Synthesis, Characterization, DNA Binding and Nuclease Activity of Cobalt(II) Complexes of Isonicotinoyl Hydrazones

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ABSTRACT *Cobalt(II) complexes of isonicotinoyl hydrazones of two series of ligands have been synthesized and characterized on the basis of elemental analyses, molar conductance, magnetic moment, mass, IR, UV spectral data. Electrochemical behavior of ligands and complexes has been investigated by using cyclic voltammetry. Cyclic voltammetric studies reveal that the oxidation/reduction potentials of all complexes are shifted towards positive or negative values than its corresponding ligands. The interactions of these complexes with calf thymus DNA have been studied by using absorption studies. Bathochromic shift and hypochromism suggests that the intercalative mode of binding of complexes with DNA. Their DNA cleavage activity of complexes was studied on double-stranded pBR322 plasmid DNA using gel electrophoresis experiments in the absence and presence of oxidant (H_2O_2) and reductant (DTT).*

KEYWORDS: *Co(II) complexes; Isonicotinoyl hydrazones; Cyclic voltammetry; DNA binding, DNA cleavage..*

INTRODUCTION

In the last few years, a renewed interest in metal-based therapy has been raised: in fact, on coordination, bioactive ligands might improve their bioactivity profiles, while inactive ligands may acquire pharmacological properties [1–5]. In addition, metal coordination is one of the most effective strategies in the design of repository, slow release or long-acting drugs [6]. In this way, the synthesis, structural investigation, and reaction of transition metal Schiff bases have received special attention, because of their biological activities as antitumoral, antifungal and antiviral activities [7]. Thus, Schiff base hydrazones are also interesting from the point of view of pharmacology. Hydrazone derivatives are found to possess antimicrobial [8], ant tubercular [9], anticonvulsant [10] and anti-inflammatory [11] activities.

Sah and *Peoples* [12] synthesized hydrazones by reacting

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isonicotinicacid hydrazide(INH) with a variety of aldehydes and ketones. These compounds are reported to have inhibitory activity in mice infected by various strains of Mycobacterium tuberculosis. Isonicotinoyl hydrazone analogs of isoniazid [13], an anti-tuberculosis drug is found to have superoxide scavenging activity [14].

Cobalt was accepted as an essential metal element widely distributed in the biological systems such as cells and body, and thus the interaction of DNA with cobalt complex has attracted much attention [15-18]. Their binding properties of cobalt with calf thymus DNA were studied by several methods, and the experimental results showed that the intercalated ligand had an important effect on the binding affinity of the cobalt complexes with DNA[19].

A few studies of Cobalt complexes of isonicotinoyl hydrazones have been observed. In this view, we are much interested to prepare a new series of cobalt complexes of Isonicotinoyl hydrazones. Here in, we reported that two series of ligands and their metal complexes have been prepared and characterized by using spectral techniques.DNA binding and DNA cleavage studies are described in this study.

EXPERIMENTAL SECTION

Materials and methods

Isoniazid, 2-hydroxybenzaldehyde, 2-hydroxyacetophenone, 2-hydroxybenzophenone, 2,4-dihydroxybenzaldehyde, 2,4-dihydroxyacetophenone, 2,4-dihydroxybenzophenone and agarose were purchased from Sigma-Aldrich. All other chemicals were of AR grade and used as provided. The solvents used for the synthesis were distilled before use. Calf –Thymus DNA (CT-DNA) was purchased from Genio Bio labs, Bangalore, India. Elemental analyses were carried out on a Heraeus Vario EL III Carlo Erba 1108 instrument. Magnetic measurements were taken at 298K using lakeshore VSM 7410 instrument. Molar conductivity measurements at 298 ± 2K in dry and purified DMF were carried out using a ELICO CM model 162 conductivity meter. The electronic spectra were recorded in DMF with a UV lamda50 (Perkin-Elmer) spectrophotometer. IR spectra were recorded in the range 4,000–400 cm^{-1} with a Perkin-Elmer spectrum100 spectrometer on KBr discs. Mass spectra of the ligands and complexes were recorded on AB SCIEX- API 400 LC/MS/MS Mass spectrometer. Samples for recording Proton NMR are dissolved in d_6-DMSO and the ^1H peak of tetramethylsilane (TMS) is used as the internal reference. ^1H NMR spectra were recorded at 500.00 MHz on a Avance-300 Bruker, Switzerland NMR spectrometer. Cyclic voltammetric measurements were taken on a CH instruments assembly equipped with an X–Y recorder. Measurements were taken on degassed (N_2 bubbling for 5 min) solutions (10^{-3} M) containing 0.1 M Bu_4NPF_6 as the supporting electrolyte. The three-electrode system consisted of glassy carbon (working), platinum wire (auxiliary) and Ag/AgCl (reference) electrodes.

Preparation of Ligands

Ligands were prepared by reacting isoniazid with carbonyl compounds. A 20 mL methanolic solutions of isonicotinylhydrazide (0.68 g; 5mmol), the required quantity of carbonyl compound (5mmol) dissolved in 20 mL of methanol were mixed in a 100-ml round bottom flask. Two drops of HCl were added to the reaction mixture and refluxed for 3-6 hours. On cooling the reaction mixture to room temperature, yellow colored crystalline products were separated. The products were collected, washed with hot water and few drops of hexane to remove impurities and dried in vacuum and recrystallized from alcohol. A general structure for ligands is shown in Fig. 1.

2-Hydroxy Benzaldehyde Isonicotinoyl hydrazone - ($C_{13}H_{11}N_3O_2$): Yield 78%, M.Pt. 246-248 $^{\circ}C$, Anal.(%) Calc. (found): C-64.73(64.42); H-4.56 (4.81); N-17.42 (17.64); HAINH: Yield 72%, M.Pt.238-240 ^0C, Anal (%) Calc. (found): C-65.88(65.96); H-5.09 (5.02); N-16.47(16.64); IR spectra,: 3346, 3180, 1683, 1613 are assigned to ν(O-H), ν(N-H), ν(C=O), ν(C=N) stretching vibrations respectively. ^{1}HNMR Spectra: (d_6-DMSO, 2.5δppm), δ:12.28 (s,1H, OH), δ:11.07(s,1H,NH), δ:8.68 (s,1H,CH), δ :7.5(m,4H,Py H) δ:6.5 (m, 4H,Ar H). Mass spectra of HBINH shows a molecular ion peak at m/e. 241.

2-Hydroxy acetophenone isonicotinoyl hydrazone - ($C_{14}H_{13}N_3O_2$): Yield 72%, M.Pt.238-240 $^{\circ}C$, Anal (%) Calc.(found): C-65.88(65.96); H-5.09 (5.02); N-16.47(16.64); IR spectra,; 3450, 3059, 1679, 1602 are assigned to ν(O-H), ν(N-H), ν(C=O), ν(C=N) stretching vibrations respectively. ^{1}HNMR Spectra: (d_6-DMSO, 2.5 δppm), δ:13.21 (s, 1H, OH), δ:11.59 (s,1H, NH), δ:8.7(m,4H,Py H), δ:7.2(m, 4H,Ar H), δ(2.50) (s,3H, CH_3). Mass spectra of HAPINH shows a molecular ion peak at 255.

