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Synthesis, characterization and cytotoxicity Study of 2-(benzylideneamino)-3-methylbutanoic acid and its coordination compounds

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Abstract
The Schiff base 2-(benzylideneamino)-3-methylbutanoic acid, **L1**, was synthesized by the reaction of 2-amino-3-methylbutanoic acid and benzaldehyde along with its coordination compounds of with Cu(II) and Co(II) metal ions. The mixed ligand complexes of these compounds with ethane-1,2-diamine, **L2,** were also synthesized. shrimp was utilized to screen these compounds for their cytotoxic activity. To characterize the ligands, infrared and electronic spectroscopy were used. Magnetic susceptibility evaluations, percentage metal compositions, and electronic and infrared spectroscopy were used to characterize the synthesized complexes. The brine shrimp lethality assay was used to screen these compounds for their cytotoxic potential. The results obtained from the characterization indicated that a Schiff base was obtained. Furthermore, the complexes exhibited a variety of geometries from octahedral to square planar. The results obtained indicated that the compounds exhibited moderate cytotoxic activity.

Keywords: Cytotoxity; Mixed ligand complexes; Anti-cancer agents; Coordination compounds; Amino acid derived Schiff base

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Introduction

Schiff bases are important compounds in organic chemistry exhibiting a wide range of pharmacological effects and biological activities, including anti–inflammatory, -cancer, -viral. -bacterial, -malarial, and pyretic properties (Wang, 2017; Zhao, 2018; Tsantis *et al.*, 2020; Şenocak, *et. al.*, 2021). Schiff bases have been instrumental in the growth of contemporary coordination chemistry (Tsantis *et al*., 2020). This is due to their highly modular synthesis that allows the control over the nature of donor sites; denticity; chelating and/or bridging abilities; and their electronic and steric characteristics (Tsantis *et al.*, 2020). Inorganic chemistry places a special emphasis on amino acid Schiff bases complexes because of their physiological and pharmacological activities (Arunadevi and Raman 2020; Şenocak, *et. al*., 2021). Co(II) complexes with vitamin B_{12} and Pd(II) complexes as anticancer and -tumor agents are two examples (Dehkhodaei, *et al.*, 2018; Sunjuk *et al*,. 2022). Cakes and other baked goods are flavored with synthetic benzaldehyde, a flavoring agent that mimics almond extract. Benzaldehyde occurs naturally in foods like oyster mushrooms and almonds. 2-amino-3-methylbutanoic acid , an alpha amino acid, is an essential amino acid. It has been established that dietary 2-amino-3-methylbutanoic acid is necessary for hematopoietic stem cell (HSC) self-renewal, as a result HSC transplants are used to treat cancer (Taya *et al.*, 2016). In view of these properties exhibited by benzaldehyde and 2-amino-3-methylbutanoic acid it was projected that the Schiff base derived from the reaction of both reagents may possess, in part, the potential to be non-toxic.

It has been reported that one of the most challenging diseases to cure and a major cause of death worldwide is cancer (Noser *et al.*, 2021). About 90.5 million people worldwide had cancer in 2015 (GBD *et al.*, 2013). Approximately 18 million new cases were reported annually as of 2019 (Sciacovelli, *et al.*, 2020). Around 8.8 million people died as a result of it worldwide (15.7% of deaths) (Sciacovelli, *et al*., 2020; Kocarnik, *et al*., 2022). Although there are many approaches to the treatment of cancer, chemotherapy has proven to be one of the most successful strategy since the discovery of cisplatin (Hanna *et al.*, 2014; Elsyana, *et al.*, 2014; Li *et. al.*, 2020). The down side of cisplatin, and other platinum based anti-cancer agents, is that they are at times ineffective against a variety of prevalent cancers. Drug side effects, such as nerve damage, hair loss, kidney malfunction and nausea are often experienced. These are also frequently accompanied by drug resistance (Li *et. al.*, 2020). Therefore, alternative coordination compounds that are efficient anti-cancer therapeutic with little or no side effects, are in demand (Li *et. al.*, 2020). Such coordination compounds include those of copper and cobalt. These constitute a less expensive and safer alternative to classical platinum-containing chemotherapy (Munteanu and Suntharalingam 2015; Molinaro *et. al.*, 2020; Ge *et. al.*, 2022; Kar *et. al.*, 2022)..

Some chemotherapy treatments include cytotoxic drugs, which are designed to prevent cell division. A substance’s capacity to kill cells is referred to as cytotoxicity (Priestman, 1989; American Cancer Society, 2021). Cell death maybe as a result of disruptions in particular biomolecular pathways, cellular systems or target site. These disruptive processes may occur in proteins, DNA or lipids. Situations in which these disruptions occur in the DNA, may hamper cell division resulting in cell death (Çelik, 2018). It should however be noted that many cytotoxic medications are unable to distinguish between malignant and normal cells: as they eradicate cancer by preventing all cell divisions (Priestman, 1989; American Cancer Society, 2021). Cytotoxicity may be measured in several ways, one of which is brine shrimp lethality assay, which is a preliminary cytotoxic analysis (Solis *et al*., 1993; Aiyelabola *et. al*., 2017).

