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Original article:

SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION, IN-VITRO ANTIBACTERIAL AND ANTIPROLIFERATIVE ACTIVITIES OF SOME METAL(II) COMPLEXES OF 3,4-DIHYDRONAPHTHALEN-1(2*H*)-ONE SCHIFF BASE

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ABSTRACT

The Schiff base, 3-hydroxy-4-{[4-(methylsulfanyl)phenyl]imino}-3,4-dihydronaphthalen-1(2*H*)-one, and its Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and Pd(II) complexes have been synthesized and characterized by microanalysis, conductance, ^{1}H NMR, infrared and electronic spectral measurements. The ligand exists in the ketoimine form in chloroform, and in the enolimine form in the solid state, as shown by ^{1}H NMR and IR spectroscopies. The ligand coordinates to the metal ions in the ratio 1:1, using *NO* chromophores forming complexes of the type $[MLNO_3]H_2O$, with the exception of the Zn(II) and Pd(II) complexes. Electronic measurements are indicative of a four coordinate square-planar geometry for all the complexes, except for the Cu(II) and Zn(II) complexes which assume a tetrahedral geometry. None is an electrolyte in nitromethane. The ligand and the metal complexes are air–stable, but decomposed on heating at 120 °C and in the range 150-156 °C respectively. The antibacterial studies reveal that the Co(II) and the Cu(II) complexes exhibit broad-spectrum activity against *Proteus mirabilis, Escherichia coli* and *Staphylococcus aureus* with inhibitory zones range of 14.0-22.0 and 13.0-25.0 mm respectively. The antiproliferative studies show that the Zn(II) complex has the best in-vitro anticancer activity against both HT-29 (colon) carcinoma and MCF-7 (human breast) adenocarcinoma with IC_{50} values of 6.46 μm and 3.19 μm, which exceeds the activity of Cis-platin by 8 % and 63 % respectively.

Keywords: antibacterial and antiproliferative activities, Cis-platin, geometry, Schiff base

INTRODUCTION

Napthoquinones are very interesting molecules because of their various uses such as precursors in the syntheses of imidazoles, phthiocols and benzophenothiazinols (Agarwal and Mital, 1976; Efimova and Efros, 1967; Srivastava et al., 1987), and as bactericides and insecticides. Natural napthoquinones extracted from *Tritonis crocosmilioro* are bactericidal to *Bacillus subtilis* (Masuda, 1987); while acylamino napthoquinone and aminochloro napthoquinone have larvicidal and insecticidal activities against Aedes *egypti* and *Plasmodium falciparum* (Lopes et al., 1977; Lucimi et al., 2010). Furthermore, they are useful as dyes in the form of dichloro napthosultam quinines (Herzberg and Hoppe, 1922), and as radiation modulators of induced lipid peroxidation in Fe(II)/Fe(III) complexes of various hydroxynapthouinone (Kumbhar et al., 1997). In addition, 7-bromo-5-cyclopropyl-5H-pyridazino[4,5-b]indol-1-amine ethanedioate derivatives and 4-anilino-5H-pyridazino[4,5-b]indoles showed good anticancer activity against human liver (Bel-7402) and human fibrosarcoma (HT-1080) cancer cell lines (Li et al., 2008). Thus, the

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aim is to synthesize and characterize the above named Schiff base and its metal(II) complexes. Their in-vitro antibacterial and antiproliferative activities are also investigated with a view to assessing their suitability as active components in disinfectants, and lead compounds in drug research for breast and colon carcinomas. The ligand and its metal(II) complexes are new, being reported here for the first time as a continuation of my group's research on the Synthesis, characterization and bioactivities of various (methylsulfanyl) metal(II) Schiff base complexes (Osowole and Daramola, 2011; Osowole, 2011).

MATERIALS AND METHODS

Reagent and chemicals

Reagent grade 2-hydroxy-1,4-napthoquinone, 4-methylthio aniline, hydrated cobalt(II) nitrate, nickel(II) nitrate, copper(II) nitrate, manganese(II)nitrate and Zinc (II) nitrate, Palladium(II) chloride were purchased from Aldrich and BDH chemicals, and were used as received. Solvents were purified by distillation.