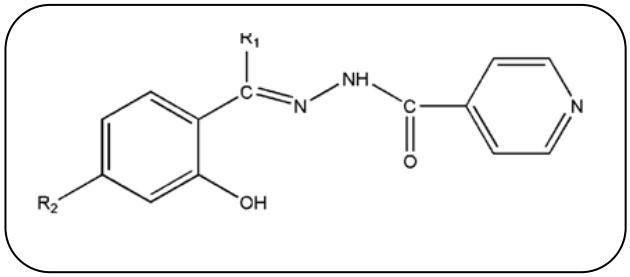

Fig.1: General structure of ligands. R_1= H, R_2= H, (HBINH-($C_{13}H_{11}N_3O_2$)), R_1= CH_3, R_2= H, (HAPINH) -($C_{14}H_{13}N_3O_2$), R_1= C_6H_5, R_2= H, (HBPINH –($C_{19}H_{15}N_3O_2$)), R_1= H, R_2= OH, (DBINH –($C_{13}H_{11}N_3O_3$), R_1= CH_3, R_2= OH, (DAPINH-($C_{14}H_{13}N_3O_3$)), R_1= C_6H_5, R_2= OH, (DBPINH –($C_{19}H_{15}N_3O_3$)).

2-Hydroxy Benzophenone isonicotinoyl hydrazone - ($C_{19}H_{15}N_3O_2$)): Yield 81%, M.Pt. 260-261°C, Anal(%) Calc (found): C-71.92(71.86); H-4.73(4.60); N-13.24(13.45); IR spectra, 3449,3095,1710,1618 are assigned to ν(O-H), ν(N-H), ν(C=O), ν(C=N) stretching vibrations respectively. ^{1}HNMR Spectra: (d_6-DMSO, 2.5 δppm), δ:10.36(s,1H,OH), δ:10.10(s, 1H,NH),δ:8.7(m,4H,PyH) δ:7.2(m,5H,ArH), δ:6.8 (s,4H,ArH). Mass spectra of HBPINH shows molecular ion peak at 317.

2,4-Dihydroxy Benzaldehyde isonicotinoyl hydrazone - ($C_{13}H_{11}N_3O_3$)): Yield 73% M.Pt.235-237°C, Anal(%) Calc (found): C-60.70(60.52); H-4.28(4.36); N-16.34(16.44); IR spectra,: 3412,3121,1750,1585 assigned to ν(O-H), ν(N-H), ν(C=O), ν(C=N) stretching vibrations respectively. ^{1}HNMR Spectra: (d_6-DMSO,2.5 δppm), δ:12.40 (s,1H,OH) & δ:10.09 (s,1H,OH), δ:11.32 (s,1H,NH), δ(8.62) (s,1H,=CH), δ:7.8(m, 4H,PyH) δ:6.6 (m, 3H, ArH). Mass spectra of DBINH shows molecular ion peak at 257.

2,4-Dihydroxy acetophenone isonicotinoyl hydrazone - ($C_{14}H_{13}N_3O_3$)): Yield 65%, M.Pt.252-254°C, Anal(%) Calc (found): C-61.99(61.83); H-4.79(4.86); N-15.49(15.53); IR spectra,: 3428,3100,1667,1603 to ν(O-H), ν(N-H), ν(C=O), ν(C=N) stretching vibrations respectively. ^{1}HNMR Spectra: (d_6-DMSO,2.5 δppm), δ:13.39 (s,1H,OH) & δ:9.93(s,1H,OH), δ:11.42 (s,1H,NH), δ:8.8(m,4H,PyH) δ(7.2) (m,3H,ArH), δ:2.50(s,3H,CH_3). Mass spectra of DAINH shows molecular ion peak at 271.

2,4-Dihydroxy benzophenone isonicotinoyl hydrazone - ($C_{19}H_{15}N_3O_3$)): Yield 84%, M.Pt. 269-270°C, Anal (%) Calc (found): C-68.46(68.34); H-4.80(4.78); N-12.61(12.68); IR spectra, 3435, 3002, 1685, 1598 assigned to ν(O-H), ν(N-H), ν(C=O), ν(C=N) stretching vibrations respectively. ^{1}HNMR Spectra: (d_6-DMSO,2.5 δppm), δ:10.19(s,1H,OH) & δ:9.78(s,1H,OH), δ:10.04(s,1H,NH), δ:8.5(m,4H,PyH), δ:7.7(m,5H,ArH),δ:6.6 (m,3H, ArH). Mass spectra of DBPINH shows molecular ion peak at 333.

Preparation of complexes

Synthesis of ($CoC_{26}H_{20}N_6O_4$)

In a clean 100-ml round bottom flask, a methanolic solution of HBINH (1.27g, 5mmol), an aqueous solution of $CoCl_2.6H_2O$(1.19g, 5mmol) in 1:1 (Ligand:Metal) molar ratio were mixed and the reaction mixture was heated under reflux for 3hr. The reaction mixture was cooled to room temperature. A fire brick coloured complex was formed. It was collected by filtration, washed with methanol followed by hexane to remove impurities and dried in vacuum. recrystallized from alcohol.

Synthesis of ($CoC_{28}H_{24}N_6O_4$)

The complex was prepared by mixing a methanolic solution of HAPINH(1.27g, 5mmol) and aqueous solution of $CoCl_2.6H_2O$(1.19g, 5mmol)) in 1:1 (Ligand:Metal) molar ratio in a clean 100-ml round bottom flask. The contents were refluxed on a water bath for 1hr cooled to room temperature, a dark olive green coloured complex which separated out was collected by filtration, washed with methanol and dried in vacuuo. recrystallized from alcohol.

Synthesis of ($CoC_{38}H_{28}N_6O_4$)

To a methanolic solution of HBPINH (1.58g, 5mmol) taken in 100-mL round bottom flask, an aqueous solution of $CoCl_2.6H_2O$(1.19g, 5mmol)) in 1:1 (Ligand:Metal) molar ratio was added and the reaction mixture was heated under reflux on a water bath for 3hr. On cooling the reaction mixture to room temperature, the gold coloured complex was separated out. It was collected by filtration, washed with methanol followed by hexane and dried in vacuo. recrystallized from alcohol.

Synthesis of ($CoC_{26}H_{20}N_6O_6$)

A methanolic solution of DBINH (1.27g, 5 mmol) and an aqueous solution of $CoCl_2.6H_2O$(1.19g, 5mmol)) in 1:1 (Ligand:Metal) molar ratio were mixed in a clean 100-ml round bottom flask. The contents were heated

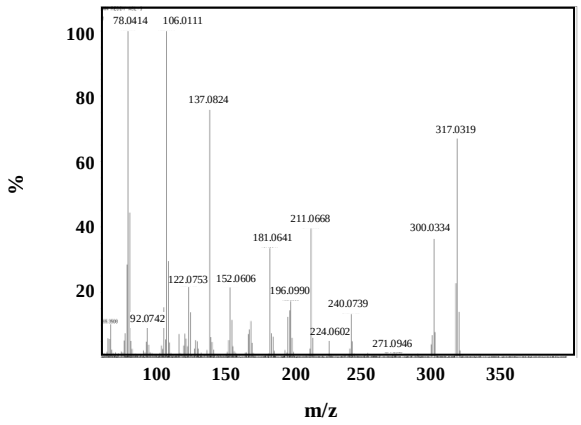

Fig. 2: Mass spectrum of HBINH.

under reflux on a water bath for 2h. The reaction mixture was cooled to room temperature. Then the olive coloured complex was separated out. It was collected by filtration, washed with methanol and dried in vacuum. recrystallized from alcohol.

