SENRA Academic Publishers, Burnaby, British Columbia
Vol. 4, No. 2, pp. 1169-1178, June 2010
ISSN: 1715-9997

Canadian Journal of pure&applied sciences

SYNTHESIS, CHARACTERISATION AND IN-VITRO BIOLOGICAL ACTIVITIES OF SOME METAL(II) COMPLEXES OF 3-(-1-(4-METHYL-6-CHLORO)-2-PYRIMIDINYLIMINO)METHYL-2-NAPTHOL

*A A Osowole[1], R Kempe[2], R Schobert [3] and S A Balogun[4]
[1]Department of Chemistry, University of Ibadan, Ibadan, Nigeria
[2]Anorganische Chemie II, Universität Bayreuth, D-95440 Bayreuth, Germany
[3]Organische Chemie I, Universität Bayreuth, D-95440 Bayreuth, Germany
[4]Department of Microbiology, Adekunle Ajasin University Akungba–Akoko, PMB 001
Akungba–Akoko, Ondo state, Nigeria

ABSTRACT

Synthesis, spectroscopic characterisation and in-vitro antibacterial and anticancer studies were done on the neutral bidentate (NO) Schiff base, 3-(-1-(4-methyl-6-chloro)-2-pyrimidinyl imino)methyl-2-napthol and its Mn(II), Co(II), Ni(II), Pd(II), Cu(II) and Zn(II) complexes. These complexes assumed a 4-coordinate tetrahedral /square-planar geometry as corroborated by room temperature magnetic data, IR and electronic spectral measurements. The conductance data confirmed their covalent nature while mass spectra and TGA data affirmed the proposed structure. The ligand was not sensitive to HL 60 (leukaemia) but resistant to 518A2 (melanoma) carcinomas. The Pd(II) complex had the best cytotoxic activities against HL 60 (Leukemia) and Melanoma (518A2) carcinomas with IC_{50} of 11.89 and 9.11 µm at 48 and 72 h respectively, which was a third as, and four times more sensitive than CDDP (Cis-platin). The complexes generally exhibited good antibacterial activities against five Gram negative (*E. cloacae, S. arizona, Serratia sp, E. Coli*) and three Gram positive (*C. violaceum, S. aureus, Bacillus sp*) bacteria with inhibitory zones range of 7-19 mm. Similar to gentamycin, the Cu(II) and Zn(II) complexes had broad-spectrum activity against the bacteria used, although much lower.

Keywords: Antibacterial and anticancer activities, cis-platin –sensitive and –resistant cell lines, Schiff base.

INTRODUCTION

The interest in metal complexes as drugs has increased in the last decade due to the search for drugs with an enhanced therapeutic effect in combination with decreased toxicity (Zhaohua *et al.,* 2001; Zhong *et. al*., 2006). It has been documented that drugs containing pyrimidine moiety are more prominent in treating solid tumours and cancers due to their good anti-proliferation activity and low toxicity e.g. bevacizumab in combination with 5-fluoro uracil is used in treatment of metastatic colorectal cancer (Ramaling and Belani, 2007) while sorafenib and sunitinib, which are small-molecule multikinase inhibitors are used for the treatment of advanced renal-cell carcinoma (Atkins *et al*., 2006; Wilhelm *et al*; 2006). Currently, the drug pazopanib (5-(4-[(2,3-dimethyl-2H-indazoyl-6-yl)methylamino]-2-pyrimidinyl]amino-2-methyl benzene sulfonamide) is undergoing clinical development for use in treating renal-cell cancer and other solid tumours (Harries *et al*., 2008). In addition, pyrimidinyl Schiff bases are widely studied due to their ease of preparation, coordination to many metal ions, and the stability of such oxidation states. Furthermore, their good solubility in DMSO and DMF makes them suitable for cell lines studies (Gorneva and Golovinsky, 2003; Shreelekha *et al*., 2006). In continuation of our studies on synthesis, characterisation and anticancer /antimicrobial properties of some metal(II) complexes of various pyrimidinyl Schiff bases (Osowole *et al*., 2009), we report our findings on anticancer and antibacterial properties of Mn(II), Ni(II), Co(II), Cu(II), Zn(II) and Pd(II) complexes of 3-(-1-(4-methyl-6-chloro-2-pyrimidinyl imino)methyl-2-napthol (derived from condensation of 2-amino-4-methyl-6-chloropyrimidine and 2-hydroxy-1-napthaldehyde) against cis-platin–sensitive (Leukemia) and –resistant (Melanoma) cell lines and, *E. cloacae, S. arizona, Serratia sp, E. Coli*) and three Gram positive (*C. violaceum, S. aureus, Bacillus sp*). Their spectroscopic, magnetic and thermal properties are also discussed. This ligand and its metal complexes are new, being reported here by our group for the first time.

MATERIALS AND METHODS

Chemicals and Instrumentation

All the chemicals used were of reagent grade. 2-amino-4-methyl-6-chloropyrimidine and 2-hydroxy-1-napthaldehyde (Across), palladium(II) chloride, hydrated manganese(II) nitrate, cobalt(II) nitrate, nickel(II) nitrate,

*Corresponding author email: aderoju30@yahoo.com

copper(II) nitrate and zinc(II) nitrate (Aldrich) were used as received. The solvents were purified using conventional methods. C, H and N analyses were carried out using a GmbH VarioEl analyser. Palladium, Manganese, cobalt, nickel, copper and zinc were determined titrimetrically (Bassett *et al*., 1978). The ^{1}H nmr spectra were recorded on a 300 MHz Oxford Varian NMR instrument in $CDCl_3$ at 295K. ^{1}H chemical shifts were referenced to the residual signals of the protons of $CDCl_3$ and are quoted in ppm. Magnetic susceptibilities were measured on Johnson Matthey magnetic susceptibility balance at room temperature (27^{o}C) all susceptibilities were corrected for the diamagnetic contributions using Pascal's constants (Earnshaw, 1980). The reflectance spectra were recorded on a Perkin-Elmer λ20 spectrophotometer equipped with an integrating sphere. The infrared spectra were measured as KBr discs on a Bruker-IFS 66V spectrometer in the range 4000-400 cm^{-1}. Thermogravimetric analyses were done in static air, using a T6 Linseis thermal analyser with a heating rate of 10°C/min in the range 30-700°C while electrolytic conductivities of the complexes (1 x 10^{-3} Mdm^{-3}) in nitromethane were determined using a Hanna HI 8828N conductometer and melting points (uncorrected) were done using a Stuart scientific melting point apparatus smp3.

