



Synthesis, spectral, magnetic and *in-vitro* biological studies of organic ligands and their corresponding heteroleptic divalent d-metal complexes

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Abstract Novel heteroleptic Co(II) and Zn(II) complexes of a Schiff base derived from 2-amino-4,6-dihydropyrimidine and 2-hydroxy-1-naphthaldehyde were synthesized and characterized by percentage metal and elemental (CHN) analyses, FTIR, UV-VIS and ¹H- and ¹³C-NMR spectroscopies, magnetic susceptibility, melting point and molar conductance measurements. The proposed molecular formula [M(L)(L')OAc] {M= Co and Zn; L=Schiff base and L'= 2,2'-Bipyridine} for the complexes were consistent with the spectral, elemental and percentage metal data. The electronic and room temperature magnetic susceptibility values were corroborative of octahedral geometry for the heteroleptic divalent complexes. In addition, the room temperature magnetic susceptibility showed a spin crossover from high to low spin within the octahedral environment for Co(II) complex, while the CHN data indicate 1:1:1 molar ratio of the metal ions to the ligands. Low values of molar conductance in dimethylsulphoxide (DMSO) established the non-electrolytic nature of the complexes. The antimicrobial studies of the Schiff base, 2,2'-bipyridine and their heteroleptic complexes screened *in vitro* against *S. aureus*, *P. aeruginosa*, *E. coli*, *B. cereus*, *P. mirabilis* and *K. oxytoca*; and *Aspergillus flavus*, *Aspergillus niger* and *R. stolonifer* displayed moderate-good activities. Remarkably, the synthesized compounds showed broad spectrum activity against tested microbes with inhibitory zones of 5.5-26.0 mm proving their potentials as broad-spectrum microbial agents. Also, the antioxidant activities of the ligands and their heteroleptic complexes were determined *in vitro* by DPPH methods. The obtained values were mostly comparable/higher to that of the standard, ascorbic acid (55.03%) suggesting a similar pathway for their antioxidant activities and proving their potentials as probable anticancer agents.

Keywords Antioxidant, heteroleptic complexes, broad-spectrum, non-electrolytic and naphthaldehyde

Introduction

Various metal ions are widely recognized due to their significant roles in human biological processes [1], a consequence of their nuclei size and electron availability to enhance interaction with organic molecules in biological systems [2]. The presence of these metal ions results in premature aging and cell breakdown prevention [3]. Enzyme-catalytic processes have also been reported to function efficiently with metal ions, as the later form enzyme active sites, stabilize tertiary/quaternary structures of the enzymes and act as catalysts triggering enzymatic reactions and vitamins in the body [4]. For instance, the human systems requires about two grams of zinc, since over one-hundred enzymes operates in the presence of zinc (*i.e.* carbonic anhydrase which is present in red blood cells and is



involved in respiration, speeds up absorption of CO₂ in muscles and tissues, helps in release of CO₂ in the lungs and regulates pH in the blood and body of humans, dehydrogenases and aldolases are involved in blood and body sugar metabolism, etc) [3,5]. Zinc is proficiently regulated by major proteins in cell signalling and similarly controls several proteins by shifting its concentration. The metal ion in the central nervous system is released from the synaptic vesicles at some glutamatergic nerve terminals to trigger signalling pathways which affects physiological functions including synaptic plasticity, potentiation and cell death. Zinc influences the productivity of nitric acid (HNO₃), changing the immune system. Zinc deficiency is widely attributed to the high phytic acid content of diets leading to poor growth, immunity impairment, and accelerates morbidity from common infections mostly yeast and fungal infections [6-7]. Also, Enzymes like ribonucleotide reductase and glutamic mutase involved in the biosynthesis of DNA and metabolism of amino acids respectively functions with the transition metal, cobalt [3]. Cobalt is a major component of vitamin B₁₂ (cobalamine) [8] which acts as a coenzyme and serves as a prosthetic group that is tightly bound to many enzymes in the body [5]. These metal ions are known to form stable Schiff base complexes [4, 9]; with improved biological activities compared to their precursors [10-13]. Nonetheless, the mechanisms of some metal ions in the body system are still a matter to be completely overhauled. The design and isolation of heteroleptic complexes with better biological applications but reduced or no toxic side effects constitutes a major challenge. Thus, this work reports the synthesis, characterization and of organic ligands and their heteroleptic Co(II) and Zn(II) complexes. Lastly, the strength of the Schiff base, 2,2'-bipyridine and their corresponding complexes as bactericidal, fungicidal and antioxidant agents will be explored. The divalent complexes and the organic ligands are new, reported for the first time.

Materials and Methods

The infrared (4000-350 cm⁻¹) and electronic (190-900 nm) spectra of the synthesised compounds were recorded using a shimadzu prestige-21 FTIR as KBr pellets and Lambda 25 UV/Visible spectrophotometers respectively. The microanalysis of carbon, hydrogen and nitrogen were determined on Eager-300 CHN elemental analyser apparatus. The proton and carbon-13 NMR spectral measurement of the Schiff base was obtained in DMSO-*d*₆ using a 300 MHz Bruker Advance III NMR spectrophotometer at 300 Kelvin temperature. The chemical shifts (δ) reported in ppm unit were obtained relative to the as internal standard, tetramethylsilane (TMS). Electrospray Ionization Mass Spectrometry (ESI-MS) for the Schiff base was performed using a reported procedure [4] with slight modifications. conductance measurements in DMSO was recorded using the conductivity meter; ETD instruments BA380, while the uncorrected melting points determination were obtained on an Electro-thermal Temp-Mel melting-point apparatus. Thermal gravimetric analysis was performed under a nitrogen atmosphere using an SDTQ 600 thermal apparatus fitted with an alumina pan. The weight of the samples were between 10 and 12 mg, while 10°C/min was maintained throughout the heating. The powdered metal (II) complexes were examined for magnetic susceptibilities on a Johnson Mathey magnetic susceptibility balance using Pascal's constants [4] according to the equation below to estimate diamagnetic corrections. The apparatus, SDTQ 600 thermal instrument fitted with alumina crucibles was used to determine the thermal properties of the compounds at a heating of 10°C/min under a nitrogen atmosphere. The percentage cobalt and zinc contents in the complexes were determined titrimetrically [14].

$$\mu_{eff} = 2.828\sqrt{XT}.$$

Analytical grade chemicals; 2-amino-4,6-dihydroxypyrimidine, 2-hydroxy-1-naphthaldehyde, 2,2'-bipyridine, Ni(CH₃CO₂)₂·4H₂O and Zn(CH₃CO₂)₂·2H₂O and N(C₂H₅)₃ were bought from Sigma Aldrich chemical company and used without further purification. The solvents; dichloromethane, dimethylsulphoxide, methanol, ethanol, acetic acid were all brought as container grades and distilled following standard procedures [15] before usage.

