

Synthesis, Characterization and *in vitro* Antibacterial Studies of Novel Transition Metal (II) Complexes of 2,5-Diamino-2-(difluoromethyl)pentanoic Acid Hydrochloride Hydrate

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Article history: Received: 20 February 2017; revised: 27 April 2018; accepted: 09 June 2018. Available online: 30 June 2018. DOI: <http://dx.doi.org/10.17807/orbital.v10i5.962>

Abstract:

The synthesis and characterization of novel transition Metal (Cu(II), Co(II), Ni(II) and Zn (II)) complexes of 2,5-diamino-2-(difluoromethyl) pentanoic acid hydrochloride hydrate (DPH) have been described. The ligand and metal complexes were characterized by Melting point, Conductivity measurement, Elemental analysis, Fourier Transform infrared (FTIR) spectroscopy, Electronic spectroscopy, Magnetic susceptibility measurement and Electrospray Ionization Mass Spectrometry (ESI-MS). The FTIR spectral data suggests that the ligand behaves as a bidentate ligand coordinates to the metal ions through an oxygen atom of the carboxylate and a nitrogen atom of amino group. The terminal amino group of the ligand is protonated to form NH_3^+ while the carboxylic moiety is deprotonated forming zwitterionic complexes. Electronic spectral and magnetic studies data suggest that the complexes of Cu(II), Co(II) and Ni(II) have octahedral geometry around metal ions while tetrahedral geometry was proposed Zn (II) ion. *In vitro* anti-bacterial activities of the ligand and metal complexes were carried out using agar diffusion method against two gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and two gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumonia*). The results obtained revealed that the metal complexes showed enhanced antibacterial activities against the four micro-organisms with lowest minimum inhibitory concentration (MIC) when compared to parent compound.

Keywords: 2,5-diamino-2-(difluoromethyl) pentanoic acid hydrochloride hydrate; metal carboxylate; antibacterial studies; minimum inhibitory concentration (MIC)

1. Introduction

Metal carboxylates have attracted much attention owing to their great biological and industrial applications [1-5]. Organic ligands containing carboxyl groups have been utilized in the preparation of many useful materials such as metal organic frameworks (MOFs) as a result of affinity of carboxylate anions to the metal ions. It is a well known fact that carboxylate or dicarboxylate ligands exhibit a versatile

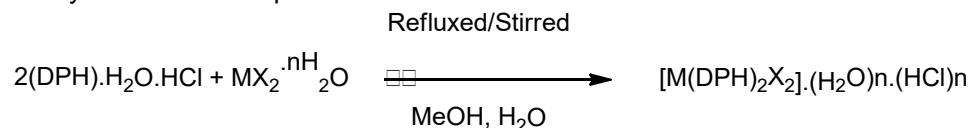
coordination behavior by displaying distinct bonding modes towards metal ions [6-12]. A literature survey has revealed the synthesis and characterization of complexes with ligand in which carboxylate anion and nitrogen atom formed coordination bonds with metal ions, some of these complexes have been found to possess good antimicrobial activities [13-21]. Transition metals are the best known cations usually used in the preparation of metal- organic complex due to their electrical, magnetic, catalytic and luminescence

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properties [22-25].

The choice of copper, cobalt, nickel, zinc in this study is as a result of their importance in biological and physiological activities that facilitate a variety of chemical reaction needed for life [20,21, 26-30].

Antimicrobial resistance is fast becoming a global concern with swift increase in multidrug-resistance bacteria and fungi [32-34]. This has necessitated continuous search for new antimicrobial compounds with particular focus on coordination compounds of biological importance [35-37]. The increased lipophilic character of the coordinated metal complexes with the resultant enhanced ability to permeate the cell membrane of the microbes has been proposed as reason for their improved activity over their parent ligands [38-39]. Chelation, which is responsible for the reduction in the polarity of the metal ions by partial sharing of its positive charge with donor group of ligands also support this concept [40-41]. Encouraging antimicrobial activities of some chelates with carboxylates groups have been reported [42-45]. To the best of our knowledge, it is interesting to state that extensive studies of 2,5-diamino-2-(difluoromethyl) pentanoic acid hydrochloride hydrate metal complexes have not



All the complexes conformed to the general formula $[\text{M}(\text{DPH})_2\text{X}_2].(\text{H}_2\text{O})_n.(\text{HCl})_n$ where $n = 1, 2, 3$ and $\text{X} = \text{Cl}, \text{CH}_3\text{COO}, \text{NO}_3$ or H_2O which was determined based on elemental analyses results. The complexes are formed by 2:1 molar condensation of ligand to transition metals and the stoichiometric reactions involved in the complex formation resemble earlier work relating to chlorpromazine hydrochloride [46]. The melting point of the metal complexes differed from the starting material (ligand), this indicates the likelihood of the formation of coordination compounds. Also, high purity of the complexes formed can also be predicted from sharp melting points obtained. The colours of the metal complexes are distinctly different from that of the ligand, thus, it can then be inferred that the colours displayed by the metal complexes are regulated by the metal ions, a possible indication to the formation of coordination compounds. All the metal complexes are insoluble in common

been reported to date.

In continuation of our studies of coordination of transition metals with biologically importance ligand, we report the synthesis, characterization and antimicrobial studies of novel transition metals (Cu(II), Co(II), Ni(II) and Zn(II)) complexes with 2,5-diamino-2-(difluoromethyl) pentanoic acid hydrochloride hydrate as ligand (DPH) (Fig. 1).

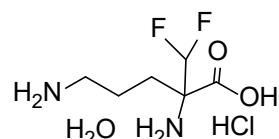


Figure 1. Molecular structure of 2,5-diamino-2-(difluoromethyl) pentanoic acid hydrochloride hydrate (DPH).

2. Results and Discussion

The preparation of DPH-metal complexes can be represented by the general formula thus:

organic solvents but soluble in DMSO and water.

The results showed the presence of water molecules in all the metal complexes either as coordinated ligand, water of crystallization outside the coordination sphere or both. The presence of uncoordinated water molecules outside the coordination sphere was confirmed using cobalt chloride paper. The droplets of colorless liquid stemmed out by gently heating of metal complexes (3-7) turned cobalt chloride paper from blue to pink confirming the presence of water molecules outside the coordination sphere while complexes 1 and 2 with no uncoordinated water molecules had no effect on cobalt chloride paper. This test further buttressed the molecular formulation proposed for each of the metal complexes.

Two amino groups are present in the ligand (DPH), the terminal one is protonated to form NH_3^+ while the second one is involved in

coordination with metal ions through nitrogen atom. The carboxylic moiety is deprotonated leading to the formation of zwitterionic structure of metal complexes. This assertion is in agreement with our earlier work where crystal structure involving the same ligand was obtained [47].

2.1 Fourier Transform Infra-red Spectra (FTIR)

The characteristic FTIR bands of the metal

complexes differed from the free ligand (DPH) either by the shift or disappearance of some characteristics frequencies and appearance of some new frequencies. This provided significant indications regarding the coordination and bonding sites of the ligand. Relevant characteristic bands of all the metal complexes together with that of the ligand are listed in Table 1. The principal bands attributed to asymmetric (ν_{as}) and symmetric (ν_s) stretching frequencies of (OCO) groups are reported in Table 2.