Biological Studies

The human HL 60 leukaemia cells were obtained from the German National Resource Centre for Biological Materials (DSMZ), Braunschweig and the human 518A2 melanoma cells were cultured in the Department of Oncology and Hematology, medical faculty of the Martin-Luther University, Halle, Germany.

Cytotoxicity (MTT) Assay

MTT[3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was used to identify viable cells which were capable of reducing it in their mitochondria by succinate dehydrogenase to a violet formazan product.

Cell lines and Culture condition

HL 60 (0.5 x 10^6 cells/mL) and 518A2 cells (1.7 x 10^5 cells/mL) were seeded out and cultured for 24h on 96-well microplates. Incubation (5% CO_2, 95% humidity, and 37°C) of cells following treatment with test compounds was continued for 24, 48 and 72h. Blank and solvent controls were incubated under identical conditions. A 5 mg/mL stock solution of MTT in phosphate-buffered saline (PBS) was then added to a final concentration of 0.05%. After 2 h the precipitate of formazan crystals was redissolved in a 10% solution of sodium dodecylsulfate (SDS) in DMSO containing 0.6% acetic acid in the case of the HL 60 cells. For the adherent melanoma (518A2) cells, the microplates were swiftly turned to discard the medium prior to adding the solvent mixture. The microplates were gently shaken in the dark for 30 min and left in the incubator overnight, to ensure a complete dissolution of the formazan. Finally the absorbance at wavelengths 570 and 630nm (background) was measured using an ELISA plate reader. All experiments were carried out in quadruplicate, and the percentage of viable cells quoted was calculated as the mean ± SD with respect to the controls set to 100%. Blank tests have shown that DMSO used in the preparation of the test compound does not have any effect on the cancer cell lines.

Antibacterial assay

The assay was carried out on the ligand and metal(II) complexes. The bacteria used were identified laboratory strains of *E. cloacae, S. arizona, Bacillus sp, S. liquefaciens, S. aureus, Klebsiella sp, Salmonella sp, Serratia sp, Pseudomonas sp, E. coli, C. violaceum*. The antibacterial susceptibility test was carried out using the Agar diffusion technique. The surface of the agar in a Petri dish was uniformly inoculated with 0.3 mL of 18 h old test bacteria culture. Using a sterile cork borer, 6 mm wells were bored into agar. Then 0.06 mL of 10 mg/mL concentration of each metal complex in DMSO was introduced into the wells and the plates were allowed to stand on bench for 30 min before incubation at 37^{0}C for 24 h after which inhibitory zones (in mm) were taken as a measure of antibacterial activity. The experiments were conducted in duplicates and gentamycin was used as a reference drug.

Synthesis

Preparation of Ligand

The ligand, {[$C_{10}H_6$(OH)CH:N($C_5H_4N_2$Cl)]} HL, was prepared by refluxing a mixture of 0.017 mol (2.50 g) of 2-amino-4-methyl-6-chloropyrimidine and 0.017 mol (3.00 g) of 2-hydoxy-1-napthaldehyde with 6 drops of acetic acid in 60 mL of absolute ethanol for 6 h. The yellow product, formed on cooling to room temperature, was filtered and recrystallized from ethanol and dried *in vacuo* over anhydrous calcium chloride. The yield of the resulting Schiff base was 3.62 g (70%). ^{1}H nmr (ppm) δ10.8 (s, OH), 9.38(s, 1H, HCN); δ 7.38-8.60 (m, 6H, $C_{10}H_6$); 6.70 (s, 1H, $CH_{pyrimidine}$); 2.50 (s, 3H, CH_3).

Preparation of the Metal(II) Complexes

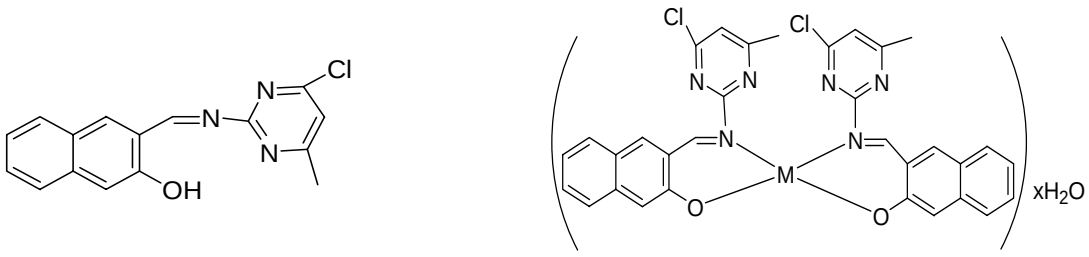
A solution of the metal(II) nitrates (0.54 mmol, 0.10-0.16 g M = Mn, Co, Ni, Cu, Zn) in 20 mL ethanol was added to a stirring solution of the ligand (1.08 mmol, 0.32 g) in 30mL ethanol at room temperature (26°C), followed by gradual addition of triethylamine (1.08 mmol, 0.15 mL). The resulting homogeneous solution was refluxed for 6h, and the products formed were later filtered, washed with ethanol and dried in *vacuo* over anhydrous $CaCl_2$.

RESULTS AND DISCUSSIONS

The reactions of the ligand with the metal (II) nitrates (Mn, Co, Ni, Cu, and Zn) gave mononuclear complexes in moderate-good yields (50-70%). The physical and analytical data, colours, % yields, melting points/decomposition temperature and room temperature magnetic moments of the compounds are presented in Table 1 and the proposed structures are shown in figure 1. The complexes were all air stable but decomposed on programmed heating and were soluble in methylene chloride, nitromethane, dimethylfornamide and dimethylsulphoxide. The elemental analysis, showed the stoichiometry of the complexes as 2L:M and confirmed the suggested molecular formula of the complexes. The decomposition pattern obtained from TGA curve corroborates the proposed formulation of the complexes. All complexes isolated adopted $[ML_2]xH_2O$ stoichiometry {Mn, Pd: x = 2; Ni, Zn: x = 1} except, the Cu(II) complex which was anhydrous. The molar conductances of the complexes ranged between 12-20 Ω^{-1} cm^2 mol^{-1} in nitromethane, showing they were non-electrolytes. A value of 60-90 Ω^{-1} cm^2 mol^{-1} is expected for a 1:1 electrolyte (Osowole *et al*., 2009). Attempts to isolate suitable crystals for single X-ray structural determination have not yet been successful.