Synthesis of 3-[(4,6-dihydroxypyrimidin-2-yl)imino]methyl]naphthalen-2-ol

The 3-[(4,6-dihydroxypyrimidin-2-yl)imino]methyl]naphthalen-2-ol Schiff base was synthesised according to a procedure reported by Festus and Osowole [16] with negligible adjustments. 2-amino-4,6-dihydroxypyrimidine (0.12 mol 1.47 g) dissolved in sodium carbonate (Na₂CO₃, 30 mL) solution was neatly added in bits to a stirring



methanolic (20 mL) solution of 2-hydroxy-1-naphthaldehyde (0.12mol, 2.0g). The entire solution was catalysed using acetic acid (2.2 mL) and was refluxed for 6 h at 60°C. The brown shade solid product in precipitate form separated out on cooling in ice, was filtered under suction, washed with hot methanol and dried in a vacuum desiccator over anhydrous calcium chloride. Percentage yield: 87% (2.0g); mp: 220-223°C; formula/mass: C₁₅H₁₁N₃O₃/281.268; color: Beaver brown; selected FTIR(KBr) data $\nu(\text{cm}^{-1})$: 3342 (OH), 3171 (Ar-CH), 1650 (C=N), 1550 (C=C), 1515, 1466 (C-N), 1366, 1290s (C-O), 990 ($\delta\text{C-H}$); UV/visible (Transition and molar absorptivity, cm^{-1}): 33710 ($\pi \rightarrow \pi^*$: $\epsilon = 1.9 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$), 27933 ($n \rightarrow \pi^*$: $\epsilon = 5.7 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$); CHN (%) - Anal (Calcd): C, 64.04 (64.15), H, 4.13 (3.94), N, 17.03, (16.94); UV/visible(cm^{-1}): 37100, 31646 ($\pi \rightarrow \pi^*$), 27933 ($n \rightarrow \pi^*$); ¹H-NMR (300 MHz, DMSO-*d*₆) δ ppm: 10.80 (s, OH); 8.92 (s, 1H, HC=N); 6.91 (s, 1H, H₅); 8.22 (d, 1H, H₁₈); 8.07 (d, 1H, H₁₁); 7.85 (d, 1H, H₁₃); 7.76 (d, 1H, H₁₆); 7.56 (d, 1H, H₁₄); 7.33 (s, 1H, H₁₅); ¹³C-NMR (75 MHz, DMSO-*d*₆) δ ppm: 138.4 (C₁₀); 129.3 (C₉); 128.8 (C_{18,11}); 127.5(C_{14,15}); 124.2 (C_{17,12}); 122.2 (C_{16,13}); and 129.3 (C₁₄); 164.1 (C₈); 131.7 (C₂); 192.8 (C_{4,6}) and 98.1 (C₅).

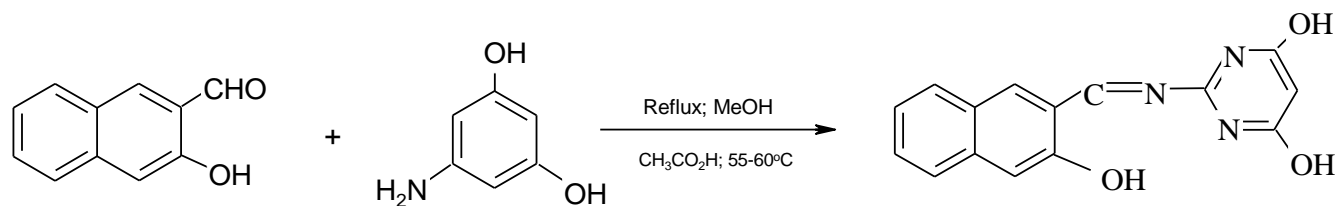


Figure 1: Scheme for the synthesis of the Schiff base ligand

Syntheses of Heteroleptic Metal (II) Complexes

In preparation of the heteroleptic complexes (Co and Zn), the metal (II) ions were reacted in 1:1:1 molar ratio with the ligands (Schiff base and 2,2'-bipyridine) in 20 mL of methanol. To a stirring 10 mL methanol Schiff base solution (0.14 mmol, 0.4 g), was added of Co(II) salt 0.14 mmol (0.36 g) in bits and stirred with slight heating (50°C). A 5 mL methanol solution of 2,2-bipyridine was added drop wisely, buffered with 2.5 mL of triethylamine to a pH of 9. The resulting mixture was refluxed for 6 h. The precipitated solid formed were allowed to cool to room temperature, filtered under suction, washed with hot ethanol and dried over anhydrous CaCl₂. Heteroleptic Zn(II) complex was also synthesized using the same procedure. Synthesized heteroleptic complexes were completely hygroscopic-less, practically soluble only in the solvents; dimethylsulphoxide and dimethylformamide and stable at room temperature

[Co(L)(L')OAC]: Molar mass (g/mol): 554.292; yield% (64.20); color: Light Brown; mp: 334-337°C; FT-IR(KBr) ν/cm^{-1} : 3342 (OH), 3169 (Ar-CH), 2924, 2852 (Acetae-CH₃), 1686 (C=O), 1634 (C=N), 1563 (C=C), 1443 (C-N), 1365, 1257 (C-O, OAC), 991 ($\delta\text{C-H}$), 563 (Co-N), 482 (Co-O); CHN(%)-Anal(Calc.d): C, 58.64 (58.50), H, 3.89 (3.85), N, 12.71 (12.64); %metal (calc.d) 10.69(10.63); UV/visible(Transition and molar absorptivity, cm^{-1}): 31348 ($\pi \rightarrow \pi^*$, $\epsilon = 1.3 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$), 26497 ($n \rightarrow \pi^*$, $\epsilon = 6.01 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$), 17285 (⁴T_{1g} → ⁴A_{2g}, $\epsilon = 29.2 \text{ M}^{-1} \text{ cm}^{-1}$), 12855 (⁴T_{1g} → ⁴T_{1g}, $\epsilon = 23.7 \text{ M}^{-1} \text{ cm}^{-1}$); μ_{eff} (B.M.): 4.71; molar conductance ($\text{ohm}^{-1} \text{ mol}^{-1} \text{ cm}^2$): 14.05.