Table 1. Major FTIR (cm^{-1}) data of the ligand and the metal complexes.

Compound	$\nu(\text{NH}_2)$ (asy/sym)	$\nu(\text{OH})$ (carbo- xylic)	$\nu(\text{OCO})$ (asy/sym)	$\nu(\text{C-N})$	$\nu(\text{H}_2\text{O}/\text{HCl})$	$\nu(\text{NH}_3)$	$\nu(\text{M-O})$ /(M-Cl)	$\nu(\text{M-N})$
DPH (L)	3254 3173	3048	1647 1499	1138	3393	-	-	-
COMPLEX 1	3200 3124	3012	1637 1456	1188	3457	3328	512	696
COMPLEX 2	3204 3125	-	1662 1452	1190	3420	-	578	698
COMPLEX 3	3214 3104	3018	1671 1452	1185	3453	3311	556	667
COMPLEX 4	3234 3157	3018	1600 1383	1197	3441	3333	553	686
COMPLEX 5	3217 3107	3054	1659 1441	1169	3453	-	583	676
COMPLEX 6	3269 3165	3073	1601 1398	1198	3404	3344	583	651
COMPLEX 7	3233 3150	3065	1620 1402	1157	3412	3352	561	652

The sharp absorption bands in the region 3352-3311 cm^{-1} observed in the spectra of metal complexes are assigned to the N-H stretching frequency due to the protonation of terminal amino group of the DPH to form NH_3^+ . This resembles similar compound of DPH earlier reported where x-ray crystal structure is obtained [47]. This may be due to the strong hydrogen bonding between water molecules and amino group [48].

Primary amino groups usually exhibit two N-H stretching frequencies hence the bands in the region 3254 and 3173 cm^{-1} could be attributed to asymmetric and symmetric stretching frequencies of NH_2 in the spectrum of the ligand. These symmetric and asymmetric N-H stretching frequencies of the DPH were shifted bathochromically due to coordination of N-donor ligand to metal ions [20], indicating involvement of NH_2 in the chelation [49-50].

The FTIR Spectrum of the ligand showed a

medium intensity band at 3048 cm^{-1} assigned to stretching vibration frequency of (-OH) of carboxylic group. On complexation with metal (II) ions, the corresponding shift bands in the spectra of the metal complexes are 3018-3012 cm^{-1} (Cu); 3037 cm^{-1} (Ni); 3073 – 3054 cm^{-1} (Co); and 3065 cm^{-1} (Zn) indicating possible coordination through the carboxylate oxygen atoms by deprotonation [7, 51-52]. The involvement of hydroxyl of carboxylate group through oxygen atom in the coordination is further supported by asymmetric and symmetric stretching frequencies of (OCO) groups. The stretching frequencies observed at 1647 cm^{-1} and 1499 cm^{-1} in the spectrum of the ligand were attributed to asymmetric (ν_{as} (OCO)) and symmetric (ν_s (OCO)) stretching frequencies respectively. The spectra of the metal complexes show bands, which could be assigned to the asymmetric (ν_{as}) and the symmetric (ν_s) stretching vibration of the carboxylate group. These data permit us to deduce that carboxylate group is involved in

coordination to metal ions. The separation $\Delta u = \Delta u_{\text{asy}}(\text{OCO}) - \Delta u_{\text{sym}}(\text{OCO})$ characterizes the nature of the metal-carboxylate bond formed. When $\Delta u_{\text{Ligand}} > \Delta u_{\text{complexes}}$, the (OCO) group is bidentately coordinated while when the differences of Ligand (Δu_L) is less than that of metal complexes ($\Delta u_{\text{complexes}}$), the (OCO) group exhibits monodentate mode of coordination [49, 53, 54]. The differences of asymmetric (Δu_{asy}) and symmetric (Δu_{sym}) stretching vibration of (OCO) group of all the metal complexes were found to be greater than that of the ligand as shown in Table 3. This confirms that carboxylate group is monodentately coordinated to the metal centers through the hydroxyl oxygen atom via deprotonation [49, 55].

The FTIR spectra of the ligand and complexes displayed broad absorption band attributed to the presence of coordinated or lattice water molecules. The stretching frequency observed at 3393 cm^{-1} in the spectrum of ligand due to water molecule vibration undergone shift in the spectra of metal complexes. The corresponding shift in the spectra of metal complexes as a result of coordination are $3457 - 3420 \text{ cm}^{-1}$ (Cu); 3441 cm^{-1}

¹(Ni); $3453 - 3404 \text{ cm}^{-1}$ (Co); 3412 cm^{-1} (Zn) [7, 51].

In the lower frequency region, new bands with medium to weak intensities which provide direct evidence for the complexation (Metal – Ligand bond) were observed in the spectra of the complexes. In the present investigation, the bands in the region $698 - 651 \text{ cm}^{-1}$ were assigned to $\nu(M - N)$ while the bands in $583 - 512 \text{ cm}^{-1}$ region were attributed to $\nu(M - O)$ and $\nu(M - Cl)$ stretching vibrations [7, 56-57].

Table 2. Principal FTIR bands (cm^{-1}) for (OCO) groups ligands and metal complexes.

Compound	$\nu_{\text{asy}}(\text{OCO})$	$\nu_{\text{sym}}(\text{OCO})$	$\Delta u = \Delta u_{\text{asy}} - \Delta u_{\text{sym}}$
L	1647	1499	148
1	1637	1456	181
2	1662	1452	210
3	1671	1452	219
4	1600	1383	217
5	1659	1441	218
6	1601	1398	203
7	1620	1402	218

