^\circ\text{C}$)

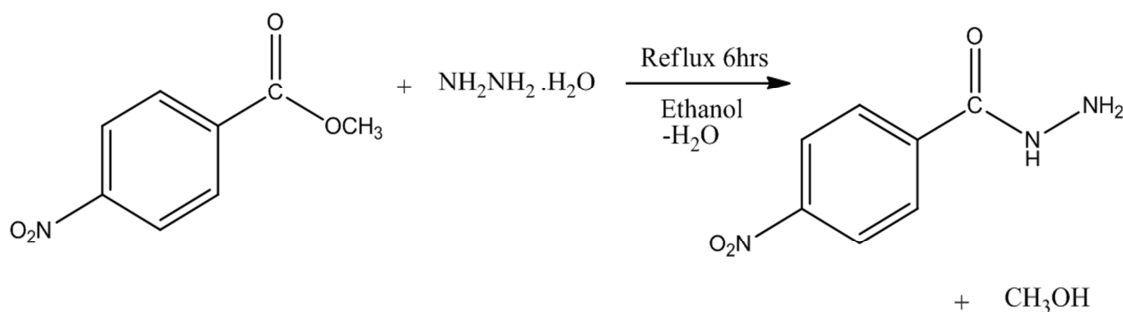


Figure 1. Synthesis of the precursor benzoyl hydrazide.

2.2.2. 3-hydroxybenzaldehyde-4-nitrobenzoylhydrazone (L)

5.0 g (27.60 mmol) of *p*-nitrobenzoylhydrazide was mixed with 50ml of ethanol in a 250 ml round bottom flask. This was heated on a water bath with continuous stirring. 3.37g (27.60 mmol) of *m*-hydroxybenzaldehyde mixed with 20 ml ethanol was then added dropwise to give a clear brownish

solution. This was thereafter refluxed for 4 hours. It was poured into a 250 ml beaker and kept over a period of 24 hours for slow evaporation after which the light brown precipitate formed was filtered under suction and washed thoroughly with ethanol, and dried over CaCl_2 (Mpt. $259\text{--}261^\circ\text{C}$)

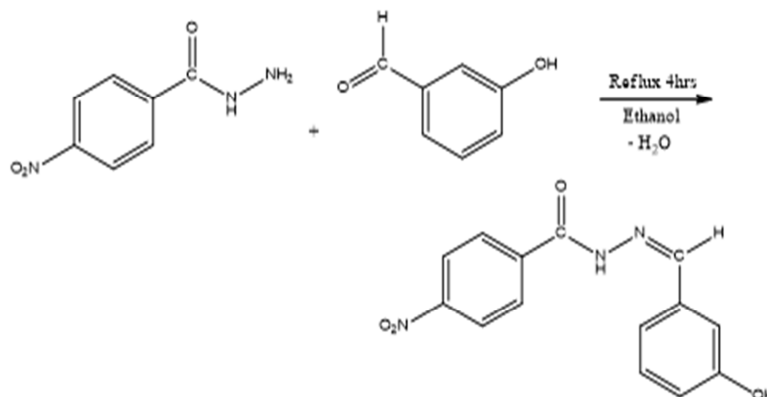
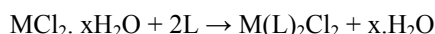


Figure 2. Synthesis of 3-hydroxybenzaldehyde-4-nitrobenzoic acid hydrazone.

2.2.3. Synthesis of Metal Complexes

The metal complexes were prepared by general procedure. An ethanolic solution of metal(II) chlorides was added to a hot solution of the ligand in ratio 1:2 (metal:ligand) in 50 ml of 1:1 acetone/ethanol and the mixture was refluxed for 4 hours. The reaction mixture was thereafter concentrated to half of its initial volume by evaporation and was kept covered in the beaker for 72 hours after which the precipitate that formed was filtered under suction, washed severally with ethanol and dried over anhydrous CaCl_2 .