[Zn(L)(L')OAC]: Molar mass (g/mol): 560.732; yield% (71.00); color: Gray; mp: 312-313°C; FT-IR(KBr) ν/cm^{-1} : 3438 (OH), 3170 (Ar-CH), 2927, 2856 (Acetae-CH₃), 1690 (C=O), 1638 (C=N), 1566 (C=C), 1465 (C-N), 1361, 1254 (C-O, OAC), 987 ($\delta\text{C-H}$), 559 (Zn-N), 486 (Zn-O); CHN(%)-Anal(Calc.d): C, 57.92 (57.83), H, 3.85 (3.78), N, 12.52 (12.49); %metal (calc.d) 11.85(11.66); UV/visible(Transition and molar absorptivity, cm^{-1}): 39525, 30487 ($\pi \rightarrow \pi^*$, $\epsilon = 1.0 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$), 25356 ($n \rightarrow \pi^*$, $\epsilon = 1 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$), 17572, 12052 (M → L); μ_{eff} (B.M.): 0.11; molar conductance ($\text{ohm}^{-1} \text{ mol}^{-1} \text{ cm}^2$): 7.15.

Antibacterial Activity

The pyrimidinyl Schiff base, 2,2'-bipyridine and their heteroleptic Co(II) and Zn(II) complexes were tested *in-vitro* against representative two gram positive bacteria species *Bacillus subtilis*, *Staphylococcus aureus*; and four gram



negative bacteria species *Klebsiella oxytoca*, *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa* by agar well diffusion technique [23]. Also, *in-vitro* antifungal screening of the compounds was examined for the species; *Aspergillus flavus*, *Aspergillus niger* and *R. stolonifer* by a well diffusion method with potato dextrose agar (PDA) as medium. Nutrient (Muller Hinton) agar was used as the microbiological medium. 0.3 mL of 18-24 h old standard test 0.5 McFarland culture of each microorganism was inoculated uniformly on the surface of the Muller Hinton's agar in petri dish. With a sterile cork borer, 7mm wells were bored into the agar. Then 0.06 mL of the 10 mg/mL solution of each metal complex in dimethyl sulphuroxide was introduced into the 7 mm well bored unto the agar. The plates were allowed to stand on the bench for 30min to solidify before incubation at 30°C for 24 h after which inhibition zone growth in diameter (mm) was taken as antimicrobial activity. The bactericide, streptomycin; and fungicide, fluconazole were used as standard drugs concomitantly with the test samples to form the positive controls, while dimethylsulphoxide served as negative control. The growth of the tested microorganisms were not affected by the concentration of DMSO used for the medium. The experiments were carried out in duplicates.

Minimum inhibitory Concentration (MIC)

The complexes were examined for minimum inhibitory concentration by the adoption of standard procedures reported in literature for bacterial and fungal organisms. The synthesised compounds were dissolved in 0.02 cm³ of dimethylsulphoxide at different concentrations (200, 100, 50, 10 µg/mL) and used for the experiment. Each of the compounds were separately added to each medium in 96-well plates. After, inoculation of each of well was carried out using 100 µL of the inoculum. The standard drugs; streptomycin and fluconazole were added to the assays as positive controls. At 30 °C and 37 °C, the plates used for the fungal and bacteria studies were incubated for 48-78h, and 24h respectively. The tested broth at 5 µL were placed on the sterile MHA plates and incubated at varied temperatures for the bacteria. At lowest concentration, the MICs of the bacteria were obtained for the compounds that inhibited the visible growth of tested cultures on the agar plates. The MIC for the fungal studies showed no visible fungal growth after incubation time of the lowest concentration.

Free radical scavenging ability by DPPH radical assay (1, 1-diphenyl-2-picryl hydrazyl)

The *in vitro* free radical scavenging prospective of the Schiff base and its heteroleptic Co(II) and Zn(II) complexes were estimated by DPPH assay procedure. The procedure was adopted to evaluate the reduction of DMSO solution of the coloured DPPH radical. Each synthesized test sample or the standard (ascorbic acid) (0.1mL) was diluted to 100µg/mL concentration in DMSO and mixed with 2.9 mL of DPPH (0.025µg/mL) solution in DMSO. A blank containing 2.9 mL of DMSO was prepared and the initial absorbance was determined. The entire reaction mixture was vigorously shaken and allowed to equilibrate in the dark for 30 minutes at room temperature. The absorbance of the mixture was recorded at 515 nm against the blank. The experiment was carried out in triplicate. The percentage reduction in absorbance was calculated from the initial and final absorbance of each mixture. Consequently, percentage scavenging ability of DPPH radical was evaluated using the expression in equation below

$$\% \text{ DPPH scavenging effect (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

where A_c is the absorbance of blank; A_s is the absorbance of test sample solution or absorbance of the standard respectively.

Discussion of Results

Synthesis

The structures of the synthesized Schiff base ligand and its heteroleptic Co(II) and Zn(II) complexes are given as figures 1 and 2. The compounds on physicochemical basis remained non-hygroscopic, unchanging at room temperature, mostly insoluble in water, fairly soluble in ethanol, methanol, methylene chloride and nitromethane but then equitably soluble in dimethylformamide and dimethylsulphoxide. Though, the analytical figures indicate 1:1:1 molar ratio of metal-to-ligands for the Co(II) and Zn(II) complex systems and different shades of colours in



expectedly good yields (64.20-87.00%) were displayed by the compounds synthesized, which were completely different from their precursors. Elemental analyses results for the synthesised compounds in combination with the percentage metal compositions in the heteroleptic complexes revealed good agreement among the experimental and theoretical data. The Schiff base and its heteroleptic compounds showed sharp and higher melting points with distinct deviation from that of the precursors. The Schiff base melted at 220-223°C while its heteroleptic metal(II) complexes had high melting points ranged 293°C to 321°C.

Mass Spectral Studies of 3-[[4,6-dihydroxypyrimidin-2-yl]imino]methyl]naphthalen-2-ol

The electrospray ionization mass spectra (ESI-MS) of the Schiff base was obtained to ascertain the molecular weight, as well as study the patterns of fragmentation involved. The mass spectrum of 3-[[4,6-dihydroxypyrimidin-2-yl]imino]methyl]naphthalen-2-ol gave the molecular ion peak at m/z 281 (Figure 2). This on loss of carbonyl (CO, $m/z = 28.0$) and $C_3O_2H_3$ ($m/z = 71$) fragments displayed peaks at m/z 255.16 and 211.03 respectively which were equivalent to the theoretical molecular weights of the fragments (M.W.=253.258 and 210.216). The molecular ion underwent additional fragmentation by loss of CN_3 molecule giving an $M+2$ fragment ion peak at m/z 130.13.