Physical measurements

The electronic and infrared spectra were recorded on a Perkin-Elmer λ25 and a Thermo Nicolet FTIR 200 spectrophotometer respectively. The ^{1}H NMR spectrum was recorded on a 300 MHz Brucker DRX-400 NMR instrument in $CDCl_3$ at 295K. ^{1}H chemical shifts were referenced to the residual signals of the protons of $CDCl_3$ and were quoted in ppm. The elemental analyses for C, H and N were recorded on Thermo Quest CE Instruments flash EA1112 analyser, while manganese, cobalt, nickel, copper, palladium and zinc were determined titrimetrically (Bassett et al., 1978). Electrolytic conductivities in nitromethane and melting points (uncorrected) were determined using a HANNA HI 991300 conductivity meter and a Mel-Temp electro thermal machine respectively.

Biological studies

The clinical isolates: *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Proteus mirabilis* were obtained from the Department of Medical Microbiology, College of Medicine, University of Ibadan, Ibadan, Nigeria; while MCF-7 (human breast adenocarcinoma) and HT-29 (colon carcinoma) cells were cultured at the Institute of Medicinal and Pharmaceutical Chemistry, Technical University, Braunschweig, Germany.

Syntheses

The ligand HL, was prepared by refluxing for 6 h at 80 °C, a homogeneous yellow solution of 11.5 mmol (1.6 g) of 4-methylthioaniline and 11.5 mmol (2.0 g) of 2-hydroxy-1,4-napthoquinone to which 6 drops of acetic acid were added in 50 mL of absolute ethanol. The yellow product, formed on cooling in ice, was filtered and recrystallized from ethanol and dried *in vacuo* over anhydrous calcium chloride. The yield of the resulting Schiff base (Figure 1) was 2.26 g (70 %). ^{1}H NMR (ppm): δ 8.09-8.14 (m, 4H, $C_{10}H_4S$), 2.40 (m, 2H, -OCH_2O-), 7.64-7.70 (m, 4H, NC_6H_4S), 2.50 (s, 3H, CH_3S).

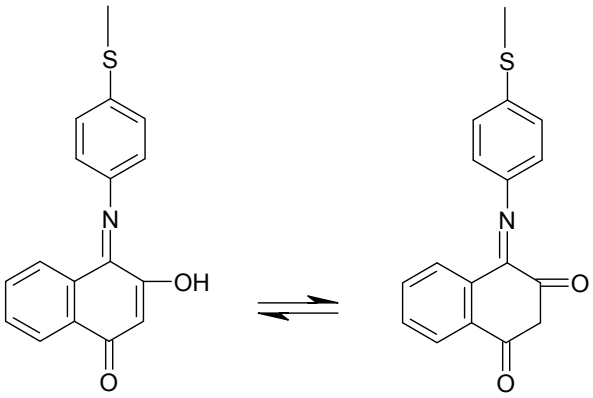

Figure 1: enolimine ↔ ketoimine tautomerism

Preparation of the metal(II) complexes

The various complexes were prepared by the addition in bits of 0.30 mmol (0.05-0.09 g) of hydrated M(II) nitrates (M = Mn, Co, Ni, Cu, Zn) to a stirring solution of 0.30 mmol (0.09 g) of the ligand in 30 mL ethanol. The resulting coloured homogeneous solution was buffered with 0.03 mmol (0.03 g) of triethylamine and refluxed for

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6 h. The products formed on cooling to room temperature were filtered, washed with ethanol, and dried *in vacuo* over anhydrous calcium chloride. The Pd(II) complex was isolated from its chloride using similar procedure.

Antimicrobial assay

The assay was carried out on the ligand and its metal(II) complexes using the Agar diffusion technique. The surface of the Muller Hinton's agar in a Petri dish was uniformly inoculated with 0.3 mL of 18 hour old *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Proteus mirabilis* cultures. A sterile cork borer was used to make 7 mm wells in the agar. A concentration of 10 mg/mL of each metal complex in DMF was then introduced into the wells and the plates allowed to stand on the bench for 30 min before incubation at 37 °C for 24 h. The inhibitory zones (in mm) were then taken as a measure of antimicrobial activity. The experiments were conducted in duplicates with sulfamethoxazole as the reference drug.