2.2.4. In vitro Anti-cholinesterase Assays

The anti-cholinesterase inhibiting activities of the ligands and metal complexes were determined by using a modified Ellman method [15]. The Compounds were prepared in a stock solution of 5% Tween 20 in buffer and was used for the cholinesterase inhibition assay, while Physostigmine hemisulfate salt (Eserine) prepared in buffer was used as the reference compound (positive control).

Procedure: In a 96-well plates was added 240 μl of buffer (50 mM Tris-HCl, pH 8.0,) and 20 μl of varying concentrations of the test samples dissolved in 5% Tween 20, 20 μl of the enzyme preparation (0.28U/ml), the reaction mixture was then incubated for 30 min at 37°C , after which 20 μl of 10 mM DTNB was added. The reaction was then initiated by the addition of 20 μl of 25 mM ATChI. The rate of hydrolysis of ATChI was then determined spectrophotometrically by measuring the change in the absorbance per minute ($\Delta A/\text{min}$) due to the formation of the

yellow 5-thio-2-nitrobenzoate anion at 412nm over a period of 4 min at 30 s interval. A solution of 2% Tween 20 was used as negative control. All assays were carried out in triplicate. Eserine ((-) physostigmine) was used as positive control.

A solution of 5% Tween 20 was used as negative control.

The percentage inhibition (% I) of oil extract was obtained using the formula:

$$I (\%) = [(V_o - V_i) / V_o] * 100$$

Where: I (%) = Percentage inhibition

V_i = enzyme activity in the presence of extract

V_o = enzyme activity in the absence of extract

3. Results and Discussion

3.1. Analytical Data

The structure of the ligand was elucidated using ^1H NMR spectroscopy. The analytical data, colours, % yields, melting points/decomposition temperatures and room temperature magnetic moments (μ_{eff}) of the complexes are given in Table 1. The reaction of nickel(II) chloride hexahydrate with the ligand (L) resulted in the formation of a light green coloured $\text{Ni}(\text{L})_2\text{Cl}_2$ while the copper(II) chloride dihydrate salt gave an orange coloured $\text{Cu}(\text{L})\text{Cl}_2 \cdot \text{H}_2\text{O}$. The complexes were stable and non-hygroscopic and could be stored for weeks without any appreciable change. The complexes have appreciable high thermal stability. There was a fair agreement between the calculated and observed % metal for the complexes.

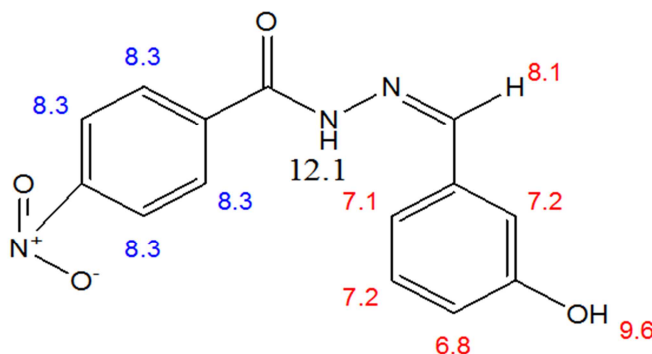
Table 1. Physical properties and analytical data for the Compounds.

Compounds	Formula FW (g)	Colour	M.Pt ($^\circ\text{C}$)	% Metal	% Yield	μ_{eff} (BM)
L	$\text{C}_{14}\text{H}_{11}\text{N}_3\text{O}_4$ (285.24)	Light Brown	259-261	-	60	-
$\text{Ni}(\text{L})_2\text{Cl}_2$	$\text{C}_{28}\text{H}_{22}\text{N}_6\text{O}_8\text{Ni}$ (700.17)	Light green	>300	8.42 (8.38)	51	2.92
$\text{Cu}(\text{L})\text{Cl}_2 \cdot \text{H}_2\text{O}$	$\text{C}_{14}\text{H}_{13}\text{N}_3\text{O}_4\text{Cu}$ (437.78)	Orange	298-300	14.16 (14.51)	60	1.65

3.2. ^1H NMR

The ^1H NMR spectrum of the ligand displayed a singlet at δ 12.10 ppm due to azomethine N-H [16, 17]. The aldehydic proton of $\text{CH}=\text{N}-\text{N}$ resonated at 8.10 ppm. The aromatic protons appeared in the range of 6.8 – 7.2 ppm for the four

proton on the ring bearing hydroxyl group and as singlet at 8.3 ppm for four proton on the ring bearing the nitro group [27]. The hydroxyl proton appeared at δ value of 9.6 ppm (Figure 1). The solvent peak appeared at 2.50 ppm and 3.33 ppm.