Typical mass spectrum of HBINH is given in Fig. 2

Synthesis of ($CoC_{28}H_{24}N_6O_6$)

To a methanolic solution of DAPINH (1.35g, 5mmol) taken in a 100-ml round bottom flask, an aqueous solution of $CoCl_2.6H_2O$(1.19g, 5mmol)) in 1:1 (Ligand:Metal) molar ratio was added and the reaction mixture was heated under reflux on a water bath for 3h. On cooling reaction mixture to room temperature, the maroon coloured complex was separated out. It was collected by filtration, washed with methanol followed by hexane and dried in vacuum. recrystallized from alcohol.

Synthesis of ($CoC_{38}H_{28}N_6O_6$)

The complex was prepared by mixing methanolic solution of DBPINH(1.66g, 5mmol) and aqueous solution of $CoCl_2.6H_2O$(1.19g, 5mmol)) in 1:1 (Ligand:Metal) molar ratio in a clean 100-ml round bottom flask. The contents were refluxed on a water bath for 3h. The reaction mixture was cooled to room temperature. Then a dark orange coloured complex which separated out was collected by filtration, washed with methanol and dried in vacuum. recrystallized from alcohol.

The analytical data of all the complexes are given in Table 1. The ES^+I mass spectrum of $Co(HBPINH)_2$ complex is shown in Fig. 4.

DNA binding experiments

The interaction of the complexes with DNA was studied in the tris-buffer medium. A solution of calf thymus DNA (CT-DNA) in (50mM NaCl/5 mM Tris-HCl; pH =7.0) buffer medium gave absorbance ratio at 260 nm and 280 nm of 1.85, indicating that the DNA was sufficiently free of proteins **[**20]. The DNA concentration per nucleotide was determined by the absorption coefficient (6600 dm^3/mol cm) at 260 nm **[**21**].** Stock solutions stored at 4 ^{0}C were used after no more than four days. The electronic spectra of metal complexes were monitored in the absence and presence of CT-DNA. Absorption titrations were performed by maintaining the metal complex concentration $2x10^{-5}$M and varying nucleic acid concentration. Absorption spectra were recorded after each successive addition of DNA solution. The intrinsic binding constant (K_b) was calculated by the equation, $[DNA]/\varepsilon_a-\varepsilon_f = [DNA]/\varepsilon_a-\varepsilon_f + 1/K_b\ (\varepsilon_a-\varepsilon_f)$, where [DNA] is the molar concentration of DNA in base pairs, ε_a, ε_b, ε_f are apparent extinction coefficient(A_{obs}/[M]), the extinction coefficient for the metal (M) complex in the fully bound form and the extinction coefficient for free metal (M) respectively. A plot of [DNA] / $(\varepsilon_a-\varepsilon_f)$ versus [DNA] gave a slope of $1/(\varepsilon_a-\varepsilon_f)$x K_b is the ratio of the intercept.

DNA cleavage studies

Cleavage experiments of supercoiled pBR322 DNA (300mg, 50μM) were carried out in presence of complex ($5X10^{-6}$M) separately in buffer solution (50mMTris-Hcl/NaCl), at pH 7.2, followed by agarose gel electrophoresis. The samples were incubated for 30 min at 37°C. A loading buffer solution containing 25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol was added and electrophoresis was carried out in Tris-HCl buffer using 0.8% agarose gel containing 100μg/mL ethidium bromide. The reaction was monitored in the presence of activators Hydrogen peroxide (H_2O_2), and Dithiothreitol (DTT). The inhibition reactions were carried out by adding the reagent prior to the addition of the complexes. The standard protocols were followed for these experiments. The samples were incubated for 30 min at 37°C. Electrophoresis was performed at 75V in TBE buffer until the bromophenol blue reached to3/4 of the gel and gels were visualized by photographing the fluorescent ethidium bromide under

Table 1: Physico-chemical properties of cobalt(II) complexes.

Complex	Melting Point °C	Mol.wt Found(calc)	Elemental analysis found(cal.)%			μ_{eff}	Λ_M[a]
			Carbon	Hydrogen	Nitrogen		
($CoC_{26}H_{20}N_6O_4$)	273-275	539.65(539)	56.98(57.88)	3.25(3.71)	15.02(15.58)	4.83	17.4
($CoC_{28}H_{24}N_6O_4$)	297-298	567.84(567)	59.54(59.25)	4.01(4.23)	15.11(14.81)	4.58	19.3
($CoC_{38}H_{28}N_6O_4$)	>300	691.11(691)	66.24(65.99)	3.77(4.05)	12.88(12.15)	4.24	4.4
($CoC_{26}H_{20}N_6O_6$)	>300	571.29(571)	55.14(54.64)	3.85(3.50)	13.89(14.71)	4.26	12.9
($CoC_{28}H_{24}N_6O_6$)	>300	599.37(599)	56.65(56.09)	3.84(4.01)	14.76(14.02)	4.45	8.1
($CoC_{38}H_{28}N_6O_6$)	>300	723.57(723)	63.55(63.07)	3.22(3.87)	10.84(11.61)	4.37	15.6