Cytotoxicity assay

The MCF-7 (human breast adenocarcinoma) and HT-29 (colon carcinoma) cells were maintained in minimum essential medium (MEM) supplemented with 10 % of fetal caw serum (FCS), and 25 mg of gentamycin at 37 °C in a humidified atmosphere with 5 % CO_2. A concentration of 100 mL of a cell suspension in culture medium [7500 cells/mL for (MCF-7) and 2500 cells/mL for (HT-29)] were plated into each of 96 well plates and incubated for three days under culture conditions. After the addition of various concentrations of the test compounds, the cells were incubated for another 96 h and 72 h respectively. The medium was then removed and the cells fixed with 1 % glutardialdehyde solution and stored under phosphate buffered saline (PBS) at 4 °C. Cell biomass was determined by a crystal violet staining, followed by the extraction of the bound dye with ethanol, and a photometric measurement at 590 nm. Mean values were calculated and the effects of the compounds were expressed as % Treated/$Control_{corr}$ values according to the following equation:

$$T/C_{corr}\ [\ \%] = (\ T\text{-}C_0/C\text{-}C_0).100$$

where (C_0 = the biomass of control cells at the tie of compound addition; C = the biomass of control cells at the time of the test end; T = the biomass of probes/samples at the time of the test end. The test compounds were prepared fresh as stock solutions in DMF and diluted with the cell culture medium to the final assay concentrations (0.1 % v/v DMF). Cis-platin was used as the reference drug. The IC_{50} value was determined as the concentration causing 50 % inhibition of cell proliferation (Scheffler et al., 2010).

RESULTS AND DISCUSSION

The formation of the ligand is confirmed by microanalyses and ^{1}HNMR measurements. The ligand exhibits ketoamine/enolimine tautomerism as seen in the IR and ^{1}H NMR measurements (Figure 1). All the complexes adopt $[MLNO_3]xH_2O$ stoichiometry, with the exception of the Zn(II) and Pd(II) complexes, which formed as $[ZnL_2]$ and $[PdLClH_2O]$ respectively, and are hygroscopic. The generalized equation for the formation of the complexes is:

$$M(NO_3)_2{\cdot}6H_2O + HL \rightarrow [MLNO_3]aH_2O + HNO_3 + bH_2O$$

(when M(II) = Mn/Co{a = 1, b= 5}); Ni(II)){a = 1.5, b= 4.5}); Cu(II) {a = 2.5, b= 3.5})

The analytical data, colors, percentage yields, melting points and molar conductivities are presented in Table 1. Attempts to isolate suitable crystals for single X-ray structural determination have not yet been successful.

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Table 1: Analytical data for the compounds

Compound (Empirical formula)	Formula mass	Color	% Yield	Λ_m^*	D. T (°C)	Analysis %C	%H	%N	(Calculated) %M
HL ($C_{17}H_{14}NSO_2$)	295.36	Dark Purple	70	-	120	68.92 (69.07)	4.53 (4.77)	4.35 (4.74)	-
$[MnLNO_3]H_2O$ ($MnC_{17}H_{15}N_2SO_6$)	429.32	Wine	50	9.0	152	47.20 (47.56)	3.51 (3.52)	3.62 (6.53)	12.73 (12.80)
$[CoLNO_3]H_2O$ ($CoC_{17}H_{15}N_2SO_6$)	433.31	Ma-roon	60	12.0	150	47.52 (47.12)	3.88 (3.49)	3.94 (6.43)	13.40 (13.60)
$[NiLNO_3]1.5H_2O$ ($NiC_{17}H_{16}N_2SO_{6.5}$)	442.10	Brown	60	13.0	156	46.19 (46.18)	3.80 (3.65)	3.95 (6.34)	13.27 (13.28)
$[CuLNO_3]2.5H_2O$ ($CuC_{17}H_{18}N_2SO_{7.5}$)	464.93	Brown	50	18.0	150	43.33 (43.92)	3.35 (3.90)	4.41 (6.03)	13.73 (13.67)
# $[ZnL_2]7H_2O$ ($ZnC_{34}H_{40}N_2S_2O_{11}$)	779.86	Red	50	15.0	150	52.25 (52.37)	3.81 (5.17)	3.60 (3.59)	8.40 (8.34)
# $[PdLClH_2O]5H_2O$ ($CuC_{17}H_{18}N_2SO_{7.5}$)	544.38	Brown	40	22.0	150	37.15 (37.51)	2.72 (4.63)	3.10 (2.57)	19.52 (19.55)

D.T = decomposition temperature; # = hygroscopic; $^*\Omega^{-1}\,cm^2\,mol^{-1}$

Conductance measurements

The molar conductivities of the complexes in nitromethane, are in the range 9.0-22.0 $ohm^{-1}\ cm^2\ mol^{-1}$, showing that they are non-electrolytes in the solvent. A value of 94.0-105.0 $ohm^{-1}\ cm^2\ mol^{-1}$ is expected for a 1:1 electrolyte (Geary, 1971).