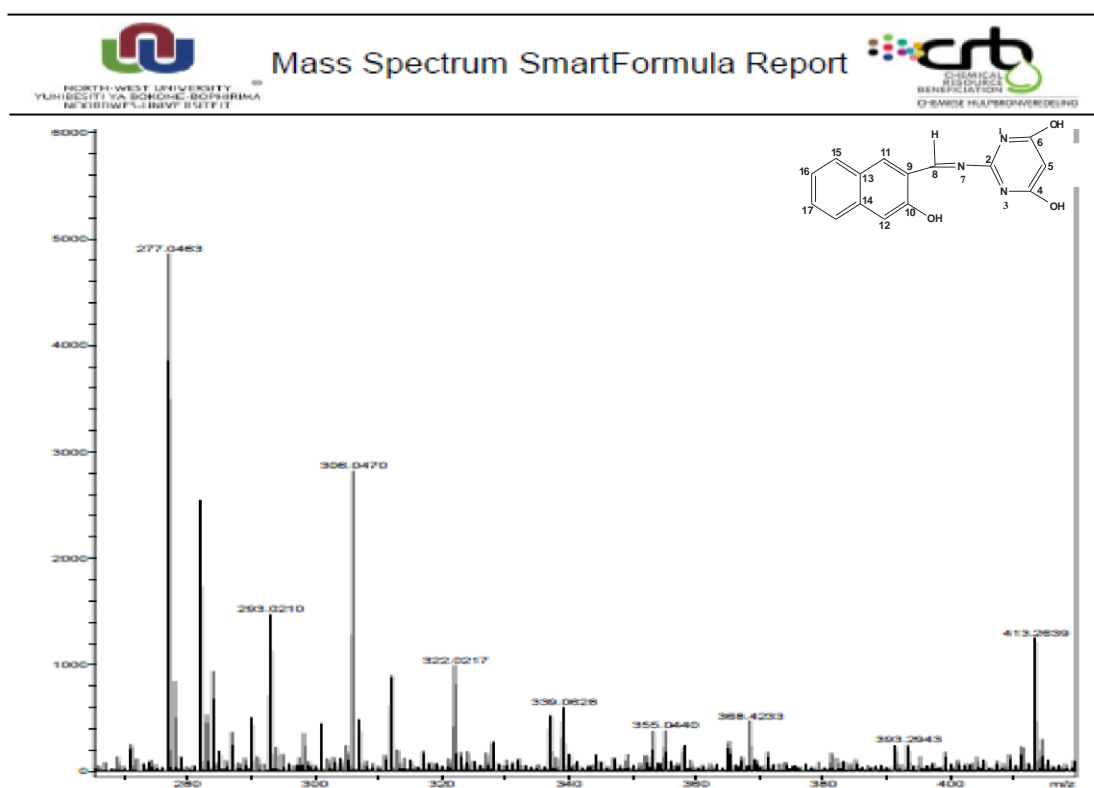


Figure 2: Mass Spectrum of 3-[[4,6-dihydroxypyrimidin-2-yl]imino]methyl]naphthalen-2-ol

NMR Spectra and Conductance Measurements

The NMR spectra of 3-[[4,6-dihydroxypyrimidin-2-yl]imino]methyl]naphthalen-2-ol were presented in Figures 3. The 1H NMR spectrum of the Schiff base displays a sharp singlet peak at 10.80 ppm due to the phenolic (O-H) protons. The azomethine (H-C=N) proton vibrated as a singlet peak at 8.92 ppm. However, observed doublet-multiplet peaks at 8.22, 8.07, 7.85, 7.76, 7.56 and 7.33 ppm were apportioned to the aromatic protons of naphthalene moiety. Also, the resonance signal at 6.40 ppm indicates the presence of pyrimidine proton at C₅.



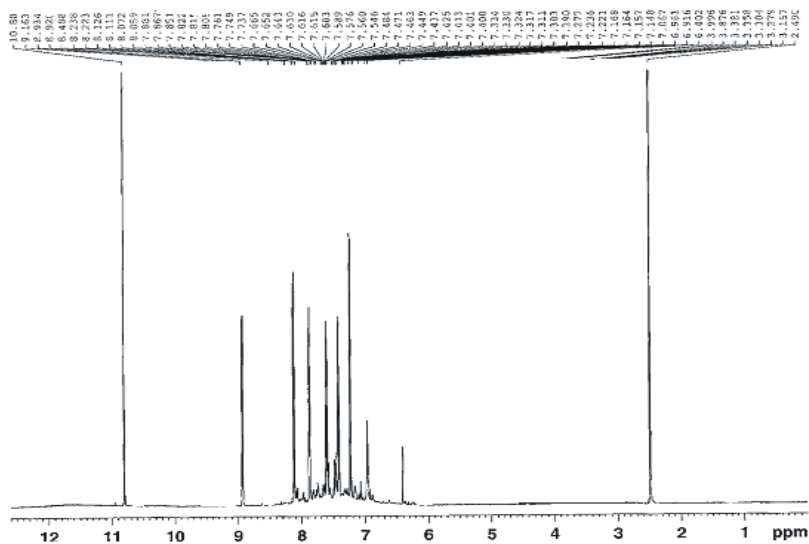


Figure 3: ^1H NMR of 3-[(4,6-dihydroxypyrimidin-2-yl)imino]methyl)naphthalen-2-ol

Observed spectral signals at 138.4, 129.3, 128.8, 127.5, 124.2, 122.2 and 118.8 ppm in the ^{13}C NMR spectrum of the Schiff base were ascribed to C_{10} , C_{14} , $\text{C}_{11\&13}$, C_{15} , $\text{C}_{17\&18}$, C_{16} and C_{12} respectively of the naphthaldehyde ring. The pyrimidine moiety carbon ($\text{C}_{4\&6}$, C_2 , C_5) atoms resonated at 192.8, 131.7 ppm and 98.1 ppm. Furthermore, the sharp peak observed at 164.1 ppm was typical of imine carbon atom and was assigned same.

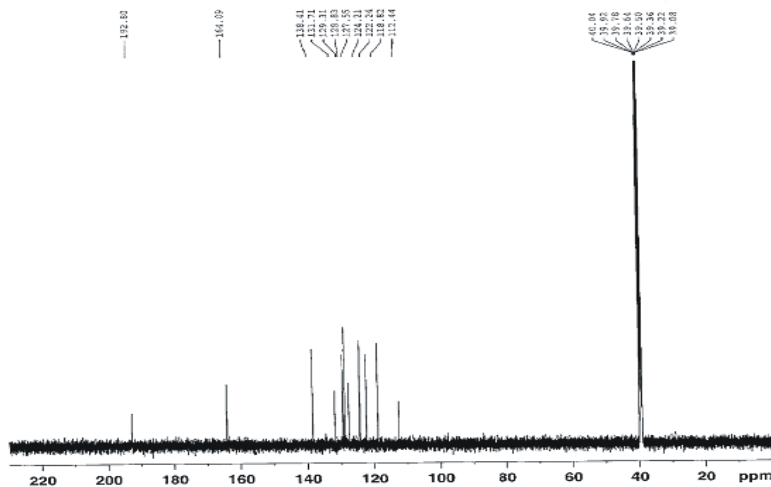


Figure 4: ^{13}C NMR of 3-[(4,6-dihydroxypyrimidin-2-yl)imino]methyl)naphthalen-2-ol