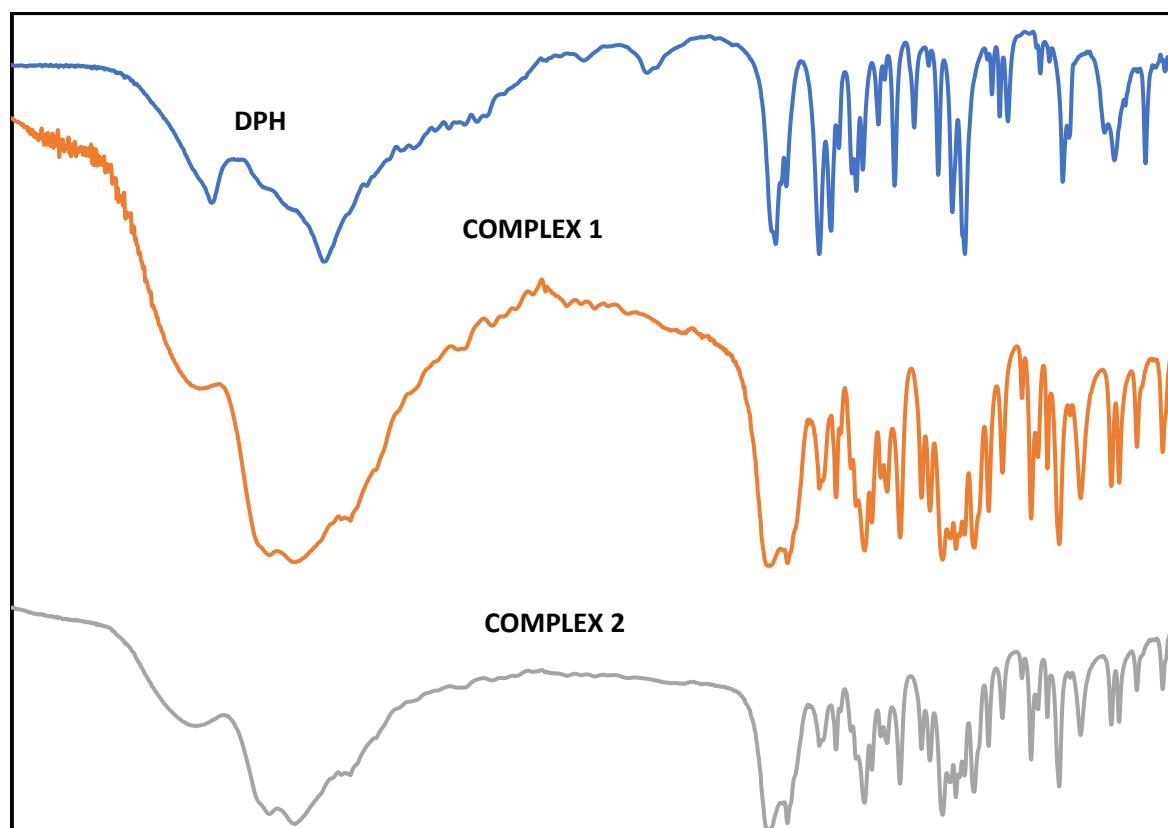


Figure 2. Comparative FTIR spectra of ligand and copper complexes.

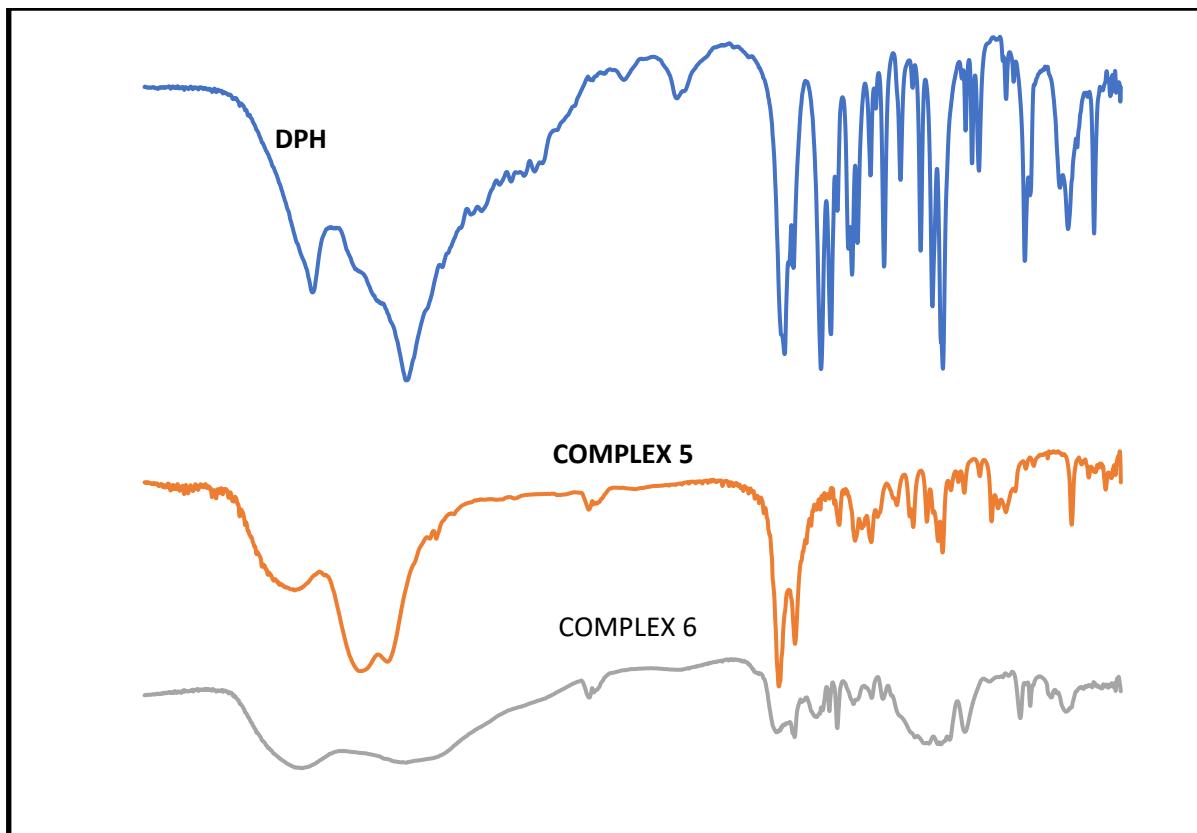


Figure 3. Comparative FTIR spectra of ligand and cobalt complexes.

2.2 Electronic Spectra and Magnetic Properties of the Complexes

The electronic spectrum of the aqueous solution of free ligand showed absorption bands in the UV-region at 228nm (43, 860 cm⁻¹), 240nm (41, 667 cm⁻¹) and 307nm (32, 573 cm⁻¹). These bands are assigned to intra-ligand transition due to n- π^* transition of the non-bonding electrons present on the oxygen of (C=O) group and nitrogen of the amine groups. The slight bathochromic shift and disappearance of the bands in the spectra of metal complexes relative to the ligand is attributable to coordination.

The Copper (II) complexes of DPH exhibited broad asymmetric bands in the region 14, 286 – 15,773 cm⁻¹ (700 – 634nm) assignable to 2E_g ${}^2T_{2g}$ \rightarrow transition. A broad band is expected for d-d transition of the copper (II) complexes in an octahedral environment. The broadness of the band could be attributed to the overlapping of several bands as a result of strong Jahn-Teller distortion expected in a d⁹ ion [56, 57]. The magnetic susceptibility measurements revealed that the copper (II) complexes have an effective magnetic moment of 1.78 – 1.84B.M. which

indicate that they are magnetically diluted and are in excess of the spin-only value of 1.73 B.M as a result of orbital contribution and spin-orbit coupling. Figure 4 showed plots of magnetic susceptibility (X_mT) versus temperature (T) for complex 2.

The absorption spectrum of Ni (II) complex showed three bands corresponding to the electronic transitions for d⁸ ion in an octahedral environment [58] similar to those earlier reported [49, 56]. The effective magnetic moment of 3.26 B.M. was obtained for the Ni (II) complex and thus strengthen the octahedral stereochemistry formulated for the complex with two unpaired electrons [57, 58]. Figure 5 showed magnetic susceptibility (X_mT) versus temperature (T) plots for complex 4.