[1]Hnmr spectra

The ligand showed the napthyl ring protons as a multiplet at δ 6.94-9.45 ppm (m, 6H, $C_{10}H_6$). The phenolic proton was observed as a singlet at 10.8 ppm(s, 1H, OH) and the proton of the imine resonated as a singlet at δ 6.70 ppm (s,1H, H=CN) while that of the pyrimidine was seen as a singlet at δ 6.67 ppm(s, 1H, $CH_{pyrimidine}$). The methyl protons resonated as a singlet at 2.50ppm(s, 3H, CH_3). On coordination to Zn(II) ion, the napthyl ring protons were deshielded and resonated as a multiplet at δ 7.01-8.24 ppm (m, 6H, $C_{10}H_6$). The phenolic proton was conspicuously absent, indicating the involvement of OH in chelating. The HC=N and CH(pyrimidine) protons resonated as singlets at δ 6.79 and δ 6.56 ppm respectively, while the methyl protons singlet shifted to δ 2.36 ppm. These shifts indicate deshielding due to coordination of the imine nitrogen atom. Similarly, Pd(II) complex napthyl ring protons were deshielded and resonated as a multiplet at δ 7.24-9.33 ppm (m, 6H, $C_{10}H_6$). The phenolic proton was conspicuously absent, indicating the involvement of OH in chelating. The HC=N proton signet, appeared at δ 6.72 ppm, while the CH of the pyrimidine appeared at δ 6.69 ppm. The methyl protons singlet shifted to δ 2.51 ppm. These downfield shifts indicate coordination of the imine nitrogen atom.



A, Schiff base; B, Metal(II) Schiff base complexes

Fig 1. Proposed structures for the ligand and its complexes.

Table 1. Analytical data for the compounds.

Compound (Empirical formula)	Formula mass	Color	μeff	% Yield	Λ_m^*	M.p (°C)	Analysis (Calculated)			
							%C	%H	%N	% M
HL ($C_{16}H_{12}N_3OCl$)	297.56	Yellow	-	70	-	188-189	64.53 (64.58)	4.13 (4.07)	13.89 (14.12)	-
$[MnL_2]2H_2O$ ($MnC_{32}H_{26}N_6O_4Cl_2$)	684.08	Brownish green	5.70	60	10.00	259-260	56.18 (56.19)	3.54 (3.83)	11.84 (12.29)	7.95 (8.03)
$[CoL_2]H_2O$ ($CoC_{32}H_{24}N_6O_3Cl_2$)	647.59	Brown	4.36	70	20.00	280-281	57.46 (57.36)	3.56 (3.61)	12.41 (12.54)	9.06 (9.10)
$[NiL_2]H_2O$ ($NiC_{32}H_{24}N_6O_3Cl_2$)	629.30	Dark Orange	3.41	70	25.00	283-284	57.16 (57.33)	3.55 (3.61)	12.51 (12.55)	9.20 (9.07)
$[CuL_2]$ ($CuC_{32}H_{22}N_6O_2Cl_2$)	616.70	Brown	1.91	70	18.00	238-239	58.67 (58.53)	3.40 (3.38)	12.50 (12.80)	10.25 (10.30)
$[ZnL_2]H_2O$ ($CuC_{32}H_{24}N_6O_3Cl_2$)	635.64	Orange	D	60	12.00	268-269	57.44 (56.90)	3.56 (3.58)	12.20 (12.40)	10.17 (10.23)
$[PdL_2]2H_2O$ ($PdC_{32}H_{26}N_6O_4Cl_2$)	733.14	Brown	D	50	15.00	183-184	52.67 (52.50)	3.40 (3.60)	11.57 (11.50)	14.24 (14.19)

D = diamagnetic; $^*\Omega^{-1}$ cm^2 mol^{-1}

Infrared spectra

The relevant infrared data are presented in table 2. The assignments of the infrared bands were made by comparing the spectra of the compounds with reported literature on similar systems (Abd El Wahab, 2008; Osowole *et al*., 2005; Osowole *et al*., 2008; Sonmez and Haciyusufoglu, 2006; Sonmez *et al*., 2004). The strong band in the ligand at 3425 cm^{-1}, which disappeared in the spectra of all the complexes, is assigned as νOH. This indicates the involvement of the enolic *O* in bonding to the metal ions. Furthermore, the ν(C-O) enolic band in the ligand at 1447 and 1360 cm^{-1} were bathochromic shifted to 1281-1192 cm^{-1} also corroborating enolic oxygen′s coordination. A new broad band, ν(OH) at 3500 cm^{-1}, in the hydrated complexes is assigned to coordinated water. The uncoordinated C=N and C=C stretching vibrations in the ligand were expectedly coupled and observed at 1631-1539 cm^{-1} (Osowole *et al*., 2005). These bands suffered bathochromic shifts to 1630-1522 cm^{-1} in the metal complexes, thus confirming the involvement of the imine *N* atom in coordination to metal(II) ion (Singh *et al*., 2001). The δC—H vibration of the ligand was observed at 967 cm^{-1} and it was bathochromic shifted to 897-776 cm^{-1} in the complexes due to the pseudo-aromatic nature of the chelates (Osowole *et al*., 2008). The bands due to ν(M—O) and ν(M—N) were observed at 499-417 and 585-501 cm^{-1} respectively in the complexes (Sonmez *et al*., 2004).

Electronic spectra and room temperature magnetic moments

In the UV region, the reflectance electronic spectra (Table 2) of all the complexes were similar, displaying strong absorption maxima between 26.50-50.00 kK, which are assigned to n- π*, π-π* and charge transfer transitions (Kwiatkowski and Kwiatkowski, 1980; Singh *et al*., 2001).

The Mn(II) complex showed two weak bands at 15.40 and 24.80 kK respectively, consistent with a four-coordinate, tetrahedral geometry and are assigned to $^6A_1 \rightarrow {}^4E_1$ (ν_1) and $^6A_1 \rightarrow {}^4A_1$ (ν_2) transitions. The effective magnetic moment of Mn(II) complexes are expected to be close to the spin-only value of 5.90 B.M. since the ground term is $^6A_{1g}$ and as such there is no orbital contribution. Consequently an observed moment of 5.70 B.M. for this complex indicates that it is high spin and complementary of tetrahedral geometry (Chohan, 2001).

The Co(II) complex exhibited two bands at 13.00 and 19.50 kK, typical of a 4-coordinate tetrahedral geometry and are assigned to $^4A_2 \rightarrow {}^4T_1(F)$, ($\nu_2$), and $^4A_2 \rightarrow {}^4T_1(P)$, ($\nu_3$), transitions. The transition, $^4A_2 \rightarrow {}^4T_2$, ($\nu_1$) in the range 5-7 KK was not observed, being outside the range covered by the instrument. An observed moment of 4.36 B.M is corroborative of tetrahedral geometry, since moments in the range 4.3-4.6 B.M are usually observed for tetrahedral Co(II) compounds (Singh *et al*., 2001; Sonmez and Haciyusufoglu, 2006; Sonmez *et al*., 2004).