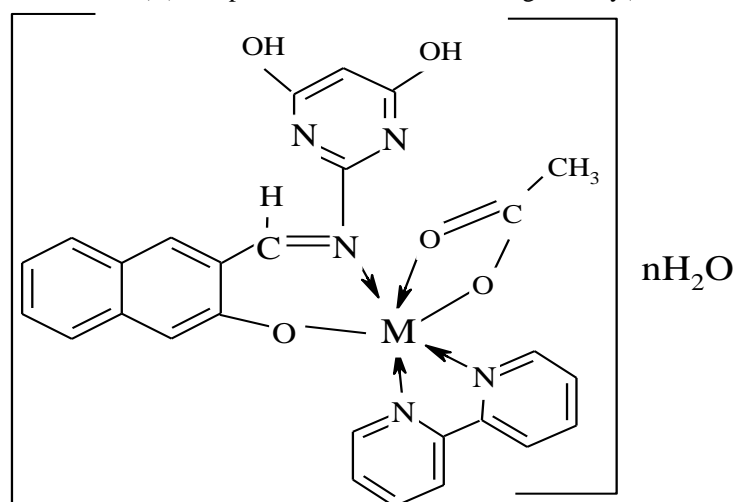
The molar conductivity values obtained in dimethylsulphoxide (DMSO) for heteroleptic Co(II) and Zn(II) complexes were generally below $15.0 \text{ Ohm}^{-1}\text{mol}^{-1}\text{cm}^2$ corroborating non-electrolytic nature of the synthesized complexes, since values not as much as $40 \text{ Ohm}^{-1}\text{mol}^{-1}\text{cm}^2$ are regularly estimated for 1:1 electrolytes [15].

Electronic Spectra and Magnetic Moment Data of Synthesized Compounds

The electronic spectra of the Schiff base, 3-[[4,6-dihydroxypyrimidin-2-yl]imino]methyl]naphthalen-2-ol, 2,2'-bipyridine and their heteroleptic Co(II) and (Zn(II) complexes were obtained. The metal(II) complexes were assigned geometry on the basis of their electronic absorptions and room temperature magnetic moment data [12, 17]. Results of electronic transitions within the ligands ($n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$), metal(II) complexes ($d-d$ transitions) and transitions of charge transfer ($L \rightarrow M$ and $M \rightarrow L$ charge transitions) are absorption bands of the compounds [18-19]. The synthesized compounds were characterized by two peaks in the ultraviolet spectra at the ranges 25356-27933 cm^{-1} and 30487-39525 cm^{-1} assigned to $\text{to } \pi^* \leftarrow n$ and $\pi^* \leftarrow \pi$ transitions respectively [11].

The d^7 Co(II) complexes of ${}^4T_2(t_2^5e^2)$ and ${}^2E(t_2^6e^1)$ configurations often undergo crossover equilibria [21]. The absorption bands around 5000 – 7000 cm^{-1} in the visible spectrum of the cobalt(II) complex were not seen as they tailed into the infrared section, but two absorption bands at 17285 cm^{-1} and 12855 cm^{-1} were observed. The bands were assigned to the transitions ${}^4T_{1g} \rightarrow {}^4A_{2g}(\nu_2)$ and ${}^4T_{1g} \rightarrow {}^4T_{1g}(P)(\nu_3)$ transitions respectively consistent of a high spin octahedral d^7 system with a 4F ground term [21-25]. Magnetic moment values of 4.20-4.60 B.M are expectedly observed for regular tetrahedral d^7 cobalt complexes [26]. Apparently, high spin octahedral cobalt(II) complexes possess three unpaired electrons, exhibits magnetic moments close to that of high spin tetrahedral divalent complexes but are often distinguished by the magnitude of effective magnetic moment deviations from the spin only value of 4.7-5.2 B.M [27]. Furthermore, several cobalt(II) complexes exhibits low-high spin equilibrium behaviour in which the complex becomes more diamagnetic in a low spin state and more paramagnetic in a high spin state [20]. The magnetic moment of 4.71 B.M. observed for the synthesized heteroleptic cobalt(II) complex was corroborative of spin crossover from high spin octahedral to low spin octahedral geometry.

The visible spectrum of the heteroleptic zinc(II) complex expectedly exhibited only charge transfer transitions from $M \rightarrow L$ at 17572 and 12052 cm^{-1} as no $d-d$ transition was expected for d^{10} zinc(II) ions [28]. Observed bands at the 25356 cm^{-1} ; and 39525 and 30487 cm^{-1} region were assigned as intraligand bands and the complex assigned octahedral geometry. Divalent zinc possesses $3d^{10}$ electron configuration and exhibits magnetic moments corresponding to zero unpaired electrons. Observed magnetic moments of 0.11 B.M. was indicative of diamagnetism for the synthesized zinc(II) complex and corroborates their geometry (Raman *et al.*, 2008).



[M = Mn and Ni (n = 1), Co and Zn (n = 0)]

Figure 5: Proposed structure of the metal complexes



FTIR Spectra Studies

The modes of coordination for the two ligands (Schiff base and 2,2'-bipyridine) to the divalent metal ions were determined from the infrared spectra of the heteroleptic divalent complexes which gave bands between 350 and 4000 cm^{-1} . The observed spectral bands were tentatively assigned by comparing the spectra of the synthesized compounds with reported literature on similar systems [28-30]. The ligands did not display bands due to amino groups observed in the spectra of the amine used for synthesis indicating condensation via the amino group with the aldehyde [31]. The infrared spectra of Schiff base had characteristic absorption band due to the phenolic -OH groups around 3342 cm^{-1} . The latter was absent both in 2,2'-bipyridine ligand and in the divalent complexes corroborating the involvement of the Schiff base naphthoxygen (O) in bonding to the metal ions [28]. The broad to medium intensity bands centred at 3342-3438 cm^{-1} in the complexes were assigned to free OH groups of the pyrimidine ring and $\nu\text{O-H}$ of coordinated/hydrated water molecules [32]. While band at 1686-1690 in the heteroleptic complexes were attributed to $\nu(\text{C=O})$ of the coordinated acetate moiety. The uncoordinated C=N stretching vibration in the free Schiff base and 2,2'-bipyridine were observed as sharp lone bands at the range 1650-1656 cm^{-1} , still remained as single absorption band but shifted to lower frequency region by 8-17 cm^{-1} in the divalent complexes exhibiting Fermi resonance [33]. The bands between 1550-1560 cm^{-1} , 1466-1517 cm^{-1} and 1364-1366 cm^{-1} in the spectra of the uncoordinated ligands were assigned to $\nu(\text{C=C})$, $\nu(\text{C-N})$ of the aromatic rings and $\nu(\text{C-O})$, while the $\delta(\text{C-H})$ vibration of the compounds were observed at the range 991-987 cm^{-1} . The mode of complexation was further supported by a lower/higher shift by 6-21 cm^{-1} of $\nu(\text{C=N})$, a shift of (C-O) vibration to positive sides by 5-7 cm^{-1} and a shift of $\nu(\text{C-N})$ to higher/lower frequencies by ± 5 cm^{-1} in the heteroleptic complexes. The appearance of new bands in the regions 559-563 cm^{-1} and 482-486 cm^{-1} were assigned to stretching vibrations of (M-N) and (M-O) respectively in the spectra of the complexes which were not observed in the spectra of the ligands were also attributed the deprotonation/involvement of naphthol oxygen and imine nitrogen atoms in complexation to the metal ions [17, 29].