Solution electronic spectra of Co (II) complexes of DPH in the visible region displayed three bands typical of octahedral geometry around cobalt (II) ions. The magnetic moments of 4.34 – 4.25 B.M. established for cobalt (II) complexes were in agreement with high-spin octahedral (with three unpaired electrons) Co (II) complexes. Thus, corroborates the proposed

stereochemistry for Co(II) complexes [57, 59].

The electronic configuration of Zn(II) ion is d^{10} hence its absorption spectra show no bands due to d-d transition, but the absorption bands in its spectrum suffered red shift with hyper or hypochromic effect. This is a natural occurrence as

there is no possibility of transition due to non-availability of empty d-orbital [59]. In view of the well known tendency of Zn(II) to form tetrahedral complexes, by considering the spectrum data and elemental analysis results, tetrahedral geometry is proposed for Zn (II) complex [60].

Table 3. Electronic spectra, magnetic moment and molar conductance data of ligand and metal complexes.

	$\mu_{\text{eff}}(\text{B.M.})$	Molar conductance ($\text{Scm}^2\text{mol}^{-1}$)	λ_{max} (nm $^{-1}$)	λ_{max} (cm $^{-1}$)	λ_{max} Energy KJmol $^{-1}$	Assignment
L (DPH)	-	115	228 240 307	43,860 41,667 32,573	525 498 390	$n - \pi^*$
COMPLEX 1	1.82	209	280 650	35,714 15,385	427 184	$n - \pi^*$ $^2E_g - ^2T_{2g}$
COMPLEX 2	1.84	172	291 634	34,364 15,773	411 189	$n - \pi^*$ $^2E_g - ^2T_{2g}$
COMPLEX 3	1.78	398	233 700	42,918 14,286	513 171	$n - \pi^*$ $^2E_g - ^2T_{2g}$
COMPLEX 4	3.26	335	278 373 651 991	35,971 26,810 15,361 10,091	430 321 184 121	$n - \pi^*$ $^3A_{2g}(F) \rightarrow ^3T_{1g}(P)$ $^3A_{2g} \rightarrow ^3T_{1g}(F)$ $^3A_{2g} \rightarrow ^3T_{2g}(F)$
COMPLEX 5	4.25	222	295 492 560 770	33,898 20,325 17,857 12,987	405 243 214 155	$n - \pi^*$ $^4T_{1g}(F) \rightarrow ^4T_{1g}(P)$ $^4T_{1g}(F) \rightarrow ^4A_{2g}(F)$ $^4T_{1g}(F) \rightarrow ^4T_{2g}(F)$
COMPLEX 6	4.34	339	293 470 520 683	34,130 21,277 19,231 14,641	408 254 230 175	$n - \pi^*$ $^4T_{1g}(F) \rightarrow ^4T_{1g}(P)$ $^4T_{1g}(F) \rightarrow ^4A_{2g}(F)$ $^4T_{1g}(F) \rightarrow ^4T_{2g}(F)$
COMPLEX 7	-	196	225 272 373	44,444 36,765 26,809	532 440 321	$n - \pi^*$ $n - \pi^*$ LMCT

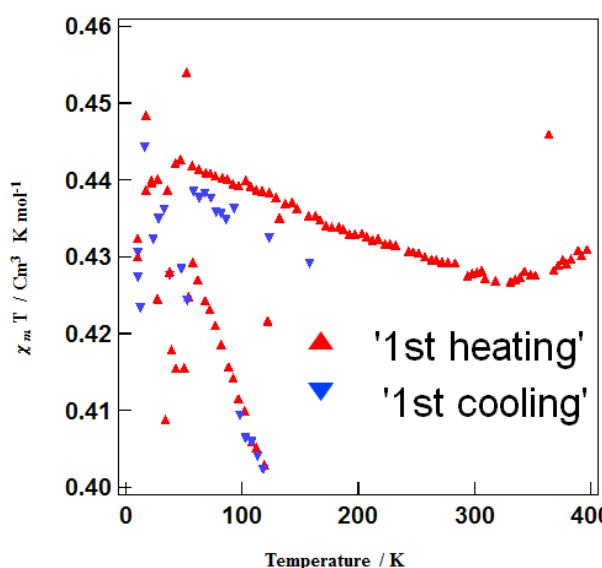


Figure 4. Magnetic susceptibility (χ_mT) versus Temperature (T) plots for complex 2: $\text{CuC}_{12}\text{H}_{27}\text{O}_5\text{N}_4\text{F}_4\text{Cl}_2$.

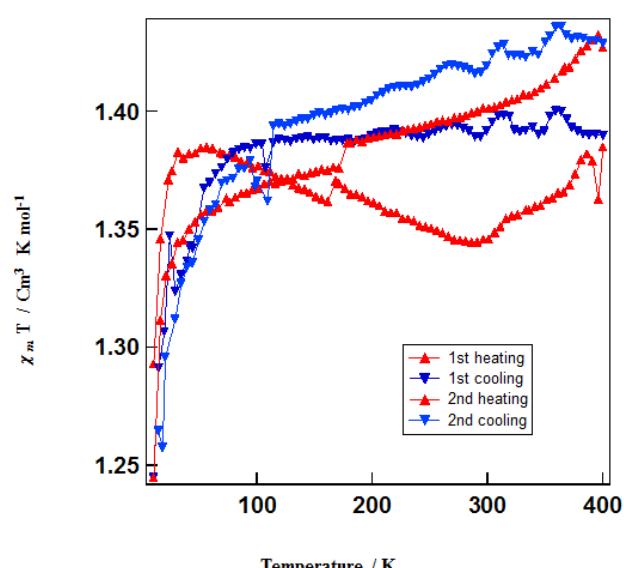


Figure 5. Magnetic susceptibility (χ_mT) versus Temperature (T) plots for complex 4: $\text{NiC}_{14}\text{H}_{38}\text{F}_4\text{N}_4\text{O}_{11}\text{Cl}_3$.

2.3 Electrospray Ionization Mass Spectrometry (ESI-MS) Results

The major fragment ions, peak assignment (theoretical and found) mass per charge ratio (m/z) values and relative abundance of metal complexes 1, 2 and 6 were shown in Tables 4 –6. The m/z values observed in each case compete

favourably well with the theoretical values; this is equally an evidence which further support the stoichiometric formulation (1:2 metal-ligand chelates). The peak noticed at $m/z = 183.10$ in all the spectra of the metal complexes is due to $[DPH + H]^+$ which signifies the presence of DPH in the coordination sphere hence suggests a proof for coordination.

Table 4. ESI-MS data for $[Cu(DPH)_2(Cl)(H_2O)] \cdot HCl$ [1].