The reflectance spectra of the Ni(II) complex showed absorption bands at 12.00 and 17.00 kK which are assigned to $^3T_1(F) \rightarrow {}^3T_2$,($\nu_2$) and $^3T_1(F) \rightarrow {}^3A_2$, ($\nu_3$) transitions, in a tetrahedral environment. Generally, square planar Ni(II) complexes are usually diamagnetic, while tetrahedral and octahedral complexes are paramagnetic with moments in the range 3.20-4.10 and 2.90-3.30 B.M respectively**.** This complex gave a moment of 3.41 B.M. and hence is tetrahedral (Abd El Wahab, 2008; Sonmez and Haciyusufoglu, 2006).

The observance of two bands at 15.70 and 24.40 kK in the Cu(II) complex indicates square planar geometry with the assignment of the bands as $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2E_{1g}$ transitions. A moment of 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

Table 2. Relevant infrared and electronic spectral data of the complexes

Compound	νOH	ν(C=N) + ν(C=C)	νPh/C—O	δC—H	ν(M—N)	ν(M—O)	Electronic spectral (kK)
[HL]	3425s	1631s 1572s 1539s	1447s 1360s	967s	-	-	28.00 32.00 39.00 42.00
$[Mn(L)_2]2H_2O$	3500b	1620s 1567s 1538s	1289s 1188s	897s 747s	537m 501m	486m 450s	15.40 24.80 27.20 31.70 44.10 47.40
$[Co(L)_2]\ H_2O$	3400b	1618s 1605s 1562s 1529s	1286m 1195m	832s 754s	568s 550m	481m 446m	11.00 17.50 29.20 34.51 42.30
$[Ni(L)_2]H_2O$	3500b	1623s 1577s 1551s	1290m 1190m	875s 786m	544m 518m	458m 423m	12.00 17.00 26.53 32.10 42.92 48.50
$[Cu(L)_2]$	-	1619s 1604s 1581s 1539s	1290m 1195m	833s 747s	525m 504m	461m 417m	15.70 24.40 26.50 30.40 42.00 50.00
$[Zn(L)_2]H_2O$	3500b	1620s 1605s 1579s 1522s	1281m 1196m	831s 755s	550m 544m	499m 456m	20.00 30.00 33.30 39.50 45.00
$[Pd(L)_2]2H_2O$	3500b	1615s 1600s 1575s 1522s	1291m 1192m	827s 749s	585m 559m	486m 452m	16.00 23.30 29.90 33.00 39.40 44.00

b = broad, s = strong, m = medium; 1kK = 1000 cm^{-1}.

coupling. [CuL_2] had a moment of 1.91 B.M. and is consequently mononuclear (Singh *et al*., 2001).

The Zn(II) complex showed only the CT transitions from M→L at 20.00 kK as no d-d transition is expected. The bands at 30.00, 33.30, 39.50 and 45.00 kK are assigned as π-π* and intraligand charge transfer transitions respectively. This complex is expectedly diamagnetic, confirming its tetrahedral geometry (Singh *et al*., 2001).

The Pd(II) complex showed absorption bands typical of a square-planar geometry at 16.00 and 23.25 kK, which are assigned to $^1A_{1g} \rightarrow {}^1B_{1g}$ and $^1A_{1g} \rightarrow {}^1E_{2g}$ transitions. Its diamagnetism is corroborative of square-planar geometry (Kwiatkowski and Kwiatkowski, 1980).

Mass spectroscopy and Thermal studies

The mass spectra of ligand and the complexes showed peaks attributed to the molecular ions m/z at 297 $[L]^+$; 647 $[MnL_2\text{-}2H]^+$; 651 $[CoL_2\text{-}3H]^+$; 652 $[NiL_2\text{-}2H]^+$; 655 $[CuL_2\text{-}3H]^+$; 658 $[ZnL_2\text{-}H]^+$ and 696 $[PdL_2\text{-}2H]^+$. The decomposition stages, temperature ranges, percentage losses in mass and assignment of decomposition moieties are given in table 3.

The ligand, HL, decomposed in three stages. Stage one was between 110-170°C and corresponds to the loss of the fragment C_2H_2N, with mass losses of (obs. = 14.00%, calc. = 13.46%). The second stage involved the loss of the fragment C_4H_4Cl, with mass losses of (obs. = 29.89%, calc. = 29.43%) within a temperature range of 170-380°C and the final stage was the loss of the organic moiety, $C_{10}H_6N_2$ at 380-700°C, with mass losses of (obs. = 58.94%, calc. = 57.19%).

The Mn(II) complex decomposed in six steps. The first

Table 3. Thermal data for the ligand and complexes.

Compound	Temperature range(°C)	TG weight loss(%) Calc.	Found	Assignments
HL ($C_{16}H_{12}N_3OCl$)	110-170	13.46	14.00	C_2H_2N
	170-380	29.43	29.89	C_4H_4Cl
	380-700	57.19	58.94	$C_{10}H_6N_2O$
[MnL_2]$2H_2O$ ($MnC_{32}H_{26}N_6O_4Cl_2$)	30-100	9.95	9.83	$2H_2O + O_2$
	100-160	1.02	1.05	¼N_2
	160-230	2.64	2.93	$CH_4 + H_2$
	230-300	6.45	6.41	C_3H_8
	300-410	7.69	7.72	C_3H_6N¾
	410-700	64.81	66.50	$C_{25}H_2N_5Cl_2$ Mn(residue)
[CoL_2]H_2O ($CoC_{32}H_{24}N_6O_3Cl_2$)	30-310	5.99	6.03	$CH_4 + 3H_2 + H_2O$
	310-410	8.37	8.41	$C_2H_4N_2$
	410-660	69.75	70.02	$C_{25}H_8N_4Cl_2O_2$ Co (residue)
[NiL_2]H_2O ($NiC_{32}H_{24}N_6O_3Cl_2$)	30-100	5.40	5.92	$CH_4 + 3H_2 + H_2O$
	100-200	10.60	10.57	Cl_2
	200-390	16.75	16.74	$C_6H_{12}N_2$
	390-700	58.57	65.68	$C_{25}H_4N_4O_2$ Ni(residue)
[CuL_2] ($CuC_{32}H_{22}N_6O_2Cl_2$)	30-170	21.05	21.24	$C_8H_{14}N_2$
	170-330	10.80	11.21	Cl_2
	330-700	58.53	66.26	$C_{24}H_8O_2N_4$ Cu(residue)
[ZnL_2]H_2O ($ZnC_{32}H_{24}N_6O_3Cl_2$)	50-100	14.62	14.72	$C_6H_6 + H_2O$
	100-230	22.54	23.23	Cl_2
	230-430	10.80	10.54	$C_{17}H_4O_2N_4$
	430-700	45.04	44.59	Zn(residue)
[PdL_2]$2H_2O$ ($PdC_{32}H_{26}N_6O_4Cl_2$)	30-80	10.66	10.72	$2H_2O + C_3H_6$
	130-360	16.53	16.71	$C_7H_9 + H_2$
	360-700	58.57	65.68	$C_{22}H_7N_4O_2Cl_2$ Pd(residue)