Antimicrobial Studies

In this study, Co(II) and Zn(II) complexes of 3-[[4,6-dihydroxypyrimidin-2-yl]imino]methyl]naphthalen-2-ol have been synthesized and screened for their antibacterial and antioxidant properties. The inhibitory activities of the compounds against the tested microorganisms are presented in Table 1. Generally, Schiff bases have been widely reported to exhibit broad-spectrum antibacterial activities against pathogenic microorganisms [34-35] but more biologically active becomes more active upon coordination with metal ions. The interaction between metal ions and biologically active ligands helps in designing new metal-based active antibacterial, antifungal, anticancer, etc [34] agents. Hence, the need to study the Schiff base, 3-[[4,6-dihydroxypyrimidin-2-yl]imino]methyl]naphthalen-2-ol and its heteroleptic Co(II) and Zn(II) complexes. The compounds were significantly active against all the screened organisms with varying degrees of inhibitory effects. The positive standard antibiotic, streptomycin was sensitive against all the bacteria organisms. Similarly, all the bacteria were inhibited by the ligands (Schiff base and 2,2'-bipyridine) with inhibitory zones of 5.5-24.5 mm and 8.5-26.0 mm respectively. The activities of the ligands against the bacterial organisms may be attributed to the presences of the heteroatoms (N, O); and -C=N- and phenol moieties which are known to enhance antimicrobial activities [36]. Largely, the heteroleptic complexes were expectedly more susceptible than the metal free ligands in one form or the other and also comparable activity to that of the positive standard antibiotic, streptomycin against *S. aureginosa* and *P. mirabilis*. With the exception of *B. cereus* (24.5 mm) and *S. aureus* (20.0 mm) where the Schiff base had higher antibacterial activities than the complexes on the individual growth of the tested microorganisms. An explanation to the observed increased activity of the complexes may be due to hyper conjugation of the coordinated aromatic Lewis bases and enhanced lipophilicity, a consequence of chelation [37] which leads to reduction in the polarity of metal ions and increases delocalization of pi-electron over the entire chelate ring [38]. Permeation of the complexes through the lipid layers of the bacterial membrane was favoured by the latter, thus killing the microorganisms [39-40]. Furthermore, chelation also neutralizes various cellular enzymes, essential for metabolic pathways in the microorganisms [41]. The complexes; Co(II) and Zn(II) were further active than the Schiff base against one/two of the tested



microorganism(s). The results of antifungal activity of the Schiff base, 2,2'-bipyridine and the heteroleptic divalent complexes are presented in Table 5 and shows that reasonable activity were displayed against screened fungal organisms. Remarkably, Co(II) complex displayed higher/comparable activity to that of streptomycin, thus indicating their importance as probable broad-spectrum antibacterial and antifungal agents *in-vitro* [42-43].

Table 1: Antimicrobial Data of the Compounds

Compounds/ Microbes	<i>B. cereus</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>K. oxytoca</i>	<i>P. aeruginosa</i>	<i>P. mirabilis</i>	<i>A. niger</i>	<i>A. flevus</i>	<i>R. Stolonifer</i>
Schiff Base	24.5±0.7	20.0±1.4	5.5±0.7	15.5±1.7	11.5±2.1	19.5±2.1	20±0.0	22±2.1	17±0.7
2,2'-Bipyridine	15.5±0.7	17.0 ± 1.2	12.0±1.4	26.0±0.7	8.5±0.7	19.5±2.1	16±1.6	19±1.4	13±0.7
[Co(L)(L')(OAC)]	15.5±0.7	16.5±2.1	14.0±1.4	17.5±0.7	18.5±2.1	23.0±0.7	21±0.0	29±0.0	16±1.4
[Zn(L)(L')(OAC)]	14.0±0.0	15.0±0.0	15.5±0.7	20.5±3.5	17.5±0.7	20.5±0.7	26±0.0	21±0.0	18±2.1
⁺ streptomycin/ ⁺ Fluconazole	33.0±1.7	29.0±2.1	32.0±1.4	36.0±2.8	26.5±0.7	23.0±1.4	36±0.3	29±0.7	38±0.3
⁻ DMSO	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Key: ⁺ = positive control, ⁻ = negative control

Table 2: Minimum Inhibitory Concentration (MIC) of the Complexes

Compounds/ Microbes	<i>B. cereus</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>K. oxytoca</i>	<i>P. aeruginosa</i>	<i>P. mirabilis</i>	<i>A. niger</i>	<i>A. flevus</i>	<i>R. Stolonifer</i>
[Co(L)(L')(OAC)]	25	50	-	25	50	75	25	75	50
[Zn(L)(L')(OAC)]	50	75	-	50	-	50	50	-	-
⁺ streptomycin/ ⁺ Fluconazole	<25	<50	<25	<25	<25	<50	<25	<25	<50
Ketoconazole	NS	NS	NS	NS	NS	NS	NS	NS	NS

Key: Values presented in µg/mL

Antioxidant Activity

Phenolic compounds are known to exhibit outstanding antioxidant potentials. Their activities are usually attributed to three mechanistic pathways; hydrogen atom donation to free radicals, single electron transfer to free radicals or/and metal chelation effect [44]. The free radical scavenging potentials of the Schiff base and 2,2'-bipyridine; and their heteroleptic complexes were examined using DPPH method. The synthesized compounds generally had good antioxidant activity and may be attributed to the presence of OH moieties [45]. The antioxidant activity of the metal free ligands at lowest concentration (50 µg/mL) were in the range 54.1-59.6% but increased significantly in the metal complexes upon complexation between the range 66.0–70.4%. The increased antioxidant activity of these complexes may be attributed to metal chelation which changes the basic chemical activities of the metal free ligands. Still, other factors such as type and charge of metal ions, increased conjugation, coordination number and size of metal complex could be involved in the increased antioxidant activity of the metal complexes. The release of protons from the O-H groups to the free radicals was also considered vital in the increased antioxidant activities of the heteroleptic complexes. Interestingly, the antioxidant activities of the complexes were mostly comparable/higher to that of the standard, ascorbic acid (55.03%) suggesting a similar pathway for their antioxidant activities [39, 46] and proving their potentials as probable anticancer agents. The data generated from the minimum inhibitory concentration of the heteroleptic complexes are presented in Table 2. The results of Table 2 which were obtained (after serial dilutions of the stock solution, i.e. 25µg/mL, 50µg/mL and 75µg/mL) at the lowest concentration indicates visible antimicrobial activity of the complexes.