Compound	Major Fragmentions	Peak assignment (m/z)		Relative Intensity (%)
		Found	Theoretical	
$[Cu(DPH)_2(Cl)(H_2O)] \cdot HCl$ $m/z = 517.81$	$[2M + 2Na]^{2+}$	540.96	540.80	47.51
	$[2M + 2NH_4]^{2+}$	535.02	535.85	58.62
	$[M + Li]^+$	524.59	524.75	91.43
	$[M - HCl + 2H]^{2+}$	241.04	241.68	40.02
	$[Cu + 2DPH + H_2O + Li]^+$	522.99	522.90	77.71
	$[Cu(DPH)_2Cl_2 + NH_4]^+$	533.02	533.04	63.43
	$[DPH + H]^+$	183.10	183.09	5.71
	$[DPH + K]^+$	221.04	221.05	100.00
	$[Cu(DPH)_2 + H]^+$	428.07	428.89	35.08

Table 5. ESI-MS data for $[Cu(DPH)_2(H_2O)_2] \cdot HCl$ [2].

Compound	Major Fragment ions	Peak assignment (m/z)		Relative intensity (%)
		Found	Theoretical	
$[Cu(DPH)_2(H_2O)_2] \cdot HCl$ $m/z = 500.37$	$[2M + 2Na + 2K + 2H]^{2+}$	563.30	563.61	0.60
	$[M + Li]^+$	506.10	506.12	17.09
	$[M + NH_4 + H + 2Li]^{2+}$	266.00	266.09	62.02
	$[M - HCl + Li + H]^+$	471.90	471.15	12.03
	$[M - H_2O + H + 2Na]^{2+}$	264.00	264.04	100.00
	$[M - H_2O + K + 2H]^{2+}$	261.10	261.52	53.16
	$[2DPH + 2H]^{2+}$	183.10	183.09	37.34
	$[M - 2H_2O + Na + 2H]^{2+}$	244.10	244.04	49.37
	$[M - HCl + 2Na]^{2+}$	254.00	254.56	33.54
	$[Cu + 2DPH + 2H]^{2+}$	215.00	214.56	53.16
	$[Cu + DPH + H_2O + K + Na + Li]^+$	331.90	331.99	71.52

Table 6. ESI-MS data for $[Co(DPH)_2(CH_3COO)(H_2O)] \cdot H_2O \cdot 2HCl$ [6].

Compound	Major Fragment ions	Peak assignment (m/z)		Relative intensity (%)
		Found	Theoretical	
$[Co(DPH)_2(CH_3COO)(H_2O)] \cdot H_2O \cdot 2HCl$ $m/z = 591.23$	$[M + 2Na + 2H]^{2+}$	319.00	319.63	6.34
	$[M + 2K + Li]^{2+}$	338.00	338.20	100.00
	$[M + Li + Na + H]^+$	559.10	559.07	16.46
	$[M - H_2O - 2HCl + H]^+$	501.00	501.34	9.24
	$[Co(DPH)_2 + 2H_2O + Na + NH_4]^+$	500.10	500.33	39.24
	$[Co(DPH)_2(CH_3COO)(H_2O) + Na + 2H]^{2+}$	261.10	261.66	36.08
	$[2DPH + 2H]^{2+}$	183.10	183.09	16.46
	$[2DPH + Co + Na + 2H]^{2+}$	224.50	224.06	6.33
	$[Co(DPH)_2 + 2H_2O + K + H]^+$	499.10	499.40	9.36

2.4 Antibacterial Screening Results

The in-vitro antibacterial activity of the ligand (DPH) and seven (7) metal complexes were evaluated using agar well diffusion technique [61, 62] against Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus substillis*, Gram-negative bacteria, *Escherichia coli*, and *Klebsiella pneumonia* and the result is presented in Table 7 and Fig 6. The Minimum Inhibitory Concentration (MIC) of the ligand (DPH) and metal complexes 2,4 and 6 is as well contained in Table 8 and Fig 7. The solvent (distilled water) showed no zone of inhibition confirming non-involvement of solvent on the activity of the metal complexes and the DPH. Generally, the results of the antibacterial screening show appreciable activity of the metal complexes when challenged with the test pathogens. The zone of inhibitions of metal complexes are much larger when compared to the free ligand [57, 62] indicating that metal complexes are able to decrease the population of bacterial species than the ligand hence they are more effective as antibacterial agents than the ligand. The synthesized copper complexes (complexes 2 and 3) were found to compete favorably well with antibiotic used particularly at higher concentration. MIC values for complexes 2, 4, and 6 are lower than that of the free ligand, indicating that complexation enhances antibacterial activity of complexes.

This process of the antibacterial activity of the metal complexes can be justified using the overtone's concept and the Tweedy chelation

theory. In relation to overtone's concept of cell permeability, the lipid membrane that surrounds the cell supports the passage of only lipid soluble materials; as a result, liposolubility is an essential factor that regulates antimicrobial activity [63]. On chelation theory, the polarity of the metal ion is reduced to a large extent due to overlap of the ligand orbital and partially sharing positive charge of the metal ion with donor groups. It equally increases the delocalization of π -electrons over the whole chelates ring and enhances the lipophilicity of the metal complexes. Consequently, this increases the lipophilic character of the metal complexes thereby favouring its permeation through the lipid layers of bacterial membrane and facilitates the blocking of metal binding sites of the enzymes of the pathogens [5, 13, 15, 57]. The variation in the effectiveness of different compound against different organisms depends either on the impermeability of the cell of the microbes or differences in the ribosomes of microbial cells [57].

It is equally established from the results that the antibacterial potency appears to be concentration dependent which generally agrees with the finding of other researchers who asserted that antimicrobial potency is usually concentration dependent. It was also observed that the most resistant pathogens to the metal complexes were *Staphylococcus aureus*, *Bacillus substillis* while the most susceptible were *Klebsiella pneumoniae* and *Escherichia coli*.

Table 7. Zone of inhibition (mm) of ligand and metal complexes on bacteria.

Compounds	<i>E. coli</i>			<i>K. pneumoniae</i>			<i>S. aureus</i>			<i>B. substillis</i>		
	100 ppm	200 ppm	300 ppm	100 ppm	200 ppm	300 ppm	100 ppm	200 ppm	300 ppm	100 ppm	200 ppm	300 ppm
1	7.0	12.1	16.1	8.2	15.6	20.7	3.1	8.4	13.7	1.1	4.5	9.0
2	6.1	12.6	18.9	9.0	16.5	23.6	1.1	10.0	15.5	0.8	4.0	8.5
3	5.0	11.2	16.1	9.4	17.0	23.7	4.3	8.1	12.4	2.9	6.0	10.1
4	3.0	9.0	13.7	5.5	12.0	19.0	NA	8.0	11.4	NA	2.7	6.5
5	5.2	9.0	14.0	8.0	15.1	20.1	NA	7.5	10.5	NA	5.3	8.6
6	6.2	9.6	15.3	9.0	15.6	21.3	NA	8.5	11.0	NA	5.6	9.3
7	2.0	6.7	12.9	4.0	8.0	14.0	NA	7.0	9.5	NA	2.0	6.0
DPH	NA	8.5	12.5	2.0	9.0	15.6	NA	6.0	10.0	NA	2.0	6.1
Ampicillin	9.0	14.1	20.5	10.0	18.6	24.2	6.0	10.0	17.0	4.0	7.5	11.5
Distilled Water	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