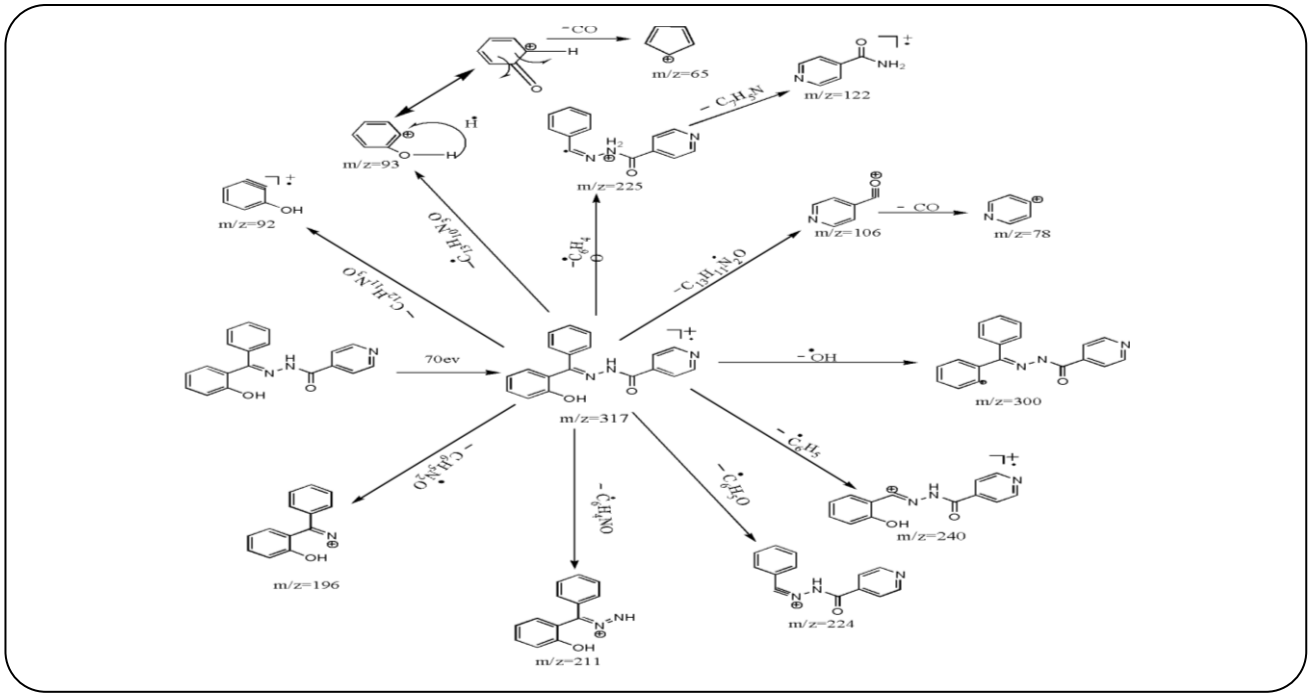

Fig. 3: Fragmentation pattern for HBINH ligand

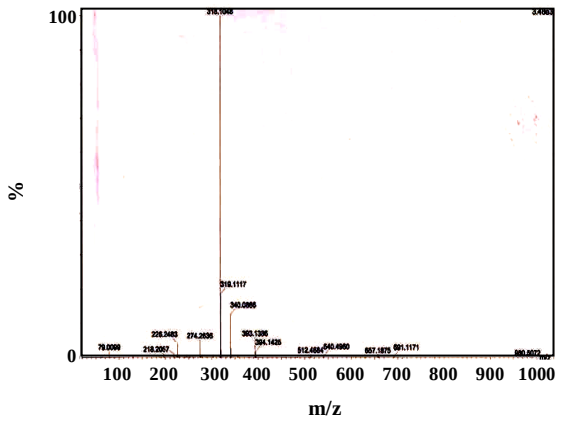

Fig. 4: ES$^+$I Mass spectrum of ($CoC_{38}H_{28}N_6O_4$).

a UV illuminator. The cleavage efficiency was measured by the ability of complex to convert supercoiled DNA (SC or Form I) to nicked circular form (NC or Form II) and linear form (LC or Form III).

RESULTS AND DISCUSSION

Elemental analysis, molar conductivity measurements and magnetic moment

The analytical data for the complexes with some physical properties are summarized in Table 1. The metal complexes are presented in the way of colored powders. All the complexes are soluble in DMF. From the analytical data of the complexes result in a stoichiometry 1:2 (metal: ligand)

Table 2: Electronic spectral data of cobalt(II) complexes

Complex	π-π*	$^4T_{1g}(F)\rightarrow{}^4T_{1g}$	$^4T_{1g}\rightarrow{}^4A_{2g}$	$^4T_{1g}\rightarrow{}^4T_{2g}$
($CoC_{26}H_{20}N_6O_4$)	37593	24390	14925	10917
($CoC_{28}H_{24}N_6O_4$)	36855	23641	14582	10893
($CoC_{38}H_{28}N_6O_4$)	36968	24106	15598	10729
($CoC_{26}H_{20}N_6O_6$)	37192	25944	15642	10661
($CoC_{28}H_{24}N_6O_6$)	37453	23205	16043	11056
($CoC_{38}H_{28}N_6O_6$)	37534	23255	14859	10833

for all the complex combinations (Table 1). The recorded conductance for 10^{-3} molar DMF solutions of the complexes indicates that all complexes are non-electrolytes due to their neutrality. The effective magnetic moments (μ_{eff}) of the cobalt(II) complexes (1-6) lie in the range 4.24- 4.83 B.M. which is consistent with three unpaired electrons and falls within the range reported for mononuclear cobalt(II) complexes[22].

Electronic spectral data

The electronic spectra of all the complexes are recorded in DMSO. The significant bands obtained from electronic spectral data are presented in Table 2. The electronic spectrum of ($CoC_{26}H_{20}N_6O_6$) is shown in Fig. 5. A Strong sharp band is observed in the region of 37593-36855cm^{-1} for the cobalt complexes is associated with π-π^* transition of the aromatic chromophore. The Co(II) complexes generally give rise to three absorption bands in the visible region under the influence of the octahedral field by the excitation of the electron from the ground state $^4T_{1g}(F)$ to the excited states $^4T_{2g}$ (F), $^4A_{2g}$ (F) and $^4T_{1g}$ (P). In the Co(II)-Schiff base complex, three bands are observed at the range of 10661-11059 cm^{-1}, 14582-16043 cm$^{-1,}$ and 23205-25944 cm^{-1} corresponding to $^4T_{1g}(F)\rightarrow{}^4T_{2g}$ (F) (υ_1), $^4T_{1g}(F)\rightarrow{}^4A_{2g}(F)$ (υ_2) and $^4T_{1g}(F)\rightarrow{}^4T_{1g}(P)$($\upsilon_3$) transitions, respectively, characteristic of octahedral of complex [23–25].

IR spectral data

The extent of shift depends on the bond strength between the atoms. The IR spectral data of the ligands & complexes is given in Table 3. For the synthesized Schiff base complexes, we observed the following changes; the high intense band due to phenolic –OH appeared in the region at 3180-3436 cm^{-1} in the ligands was disappeared in the complexes [26,27]. The H-bonded –OH groups

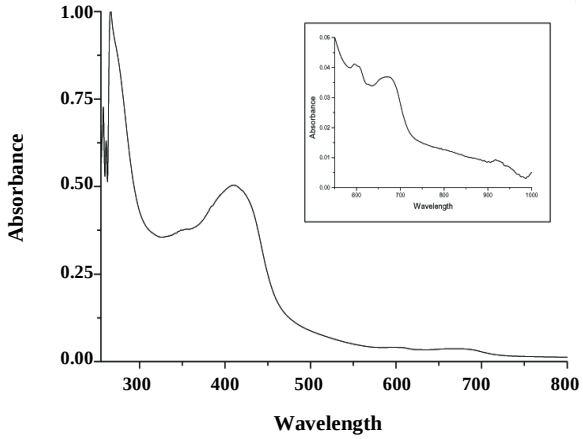

Fig. 5: Electronic spectrum of ($CoC_{26}H_{20}N_6O_6$)in DMF.

have been replaced by the metal ion. This is strongly supported by the observation of υ(C-O) stretching vibrations in the region of 1226-1274 cm^{-1} undergoes shift to higher wave number at 1239-1278 cm^{-1} compared with the complex band. These observations support the formation of M–O bonds via deprotonation [28]. This is further evidenced by the appearance of a band in the complexes in the region 453–496 cm^{-1} assigned as υ(M–O) bands [29]. So the medium intense band in the range 1609-1631 cm^{-1} are observed due to ν(C=N) which has been shifted towards lower region at around 1571-1604 cm^{-1} in the complexes indicating the participation of the azomethine group in the complexes formation this shift is also due to the reduction of double bond character of the carbon-nitrogen bond of the azomethine group and indicates that C=N of the ligand coordinates to the metal through nitrogen and is further reflected by the appearance of a new band at 409–420 cm^{-1} (lower wavenumber region) due to υ(M–N).