Infrared and electronic spectra

The relevant infrared data are presented in Table 2. The band at 3433 cm^{-1} in the ligand is assigned as νOH and its broadness is attributed to intramolecular hydrogen bonding (Derebe et al., 2002). The absence of this band in the complexes indicates the involvement of the napthaquinol *O* in bonding to the metal ions. The new broad band at 3500 cm^{-1} in the complexes is assigned to ν(OH) of crystallization water. The uncoordinated C=N vibrations in the ligand are observed as four bands in the range 1677-1509 cm^{-1}. These bands are observed as three to four bands in the complexes, and are hypsochromic/bathochromic shifted to 1681-1506 cm^{-1}; confirming the involvement of the imine *N* atom in coordination to the metal(II) ion. The appearance of the bands due to ν(M—O) and ν(M—N) in the complexes, at 471-428 and 572-534 cm^{-1} is further evidence of coordination. These bands are absent in the ligand spectrum (Abd El-Wahab, 2008).

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

Compound	vOH	v(C=N)	v(M—N)	v(M—O)	Electronic transitions (kK)
HL	3433b	1677s 1594s 1568s 1509s	-	-	29.41, 35.84, 40.98
$[MnLNO_3]H_2O$	3500b	1681s 1594s 1567s 1506s	572s 500s	471s 450m	20.41, 30.03, 35.34, 40.32
$[CoLNO_3]H_2O$	3500b	1680s 1593s 1567s 1507s	570s 501s	471m 428m	21.51, 29.85, 35.21, 40.65.
$[NiLNO_3]1.5H_2O$	3500b	1681s 1592s 1566s	572s 513s	471s 459m	21.19, 30.12, 35.21, 40.49.
$[CuLNO_3]2.5H_2O$	3500b	1667s 1605s 1590s 1570s	570s 514s	443m 428m	20.0, 30.03, 35.34
$[ZnL_2]7H_2O$	3500b	1681s 1594s 1567s 1508s	571m 514s	471m 428s	21.05, 30.12, 35.34, 40.49
$[PdLClH_2O]5H_2O$	3500b	1676m 1608m 1592s 1570s	572s 501m	471m 441m	21.28, 30.12, 35.34

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

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The electronic spectra are presented in Table 2. The Mn(II) and Co(II) complexes both exhibit a single band each at 20.41 kK and 21.51 kK respectively, suggestive of a 4-coordinate, square-planar geometry (Osowole et al., 2009). Similarly, the Ni(II) and Pd(II) complexes have an absorption band each at 21.19 kK and 21.28 kK, indicative of a 4- coordinate square-planar geometry; and are assigned to $^1A_{1g} \rightarrow {}^1E_{1g}$ transition (Sonmez and Haciyusufoglu, 2006). The Cu(II) complex has a single band at 20.0 kK, suggestive of a distorted tetrahedral geometry with the assignment $^2T_2 \rightarrow {}^2E$ (Gaber et al., 2001). As expected, the Zn(II) complex has no d-d band. However, the presence of a M→L CT band at 21.05kK is indicative of a tetrahedral geometry. The ligand bands are observed at 29.41, 35.84 and 40.98 kK, and are assigned to n→π*, π→π* and CT transitions. These bands are mostly bathochromic shifted in the complexes to 29.85-30.12, 35.21-35.34 and 40.32-40.65 kK due to coordination (Pandya and Shah, 2009).

^{1}H NMR spectra

The phenyl napthoquinone protons are observed as a multiplet at 8.09-8.14 ppm, while the CH_2 protons are observed as a singlet at 2.40 ppm. The four phenyl protons in NC_6H_4S moiety resonate as a multiplet at 7.64-7.70 ppm, while the methyl group in the CH_3S moiety is seen as a singlet at 2.51 ppm.