Research findings, showed that coordination compounds of the amino acid Schiff bases exhibited significant cytotoxic activity (Sunjuk *et al*., 2022). In previous articles, it has been suggested that complexation may be beneficial to improve the biological activity, boosts bioavailability, and lower toxicity of biologically important organic compounds (Sakurai, *et* *al*., 2002; Chohan *et al*., 2002; Zhang, *et al*., 2002; Chang, 2010; Aiyelabola *et al*., 2017; Manolov, *et al* 2020). Therefore, Schiff base 2-(benzylideneamino)-3-methylbutanoic acid (**L1; Fig. 1)** was synthesized by the condensation reaction of 2-amino-3-methylbutanoic acid **(Fig. 2)** and benzaldehyde **(Fig. 3)**. The Cu(II) and Co(II) complexes of ligand **L1** and its mixed ligand complexes with ethane-1,2-diamine **L2 (Fig. 4) w**ere also synthesized. `The ligands were characterized using electronic and infrared spectrophotometry. The synthesized complexes were characterized using electronic and infrared spectroscopy, magnetic susceptibility measurements and percentage metal compositions. The synthesized compounds were further assessed for their cytotoxic activity using brine shrimp lethality assay.

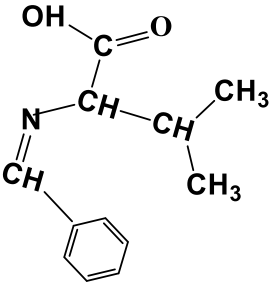

Figure 1: 2-(benzylideneamino)-3-methylbutanoic acid **(L1**)

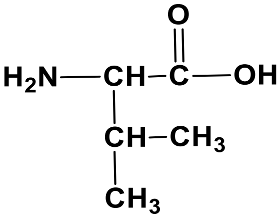

Figure .2: 2-Amino-3-methylbutanoic acid

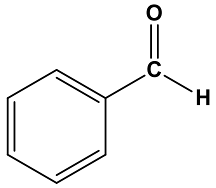

Figure .3: Benzaldehyde

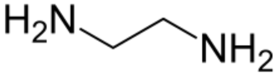

Figure 4: Ethane-1,2-diamine (**L2)**

Materials and Methods

All starting materials and solvents purchased from Aldrich and Fluka were used without further purification. Melting points were determined in an open capillary tube on a Gallenkamp (Variable heater) melting point apparatus. The infrared spectra of all synthesized products were obtained using Agilent Cary 630 FTIR. The electronic spectra, of all the compounds, were obtained in aqueous solution, in the wavelength range 400-1000 nm using 1800 Shimadzu ultra-violet spectrophotometer. Magnetic susceptibility measurement of the metal complexes was obtained using a MSB Mk1 magnetic susceptibility balance, Sherwood Scientific with [$HgCo(SCN)_4$] as standard. The metal analyses for the complexes were obtained using titrimetric method using ethylenediamine tetraacetic acid.EDTA. The equations of the reactions for the coordination compounds are given in equations (1)-(5).

$2CoCl_2.6H_2O$ + 4**L1** → $2Co($**L1**$)_2H_2O$ + $4H_2O$……………………(1)
COMPOUND 1

$CoCl_2.6H_2O$ + **L1** + **L2** → [Co**L1L2**]Cl + $6H_2O$…………………..(2)
COMPOUND 2

$2CuCl_2.2H_2O$ + 4**L1** → $2Cu($**L1**$)_2H_2O$ …..……………………….(3)
COMPOUND 3

$CuCl_2.2H_2O$ + **L1** + **L2** → [Cu**L1L2**$(H_2O)_2$]Cl,,,,,,,,,,,,,,,,,,,,,,,,,,,,(4)
COMPOUND 4

Synthesis of Schiff base 2-(benzylideneamino)-3-methylbutanoic acid L1

A solution of benzaldehyde (1.13 g, 0.01 mol) was added to a solution of 2-amino-3-methylbutanoic acid (1.25 g, 0.01 mol) in 250 mL of absolute ethanol, with three drops of glacial acetic acid. The reaction mixture was then heated for 2 h. The mixture was cooled to room temperature and a white precipitate was obtained. The product was filtered, washed and dried to give **L1**.

Syntheses of Metal Complexes

Compound **1**: Cobalt(II) chloride hexahydrate (1.21 g, 0.005 mol) was dissolved in absolute ethanol. This was added to a refluxing solution of benzaldehyde (1.09 g, 0.01 mol) and 2-amino-3-methylbutanoic acid (1.23 g, 0.01 mol) in absolute ethanol (250 mL) with the addition of glacial acetic acid (2 mL). The reaction mixture was refluxed for 2 h. A purple precipitate was obtained. This was recrystallized using methanol/ethanol mixture (70/30, 10 mL).

Yield: 1.04 g, 63.10%, mp: 225-226 (d) ˚C, metal composition (%.): 12.19 (found); 12.11 (calcd), μ_{eff}: 2.66 BM.

Compound **2**: A mixture of cobalt(II) chloride hexahydrate (1.26 g, 0.005 mol), benzaldehyde (1.12 g, 0.01 mol) and 2-amino-3-methylbutanoic acid (1.21 g, 0.01 mol) and glacial acetic acid (2 mL) in absolute ethanol (250 mL) was heated. Ethanolic solution of ethane-1,2-diamine (0.89 g, 0.01 mol) were then added drop wisely to the solution. This was heated for an hour and a scum was formed. The resultant mixture was filtered washed and dried to give an orange coloured precipitate.

Yield: 2.62 g (69.40%), mp: 230-231 (d) ˚C, metal composition (%.): 15.42 (found); 15.65 (calcd), μ_{eff}: 1.96 BM.

Similar procedure was used for the preparation of the copper(II) complexes.