These significant shifts of free ligand ν(C=N) to lower wavenumber region and υ(C-O) towards higher wavenumber region in metal complexes point to the bonding

Table 3: IR spectral data(cm^{-1}) of cobalt(II) complexes

Compound	ν(O-H)	ν(N-H)	ν(C=O)	ν(C=N)	ν(C-O)	ν (M-O)	ν (M-N)
($C_{13}H_{11}N_3O_2$)	3180	3003	1698	1613	1274	-	-
(Co-$C_{26}H_{20}N_6O_4$)	-	3014	1675	1602	1246	496	420
($C_{14}H_{13}N_3O_2$)	3234	3015	1645	1609	1258	-	-
(Co-$C_{28}H_{24}N_6O_4$)	-	3016	1671	1576	1263	478	417
($C_{19}H_{15}N_3O_2$)	3398	3104	1669	1615	1278	-	-
(Co-$C_{38}H_{28}N_6O_4$)	-	3055	1625	1600	1231	457	409
($C_{13}H_{11}N_3O_6$)	3181	3017	1629	1609	1239	-	-
(Co-$C_{26}H_{20}N_6O_6$)	3178	3049	1614	1571	1237	453	412
($C_{14}H_{13}N_3O_3$)	3436	3023	1647	1631	1254	-	-
(Co-$C_{28}H_{24}N_6O_6$)	3429	3032	1628	1603	1267	474	418
($C_{19}H_{15}N_3O_3$)	3435	3002	1658	1602	1226	-	-
(Co-$C_{38}H_{28}N_6O_6$)	3432	3055	1641	1604	1248	480	411

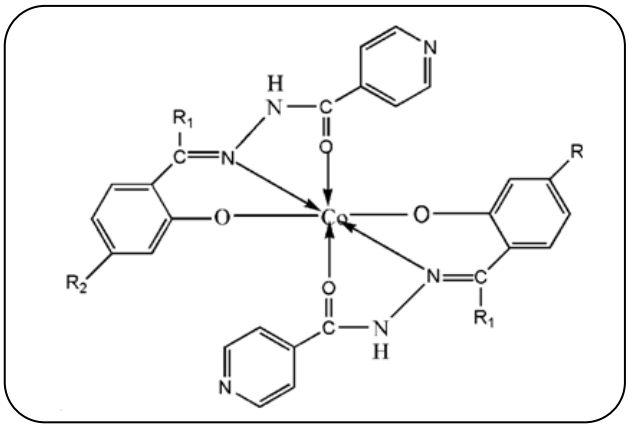

Fig. 6: Structure of metal complexes.

of the ligand to the metal ion through phenolic oxygen and azomethine nitrogen and support for the coordination sites of the ligand.

Based on the above spectral data we suggest the general structure of the metal complexes. It is shown in Fig. 6.

Cyclic voltammetry

The electrochemical behavior of Co(II) complexes was examined by cyclic voltammetry in DMF and TBHP as supporting electrolyte. The cyclic voltammetric profile of ($CoC_{26}H_{20}N_6O_6$)complex is given in Fig. 7. The electrochemical data of cobalt(II) complexes are presented in Table 4. The data reveal that cobalt complexes have a single cathodic wave, corresponding to one electron Co(II)→Co(I) [30,31]. The reduction is reversible which occurs in the range -1.275 to 0.012 V. The separation between cathodic and anodic peaks (ΔE = 176-268 mV) indicates quasi-reversible character. The potential difference ΔEp = Ep_c-Ep_a in all the complexes exceeds the Nerstian requirement of 59/n mV (n = number of electrons involved in oxidation-reduction) which suggests the quasi-reversible character of the electron transfer reaction.

DNA Studies

DNA binding studies

The interaction of the complexes with DNA was performed in tris-buffer solution of calf thymus DNA (CT-DNA) in 50mM NaCl/5 mM Tris-HCl; pH =7.0. The electronic spectra of metal complexes were monitored in the absence and presence of CT-DNA. Fig. 8 shows the UV-visible spectrum of ($CoC_{28}H_{24}N_6O_4$) compound interaction with different concentrations of DNA. It has been observed that for each addition of CT-DNA to all the complexes shows a decrease in molar absorptivity (hypochromism ,Δε, +10.18 to +47.55%, Table 5) of the π-π* absorption band as well as a bathochromic shift of a few nm (3-7nm). The intrinsic binding constant values are shown in Table 5.

Hyperchromic effect and hypochromic effect are the special features of DNA concerning its double helix structure. Hypochromism results from the contraction of

Table 4: Cyclic voltammetric data of cobalt(II) complexes

Complex	Redox couple	E_{pc}V	E_{pa}V	ΔE (mV)	$E_{1/2}$	$-i_c$	I_a	$-i_c/i_a$	Log K_c[a]	$-\Delta G^o$
(Co-$C_{26}H_{20}N_6O_4$)	II/I	-0.587	-0.319	268	-0.453	3.2021	3.1702	1.01	0.125	717
(Co-$C_{28}H_{24}N_6O_4$)	II/I	-1.224	-1.033	191	-1.128	2.644	3.887	0.68	0.175	1004
(Co-$C_{38}H_{28}N_6O_4$)	II/I	-0.260	0.012	272	-0.136	2.341	4.777	0.49	0.123	706
(Co-$C_{26}H_{20}N_6O_6$)	II/I	-1.275	-1.099	176	-0.839	5.987	4.370	1.37	0.198	1136
(Co-$C_{28}H_{24}N_6O_6$)	II/I	-0.329	-0.085	244	-0.207	8.656	6.318	1.86	0.143	820
(Co-$C_{38}H_{28}N_6O_6$)	II/I	-0.354	-0.107	247	-0.230	2.200	3.014	0.73	0.136	780

[a]*log K_c = 0.434ZF/RTΔE_p;* [b]*ΔG^o = -2.303RTlog K_c*

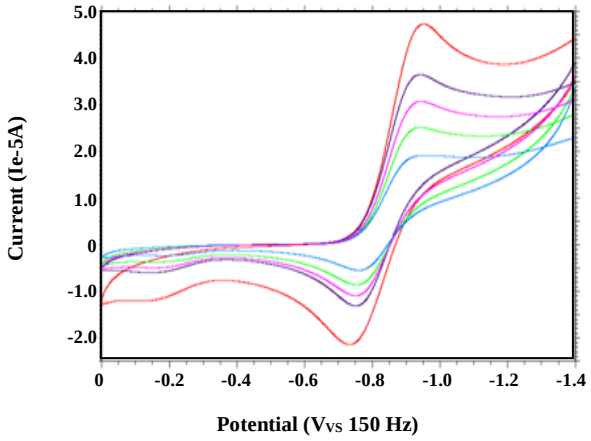

Fig. 7: Cyclic voltammogram of ($CoC_{26}H_{20}N_6O_6$) at different scan rates 25,50,75,100 mVs^{-1}.

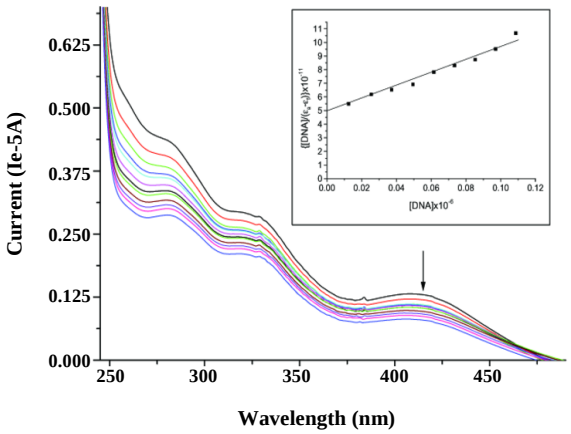

Fig. 8: Absorption spectra of ($CoC_{28}H_{24}N_6O_4$) complex in the absence and in the presence of increasing concentration of CT-DNA; top most spectrum is recorded in the absence of DNA and below spectra on addition of 10 µL DNA each time; A plot of [DNA]/ (ε_a- ε_f) vs. [DNA] is shown in the inset.