step was the loss of two molecules of water and oxygen with mass losses of (obs. = 9.95%, calc. = 9.83%) in the temperature range 30-100°C. The second step involved the loss of 0.25 mole N_2, with mass losses of (obs. = 1.05%, calc. = 1.02%) between 100-160°C. The third step corresponds to the loss of methane and hydrogen at 160-230°C with, mass losses of (obs. = 2.93%, calc. = 2.64%). The fourth step involved the loss of propane, with mass losses of (obs. = 6.41%, calc. = 6.45%) at 230-300°C. The fifth was the loss of the fragment $C_3H_6N_{¾}$ in the temperature range 300-410°C, with mass losses of (obs. = 7.72%, calc. = 7.69%). The final step involved loss of the organic moiety $C_{25}H_2N_5Cl_2$ at 410-700°C, with mass losses of (obs. = 66.50 %, calc. = 64.81%), leaving behind Mn as the residue.

The thermal degradation of the Co(II) complex occurred in three steps. The first step was within the temperature range of 30-310°C and corresponds to the loss of a molecule of water and methane, and three molecules of hydrogen with mass losses of (obs. = 6.03%, calc. = 5.99%). The second step occurred between 310-410°C and was characterized by loss of the fragment, $C_2H_4N_2$, with mass losses of (obs. = 8.41%, calc. = 8.37%). The final step involved the loss of the organic moiety $C_{25}H_8O_2N_4Cl_2$, within a temperature range of 410-700°C with corresponding mass losses of (obs. = 70.02 %, calc. = 69.75%). The final product was cobalt.

The Ni(II) complex decomposed in four steps. The first step was the loss of a molecule of water, methane and hydrogen at 30-100°C, with mass losses of (obs. = 5.92%, calc. = 5.40%). The second step was in the temperature range of 100-200 °C, and is attributed to the loss of Cl_2 (obs. = 10.57%, calc. = 10.60%). The third step involved the loss of the fragment $C_6H_{12}N_2$, at 200-390°C, with mass losses of (obs. = 16.74%, calc. = 16.75%). The final step was within the temperature range of 390-700°C and is assigned to the loss of the organic moiety $C_{25}H_4N_4O_2$, (obs. = 66.10%, calc. = 58.57%). The remaining residue was Ni.

The Cu(II) complex decomposed in three ways. The first decomposition, was the loss of the fragment, $C_8H_{14}N_2$ at 30-170°C, with mass losses of (obs. = 21.24%, calc. = 21.05 %). The second step occurred within a temperature range of 170-330°C and is attributed to the loss of Cl_2 (obs. = 11.21%, calc. = 10.80%). The final stage was within a temperature range of 460-700°C, assigned to the loss of the organic moiety $C_{24}H_8O_2N_4$ (obs. = 66.26%, calc. = 58.53%). The remaining fraction was Copper.

The Zn(II) complex decomposed in four stages. The stage one was between 50-100°C, which indicated the loss of a mole of water and benzene respectively, with mass losses of (obs. = 14.72%, calc. = 14.62%). The second stage was from 100-230 °C and is assigned to the loss of the fragment, $C_9H_{12}N_2$, with mass losses of (obs. = 23.32 %, calc. = 22.54%). The third stage involved the loss of a mole of chlorine at 230-430°C, with mass losses of (obs. = 10.54%, calc. = 10.80%). The final stage was within a temperature range of 430-700°C, attributed to the loss of the organic moiety $C_{17}H_4N_4O_2$, (obs. = 44.59%, calc. = 45.04%). The remaining residue was Zn.

The Pd(II) complex decomposed in three steps. The first step involved the loss of two molecules of water and a molecule of propene at 30-130°C, with mass losses of (obs. = 10.72%, calc. = 10.66 %). The second was within a temperature range of 130-360°C, which corresponds to the loss of the fragment, $C_7H_9N_2$ with mass losses of (obs. = 16.71%, calc. = 16.53%). The final stage was within a temperature range of 360-700°C and corresponds to the loss of the organic moiety $C_{22}H_7O_2N_4Cl_2$ with mass losses of (obs. = 65.68%, calc. = 58.70%). The remaining fraction was Pd. The high mass residue (~7%) observed in

Table 4. IC_{50} values of the ligand and its complexes against Melanonoma (518A2) and Leukemia (HL60) cells.

Compound	IC_{50} [µM] Melanoma cells(518A2)			IC_{50} [µM] Leukaemia cells (HL 60)		
	24h	48h	72h	24h	48h	72h
CDDP	35	-	-	3.5	-	-
H_2L	74.12±15.82	78.01±15.56	56.43 ±7.33	>100	>100	>100
$[Mn(L)_2]2H_2O$	>100	37.02±4.29	31.93±3.96	32.39	29.58	18.80
$[Co(L)_2]H_2O$	>100	>100	>100	>100	35.35	26.91
$[Ni(L)_2]H_2O$	>100	>100	>100	>100	16.43	28.48
$[Cu(L)_2]$	>100	>100	>100	14.04	75.33	39.29
$[Zn(L)_2]H_2O$	>100	>100	>100	>100	>100	>100
$[Pd(L)_2]2H_2O$	10.68±1.47	12.51±0.88	9.11±0.55	20.56	11.89	22.55

<5 µm = super active; 5-10µm = strongly active; 11-19µm = moderately active; 20-30µm = weakly active; >30µm = resistant; >100 µm = not active; CDDP = cis-platin; - = Not determined