Compound **3**: A mixture of copper(II) chloride dihydrate (0.42 g, 0.005 mol), benzaldehyde (1.12 g, 0.01 mol) and 2-amino-3-methylbutanoic acid (1.22 g, 0.01 mol) in absolute ethanol (250 mL) with the addition of glacial acetic acid (2 mL) was refluxed. This gave a royal blue product.

Yield: 2.69 g (54.58%), mp: 234-235 (d) ˚C, metal composition (%.): 13.12 (found); 13.01

(calcd), μ_{eff}: 1.53 BM.

Compound **4**: A mixture of copper(II) chloride dihydrate (0.93 g, 0.005 mol), benzaldehyde (1.16 g, 0.01 mol) and 2-amino-3-methylbutanoic acid (1.25 g, 0.01 mol) in absolute ethanol (250 mL), ethane-1,2-diamine (0.90 g, 0.01 mol) and glacial acetic acid (2 mL) was refluxed. This gave a blue product.

Yield: 2.90 g (69.41%), mp: 257-258 ˚C, metal composition (%.): 15.42 (found); 15.31 (calcd), μ_{eff}: 1.80 BM.

Cytotoxicity

The procedure used was modified from the assay described by Solis *et al.*, 1993 Brine shrimp (*Artemia salina*) were hatched from shrimp eggs in a conical shaped vessel (1 L). Subsequently the vessel was filled with sterile, artificial seawater under continuous aeration for 48 h. After hatching, active nauplii free from eggshells were collected from brighter portion of the hatching chamber. These were employed for the assay. Ten nauplii were drawn through a Pasteur pipette and placed in each vial containing 4.5 mg/L of brine solution. In each experiment, different volumes of the sample were added to 4.5 mL of brine solution. This produced different concentrations of 20, 40, 60, 80 and 100 µg/mL. Solutions were maintained at room temperature for 24 h under light. The surviving larvae were counted. Experiments were conducted along with the control (vehicle treated), of the test substances in a set of three tubes per dose. Estimation of the LC_{50} values was estimated using probit® analysis on a USEPA computer program.

Results and Discussion

Ligands

L1: The infrared spectrum (**Fig. 5**) of **L1** elicited a strong band at 1591 cm^{-1} (**Table 1**) which was attributed to the ν(C=N), and is characteristic of the azomethine functional group (Nakamoto, 2009; Kemp, 1999). Therefore, the appearance of this band is indicative of the formation of a Schiff base. The fact that this stretching frequency was absent in the spectra of both starting reagents further corroborates this. Furthermore, the appearance of this stretching vibrational frequency is in agreement with previous reports for similar compounds (Alturiqi *et al.,* 2018; Vhanal,*et al.,* 2019; Hanan *et al.,* 2021; Pavia *et al.,* 2021). Additional evidence for the formation of the Schiff base was provided by the absence of vibrational stretching frequencies characteristic of amino and carbonyl (aldehyde) substituents. Interestingly, both frequencies were observed in the spectrum of both 2-amino-3-methylbutanoic acid and benzaldehyde respectively, both starting materials in the synthesis of ligand **L1**. A band observed at 1356 cm^{-1} was ascribed to $\nu(C \overset{...}{-} C \overset{...}{-} N)$. The appearance of this stretching frequency also gives credence to the formation of the Schiff base. Since such bands are absent in the spectra of the both reactants. It also suggests the formation of the imine unctional group. The aromatic **ν**(C=C), 1603 cm^{-1}, was pretty much masked by the ν(C=N) band and was observed as a weak band by the side of this band. Peaks at 1016 and 1038 cm^{-1}, was ascribed to δ(O-H) from the carboxylic acid end of ligand **L1.** It therefore, served as an indication that the carboxylic acid end of the ligand **L1** was protonated. Supporing this further was the band at 3116 cm^{-1} attributable to *v*(O-H) of the carboxylic acid moiety. This was observed to be lower that expected and this was suggested to be as a result of intramolecular hydrogen bonding.

The electronic spectrum of ligand **L1** showed three bands in the ultraviolet region at 212, 260 and 374 nm and were assigned to n→ σ*, n→π* and π→π* transitions of the major chromophores present in the ligand (Kemp, 1999; Pavia *et al*., 2021).