DNA in the helix axis as well as from the change in conformation on DNA. Hypochromism was observed due to intercalative mode involving strong stacking interactions between aromatic chromophore of metal complexes and nitrogenous bases of DNA[32]. The hypochromism(H%) of complexes are calculated by using the following equation.

$$H\% = \frac{A_{Free} - A_{Bounded}}{A_{Free}}$$

DNA cleavage studies

The nuclease activity of binuclear complexes derived from tridentate Schiff base ligands has been studied by agarose gel electrophoresis using pBR 322 plasmid DNA in Tris–HCl/NaCl(50 mM/5 mM) buffer (pH-7) in the presence and absence of H_2O_2 and DTT after 30 min incubation period at 37°C. Figs. 7(a) & 7(b) show the cleavage activity of cobalt (II) complexes. Percentage of cleavage activity of all the complexes is given Table 6(a) & 6(b) & 6(c). All the complexes show good nuclease activity except ($CoC_{38}H_{28}N_6O_4$). Cleavage activity increases by adding H_2O_2 to metal complex in the case of ($CoC_{38}H_{28}N_6O_4$), ($CoC_{26}H_{20}N_6O_6$)and ($CoC_{28}H_{24}N_6O_6$). In the presence of H_2O_2 the complex cleaves the supercoiled DNA (Form I) into nicked DNA (Form II) and further cleaves to linear DNA (Form III); It is due to the enhanced reaction of cobalt ion with H_2O_2 thereby producing diffusible hydroxyl radicals which are capable of damaging DNA by two well-known pathways: (1) the Fenton and (2) the Haber–Weiss mechanisms [33,34]. The requirement for a reductant for the cleavage of DNA by cobalt complexes indicates that Co(II) ions are being reduced to Co(I) ions, which further react with H_2O_2 and produce hydroxyl radicals [35,36]. These OH^- free

Table 5: Electronic absorption data upon addition of CT-DNA to the complexes.

Complex	λ_{max} (nm)		Δλ/nm	H%	K_b (M^{-1})
	Free	Bound			
($CoC_{26}H_{20}N_6O_4$)	391	397	6	+19.10	$7.43x10^6$
($CoC_{28}H_{24}N_6O_4$)	405	409	4	+28.43	$6.78x10^6$
($CoC_{38}H_{28}N_6O_4$)	329	333	4	+10.18	$2.91x10^6$
($CoC_{26}H_{20}N_6O_6$)	382	385	3	+30.21	$9.33x10^6$
($CoC_{28}H_{24}N_6O_6$)	398	405	7	+47.55	$7.82x10^6$
($CoC_{38}H_{28}N_6O_6$)	411	416	5	+16.97	$5.41x10^6$

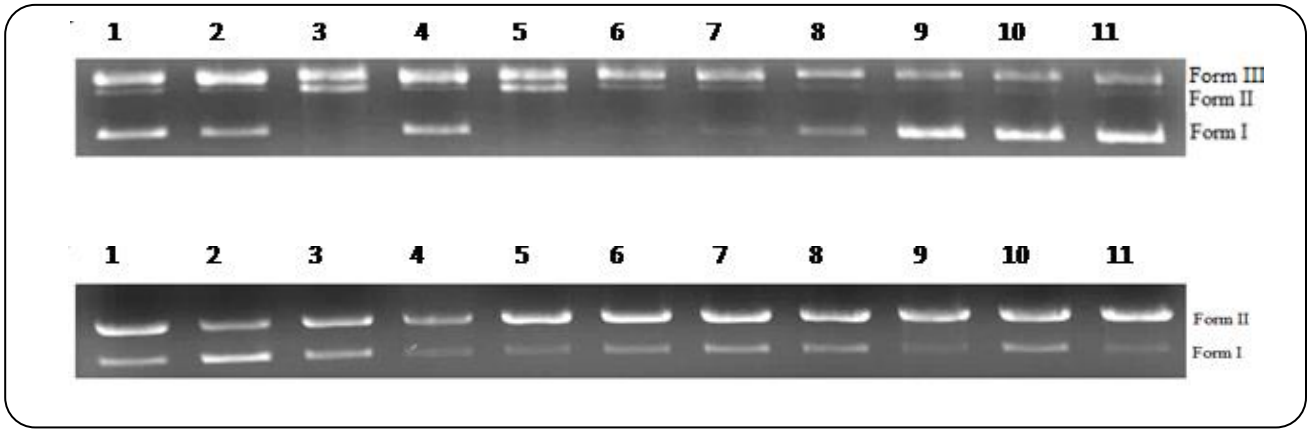

Fig. 9: (a) Agarose gel (0.8%) showing results of electrophoresis of 1 µL of pBR 322 Plasmid DNA; 4 µL of Tris–HCl/NaCl (50 mM/5 mM) buffer (pH-7); 2 µL of complex in DMF($1x10^{-3}$ M); 11 µL of sterilized water; 2 µL of H_2O_2 (total volume 20 µL) were added, respectively, incubated at 37°C (30 min); Lane 1:DNAcontrol; Lane 2: DNA control + H_2O_2; Lane 3: ($CoC_{26}H_{20}N_6O_4$)+ DNA; Lane 4: $CoC_{26}H_{20}N_6O_4$)+ DNA + H_2O_2; Lane 5: $CoC_{26}H_{20}N_6O_4$)+ DNA +H_2O_2+ DTT; Lane 6: ($CoC_{28}H_{24}N_6O_4$)+ DNA; Lane 7: ($CoC_{28}H_{24}N_6O_4$)+ DNA+H_2O_2; Lane 8: ($CoC_{28}H_{24}N_6O_4$)+ DNA+H_2O_2+DTT; Lane 9: ($CoC_{38}H_{28}N_6O_4$)+ DNA ; Lane 10: ($CoC_{38}H_{28}N_6O_4$)+ DNA + H_2O_2; Lane 11: ($CoC_{38}H_{28}N_6O_4$)+ DNA + H_2O_2+ DTT. (b) Agarose gel (0.8%) showing results of electrophoresis of 1 µL of pBR 322 Plasmid DNA; 4 µL of Tris–HCl/NaCl (50 mM/5 mM) buffer (pH-7); 2 µL of complex in DMF($1x10^{-3}$ M); 11 µL of sterilized water; 2 µL of H_2O_2 (total volume 20 µL) were added, respectively, incubated at 37°C (30 min); Lane 1:DNAcontrol; Lane 2: DNA control + H_2O_2; Lane 3: ($CoC_{26}H_{20}N_6O_6$)+ DNA; Lane 4: ($CoC_{26}H_{20}N_6O_6$)+ DNA + H_2O_2; Lane 5: ($CoC_{26}H_{20}N_6O_6$)+ DNA +H_2O_2+ DTT; Lane 6: ($CoC_{28}H_{24}N_6O_6$)+ DNA; Lane 7: ($CoC_{28}H_{24}N_6O_6$)+ DNA+H_2O_2; Lane 8($CoC_{28}H_{24}N_6O_6$)+ DNA+H_2O_2+DTT; Lane 9: ($CoC_{38}H_{28}N_6O_6$)+ DNA ; Lane 10: ($CoC_{38}H_{28}N_6O_6$)+ DNA + H_2O_2; Lane 11: ($CoC_{38}H_{28}N_6O_6$)+ DNA + H_2O_2+ DTT.

radicals participate in the oxidation of the deoxyribose moiety, followed by the hydrolytic cleavage of the sugar-phosphate backbone [37]. The mechanistic pathway for the above reaction is as follows:

$$Co^{2+} + H_2O_2 \rightarrow Co^{+} + HOO^{\bullet} + H^{+} \quad (1)$$

$$Co^{+} + H_2O_2 \rightarrow Co^{2+} + OH^{-} + {}^{\bullet}OH \quad (2)$$

DNA cleavage Forms such as Form I, Form II & Form III are calculated by the following equations:

% Form I= Intensity of Band Form I x Area

% Form II= Intensity of Band Form II x Area

% Form III= Intensity of Band Form III x Area

The cleavage rate is enhanced in the presence of both oxidant (H_2O_2) & reductant(DTT). The Co(II) formed in the second step is reduced to Co (I) by DTT, and reduced Co (I) ion to react with H_2O_2 and produces more hydroxyl radicals[38]. Then the rate of DNA cleavage further increases due to the catalytic reaction (Lanes 4,7,10). The plausible mechanism is given here.