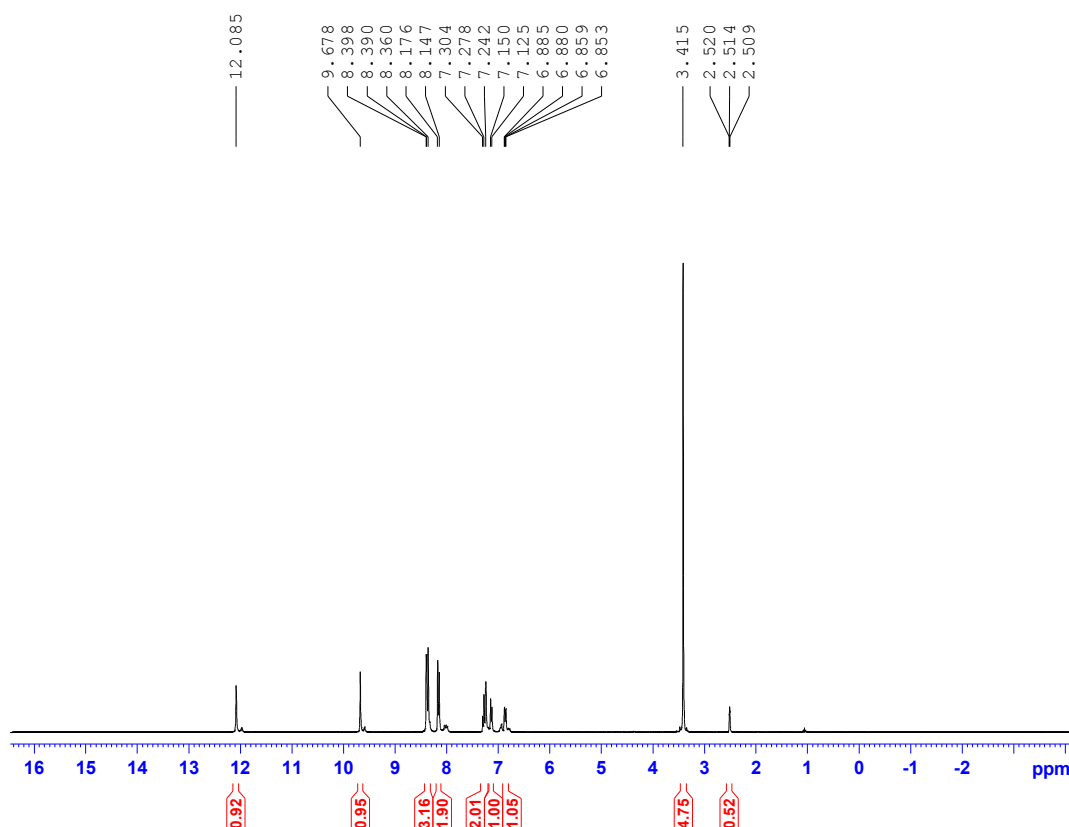


Figure 3. ^1H NMR spectrum of 3-hydroxybenzaldehyde-4-nitrobenzoic acid Hydrazone.