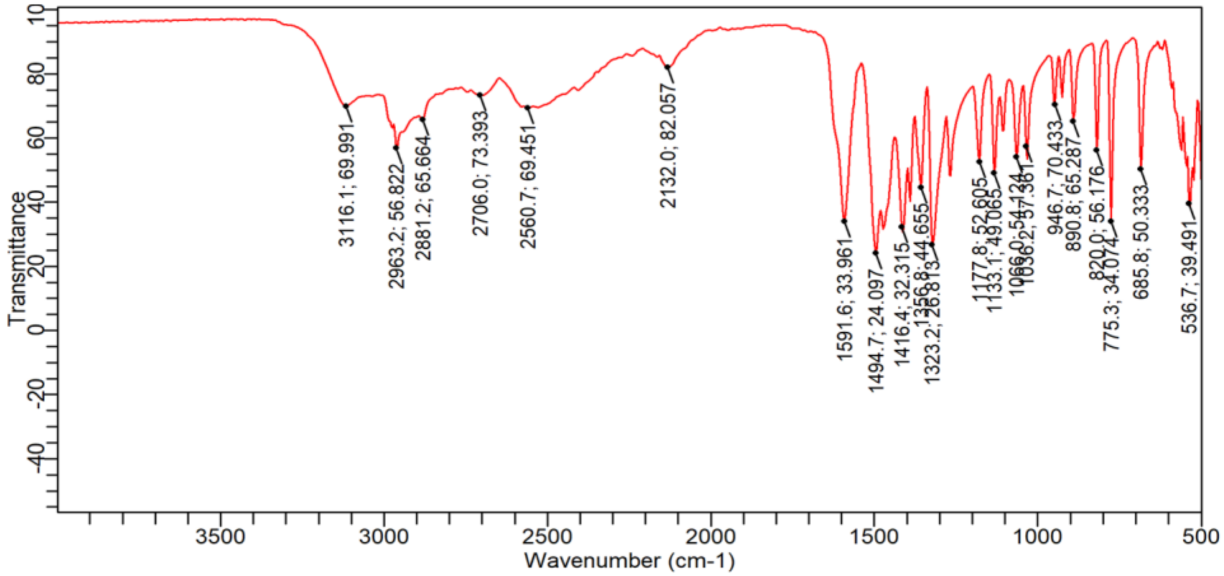

Figure 5: Infra red spectrum of ligand **L1**

Table 1: Relevant Infrared Spectra Bands for the Ligands and Complexes (cm^{-1}).

	L1	**L2**	**1**	**2**	**3**	**4**
ν(O-H)	3116	-	3332,3272	3447,3339	3278,3250	3298
ν(N-H)	-	3373	-	3224,3153	-	3250
ν(C=O)	-	-	1468	1654	1464	1464
ν(C=N)	1591	-	1576	-	1613	1613
ν(C=C)Ar	1475	-	1532	1606	1601	1604
ν(C=O)	1323	-	1319	1580,1326	1590,1312	1580,1312
ν(C⎓C⎓N)	1356	-	1358	1565,1364	1532,1356	1536,1360
ν(-CH_3)	-	-	-	1375	1371	1371

δ(N-H)	-	-	-	1207	1215	1215
δ(C-N)	-	-	-	1326	1278	1278
δ(O-H)	-	-	-	1058,1013	-	-
ν(M-O)	-	-	682,626	648,574	641,581	641,581
ν(M-N)	-	-	540,488,421	506,473	508,424	560,443

L2: The infrared spectrum revealed a band at 3373 cm^{-1} which was ascribed to the *ν*(N–H) of the amine substituent of the ligand. Two other sharp bands were also observed at 1567 and 1403 cm^{-1}, (**Table 2)** and were ascribed to *ν*(C–C–N) and *ν*(C–N) of C–C–NH_2 bond vibration (Nakamoto, 2009; Kemp, 1999).

The ultra-violet spectrum revealed a band at 495 nm attributable to $n \rightarrow \sigma^*$ transition.

Table 2: Relevant Electronic Spectra Bands (nm), for the Ligands and Complexes.

Compound	Bands (nm)	d-d (nm)
L1	212, 260, 374	-
L2	234, 298	-
1	214, 231, 280	532, 763. 862
2	sh214, 256	482, 687, 826
3	219, 246	668 and 720
4	206, 226, 347	545, 590shld, 610

Coordination Compounds

Compound 1

The infrared spectrum (**Fig. 6**) of compound **1** revealed bands at 1468 and 1532, 1329 cm^{-1} assigned to asymmetric and symmetric carboxylate stretching frequencies. This suggested that the carboxylic acid substituent end of ligand **L1** was deprotonated in compound **1**. Bands at 3332 and 3272 cm^{-1} were assigned to the vibrational stretching frequency attributable to O-H functional group. Since template synthesis was used to obtain compound **1**, the presence of a band at 1575 cm^{-1} attributed to ν(C=N) suggest the formation of the Schiff. In addition to this, the *ν*(C⎓C⎓N) observed at 1358 cm^{-1} served to corroborate this further. Evidence of coordination was provided by bands observed at the lower energy end of the spectrum. Bands at 682 and 626 cm^{-1} were ascribed to ν(Co-O). On the hand bands at 540, 488 and 421 cm^{-1} were assigned to ν(Co-N).

The electronic spectrum of compound **1** showed bands at the ultraviolet region at 214, 231 and 280 nm. Attributable to n→ σ*, n→π* and π→π* transitions. Shifts to higher energy for the n→π* and π→π* transition in comparison with **L1** suggested coordination. The visible region of the spectrum showed bands at 532, 763 and 862 nm and were assigned to *d-d* transitions: $^4A_2(F) \rightarrow {}^4T_1(P)$, $^4A_2(F) \rightarrow {}^4T_2(F)$ and $^4A_2(F) \rightarrow {}^4T_1(F)$, respectively. Compound **1** exhibited a magnetic moment of 2.66 BM. According to previous reports this is indicative of a diametric, square planar geometry. This they argued may be as a consequence of interaction between electron spins on neighbouring metal ions or polymerization. Therefore, it was suggested that the mechanism involved in dimerization of compound **1** to be due to both mutual pairing of electron spins via orbital overlap, metal-metal interaction and super exchange moment (Greenwood and Earnshaw, 1997).