Table 3: Antioxidant data of the Schiff Base, 2,2'-Bipyridine and their heteroleptic metal complexes

Compounds	Absorbance	% Inhibition
	0.573	85.60
Schiff Base	0.575	85.80
	0.567	84.60
	0.501	74.76
2,2'-Bipyridine	0.510	76.23
	0.518	77.40
	0.335	50.03
[Co(L ²)(Bipy)(OAc)]	0.661	98.70
	0.669	99.86
	0.668	99.73
Zn(L ²)(Bipy)(OAc)]	0.658	98.33
	0.646	96.4
Standard	0.578	86.26
Ascorbic Acid	0.587	87.66
	0.594	88.67

Conclusion

Heteroleptic Co(II) and Zn(II) complexes of 3-[[4,6-dihydroxypyrimidin-2-yl]imino]methyl}naphthalen-2-ol Schiff base were synthesized and characterized. Percentage metal and elemental (CHN) analyses, FTIR, UV-VIS and ¹H- and ¹³C-NMR spectroscopies, magnetic susceptibility, melting point and molar conductance measurements indicates that all the complexes assumed six coordinate octahedral geometry, were generally mononuclear and covalent in nature. The *in-vitro* antibacterial and antioxidant studies showed that the ligands and their heteroleptic complexes displayed very moderate to good properties proving their potentials as broad-spectrum antibacterial and probable anticancer agents.

References

- [1]. M. O. Agwara, P. T. Ndifon, N. B. Ndosiri, A. G. Paboudam, D. M. Yufanyi, and A. Mohamadou, (2010) "Synthesis, characterisation and antimicrobial activities of cobalt(II), copper(II) and zinc(II) mixed-ligand complexes containing 1,10-phenanthroline and 2,2'-bipyridine," *Bulletin of the Chemical Society of Ethiopia*, 24 (3), 383–389.
- [2]. G. F. Cesar, (2005). Relevance, essentiality and toxicity of trace elements in human health. *Molecular Aspects of Medicine* 26:235-244.
- [3]. P. Satyl, G. D. Tuli, S. K. Basu, and R. D. Madan, (2004). *Advanced Inorganic Chemistry, Vol: II*. S.Chand and company Ltd. Ram Nagar, New Delhi.
- [4]. C. Festus, (2017). Synthesis, characterization and antibacterial studies of heteroleptic Co(II), Ni(II), Cu(II) and Zn(II) complexes of *n*-(2-hydroxybenzylidene)pyrazine-2-carboxamide. *International Journal of Chemistry, Pharmacy & Technology* 2(5), 202-211.
- [5]. J. D. Lee, (1999). *Concise Inorganic Chemistry: 5th Ed.* Blackwell Science Ltd, Oxford London.
- [6]. U. Melaku, E. W. Clive, and F. Habtamon, (2005). Content of zinc, iron, calcium and their absorption inhibitors in foods commonly consumed in Ethiopia. *J. Food composite Anal.* 18:803-817.
- [7]. H. C. Michael, (2011). *Bacteria: Encyclopedia of earth*. Eds. Sidney, D. and Devland, C. J. National Council for Science and the Environment. Washington DC.
- [8]. M. Kobayashi and S. Shimizu (1999). Cobalt proteins. *Eur. J. Biochem.* 261, 1-9.
- [9]. M. Ali, and H.R. Ali, (2014). One-Pot synthesis of metallopyrazinoporphyrazines using 2,3-diaminomaleonitrile and 1,2-dicarbonyl compounds accelerated by microwave irradiation. *Organic Chemistry International*, 2014.