Table 6: (a) Selected SC pBR 322 DNA cleavage data of cobalt complexes in the absence of H_2O_2 &DTT. (b) Selected SC pBR 322 DNA cleavage data of cobalt complexes in the presence of H_2O_2. (c) Selected SC pBR 322 DNA cleavage data of cobalt complexes in the presence of H_2O_2 & DTT

Figure	Lane No	Complex	Percentage of		
			Form I	Form II	Form III
(a)					
7(a)	3	(Co-$C_{26}H_{20}N_6O_4$)	1.06	32.18	66.76
7(a)	6	(Co-$C_{28}H_{24}N_6O_4$)	8.54	12.53	78.93
7(a)	9	(Co-$C_{38}H_{28}N_6O_4$)	64.87	9.53	25.60
7(b)	3	(Co-$C_{26}H_{20}N_6O_6$)	42.91	57.09	-
7(b)	6	(Co-$C_{28}H_{24}N_6O_6$)	23.43	76.57	-
7(b)	9	(Co-$C_{38}H_{28}N_6O_6$)	11.25	88.75	-
(b)					
7(a)	4	(Co-$C_{26}H_{20}N_6O_4$)	0.17	37.38	62.45
7(a)	7	(Co-$C_{28}H_{24}N_6O_4$)	5.29	15.88	78.83
7(a)	10	(Co-$C_{38}H_{28}N_6O_4$)	62.16	7.02	30.82
7(b)	4	(Co-$C_{26}H_{20}N_6O_6$)	35.64	64.36	-
7(b)	7	(Co-$C_{28}H_{24}N_6O_6$)	30.35	69.65	-
7(b)	10	(Co-$C_{38}H_{28}N_6O_6$)	38.61	61.39	-
(c)					
7(a)	5	(Co-$C_{26}H_{20}N_6O_4$)	0.59	32.82	66.59
7(a)	8	(Co-$C_{28}H_{24}N_6O_4$)	25.37	11.59	63.04
7(a)	11	(Co-$C_{38}H_{28}N_6O_4$)	70.18	6.47	23.35
7(b)	5	(Co-$C_{26}H_{20}N_6O_6$)	31.61	68.39	-
7(b)	8	(Co-$C_{28}H_{24}N_6O_6$)	34.29	65.71	-
7(b)	11	(Co-$C_{38}H_{28}N_6O_6$)	10.35	89.65	-

$$2CO^{2+} + R(SH)_2 \rightarrow 2CO^{+} + R(SS) + 2H^{+}$$

$$2CO^{+} + H_2O_2 \rightarrow CO^{2+} + OH^{-} + {}^{\bullet}OH$$

Where $R(SH)_2$ is DTT(Dithiothreitol)

Finally, we conclude that the hydroxyl radical production becomes catalytic in the presence of H_2O_2 & DTT reagents.

CONCLUSIONS

Cobalt (II) complexes of a series of six isonicotinoyl hydrazones have been synthesized and characterized based on various physicochemical and spectral techniques. These studies revealed that the complexes have general formula CoL_2 (where L = hydrazone). The hydrazones act as uni-negative tridentate ligand. Electronic spectral data suggest that the complexes have octahedral geometry. Absorption studies reveal that the complexes bind DNA via intercalation involving strong π-stacking interaction of aromatic moiety of the complex between base pairs of DNA. In the presence of H_2O_2, the complexes cleave DNA effectively. It may be due to the reaction of hydroxyl radical with DNA. In the presence of DTT and H_2O_2, complexes cleave DNA more effectively suggesting that the complexes cleave DNA by oxidative path.

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REFERENCES

[1] Sa´nchez-Delgado R.A., Navarro M., Pe´rez H., Urbina J.A., Toward a Novel Metal-Based Chemotherapy against Tropical Diseases. 2. Synthesis and Antimalarial Activity in Vitro and in Vivo of New Ruthenium− and Rhodium−Chloroquine Complexes , *J. Med. Chem.*, **39**: 1095-1099 (1996).

[2] Navarro M., Pe´rez H., Sa´nchez-Delgado R.A., Toward a Novel Metal-Based Chemotherapy Against Tropical Diseases. 3. Synthesis and Antimalarial Activity in Vitro and in Vivo of the New Gold−Chloroquine Complex $[Au(PPh_3)(CQ)]PF_6$, *J. Med. Chem.*, **40**: 1937-1939 (1997).

[3] Chohan Z.H., Rauf A., Studies on Biologic Ally Active Complexes of Cobalt(II) and Nickel(II) with Dithiooxamide-Derived Ligands, *J. Inorg. Biochem.*, **46**: 41-48 (1992).

[4] Malhotra R., Singh J.P., Dudeja M., Dhindsa K.S., Ligational Behavior of N-Substituted Acid Hydrazides Towards Transition Metals and Potentiation of Their Microbiocidal Activity, *J. Inorg. Biochem.*, **46**: 119-127 (1992).

[5] Lebon F., Ledecq M., Benatallah Z., Sicsic S., Lapouyade R., Kahan O., Garcon A., Reboud-Ravaux M., Durant F.J., Metal-Organic Compounds: A New Approach for Drug Discovery. N1-(4-methyl-2-pyridyl)-2,3,6-trimethoxybenzamide Copper(II) Complex as an Inhibitor of human Immunodeficiency Virus 1 Protease, *J. Chem. Soc. Perkin Trans.*, **2**: 795-800 (1999).

[6] Bharti N., Maurya M.R., Naqvi F., Bhattacharya A., Bhattacharya S., Azam A., Palladium(II) Complexes of NS Donor Ligandsderived from S-methyl-dithiocarbazate, S- benzyldithiocarbazate and Thiosemicarbazide as Antiamoebic Agents, *Eur. J. Med. Chem.*, **35**: 481-486 (2000).

[7] Sridhar S.K., Pandeya S.N., Stables J.P., Ramesh A., Anticonvulsant Activity of Hydrazones, Schiff and Mannich Bases of Isatin Derivatives, *Eur. J. Pharm. Sci.*, **16**: 129-132 (2002).

[8] Vicini P., Zani F., Cozzini P., Doytchinova I., Hydrazones of 1,2-benzisothiazole Hydrazides: Synthesis, Antimicrobial Activity and QSAR Investigations, *Eur. J. Med. Chem.*, **37**: 553-564 (2002).

[9] Kaymakcioglu B.K., Rollas S., Synthesis, Characterization and Evaluation of Antituberculosis Activity of Some Hydrazones, *Farmaco.*, **57**: 595-599 (2002).

[10] Ragavendran J.V., Sriram D., Patel S.K., Reddy I.V., Bharathwajan N., Stables J., Yogeeswari P., Design and Synthesis of Anticonvulsants from a Combined Phthalimide–GABA–Anilide and Hydrazone Pharmacophore, *Eur. J. Med. Chem.*, **42**: 146-151 (2007).