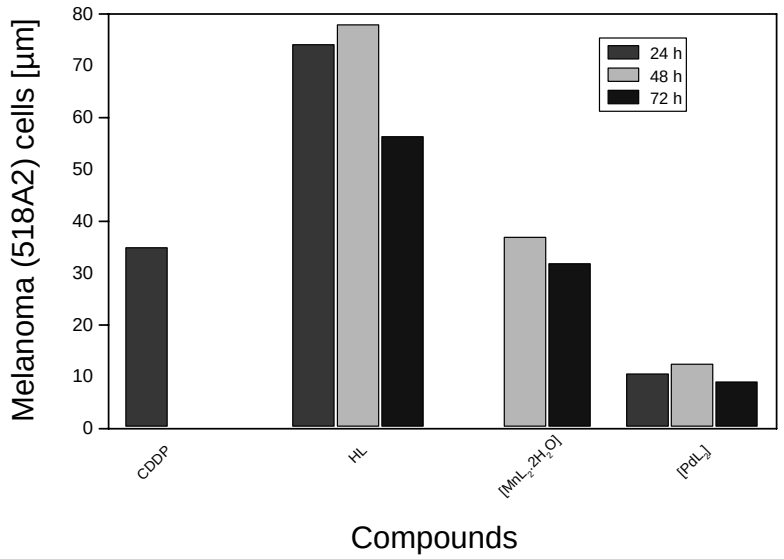


Fig 2. The inhibitory effect of the ligand, Mn(II) and Pd(II) complexes against Melanoma cells.

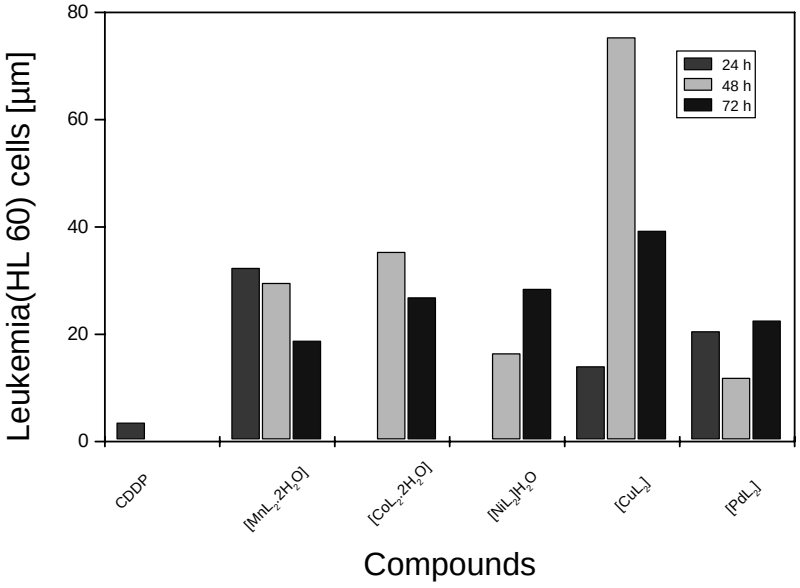


Fig 3. The inhibitory effect of some of the complexes against Leukemia cells.

the Ni(II), Cu(II) and Pd(II) complexes were attributed to carboneous material (Sharma and Srivastava, 2006).

Biological studies

The growth inhibitory effects of the ligand and its complexes on the cis-platin (CDDP) resistant 518A2 (Melanoma) and (CDDP) sensitive HL 60(Leukemia) cell lines are shown in table 4, figures 2 and 3. The ligand, HL (74.12-78.01µm) was about twice as resistant as CDDP (35.0µm) against Melanoma cells during the first 48h of experiment, while its resistance dropped by about 20% at 72 h of exposure. In contrast, the Co(II), Ni(II), Cu(II) and Zn(II) complexes showed no anti-tumor activity throughout the duration of experiment. It is important to

Table 5. Inhibitory zones(mm) of the ligand and complexes against various bacterial isolates.

Complexes	*E. Cloacae*	*S.arizona*	*Bacillus sp*	*S.Liquefaciens*	*S. aureus*	*Klebsiella sp*	*Serratia sp*	*Pseudomonas sp*	*E.Coli*	*C.violaceum*
HL	7.0±0.1	R	R	8.0±0.3	R	R	7.0±1.2	R	11.0±0	11.0±0
$[Mn(L)_2]2H_2O$	10.0±0.5	7.0±0.3	8.0±0.1	10.0±0.1	10.0±0.01	9.0±1.2	14.0±1.2	R	15.0±0	7.0±0.2
$[Co(L)_2]H_2O$	7.0±0.01	10.0±0.5	R	R	8.0±0.01	R	7.0±0.4	R	19.0±0.1	12.0±0.6
$[Ni(L)_2]H_2O$	8.0 ±0.03	15.0±0.2	8.0±0.01	R	8.0±0.1	R	9.0±2.0	R	18.0±0.2	8.0±0
$[Cu(L)_2]$	11.0±0.1	10.0±0.1	9.0±0.2	11.0±0.1	13.0±0.1	11.0±2.0	14.0±2.1	10.0±0.6	15.0±1.1	12.0±1.1
$[Zn(L)_2]H_2O$	12.0±0.3	12.0±0.1	7.0±1.0	13.0±0.1	7.0±0	9.0±0.6	15.0±1.1	7.0±0.3	19.0±0.1	7.0±1.2
$[Pd(L)_2]2H_2O$	8.0±0.1	10.0±0.4	13.0±1.1	13.0±0.2	9.0±0.3	7.0±2.1	19.0±1.4	R	16.0±0.3	10.0±0
DMSO*	R	R	R	R	R	R	R	R	R	R
Gentamycin$^+$	40.0±0.1	30.0±2.0	28.0±1.2	26.0±1.4	40.0±0.8	30.0±1.3	29.0±1.2	40.0±1.6	45.0±1.2	43.0±1.6

R = Resistant, * = Negative standard, $^+$ = Positive standard, *E. cloacae* = *Enterococcus cloacae*; *S. arizona* = *Salmonella arizona*; *S. liquefaciens* = *Serratia liquefaciens*; *S. aureus* = *Staphyloccus aureus*; *E. Coli* = *Escherichia coli*; *C. violaceum* = *Chromobacter violaceum*

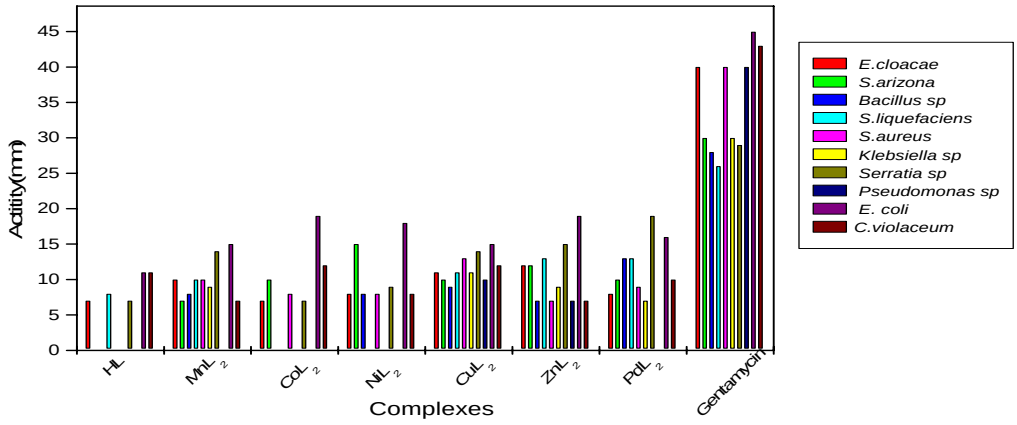


Fig. 4. The comparative activities of the complexes against bacteria strains and standard antibiotic.