Antibacterial activity

The results of antibacterial activities are shown in Figure 2 and presented in Table 3. The ligand is active against *Staphyloccous aureus* and *Proteus mirabilis* with inhibitory zones range of 17.0 and 20.0 mm but it is inactive against *Klebsiella pneumoniae* and *E.coli*. As expected, the complexes are more susceptible to *Proteus mirabilis*, a gram negative bacterium, due to its thin peptidoglycan layer, which makes it more permeable to the complexes with inhibitory zones range of 19.0-26.0 mm (Thangadurai and Natarajan, 2001). Furthermore, none of the complex is active against *Klebsiella pneumoniae*. All the metal complexes are active against *S. aureus* with inhibitory zones range of 13.0-25.0 mm, except for the Pd(II) complex, which has no activity.

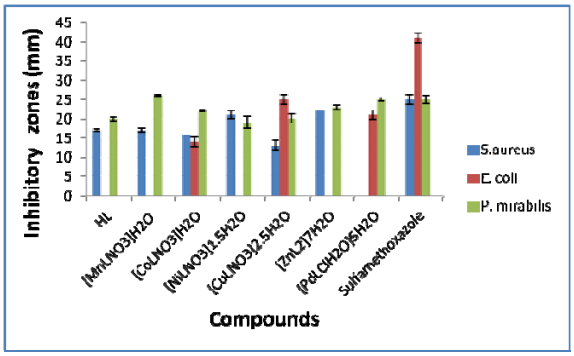

Figure 2: Histogram of the antibacterial activities of the ligand and its complexes

The resistance of *Klebsiella pneumoniae* to the ligand and the metal complexes, and the insensitivity of *E. coli* to the Ni(II), Mn(II) and Zn(II) complexes is attributed to the production of extended-spectrum beta-lactamases (ESBL) by these bacteria, which inactivates the compounds (Kamalakannan and Venkappayya, 2002; Vitkauskiene et al., 2006). Surprisingly *E. coli* is sensitive to Co(II), Pd(II) and Cu(II) complexes with inhibitory zones range of 14.0-25.0 mm. As expected, the complexes are mostly more active than the ligand against *P. mirabilis* and *E. coli*. This is due to the partial sharing of their positive charge with donor groups of the ligand and possible π-electron delocalisation on the aromatic rings which in turn increased the lipophilic character, favouring its permeation into the bacterial membrane, thus causing the death of the organisms (Mostafa and Badria, 2008). The ligand has the same activity of 17.0 and 20.0 mm as the Mn(II) and Pd(II) complexes against *S. aureus* and *P. mirabilis*. This observation could not be explained. The lower activities of the Co(II) and Cu(II), and Ni(II) complexes relative to the ligand against *S. aureus* and *P. mirabilis* may be attributed to low degree of permeability of the cells of the bacteria, or the difference in the bacteria ribosome (Rafique et al., 2010).

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Table 3: Zones of inhibition (mm) of the compounds against various bacteria isolates

Compounds	*S.aureus*	*E. coli*	*P. mirabilis*	*K.pneumoniae*
HL	17.0 ± 0.2	R	20.0 ± 0.5	R
$[MnLNO_3]H_2O$	17.00 ± 0.4	R	26.0 ± 0.2	R
$[CoLNO_3]H_2O$	16.0 ± 0.0	14.0 ± 1.2	22.0 ± 0.3	R
$[NiLNO_3]1.5H_2O$	21.0 ± 1.0	R	19.0 ± 1.6	R
$[CuLNO_3]2.5H_2O$	13.0 ± 1.4	25.0 ± 1.2	20.0 ± 1.2	R
$[ZnL_2]7H_2O$	22.0 ± 0.0	R	23.0 ± 0.6	R
$[PdLClH_2O]5H_2O$	R	21.0 ± 1.2	25.0 ± 0.4	R
Sulfamethoxazole$^+$	25.0 ± 1.2	41.0 ± 1.2	25.0 ± 1.0	R