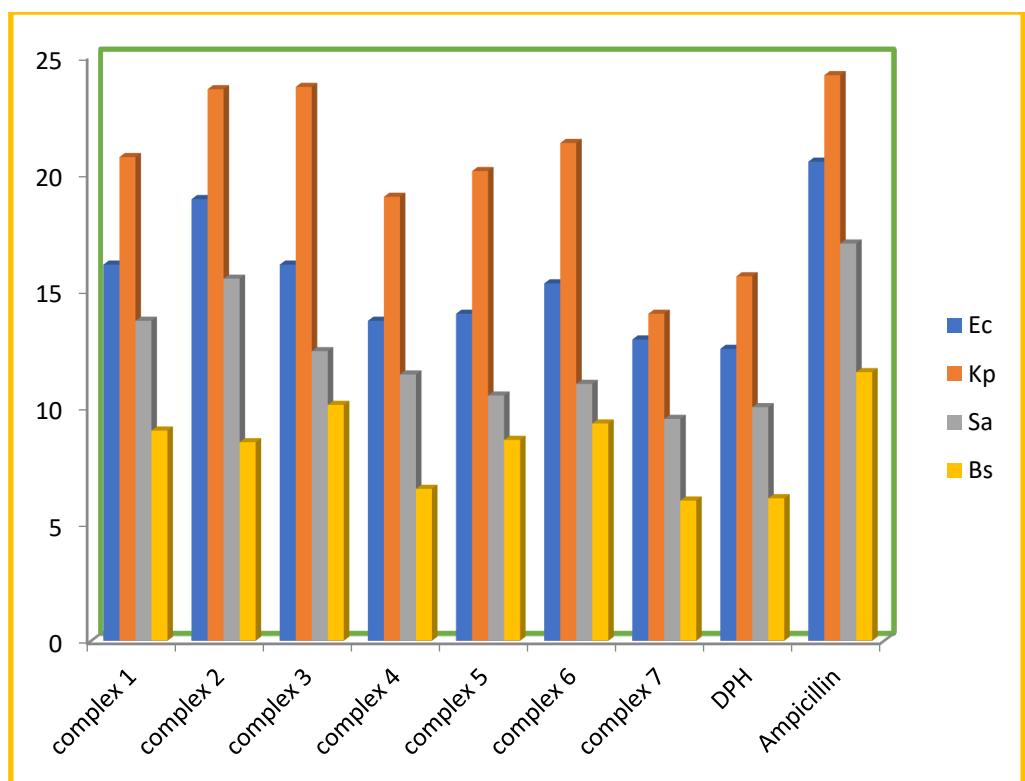


Figure 6. Inhibitory level of the ligand and metal complexes on bacteria at concentration of 300 ppm.

Table 8. Minimum inhibitory concentration (MIC) of the ligand and metal complexes against bacteria.

TEST ORGANISMS	Minimum Inhibitory Concentration ($\mu\text{g/mL}$)			
	L(DPH)	Complex 2	Complex 4	Complex 6
<i>E. coli</i>	1.2×10^1	3.4×10^{-1}	4.1×10^{-1}	4.0×10^{-1}
<i>K. pneumoniae</i>	1.7×10^1	4.3×10^{-2}	4.6×10^{-2}	6.3×10^{-2}
<i>S. aureus</i>	3.3×10^1	2.3	3.0	5.1
<i>B. subtilis</i>	5.4×10^1	1.1×10^1	1.4×10^1	1.6×10^1

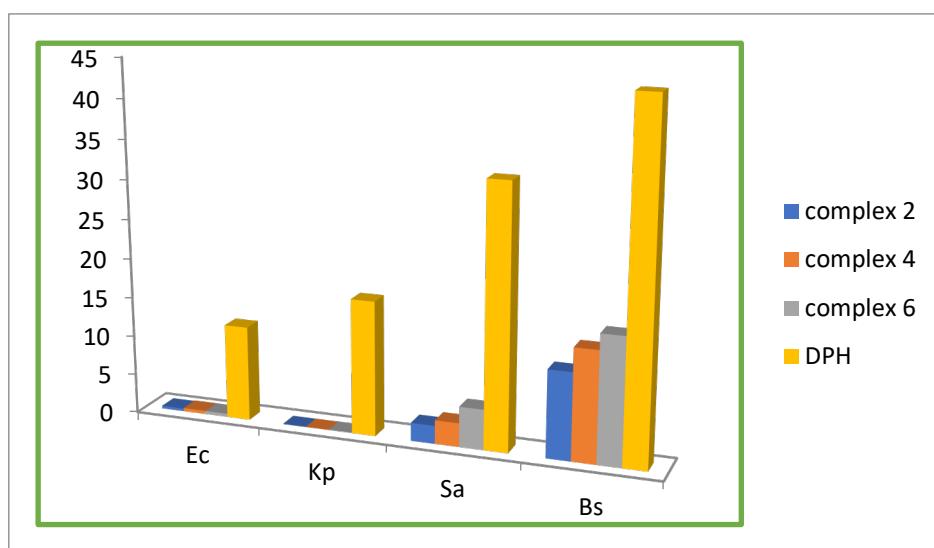


Figure 7. Graph showing Minimum Inhibitory Concentration of the Ligand and the selected metal complexes. *E. coli* – *Escherichia coli* (Ec); *K. pneumoniae* – *Klebsiella pneumoniae* (Kp); *S. aureus* – *Staphylococcus aureus* (Sa); *B. subtilis* – *Bacillus subtilis* (Bs)

3. Material and Methods

3.1 Materials

The ligand, 2,5-diamino-2-(difluoromethyl) pentanoic acid hydrochloridehydrate (DPH) was commercially obtained and used without further purification. Hydrated metal salts used for complexation ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$; $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$; $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$; $\text{Ni}(\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$; $\text{Co}(\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$; $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$) were obtained from British Drug House Chemical Limited Co. Poole England. Isolates of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Bacillus substillis* were obtained from University of Ilorin Teaching Hospital through Microbiology Department, University of Ilorin, Nigeria.

3.2 Physical Measurements

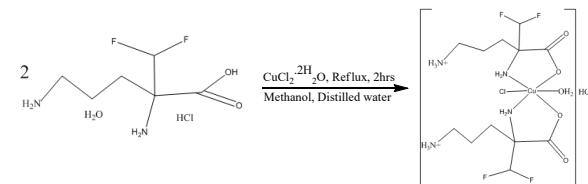
The elemental (CHN) analyses were performed on Thermo Flask 112 CHNSO elemental analyzer from Micro analytical Laboratory at Medac Limited, Surrey, United Kingdom. The FTIR spectra were collected on FTIR -8501 Shimadzu spectrophotometer over 4000-400cm⁻¹ using KBr pellets. Melting points were determined using MPA100 OptiMelt Automated Melting Point system. Solution electronic absorption spectra of the ligand and complexes were ran in the range of 180-400 nm and 180-1100 nm respectively on Jenway 6405uv/vis. The conductivity of the ligand and their complexes were determined in distilled water using EC214 conductivity meter Hanna instrument with cell constant of 1.013. The electrospray ionization mass spectra were recorded on Micromass Autoseptic Premier/Agilent HP6890GC at Medac Limited, UK. The magnetic susceptibility measurements ($\chi_m\text{T}$) for the transition metal complexes between 5 and 400 K were measured with a superconducting quantum interference device (SQUID) magnetometer (Quantum Design MPMS - 5S) available at Chemistry department, Kumamoto University, Japan.