note that though the Mn(II) complex does not show antitumor activity at 24 h, it exhibited comparable activity to CDDP (35.0µm) at 48 and 72 h, with IC_{50} values of 37.02 and 31.93µm respectively. Notably, $[PdL_2]$ was about three times more sensitive than CDDP with IC_{50} of 10.68 and 12.51 µm, at 24 and 48 h of experiment respectively. Further exposure of the cell line to the metal complex at 72 h, showed that the sensitivity increased to four times that of CDDP. Consequently, for further studies with the Pd(II) complex in drug development against melanoma cancers, the most effective time for exposure is 72 h.

The ligand and the Zn(II) complex were not sensitive to CDDP (3.5 µm) sensitive HL 60 Leukemia cells throughout the duration of experiment (Fig. 3). The Mn(II) complex was resistant with IC_{50} of 32.39 µm at 24 h. Further exposure at 48 and 72 h showed improved weak and moderate activity with IC_{50} of 29.58 and 18.80µm respectively. Similarly, the Co(II) complex had no activity at 24 h, but became resistant at 48 h (35.35 µm) and weakly active (26.91µm) at 72 h respectively. The Ni(II) complex was not effective at 24 h but showed moderate activity at 48 h (16.43 µm) and became weakly active at 72 h (28.48 µm). On the other hand, the Cu(II) complex exhibited moderate activity at 24 h (14.04 µm), which was a quarter of that of CDDP (3.5 µm). However it became resistant at 48 (75.33 µm) and 72 (39.29 µm) h respectively, with the latter having overcome the former's resistance by about 50 %. The Pd(II) complex was sensitive throughout the duration of the experiment. It was weakly active (20.56 µm) at 24 h. It then became moderately active (11.89 µm) at 48 h and finally at 72 h, activity dropped back to weakly active (22.55 µm). The activity at 48 h was a third of that of CDDP (3.5 µm).

It was evident from this cytotoxic studies, that the Pd(II) complex had the best anticancer activity against both

Melanoma (518A2) and HL60 (Leukemia) carcinomas in-vitro with IC_{50} values of 9.11 and 11.87 µm at 72 h and 48 h of exposure respectively, which was four times more sensitive than, and a third as sensitive as CDDP. The activities of the Cu(II) and Pd(II) complexes may be attributed to their planar structure. It has been documented that complexes with such geometry avoid possible steric hindrance during physiological actions, and are consequently more active than complexes of other geometries (Bolos *et al*., 1998).

Antibacterial studies

The results of the antibacterial activities of the compounds are presented in figure 4 and Table 5. Five organisms, *E. cloacae, S. liquefaciens, Serratia sp, E. coli, S. aureus* and *C. violaceum* were sensitive to the ligand with inhibitory zones range of 7-11 mm. The metal complexes are generally more active than the ligand against the organisms with the exception of $[CoL_2]H_2O$ which had the same activity (7 mm) as the ligand against *E. cloacae* and *Serratia sp*. $[MnL_2]2H_2O$, $[NiL_2]H_2O$ and $[ZnL_2]H_2O$ had lower activities (7<11 mm) against *C. violaceum*. Improved activities of the complexes were attributed to chelation, which reduced 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 delocalisation on the aromatic rings. This increased the lipophilic character, favouring its permeation into the bacterial membrane, causing the death of the organisms (Thangadurai and Natarajan, 2001). Furthermore, *E. cloacae*, *S.arizona, S. aureus*, *Serratia sp*, *E. coli* and *C. violaceum* were sensitive to all the complexes with inhibitory zones in the range of 8-11, 7-15, 8-13, 9-15, 15-20 and 7-12 mm respectively. *Pseudomonas sp* was resistant to all the complexes except $[CuL_2]$ and $[ZnL_2]H_2O$ with inhibitory zones of 7 and 10 mm respectively. This was attributed to its very versatile nutritional capability and adaptability to various hydrocarbon rings, and the possession of pump mechanism which ejects metal complexes as soon as they enter the cell (Pelczar *et al*., 1996). It is not clear why *E. coli* showed a high sensitivity (15-19 mm) to the complexes. In addition, *Klebsiella sp* and *S.liquefaciens* were sensitive to all the complexes with the exception of $[CoL_2]H_2O$ and $[NiL_2]H_2O$ with inhibitory zones of 7-11 and 10-13 mm respectively. A look at the antibiotic, gentamycin, activities (26-45 mm) against the various bacterial isolates relative to the metal complexes (7-19 mm) showed that the activities of the latter were much lower, with optimum activity being about half of gentamycin with *Serratia sp*{$[ZnL_2]H_2O$}, *S. liquefaciens* {$[ZnL_2]H_2O$}, {$[PdL_2]2H_2O$}, *S. arizona* {$[NiL_2]H_2O$} and *Bacillus sp,* {$[PdL_2]2H_2O$}. The complexes, $[CuL_2]$ and $[ZnL_2]H_2O$ had broad-spectrum activities like gentamycin against the organisms used, although much lower.

CONCLUSION

The coordination of the Schiff base, 3-(-1-(4,6-dimethyl-2-pyrimidinylimino)methyl-2-napthol, to Mn(II), Co(II), Ni(II), Mn(II), Zn(II), Cu(II) and Pd(II) ions with N_2O_2 chromophores resulted in tetrahedral/square planar geometry for the complexes. The complex, $[PdL_2]2H_2O$ had the best anticancer activities against HL 60 (Leukaemia) and Melanoma (518A2) carcinomas while $[CuL_2]$ and $[ZnL_2]H_2O$ had broad-spectrum activity similar to gentamycin against the bacteria used, although much lower.

ACKNOWLEDGEMENT

AAO thanks the Alexander von Humboldt (AvH) Foundation for a Georg Forster fellowship.