Key: R = Resistance; $^+$ = positive standard

The activities of sulfamethoxazole (25.0-41.0 mm) against the various bacterial isolates, when compared to the metal complexes (13.0-26.0 mm), indicate that most of the metal complexes have lower activity. However, the Zn(II) complex shows optimum activity of about 90 % that of sulfamethoxazole against *S. aureus* and *P. mirabilis*. It is note worthy that the Pd(II) complex has the same activity of 25.0 mm as the antibiotic against *P. mirabilis*; while the Mn(II) complex's activity (26.0 mm) against *P. mirabilis,* is marginally higher than that of the antibiotic (25.0 mm). Thus, the Co(II) and Cu(II) complexes exhibit broad-spectrum antibacterial activity against *Staphyloccous aureus*, *Escherichia coli* and *Proteus mirabilis,* with inhibitory zones range of 14.0-22.0 and 13.0-25.0 mm respectively; proving their usefulness as potential broad-spectrum antibacterial agents.

Anticancer activity

The results of the anticancer activities are presented in Table 4. The metal complexes are more sensitive to the HT-29 (colon carcinoma), than the MCF-7 (human breast adenocarcinoma) cells. The activities of the Zn(II), Cu(II) and Pd(II) complexes are 63 %, 58 % and 44 % Cis-platin activity respectively, against MCF-7 cells; while the ligand has an activity of 29 % that of Cis-platin. On the contrary, the Zn(II) complex has the best activity against HT29 cells, exceeding that of Cis-platin by 8 %. The Cu(II) complex has about the same activity as Cis-platin, while the activities of the Pd(II) complex and the ligand are 65 % and 56 % of Cis-platin activity respectively. Thus, in both cases chelation enhances the antiproliferative activity of the complexes.

Table 4: IC_{50} values of the ligand and its Co(II), Cu(II), Pd(II) complexes against MCF-7 and HT-29 cells

Compounds	MCF-7 (human breast adenocarcinoma) [μM]	HT-29(colon carcinoma cells) [μM]
CDDP	2.0	7.0
HL	6.92 ± 0.1	12.53 ± 0.1
$[CuL_2]7H_2O$	3.46 ± 0.1	7.27 ± 0.1
$[ZnL_2]3H_2O$	3.19 ± 0.2	6.46 ± 0.2
$[PdL_2]3H_2O$	4.53 ± 0.1	10.81 ± 0.1

Results are expressed as means (± error) of at least two independent experiments

CONCLUSION

The ligand exhibits enolimine ↔ ketoimine tautomerism as shown by ^{1}H NMR and IR spectroscopies. All the metal(II) complexes assume a 4-coordinate square-planar geometry with the exception of the Cu(II) and Zn(II) complexes, which are tetrahedral, as corroborated by electronic spectral measurements. The Co(II) and the Cu(II) complexes exhibit broad-spectrum antibacterial activities against *Staphylococcus aureus*, *Escherichia coli* and *Proteus mirabilis* with inhibitory zones range of 14.0-22.0 and 13.0-25.0 mm respectively. The in-vitro antiproliferative studies show that the Zn(II) complex has the best antiproliferative activity against both HT-29 (colon) carcinoma and MCF-7 (human breast) adenocarcinoma with IC_{50} values of 6.46 µm and 3.19 µm, which exceeds the activity of Cis-platin by 8 % and 63 % respectively.

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REFERENCES

Abd El-Wahab ZH. 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 2008; 61:1696-709.

Agarwal NL, Mital RL. Synthesis of 6-anilino-12H-benzo[a]phenothiazin-5-ols. Ind J Chem 1976;14B:381-2.

Bassett J, Denney RC, Jeffery GH, Mendham J. Vogel's textbook of quantitative inorganic analysis (pp 325-61). London: ELBS, 1978.

Derebe MG, Raju VJT, Retta N. Synthesis and characterization of some metal complexes of a Schiff base derived from ninhydrin and L-alanine. Bull Chem Soc (Ethiopia) 2002;16:53-64.

Efimova GA, Efros LS. Heterocyclic derivatives of substituted 1,4-naphtho-quinones. IV Condensation of 2,3-diamino-1,4-naphthoquinone and its monomethyl derivative with 1,3-diketones. Zhurnal Organicheskoi Khim 1967;3:162-8.

Gaber M, Mabrouk HE, Al-Shihry S. Complexing behaviour of naphthylidene sulfamethazine Schiff base ligand towards some metal ions. Egyptian J Chem 2001;44:191-200.