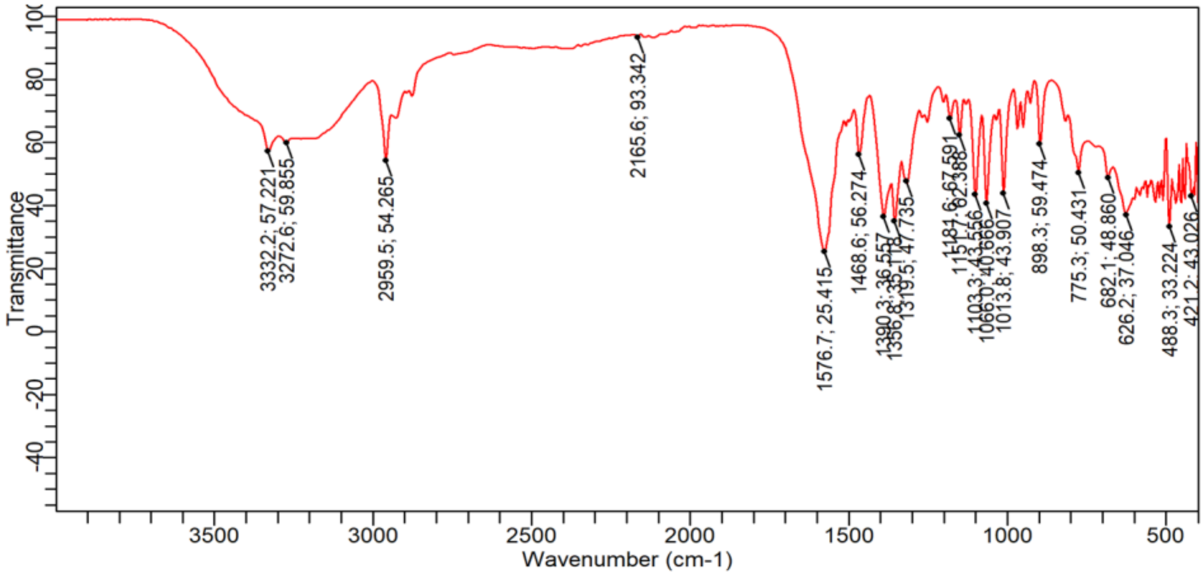

Figure 6: Infra red spectrum of **Compound 1**

Compound 2

The infrared spectrum of this compound (Figure 7) elicited a broad band at the high energy end of the spectrum at 3447 and 3339 cm^{-1}. Both which were attributed to the O-H vibrational stretching frequency. The presence of the δ(O-H) at 1058 and 1013 cm^{-1} further supported this (Pavia *et al*., 2021). Bands observed at ν(COO^{-}) 1654 and 1580, 1326 cm^{-1} were ascribed to ν(COO^{-}) asymmetric and symmetric stretching frequencies. This in addition with the bands at 1565 and 1364 cm^{-1}, assigned to *v*(C=C=N), suggested the formation of the Schiff base. Evidence of coordination of the Schiff base was provided by bands at observed at 648 and 574 cm^{-1}, which was assigned to ν(Co-O). Additionally, the band at 506 cm^{-1} attributed to ν(Co-N) (Nakamoto, 2009). Two bands were observed at 3224 and 3153 cm^{-1} which were assigned to both asymmetric and asymmetric stretching frequencies of the (N-H) of -NH_2. This is ascribable to the NH_2 substituent of the secondary ligand, ethane-1,2-diamine (Nakamoto, 2009). This therefore served as evidence for the coordination of the primary ligand as well as the formation of the mixed ligand complex. It should be noted that this stretching frequency was absent in the spectrum of compound **1**, which is the traditional coordination counterpart of compound **2**. This was also corroborated by the band at 1207 cm^{-1} ascribed to δ(N-H). The band at 473 cm^{-1} attributable to ν(Co-N), indicate the coordination of the secondary ligand with the central metal ion.

The electronic spectrum, 214(sh) and 256 nm ascribable to n→ σ* and n→π* intraligand transitions of the ligand. In the visible region of the spectrum exhibited bands at 482, 687 and 826 nm. Thes were attributed to $^{4}A_{2}(F) \rightarrow {}^{4}T_{1}(P)$, $^{4}A_{2}(F) \rightarrow {}^{4}T_{2}(F)$ and $^{4}A_{2}(F) \rightarrow {}^{4}T_{1}(F)$ *d-d* transitions (Greenwood and Earnshaw, 1997) .

The effective magnetic moment for the complex was 1.96 BM. This observed magnetic moment was much lower than that expected for high spin cobalt(II) complex with d^7 configuration. This may be interpreted according to previous reports to be attributable to four-coordinate, square planar geometry for cobalt(II) with antiferromagnetism. This feature was studied previously by other workers and they suggested that the subnormality in the magnetic moments may be ascribed to antiferromagnetism which is due to the interaction between electron spins on neighbouring metal ions (Aiyelabola *et al*., 2020).