REFERENCES

Abd El-Wahab, ZH. 2008. Complexation of 4- amino -1,3 dimethyl-2,6-pyrimidinedione derivatives with cobalt(II) and nickel(II) ions: synthesis, spectral, thermal and antimicrobial studies. J. Coord. Chem. 61(11):1696-1709.

Atkins, M., Jones, CA. and Kirkpatrick P. 2006. Sunitinib maleate. Nat. Rev. Drug Discov. 5:279-280.

Bassett, J., Denney, RC., Jeffery, GH. and Mendham, J. 1978. Vogel's Textbook of Quantitative Inorganic Analysis. ELBS. London. 325-361.

Bolos, CA., Nikolov, G St., Ekateriniadour, L., Kortsaris, A. and Kyriakidis, DA. 1998. Structure-activity relationships for some diamine, triamine and Schiff base derivatives and their Cu(II) complexes. Metal based drugs. 5 (6):323-332.

Chohan, ZH. 2001. Synthesis, characterization and biological properties of bivalent transition metal complexes of Co(II), Cu(II), Ni(II) and Zn(II) with some acylhydrazine derived from furanyl and thienyl ONO and SNO donor Schiff base ligands. Synth. React. Inorg. Met.-Org. Chem. 31 (1):1-16.

Earnshaw, A. 1980. The Introduction to Magneto-chemistry. Academic Press. London. 80-88.

Gorneva, GA. and Golovinsky, E. 2003. Comparative study of the apoptogenic effect of fluoro pyrimidines on murine leukaemia cells. Nova Acta Leopoldina. 87 (329):183-189.

Harris, PA., Boloor, A., Cheung, M., Kumar, R., Crosby, RM., Davis-Ward, RG., Expertly, AH., Hinkle, KW., Hunter, RN., Johnson, JH., Knick, VB., Lindeman, CP., Luttrell, DK., Mooch, RA., Nolte, RT., Rudolph, SK., Szewczyk, JR., Truesdale, AT., Veal, JM., Wang, L. and Stafford, JA. 2008. Discovery of 5-[4-[(2,3-

dimethyl-2H-indazol-6-yl)methylamino]-2-pyrimidinyl]-amino]-2-methyl-benzenesulfonamide (Pazopanib), a novel and potent vascular endothelial growth factor receptor inhibitor. J. Med. Chem. 51: 4632-4640.

Kwiatkowski, E. and Kwiatkowski, M. 1980. Unsymmetrical Schiff base complexes of Ni(II) and Pd(II). Inorg. Chim. Acta. 47 (2):197-202.

Lever ABP. 1980. Inorganic Electronic Spectroscopy. (4th ed.) Elsevier. London. 481-579.

Osowole, AA, Kolawole GA. and Fagade, OE. 2005. Synthesis, Physicochemical and Biological Properties of Ni(II), Cu(II) and Zn(II) Complexes of an Unsymmetrical Tetradentate Schiff-base and their Adducts. Synth. React. Inorg. Met. Org. Chem. & Nano-Met. Chem. 35:829-836.

Osowole, AA., Kolawole, GA. and Fagade, OE. 2008. Synthesis, Characterization and Biological Studies on unsymmetrical Schiff-base Complexes of Ni(II), Cu(II), Zn(II) and Adducts with 2, 2'-Dipyridyl and 1,10-phenanthroline. J. Coord. Chem. 61(7):1046-1055.

Osowole, AA., Kolawole, GA., Kempe, R. and Fagade, OE. 2009. Spectroscopic, magnetic and biological studies on some metal(II) complexes of 3-(4,6-dimethyl-2-pyrimidinylamino)-1-phenyl-2-butenone and their adducts with 2,2'-bipyridine and 1,10-phenanthroline. Synth. React. Inorg. Met. Org. Chem .& Nano-Met. Chem. 39:165-174.

Pelczar, MJ Jr., Chan, ECS. and Krieg, NR.. 1996. Microbiology. McGraw-Hill. New York, USA. 469-510.

Ramaling, S. and Belani, CP. 2007. Role of bevacizumab for the treatment of non-small-cell lung cancer. Future oncology. 3:131-139.

Sharma, VK. and Srivastava, S. 2006. Template synthesis and structural characterisation of Homo Binuclear Cr(III), Mn(II), Fe(III), Co(III) and Ru(III) complexes with octamacrocyclic ligands. Turk J. Chem. 30:755-767.

Shreelekha, A., Vivek, B., Di, C., Fakhara, A., Ping Dou, Q., Subhash, P. and Fazlul, HS. 2006. Novel Schiff Base Copper Complexes of Quinoline-2-Carboxaldehyde as Proteasome Inhibitors in Human Prostate Cancer Cells. J. Med. Chem., 49 (24):7242 -7246.

Singh, KN., Singh, DK. and Singh, SB. 2001. Synthesis, characterization and biological studies on Co(II), Ni(II), Cu(II) and Zn(II) complexes with N-picolinoyl-N-thiobenzoyl hydrazine. Synth. React. Inorg. Met.-Org. Chem. 32 (4):703-720.

Soenmez, M., Levent, A. and Sekerci, M. 2004. Synthesis, characterization, and thermal investigation of some metal complexes containing polydentate ONO-donor heterocyclic Schiff base Ligand. Russian J. Coord. Chem. 30 (9):655-659.

Sonmez, M. and Haciyusufoglu, ME. 2006. Synthesis and characterization of Co(II), Ni(II), Cu(II) and Zn(II) Schiff base complexes derived from acetylacetone with 1- amino -5-benzoyl-4-phenyl-1H pyrimidine -2-one. Asian J. Chem. 18(3):2032-2036.

Thangadurai, TD. and Natarajan, K. 2001. Mixed ligand complexes of ruthenium(II) containing α,β-unsaturated-β-ketoamines and their antibacterial activity. Trans. Met. Chem. 26: 500–504.

Wilhelm, S., Carter, C., Lynch, M., Lowinger, T., Dumas, J., Smith, RA., Schwartz, B., Simantov, R. and Kelley, S. 2006. Discovery and development of sorafenib: a multikinase inhibitor for treating cancer 1. Nat. Rev. Drug Discov. 5(10):835-844.

Zhaohua, H., Zhaoliang, L. and Huang, J. 2001. A novel kind of antitumor drugs using sulfonamide as parent compound. Eur. J. Med. Chem. 36 (11-12):863-867.

Zhong, X., Yi, J., Sun, J., Wei, HL., Liu, WS. and Yu, KB. 2006. Synthesis and crystal structure of some transition metal complexes with a novel bis-Schiff base ligand and their antitumor activities. Eur. J. Med. Chem. 41 (9):1090-1092.

Received: April 10, 2010; Accepted: May 29, 2010