3.3. Infrared Spectra

The infrared spectra data of the synthesised compounds are shown in Table 2. Characteristically, four bands have been useful in the interpretation of infrared spectra of hydrazone compounds. These are due to: ν (N-H), ν (C=O), ν (C=N) and ν (N-N) vibrational frequencies [18, 20]. These bands usually undergo bathochromic or hypsochromic shifts on complexation of the ligands to metal ions, and this provides an evidence of coordination. Furthermore, appearance of bands due to metal-oxygen and metal-nitrogen bonds in the spectra of complexes give credence to coordination. The ligand (L), had a band of weak intensity at 3280 cm^{-1} due to ν (N-H) vibrations. This band had a bathochromic shift to lower energy at 3208 cm^{-1} in the Ni(II) complex and to 3210 cm^{-1} in Cu(II) complex [25, 28]. The ν (C=O) band appeared at 1664

cm^{-1} in the free ligand. This band remained in the same position in the Ni(II) complex but shifted to lower frequency at 1655 cm^{-1} in the Cu(II) complex. The ν (C=N) occurred at 1563 cm^{-1} in the L and had hypsochromic shift to higher wave number at 1594 cm^{-1} and 1595 cm^{-1} for nickel and copper complexes respectively. These shifts are indicative of interaction between the ligands and the metal ions. The metal-oxygen and metal-nitrogen bonds [13, 17, 25] which hitherto were absent in the free ligand appeared in the nickel(II) complexes at: 692 cm^{-1} (M-O) and 559 cm^{-1} (M-N), and in copper(II) complex at 689 cm^{-1} (M-O) and 572 cm^{-1} (M-N). The ν (H-O) band appeared in the free ligand at 3472 cm^{-1} . This band remained in the same position in the infrared spectra of the metal complexes suggesting that the hydroxyl group did not partake in bonding.

Table 2. Infrared data for the compounds.

Compounds	ν (O-H/ H_2O)	ν (N-H)	ν (C=O)	ν (C=N)	ν (N-N)	M-O	M-N
L	3472w	3281m	1664s	1563m	995m	-	-
Ni(L) $_2$ Cl $_2$	3472m	3208w	1664w	1594 m	957w	692m	559m
Cu(L)Cl $_2$. H_2O	3521m	3210m	1655m	1595m	863m	689w	572w

3.4. Electronic Spectra and Magnetic Measurements

The solution electronic spectra data as measured in methanol for the ligand and metal(II) complexes are presented in Table 3. The electronic spectrum of the ligand displayed two absorption bands in the range of 47169 to 30303 cm^{-1} and these are assigned to the intraligand π - π^* transition in the aromatic ring and n- π^* transition of the C=O

functional group [29, 30, 31]. These bands had a hypsochromic shift to a higher wave number in the Ni(II) complex in the range 49504 to 35850 cm^{-1} , while in the Cu(II) complex, there was red shift to lower wave number between 41736 to 32446 cm^{-1} indicating the interaction of the metal ions with the ligand. In the visible region, the nickel(II) complexes showed three bands which are expected for a d^8 ion in an octahedral environment. These bands occur at

23510 cm^{-1} and was assigned to ${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{2g}(\text{v}_1)$; 15873 cm^{-1} due ${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{1g}(\text{v}_2)$ and 11240 cm^{-1} assigned to ${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{1g}(\text{P})(\text{v}_3)$. The visible spectra of the synthesized copper(II) complex had a single broad band between centred at 15610 cm^{-1} which is consistent with a square pyramidal geometry [21].

The room temperature magnetic moment for the nickel(II) complex is 2.92 B.M and this showed that the complex is

paramagnetic with two unpaired electrons, suggesting an octahedral geometry for the complex [25]. Cu(II) complex had a moment of 1.65 B.M which is lower than the spin only value of 1.73 B.M. expected for a d^9 ion with one unpaired electron. The lower value could be as a result of antiferromagnetic interaction between adjacent magnetic centres. A square pyramidal geometry has been proposed for the complex.

Table 3. The electronic spectral data for the compound.

Compounds	Intraligand Transitions (cm^{-1})	d-d bands (cm^{-1})	Assignments	Proposed geometry
L	47169, 30303	-	-	-
Ni(L) ₂ Cl ₂	49504, 35850	11240 15873 23510	${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{1g}(\text{P})$ ${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{1g}$ ${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{2g}$	Octahedral
Cu(L)Cl ₂ .H ₂ O	41736, 32446	15610	${}^2\text{B}_{1g} \rightarrow {}^2\text{A}_{1g}$	Square Pyramidal