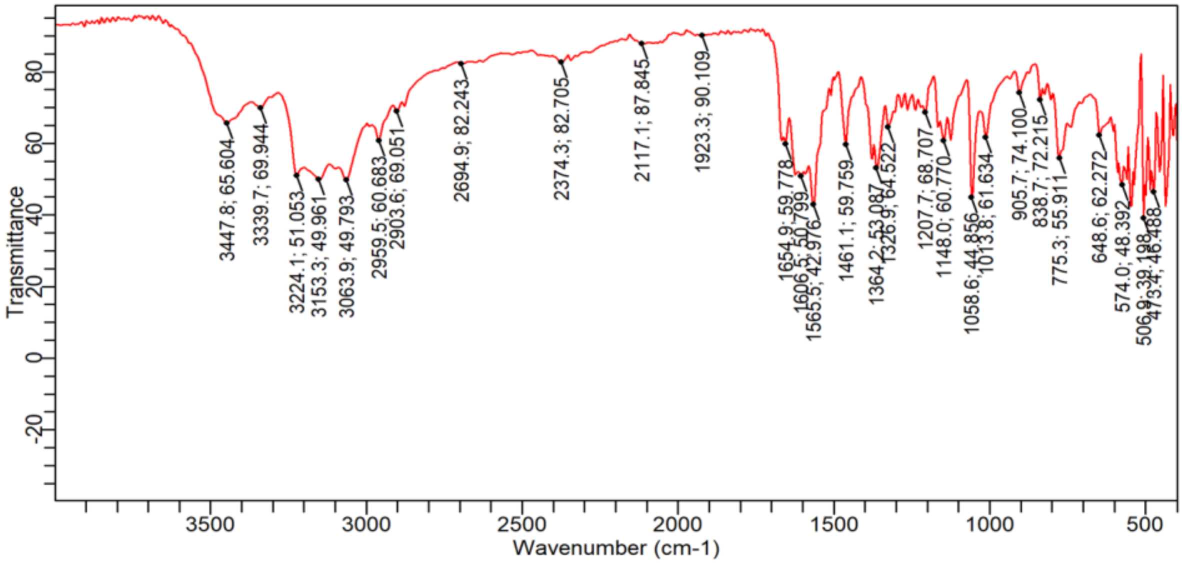

Figure 7: Infra red Spectrum of Compound 2

Compound 3

Similar to its cobalt complex counterpart, the infrared spectrum (FIG.8) of compound 3 exhibited two bands at 3278 and 3250 cm^{-1} assigned to ν(O-H). The presence of bands at 1613 cm^{-1}, attributed to ν(C=N), and 1532 and 1356 cm^{-1} ascribed to *ν*(C═C═N), suggested the formation of the Schiff base. In compound 3 the asymmetric carboxylate stretching frequency was observed at 1464 cm^{-1} (Table 1). The symmetric carboxylate stretching frequencies was observed at 1590 and 1312 cm^{-1}. Cu-O stretching frequencies was observed at 641 and 581 cm^{-1}. The ν(Cu-N) was observed at 508 and 424 cm^{-1}. Both the former and latter stretching frequencies suggested coordination of the central metal ion, copper(II) with the ligand.

The visible spectrum of compound **3** displayed broad bands at 668 and 720 nm assigned to $^2B_{1g} \rightarrow {}^2E_{1g}$ and $^2B_{1g} \rightarrow {}^2B_{2g}$ transitions. Its magnetic moment of 1.53 BM is indicative of an antiferromagnetic spin-spin interaction through molecular association with possible Cu–Cu interaction or dimerization. Similar results have been reported earlier for copper(II) acetate complex. It was suggested that the possible mechanism involves the single unpaired electrons on the copper atoms interact as a couple, antiferromagnetically to produce a low-lying singlet (diamagnetic), excited but thermally accessible triplet (paramagnetic) level. The separation is therefore only a few KJ mol^{-1}. The simplest interpretation is to assume that singlet and triplet levels arise from a single interaction between the unpaired spins of the copper atoms (Greenwood and Earnshaw, 1997; Aiyelabola *et al.*, 2017) .