- [10]. A. Osowole and C. Festus, (2015). Synthesis, Characterization, Antibacterial and Antioxidant Activities of Some Heteroleptic Metal (II) Complexes of 3-[-(Pyrimidin-2-yl) Imino]Methyl} Naphthalen-2-ol, 6, 080-089.
- [11]. Z.H. Chohan, (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, 1-6.
- [12]. S. Delaney, M. Pascaly, P.K. Bhattacharya, K. Han, and J.K. Barton, (2002). "Oxidative damage by ruthenium complexes containing the Dipyridophenazine ligand or its derivatives: A focus on intercalation," *Inorganic Chemistry*, 41, 1966-1974.
- [13]. M. Goldstein, J.K. Barton, J.M. Goldberg, C.V. Kumar, and N.J. Turro, (1986). *J. Am. Chem. Soc.*, 10, 2081-2088.
- [14]. J. Bassett, R.C. Denney, G.H. Jeffery, and J. Mendham, (1978). Vogel's textbook of quantitative inorganic analysis. ELBS, London, 325-361.
- [15]. W.J. Geary, (1971). The use of conductivity measurements in organic solvents for the characterization of coordination compounds. *Coord. Chem. Rev.*, 7, 81.
- [16]. A. Osowole and C. Festus, (2013). Synthesis, characterization and antibacterial activities of some metal(II) complexes of 3-(-1-(2-pyrimidinylimino)methyl-2-naphthol. *Elixir Appl. Chem.* 59 15843-15847.
- [17]. G.B. Bagihalli, S.A. Patil, and P.S. Badami, (2009). Synthesis, physicochemical investigation and biological studies of Zinc(II) complexes with 1,2,4-triazole Schiff bases. *Journal of the Iranian Chemical Society*.6, 259-270.
- [18]. T.L. Yang, and W.W. Qin, (2001). "Transition metal manganese(II), nickel(II), copper(II) and zinc(II) complexes of a new Schiff base ligand: synthesis, characterization and antitumor activity studies," *Polish Journal of Chemistry*, 80, 1657-1662.
- [19]. M.G. Abd El-Wahed, M.S. Refat, and S.M. El-Megharbel, (2008). "Metal complexes of antiurethemic drug: synthesis, spectroscopic characterization and thermal study on allopurinol complexes," *Journal of Molecular Structure*, 888, 416-429.
- [20]. F.A. Cotton, and G. Wilkinson, (1978). *Advanced Inorganic Chemistry*. Wiley Eastern Ltd, New Delhi.
- [21]. A. Earnshaw, (1980). *The introduction to magnetochemistry*, Academic Press, London, UK.
- [22]. F.A. Cotton, and G. Wilkinson, (1972). *Advanced Inorganic Chemistry*. A comprehensive text. 3rd U.S. Ed. Wiley Eastern Limited, New Delhi.
- [23]. K.F. Purell, and J.C. Kotz, (1997). *Inorganic Chemistry*, Holt-Saunders International Edition W.B. Saunders Company, Philadelphia London.
- [24]. F.A. Cotton, G. Wilkinson, C.A. Murillo, and M. Bochmann, (1999). *Advanced Inorganic Chemistry*, 6th.Ed. John Wiley. New York, 857-859.
- [25]. M. Tuncel, and S. Selahattin, (2005). Synthesis and characterization of Cu(II), Ni(II) and Co(II) complexes with azo-linked Schiff base ligands. *Synth. React. Inorg. Org. Chem. and Nano-Met. Chem.*, 35, 203-212.
- [26]. M. Sonmez, A. Levent, and M. Sekerci, (2004). "Synthesis, characterization, and thermal investigation of some metal complexes containing polydentate ONO-donor heterocyclic Schiff base ligand," *Russian Journal of Coordination Chemistry*, 30, 655-660.
- [27]. S. Yamada, (1966). Recent aspects of the stereochemistry of Schiff base metal complexes, *Coord. Chem. Rev.* 1, 415.
- [28]. K.M. Atmaram, and V.M. Kirian, (2011). Synthesis, characterization and antimicrobial activity of mixed Schiff base ligand complexes of transition metal(II) ions. *Intl J. ChemTech Research*, 3, 477-482.
- [29]. K. Ramana, A. Kumar, G.P. Raghavendra, V. Srilalitha, G.S. Narayana, and L.K. Ravindranath, (2012). Synthesis and characterization of Iron complexes of resacetophenone salicyloyl hydrazone. *Chem. Bull. "POLITEHNICA" Univ. (Timisoara)*, 57, 7-14.
- [30]. G. Suparna, (2013). Synthesis and pharmacological studies of some bivalent metal complexes with Schiff based ligand derived from xipamide. *Der Pharma Chemica*. 5, 232-235.



- [31]. G. Valarmathy, and R. Subbalakshmi, (2014). Synthesis, spectral characterisation, electrochemical, and uorescence studies of biologically active novel Schiff base complexes derived from E-4-(2-hydroxy-3-methoxybenzlideneamino)-N-(pyrimidin-2-yl)benzenesulfonamide. *Turkish Journal of Chemistry*. 38, 521-530.
- [32]. J.T Makode, and A.S. Aswar, (2004). Synthesis, characterization, biological and thermal properties of some new Schiff base complexes derived from 2-hydroxyl-5-chloroacetophenone and s-methylthiocarbazate. *Indian Journal of Chem.* 43, 2120-2125.
- [33]. P.S. Kalsi, (2004). Spectroscopy of organic compounds. 6th edition. New age international publishers, India 71-7249.
- [34]. Z. Huang, Z. Lin, and A. Huang, (2001). A novel kind of antitumor drugs using sulfonamide as parent compound. *Eur. J. Med. Chem.*, 36, 863-872.
- [35]. M.N. Patel, H.N. Joshi, and C.R. Patel, (2012). Copper(II) complexes with norfloxacin and neutral terpyridines: cytotoxic, antibacterial, superoxide dismutase and DNA-interaction approach. *Polyhedron*. 40, 159.
- [36]. T. Nogrady, (1988). Medical Chemistry. A biochemistry approach, Oxford University Press, New York, 100.
- [37]. A.A. Osowole, A.C. Ekennia, O.O. Olubiyi, and M. Olagunju, (2016). Synthesis, spectral, thermal, antibacterial and molecular docking studies of some metal(II) complexes of 2-(1,3-benzothiazol-2-ylamino)naphthalene-1,4-dione.
- [38]. A.A. Ahmed, K.A. Yasmien, H. I.Heba, and A.A. Ali. (2012). Antioxidant, antimicrobial, and theoretical studies of the thiosemicarbazone derivative Schiff base 2-(2-imino-1-methyl imidazolidin-4-ylidene) hydrazine carbothioamide (IMHC). *Org Med ChemLett*. 2, 4.
- [39]. A.A. Al-Amiery, A.A.H. Kadhum, and A.B. Mohammed, (2012). "Antifungal and antioxidant activities of pyrrolidone thiosemicarbazone complexes," *Bioinorganic Chemistry and Applications*, 2012, 6.
- [40]. P.A. Ajibade, and O.G. Idemudia, (2013). Synthesis, characterization and antibacterial studies of Pd(II) and Pt(II) complexes of some diaminopyrimidine derivatives. *Bioinorg. Chem. Appl.* 2013, 1–8.
- [41]. K.S. Abuo–Melha, H. Faruk, (2008). Bimetallic complexes of Schiff base bis-[4-hydroxy coumarin-3-yl]-¹N,⁵N thiocarbohydrazone as a potentially diabasic pentadendate ligand: synthesis, spectral, and antimicrobial properties. *J. Iran. Chem..Soc.*, 5, 122-134.
- [42]. A.A. Osowole, and I. Ott, (2012). Synthesis, characterization, *in-vitro* anticancer and antimicrobial properties of some metal (II) complexes of 4-[(2,3-dihydro-1*H*-inden-4- ylimino) methyl] benzene-2,4-diol. *International Research Journal of Pure and Applied Chemistry*, 2, 156-169.
- [43]. P.A. Ajibade, and N.H. Zulu, (2011). Metal complexes of diisopropylthiourea: Synthesis, characterization and antibacterial studies. *Int. J. Mol. Sci.*, 12, 7186-7198.
- [44]. K.P. Qadeer, and M. Shahabuddin, (2014). Synthesis, characterization and antioxidant activity of Rutin complexes. *Pak. J. Anal. Environ. Chem.* 15.
- [45]. M. Kfoury *et al.* (2014). Effect of cyclodextrin complexation on phenylpropanoids' solubility and antioxidant activity. *Beilstein Journal of Organic Chemistry*; 10(1): 2322-2331.
- [46]. A. Choudhary, R. Sharma, M. Nagar, M. Mohsin, and H.S. Meena, (2011). "Synthesis, characterization and antioxidant activity of some transition metal complexes with terpenoid derivatives," *Journal of the Chilean Chemical Society*, 56, 911– 917.