3.5. Acetylcholinesterase Inhibitory Activity

The acetylcholinesterase enzyme has been characterised and is believed to have three major domains [22]. These are the esteratic site, which contains the active serine; the anionic site, which is about 4.7 Å from the esteratic site and binds the quaternary ammonium group of the substrate acetylcholine and a hydrophobic region that is adjacent to or near the esteratic and anionic sites. This is believed to be important in binding aryl substrates and active site ligands [22]. Research involving the three main classes of cholinesterase inhibitors point to the importance of hydrophobicity among the physostigmine and benzylamine derived inhibitors, electronic effects among benzylamine derivatives, and the contribution of steric effects [23]. These observations were supported by structure activity relationship studies on the inhibitory action of phenserine, a physostigmine derivative, against acetylcholinesterase and butyrylcholinesterase [24].

The ligand and metal(II) complexes tested against cholinesterase activity (Table 4, Figure 2) showed that they all had moderate anticholinesterase activity when compared to the positive control, Eserine (physostigmine). Eserine had the highest activity with IC_{50} of $68 \pm 1.13 \mu\text{g/mL}$ compared to the ligand which had IC_{50} of $190 \pm 20 \mu\text{g/mL}$, while the nickel(II) and copper(II) complexes displayed activities with an IC_{50} of $390 \pm 80 \mu\text{g/mL}$ and $220 \pm 20 \mu\text{g/mL}$ respectively.

It is apparent that the hydrophobic nature of the compounds enhanced their moderate cholinesterase inhibitory activity. It can also be deduced that there are structural similarities between the hydrazone (L) and the positive control, physostigmine, such as the presence of a carbonyl ($\text{C}=\text{O}$) group, and an aromatic ($-\text{C}_6\text{H}_6$) group near the $-\text{C}=\text{O}$ group. The distinguishing structural characteristics of the active hydrazone are the presence of the free $-\text{C}=\text{O}$ group and a $-\text{C}_6\text{H}_6$ group. The ability of the N-N bond to rotate and render it flexible in compounds, could contribute to a better enzyme-inhibitor interaction that resulted in its

observed activity for the free ligand [32]. The Nickel(II) and Copper(II) complexes, do not have the free carbonyl group having been used for bonding with the metal ion, which could be responsible for their lower anticholinesterase activity compared to the hydrazone ligand.

Table 4. The IC_{50} ($\mu\text{g/mL}$) values of Acetylcholinesterase inhibitory Activities of the compounds.

Compounds	IC_{50} ($\mu\text{g/mL}$)
L	$190 \pm 20 \mu\text{g/mL}$
Ni(L) ₂ Cl ₂	$390 \pm 80 \mu\text{g/mL}$
Cu(L)Cl ₂ .H ₂ O	$220 \pm 20 \mu\text{g/mL}$
Eserine	$68.5 \pm 1.13 \mu\text{g/mL}$

4. Conclusion

Investigations into the synthesis, characterisation and acetylcholinesterase inhibitory activity of 3-hydroxybenzaldehyde-4-nitrobenzoic acid hydrazone, its Nickel(II) and Copper(II) complexes have been carried out in this study. The synthesised compounds were characterised by various physicochemical methods such as ${}^1\text{H}$ NMR, IR, UV-Vis and magnetic measurement. The ligand coordinated to the metal ion in a neutral bidentate form through the carbonyl oxygen ($\text{C}=\text{O}$) and the azomethine nitrogen ($\text{C}=\text{N}$). The magnetic moments observed for the nickel(II) (2.92 B.M.) and copper(II) (1.65 B. M.) are close the spin only values and suggested octahedral geometry for the compounds. The free ligand had an IC_{50} value of $190 \pm 20 \mu\text{g/mL}$ while the copper(II) complex had a value of $220 \pm 20 \mu\text{g/mL}$, and the Nickel(II) complex had the least inhibitory ability with a value of $390 \pm 80 \mu\text{g/mL}$. the positive control, Eserine exhibited the highest acetylcholinesterase activity with a value of $68 \pm 1.13 \mu\text{g/mL}$. It can be concluded that free organic chelators have better orientation and are unhindered to have a better enzyme-inhibitor interaction than the metal complexes.