3.3 Synthesis of complexes

3.3.1 Synthesis of $[\text{Cu}(\text{DPH})_2(\text{Cl})(\text{H}_2\text{O})]\text{HCl}$ (1):

The complex was synthesized by adding (DPH)

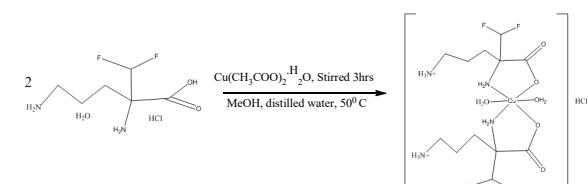
(0.473g, 2mmol) dissolved in distilled (15 mL) to stirring methanolic solution (10 mL) of copper(II)chloride dihydrate (0.1705g, 1mmol). The resulting mixture was refluxed for 2 hours. The blue coloured solution was filtered hot and the filtrate kept at room temperature for slow evaporation. The skye-blue powdered formed after three days was washed thrice with methanol, recrystallized in methanol: water (3:1) and dried in a desiccator over silical gel. Mol. Wt: 517.81 gmol⁻¹; Yield: 0.245g (94.37%), M.pt 215-216 °C; Colour: Skye blue; Elemental Analysis for $[\text{Cu}(\text{DPH})_2(\text{Cl})(\text{H}_2\text{O})]\text{HCl}(\text{C}_{12}\text{H}_{27}\text{F}_4\text{N}_4\text{O}_5\text{Cl}_2\text{Cu})$ calcd (found)%: C 27.83 (27.80), H 5.26 (5.09); N 10.82 (11.04).



Scheme 1. Synthesis of complex 1.

3.3.2 Synthesis of $[\text{Cu}(\text{DPH})_2(\text{H}_2\text{O})_2]\text{HCl}$ (2):

This coordination compound was prepared by dissolving the ligand (0.4734 g, 2 mmol) in 20 mL distilled water followed by slow addition of copper(II) acetate monohydrate $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ (0.1996 g, 1 mmol) dissolved in 10 mL methanol. There was instant formation of violet-blue coloured solution which was stirred continuously for 3 hours at 50 °C. The solution was cooled, precipitate formed filtered, washed with methanol and dried over silica gel. M.wt: 500.37 gmol⁻¹; Yield: 0.307g (63.25%); M.pt 205 °C (dec.); Colour: Violet-blue; Elemental analysis for $[\text{Cu}(\text{DPH})_2(\text{H}_2\text{O})_2]\text{HCl}(\text{C}_{12}\text{H}_{29}\text{F}_4\text{N}_4\text{O}_6\text{ClCu})$ calcd (found)%: C 28.80 (28.63), H 5.84 (5.76), N 11.20 (11.17).

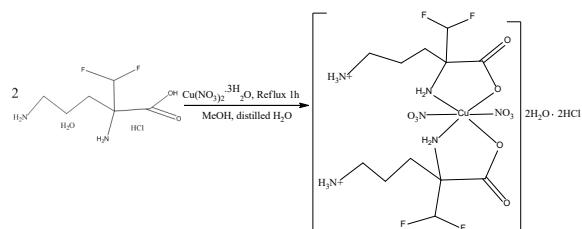


Scheme 2. Synthesis of complex 2.

3.3.3 Synthesis of $[\text{Cu}(\text{DPH})_2(\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}]\text{HCl}$ (3):

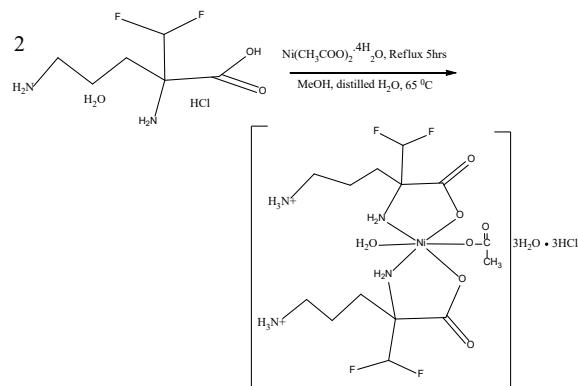
$\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ (0.2416 g, 1 mmol) dissolved

in 15 ml of methanol was slowly added to (0.473 g, 2 mmol) of DPH dissolved in 15 mL of distilled water. This resulted into the formation of blue coloured solution, which was refluxed for 1 h. The concentration of the solution led to the formation of deep-blue precipitate that was harvested by decanting, washed thoroughly with methanol and dried over silica gel. M.wt 660.85 gmol⁻¹, Yield: 0.265g (60.15%); M.pt 210-212 °C; Colour: Deep-blue; Elemental analysis for $[\text{Cu}(\text{DPH})_2(\text{NO}_3)_2] \cdot 2\text{H}_2\text{O} \cdot \text{HCl}$ ($\text{C}_{12}\text{H}_{30}\text{F}_4\text{N}_6\text{O}_{12}\text{Cl}_2\text{Cu}$) calcd(found) (%): C 21.81(22.05), H 4.58(4.12), N 12.72(12.66).



Scheme 3. Synthesis of complex 3.

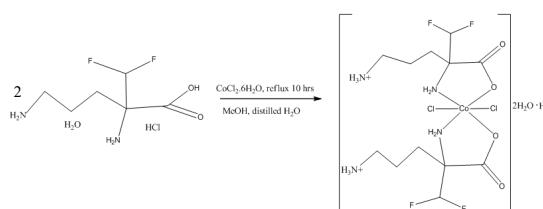
3.3.4 Synthesis of $[\text{Ni}(\text{DPH})_2(\text{CH}_3\text{COO})(\text{H}_2\text{O})] \cdot 3\text{H}_2\text{O} \cdot 3\text{HCl}$ (4): The ligand (DPH) (0.4734 g, 2 mmol) was dissolved in 10 mL of distilled water followed by slow addition of methanolic solution of (0.2489 g, 1 mmol) of nickel(II) acetate tetrahydrate $\text{Ni}(\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$ which resulted into bluish green solution. The mixture was refluxed for 5 hours at 65 °C, cooled and allowed to evaporate slowly at room temperature. The light green precipitate formed was filtered, washed severally with acetone and dried over silica gel. M.wt: 663.52 gmol⁻¹; Yield: 0.537g (89.32%); M. pt 211-213 °C; Colour: Light-green; Elemental analysis for $[\text{Ni}(\text{DPH})_2(\text{CH}_3\text{COO})(\text{H}_2\text{O})] \cdot 3\text{H}_2\text{O} \cdot 3\text{HCl}$ ($\text{C}_{14}\text{H}_{38}\text{F}_4\text{N}_4\text{O}_{10}\text{Cl}_3\text{Ni}$) calcd (found) %: C 25.34(24.99), H 5.77 (5.35), N 8.44 (8.74).