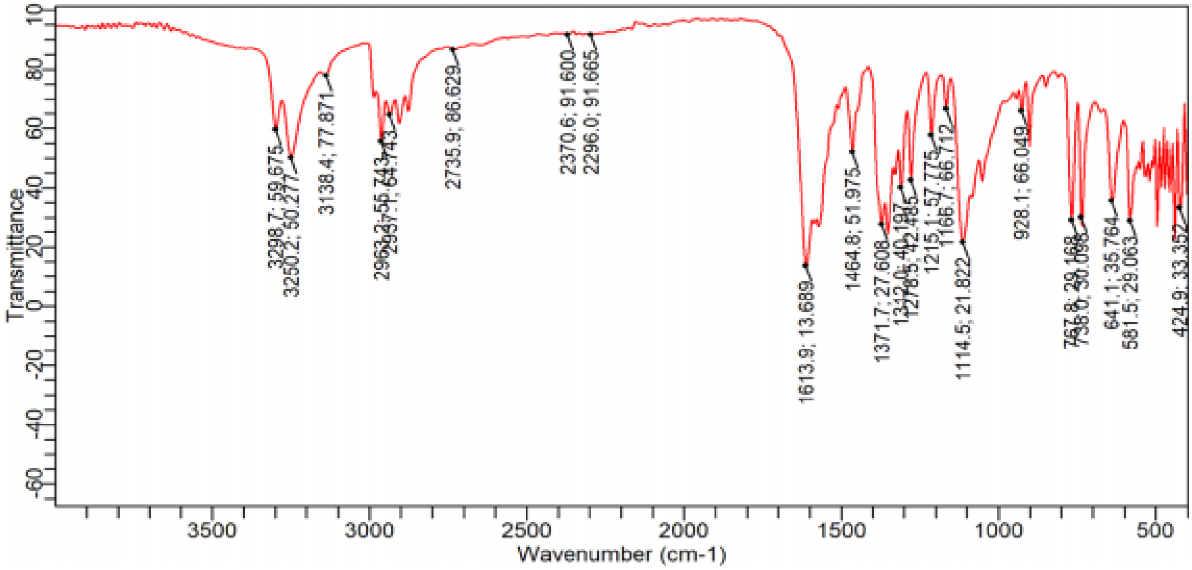

Figure 8: Infra red spectrum of **Compound 3**

Compound 4

In the infrared spectrum of compound **4** evidence for the formation of **L1** was provided by the observance of bands at 1613 and 1536, 1360 cm^{-1} (Fig. 9) which were attributable to ν(C=N) and ν(C⎓C⎓N) respectively. A broad band at 3298 cm^{-1} was assigned to ν(O-H). Another band at the high energy region of the spectrum at 3250 cm^{-1} was ascribed to (N-H) vibrational stretching frequency (Pavia *et al.*, 2021). It is suggested that this is from the amino substituent of the secondary ligand. The asymmetric and symmetric stretching frequencies of the carboxylate substituent of ligand **L1** was observed at 1464 and 1580, 1312 cm^{-1}. In the infrared spectrum of compound **4** evidence for the formation of a complex was provided by the presence of bands at lower energy region of the spectrum attributable to metal-oxygen and metal-nitrogen vibrational frequencies. Bands observed at 641 and 581 cm^{-1} were assigned to Cu-O vibrational frequencies (Nakamoto, 2009). This is indicative of coordination of oxygen atom of ligand **L1** and that from the O-H substituent, whose band was observed at the high energy end of the spectrum. In addition to this, the bands observed at 560 and 443 cm^{-1} were ascribed to copper-nitrogen vibrational frequencies. The observance of both bands suggests the coordination of both the primary ligand, ligand **L1** and the secondary ligand **L2**. Therefore, this serves to corroborate the formation of the mixed ligand complex.

The electronic spectrum exhibited bands in the ultra-violet region at 206, 226 and 347 nm attributed to intraligand transfer transitions. The d-d transition were observed in this case at 545, 590 (shld) and 610 nm ascribed to $^2B_{1g} \rightarrow {}^2A_{1g}$, $^2B_{1g} \rightarrow {}^2E_{1g}$ and $^2B_{1g} \rightarrow {}^2B_{2g}$ transitions, which suggested an octahedral geometry. This proposed geometry was corroborated by its magnetic moment of 1.80 BM, indicative of a tetragonally distorted octahedral geometry. This is in agreement with that proposed by previous workers for similar compounds (Greenwood and Earnshaw, 1997; Aiyelabola *et al*., 2020).

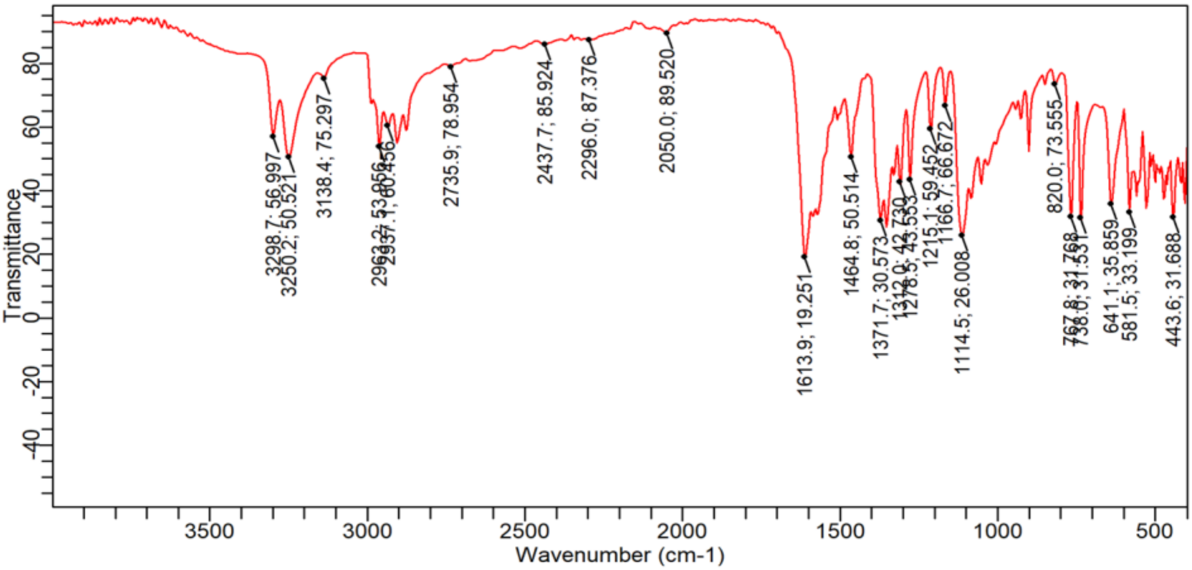

Figure 9: Infra red spectrum of Compound 4

Based on the results obtained it is proposed that ligand L1 coordinated in a bidentate manner, using the nitrogen atom of the imine moiety and an oxygen atom of the carboxylate substituent. The result obtained further indicated that although in L1 the carboxylic acid end was protonated, prior to coordination deprotonation occurred to give the carboxylate anion. The results further suggested that the secondary ligand, L2 coordinated in a bidentate fashion as well, making use of both nitrogen atoms of the amino substituent as donor atoms in coordination. It is suggested that compound 1 exists as a dimeric cobalt complex, with a water molecule coordinated with each cobalt atom (Fig. 10). On the other hand, it is suggested that compound 2 consists of a square planar cobalt complex with antiferromagnetism (Fig. 11). For compound 3 a dinuclear copper complex with four molecules of ligand L1 shared by two copper ion centers and two molecules of water in the axial region is proposed (Fig. 12). An octahedral geometry is proposed for compound 4, with one molecule of ligand L1, and one molecule of the secondary ligand, ethane-1,2-diamine, L2. In addition to this are two molecules of water (Fig. 13).