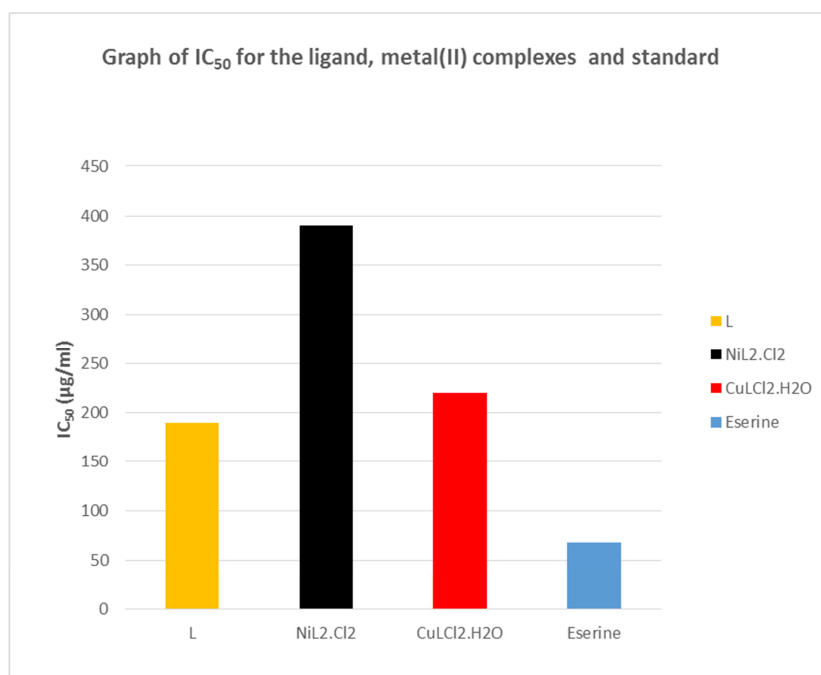


Figure 4. Graphical representation of IC₅₀ for the compounds.

5. Proposed Structure

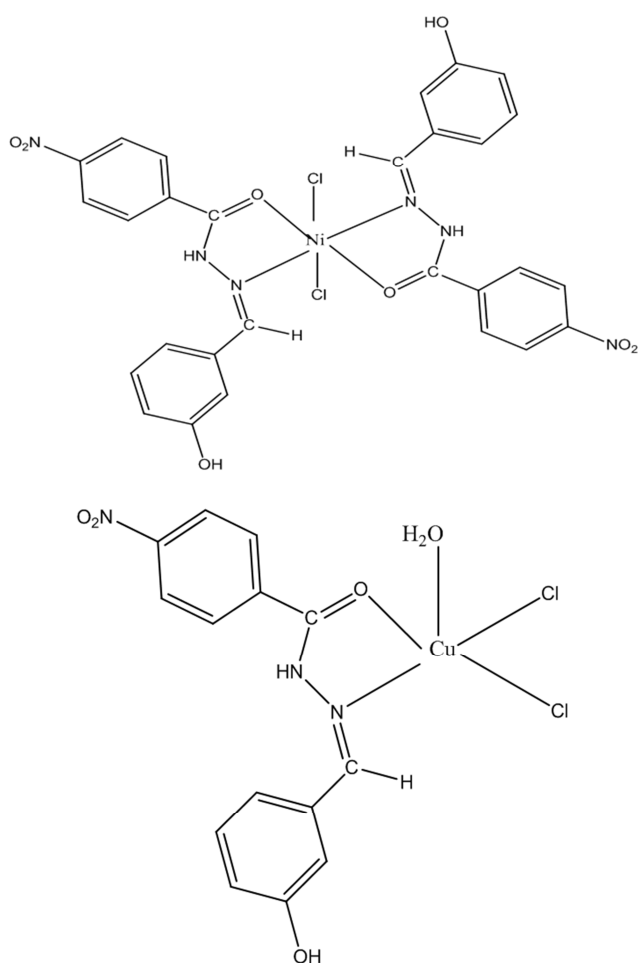


Figure 5. Proposed structure for nickel(II) and copper (II) complexes.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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