Geary WJ. The use of conductivity measurements in organic solvents for the characterization of coordination compounds. Coord Chem Rev 1971;7:81-122.

Herzberg W, Hoppe G. Dyes. 1922; US 1419502.

Kamalakannan P, Venkappayya D. Synthesis and characterization of cobalt and nickel chelates of 5-dimethylaminomethyl-2-thiouracil and their evaluation as antimicrobial and anticancer agents. J Inorg Biochem 2002;21:22–37.

Kumbhar AS, Padhye SB, Jitender KRK. Naturally occurring hydroxy napthquinones and their iron complexes as modulators of radiation induced lipid peroxidation in synaptosomes. Metal-Based Drugs 1997;4: 279-85.

Li R, Huang P, Qiao J. Design, synthesis and antitumor activities of novel 4- anilino-5H-pyridazino[4,5-b]indoles. Zhongnan Yaoxue 2008;6;144-8.

Lopes JNC, Johnson AW, Grove JF, Bulhoes MS. Synthesis of 2-amino-1,4-napthoquinone derivatives and the evaluation of their larvicidal and molluscidal activities. Ciencia e Cultura 1977;29:1145-9.

Lucumi E, Darling C, Jo H, Napper AD, Chandramohanadas R, Fisher N et al. Discovery of potent small-molecule inhibitors of multidrug-resistant Plasmodium falciparum using a novel miniaturized high-throughput luciferase-based assay. Antimicrob Agents Chemother 2010;54:3597-604.

Masuda K. Isolation of antimicrobial 5,8-dihydroxy-2,3-dimethoxy-6,7-methylenedioxy-1,4-napthoquinone and its isomers from Tritonia crocosmaeflora (Iridaceae). Eur Pat Appl 1987;13.

Mostafa SI, Badria FA. Synthesis, spectroscopic, and anticancerous properties of mixed ligand palladium(II) and silver(I) complexes with 4,6-diamino-5-hydroxy-2-mercaptopyrimidine and 2,2′-bipyridyl. Metal-Based Drugs 2008;1-7.

Osowole AA. Synthesis, characterization, in-vitro antibacterial and anticancer studies on some metal(II) complexes of (methylsulfanyl)chromenol Schiff base. Elixir Appl Chem 2011;39:4827-31.

Osowole AA, Daramola AO. Synthesis, physicochemical and biological activities of some metal(II) complexes of (methylsulfanyl)-2,4-benzenediol Schiff base. Elixir Appl Chem 2011;39:4876-9.

Osowole AA, Kolawole GA, Kempe R, Fagade OE. Spectroscopic, magnetic and biological studies on some metal(II) complexes of 3-(4,6-dimethyl-2-pyrimi-dinyl-amino)-1-phenyl-2-butenone and their adducts with 2,2′-bipyridine and 1,10-phenanthroline. Synth React Inorg Met Org Chem Nano-Met Chem 2009;39:165-74.

Pandya JH, Shah MK. Synthesis and antimicrobial properties of transition metal complexes of novel Schiff base ligand derived from 5-bromosalicyldehyde. J Ind Council Chem 2009;26:109-12.

Rafique S, Idrees M, Nasim A, Akbar H, Athar A. Transition metal complexes as potential therapeutic agents. Biotechnol Mol Biol Rev 2010;5(2):38-45.

Scheffler H, You Y, Ott, I. Comparative studies on the cytotoxicity, cellular and nuclear uptake of a series of chloro gold(I) phosphine complexes. Polyhedron 2010; 29:66-9.

Sonmez M, Haciyusufoglu ME. 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 2006;18:2032-6.

Srivastava VK, Kaushik NK, Khera B. Phthiocol chelates of bis(cyclopentadienyl titanium(IV). Synth React Inorg Met Org Chem 1987;17:15-24.

Thangadurai TD, Natarajan K. Mixed ligand complexes of ruthenium(II) containing α, β unsaturated-β-ketoamines and their antibacterial activity. Trans Met Chem 2001; 26:500–4.

Vitkauskiene A, Dudzevicius V, Ryskus L, Adukauskiene D, Sakalauskas R. [The rate of isolation of Klebsiella pneumoniae producing extended-spectrum beta-lactamases and resistance to antibiotics]. (Article in-Lithuanian). Medicina (Kaunas) 2006;42: 288-93.