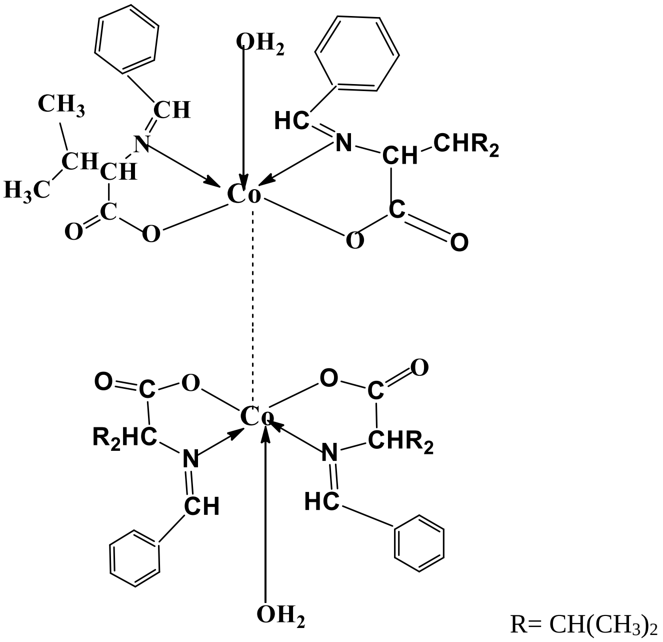

Figure 10: Pictorial representation for the proposed structure for Compound 1

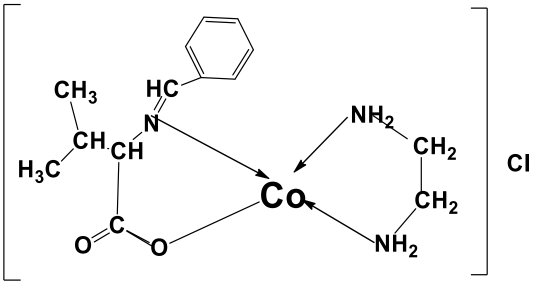

Figure 11: Pictorial Representation for the Proposed Structure for Compound 2

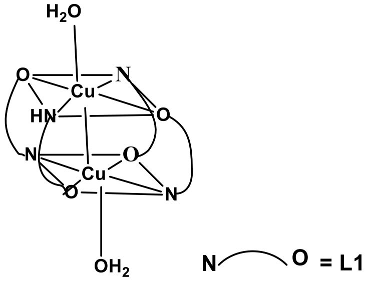

Figure 12: Pictorial representation for the proposed structure for Compound 3

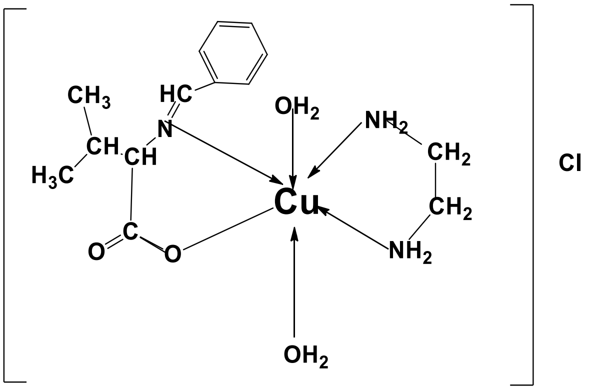

Figure 13: Pictorial representation for the proposed structure for Compound 4

Cytotoxicity

The results obtained from the brine shrimp lethality assay for the synthesized compounds are presented in Table 3. The results obtained indicated that compound 3 had the highest cytotoxic activity, followed by compound 2, with compound 4 eliciting the least activity. The activity of compound 2 is two fold greater than that of compound 4 (Table 3), although both compounds were proposed to be ionic, differences in their geometry and the metal ion size (the variables in this compounds) are suggested to be possible factors on which their activity is dependent. This is more evident in the case of compounds 1 and 3, both having similar geometry, but compound 3 is three-fold more active than compound 1. The data obtained from the assay also showed that compounds 2 and 3 were more active than ligand L1 (LC_{50} 39.23). This suggests that coordination may enhance the cytotoxic property of a compound. However, this is not conclusive as both traditional and mixed ligand complexes of ligand L1 involving both metal ions used in this work had lower activity on complexation relative to L1. In comparison of the activity of the complexes with the metal salts used, for the cobalt complexes, coordination enhanced the potential nontoxic nature, on a cellular level, of the complexes. The cobalt salt (LC_{50} 30.51 g/ml) had better activity ($P< 0.05$) than the complexes. The fact that both the metal salt and compound 2 are ionic, may be a contributing factor to the better activity of compound 2 relative to compound 1. Although more data is required to ascertain this. On the other hand the copper(II) complexes exhibited better activity than the metal salt (LC_{50} 98.34 g/ml) used. The standard $K_2Cr_2O_7$ (LC_{50} 5.56 g/ml) exhibited significantly higher cytotoxic activity compared with that of the ligand and synthesized compounds.

T**able 3:** LD_{50} (µg/mL) of the Compounds

COMPOUND	**LD_{50} (µg/mL)**
L1	39.23
1	64.48
2	35.42
3	22.83
4	71.33

Conclusion

In this study the condensation reaction of 2-amino-3-methylbutanoic acid and benzaldehyde gave a Schiff base: 2-(benzylideneamino)-3-methylbutanoic acid, L1. This was coordinated with Co(II) and Cu(II) ions. Mixed ligand complexes using ethane-1,2-diamine (L2) as the secondary ligand were also synthesized. The result obtained from the characterization of the coordination compounds indicated that a variety of geometries were displayed by the complexes. The synthesized compounds exhibited good cytotoxic activity, although not as effective as the standard.

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