Scheme 4. Synthesis of complex 4.

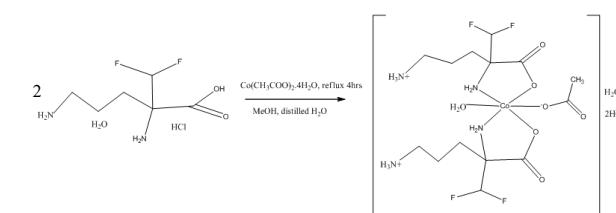
3.3.5 Synthesis of $[\text{Co}(\text{DPH})_2\text{Cl}_2] \cdot 2\text{H}_2\text{O} \cdot \text{HCl}$ (5)

This cobalt complex was prepared by dissolving the (0.4734 g, 2 mmol) DPH in 10 mL of distilled water followed by slow addition of (0.2379 g, 1 mmol) cobalt (II) chloride hexahydrate, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ in 10 mL methanol. The solution which turned to brown colour and later to wine colour was heated under reflux for 10 h until stable purple solution was obtained. The resulting purple coloured solution formed was cooled and left to evaporate slowly at room temperature. The purple precipitate formed was separated out by filtration, washed with methanol and dried over silica gel. M.wt: 566.67 gmol⁻¹; Yield: 0.102g (50.12%); M.pt 214 °C (dec.); Colour: Purple; Elemental analysis for $[\text{Co}(\text{DPH})_2\text{Cl}_2] \cdot 2\text{H}_2\text{O} \cdot \text{HCl}$ ($\text{C}_{12}\text{H}_{29}\text{F}_4\text{N}_4\text{O}_6\text{Cl}_3\text{Co}$) calcd (found) %: C 25.43 (25.86), H 5.16 (5.29), N 9.89 (10.02).



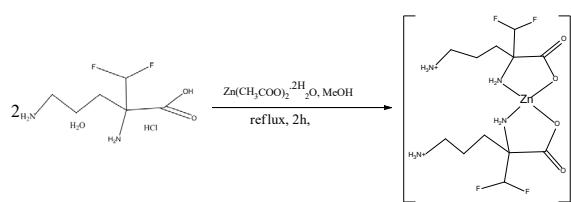
Scheme 5. Synthesis of complex 5.

3.3.6 Synthesis of $[\text{Co}(\text{DPH})_2(\text{CH}_3\text{COO})(\text{H}_2\text{O})] \cdot \text{H}_2\text{O} \cdot 2\text{HCl}$ (6): DPH (0.4734 g, 2 mmol) was dissolved in 15 mL of distilled water followed by a slow addition of (0.2491 g, 1 mmol) of cobalt(II) acetate tetrahydrate, $\text{Co}(\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$ dissolved in 10 mL of methanol. The resulting pinkish solution was heated under reflux for 4 hours. The product formed was separated out by filtration, dried, redissolved in methanol, allow to evaporate slowly at room temperature. The pinkish precipitate obtained was then dried over silica gel. M.wt: 591.23 gmol⁻¹; Yield: 0.426g (73.26%); M.pt 177-178 °C; Colour: Pink; Elemental analysis for $[\text{Co}(\text{DPH})_2(\text{CH}_3\text{COO})(\text{H}_2\text{O})] \cdot \text{H}_2\text{O} \cdot 2\text{HCl}$ ($\text{C}_{14}\text{H}_{33}\text{F}_4\text{N}_4\text{O}_8\text{Cl}_2\text{Co}$) calcd(found) (%): C 28.44 (28.21); H 5.63 (5.78); N 9.48 (10.04).



Scheme 6. Synthesis of complex 6.

3.3.7 Synthesis of $Zn(DPH)_2 \cdot 2H_2O \cdot 2HCl$ (7): Zinc acetate dihydrate, $Zn(CH_3COO)_2 \cdot 2H_2O$ (0.2195 g, 1 mmol) dissolved in 10 mL methanol was slowly added to 0.4734 g (2 mmol) methanolic solution of DPH. The mixture was refluxed for 2 hours. The colorless clear solution obtained was subjected to slow evaporation at room temperature. The white precipitate harvested was re-dissolved in methanol to remove excess ligand, filtered and allowed to evaporate slowly at room temperature. The white precipitate obtained was the dried over silica gel. M.wt: 538.08 gmol⁻¹, Yield 0.469 g (87.33%); M.pt 174-176°C; Colour: White; Elemental analysis for $[Zn(DPH)_2] \cdot 2H_2O \cdot 2HCl$ (C₁₂H₃₀F₄N₄O₆Cl₂Zn) calcd (found) (%): C 26.75 (27.24); H 5.61 (5.90); N 10.40 (10.86).



Scheme 7. Synthesis of complex 7.

3.4 Antibacterial susceptibility testing

The four (4) clinical bacteria isolates: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Bacillus substillis* obtained from University of Ilorin Teaching Hospital (UITH) through Microbiology Department, University of Ilorin, Nigeria, were challenged with varying concentrations of ligand and metal complexes samples using the agar well diffusion technique. Standardized sensitivity text agar (Lab M, UK) was used for antibacterial assay. Sample solutions were prepared in distilled water at concentration 300, 200 and 100 ppm. The drug solution of ampicillin was used as control. The antibacterial activity tests were carried out in triplicate. The zones of inhibition were measured and interpreted using standard recommendation of National Committee for Clinical Laboratory Standards (NCCLS, 2006) [61]. The activities of the metal complexes were compared with known standard antibiotics, ampicillin. Minimum inhibitory concentration (MIC) tests using the broth dilution method were also conducted against bacteria isolates to demonstrate the effect of complexation on their activity.

4. Conclusions

In summary, we have synthesized novel transition metal (Cu(II), Co(II), Ni(II) and Zn (II)) complexes containing 2,5-diamino-2-(difluoromethyl) pentanoic acid hydrochloride hydrate (DPH) as ligand. The ligand and metal complexes were characterized by melting point, conductivity measurement, CHN elemental analysis, FTIR and electronic spectroscopies, magnetic susceptibility measurements and ESI-MS. Spectroscopic studies revealed that the DPH behaves as a bidentate ligand coordinates to the metal ions through hydroxyl of the carboxylate moiety and a nitrogen atom of amino group. Octahedral and tetrahedral geometries were proposed for Cu(II), Co(II), Ni(II) and Zn(II) respectively. The screening data of antibacterial studies revealed that the metal complexes exhibited enhanced activities than the free ligand indicating that metal complexes are more efficient antibacterial agent. These studies may lead to discovery of new chemotherapeutic agents that are metal based.

Supporting Information

FTIR and ESI-MS spectra of complexes.

Acknowledgments

WAO and JAO appreciate the financial support of Science and Technology Education Post Basic Project (Step B), University of Ilorin, Ilorin.

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