[11] Rollas S., Gulerman N., Erdeniz H., Synthesis and Antimicrobial Activity of Some New hydrazones of 4-Fluorobenzoic Acid Hydrazide and 3-acetyl-2,5-disubstituted-1,3,4-oxadiazolines, *Farmaco*, **57**: 171-174 (2002).

[12] Sah P.P.T., Peoples S.A., Isonicotinoyl Hydrazones as Antitubercular Agents and Derivatives for Identification of Aldehydes and Ketones. *J. Pharm. Sci.*, **43**: 513-524 (1954).

[13] Alexandrou N.E., Vasilikiotis G.S., Infra-Red Spectra of Isoniazid Hydrazones, *Spectrochim. Acta Part A*, **23**: 677-679 (1967).

[14] Georgevia N., Gadjeva V., Isonicotinoylhydrazone Analogs of Isoniazid: Relationship between Superoxide Scavenging and Tuberculostatic Activities, *Biochemistry (Moscow)*, **67**: 588-591 (2002).

[15] Shimakoshi H., Kaieda T., Matsuo T., Sato H., Hisaeda Y., Syntheses of New Water-Soluble Dicobalt Complexes having Two Cobalt-Carbon Bonds and Their Ability for DNA Cleavage, *Tetrahedron Lett.*, **44**: 5197-5199 (2003).

[16] Vaidyanathan V. G., Nair B.U., Photooxidation of DNA by a Cobalt(II) Tridentate Complex, *J. Inorg. Biochem.*, **94**: 121-126 (2003).

[17] Zhou X.F., Biomimetic TCF Bleaching of Pulp by Simple Inorganic Complexes of Cupric/Cobalt Acetate, *Iran. J. Chem. Chem. Eng. (IJCCE)*, **33**: 99-105 (2014).

[18] Muhammad A.A., Mohd J.M., Ismail Y., Synthesis, Characterization and Biological Studies of 2-(4-Nitrophenylaminocarbonyl)Benzoic Acid and Its Complexes with Cr(III), Co(II), Ni(II), Cu(II) and Zn(II), *Iran. J. Chem. Chem. Eng. (IJCCE)*, **31**: 9-14 (2012).

[19] Jiao K., Wang Q.X., Sun W., Jian F.F., Synthesis, Characterization and DNA-Binding Properties of a New Cobalt(II) Complex: $Co(bbt)_2Cl_2$, *J. Inorg. Biochem.*, **99**: 1369-1375 (2005).

[20] Tu C., Wu X., Liu Q., Wang X., Xu Q., Guo Z., Crystal Structure, DNA-Binding Ability and Cytotoxic Activity of Platinum(II) 2,2′-Dipyridylamine Complexes, *Inorg. Chim. Acta*, **357**: 95-102 (2004).

[21] Wu B.Y., Gao L.H., Duan Z.M., Wang K.Z., Syntheses and DNA-Binding Studies of Two Ruthenium(II) Complexes Containing one Ancillary Ligand of Bpy or Phen: $[Ru(bpy)(pp[2,3]p)_2](ClO_4)_2$ and $[Ru(phen)(pp[2,3]p)_2](ClO_4)_2$, *J. Inorg. Biochem.*, **99**: 1685-1691 (2005).

[22] Dutta R.L., Syamal A., "Elements of Magnetochemistry". 2nd ed. New Delhi: Affiliated East-West Press Pvt. Ltd; (1993).

[23] Singh V.P., Katiyar A., Synthesis, Spectral Characterization and Antimicrobial Activity of Some Transition Metal(II) Complexes with Acetone p-Amino Acetophenone Benzoylhydrazone, *Pestic. Biochem. Physiol.*, **92**: 8–14 (2008).

[24] Chandra S., Sharma S., Chromium(III), Manganese(II), Cobalt(II), Nickel(II), Copper(II) and Palladium(II) Complexes of a 12-Membered Tetraaza $[N_4]$ Macrocyclic Ligand, *Trans. Met. Chem.*, **27**: 732-735 (2002).

[25] Grag B.S., Singh P.K., Grag S.K., Synthesis and Spectral Characterisation of Complexes of Acenaphthaquinonemono(Lepidyl)Hydrazone with Various Salts of Cobalt(II), *Synth. React. Inorg. Met.-Org. Chem.*, **23**: 17-28 (1993).

[26] Magdy Shebl, Saied M.E. Khalil, Saleh A. Ahmed, Hesham A.A. Medien, Synthesis, Spectroscopic Characterization and Antimicrobial Activity of Mono-, bi- and Tri-Nuclear Metal Complexes of a New Schiff Base Ligand, *J. Mol. Struct.*, **980**: 39–50 (2010).

[27] Emara A.A.A., Abou-Hussen A.A.A., Spectroscopic Studies of Bimetallic Complexes Derived from Tridentate or Tetradentate Schiff Bases of Some di- and Tri-Valent Transition Metals, *Spectrochim. Acta Part A*, **64**: 1010–1024 (2006).

[28] Ravanasiddappa M., Sureshg T., Syed K., Radhavendray S.C., Basavaraja C., Angadi S.D., Transition Metal Complexes of 1, 4 (2-Hydroxyphenyl - 1-yl) Diimmino Azine, Synthesis, Characterization and Antimicrobial Studies, *E-J. Chem.*, **5**: 395-403 (2008).

[29] Raju M. Patil, Synthetic, Structural and Biological Properties of Binuclear Complexes with Some Schiff Bases, *Acta Poloniae Pharm. Drug Res.*, **64**: 345-353 (2007).

[30] Cibian M., Garry S. H., Geometry and Spin Change at the Heart of a Cobalt(II) Complex: A Special Case of Solvatomorphism, *Chem. Eur. J.*, **21**: 9474-9481 (2015).

[31] Kılıc A., Durgun M., Tas E., Yılmaz I., Novel *vic*-Dioxime Ligands and Their Poly-Metal Complexes Bearing 1,8-diamino-3,6-dioxaoctane: Synthesis, Characterization, Spectroscopy and Electrochemistry, *Transition Met. Chem.* **33**: 29-37(2008).

[32] Khan T.A., Shagufta M., Transition Metal Ion Directed Bimetallic Macrocyclic Complexes, *Trans. Met. Chem.*, **24**: 669-671 (1999).

[33] Kaushik M.,, Woongki K., Scott Daniels J., Kent S. G., Oxidative DNA Cleavage by the Antitumor Antibiotic Leinamycin and Simple 1,2-Dithiolan-3-one 1-Oxides: Evidence for Thiol-Dependent Conversion of Molecular Oxygen to DNA-Cleaving Oxygen Radicals Mediated by Polysulfides, *J. Am. Chem. Soc.*, **119**: 11691-11692(1997).

[34] YingY. K ., Jin-Lei T., Dong-Dong L., , Hui L., , Wen G., Shi Ping Y., Oxidative DNA Cleavage by Cu(II) Complexes of 1,10-phenanthroline-5,6-dione, *J. Coord. Chem.*,**62**: 2182-2192 (2009).

[35] Louie A.Y., Meade T.J., Metal Complexes as Enzyme Inhibitors, *Chem. Rev.*, **99**: 2711-2734 (1999).

[36] Vidyanathan V.G., Nair B.U., Oxidative Cleavage of DNA by Tridentate Copper (II) Complex, *J. Inorg. Biochem.*, **93**: 271-276 (2003).

[37] Pogozelski W.K., Tullius T.D., Oxidative Strand Scission of Nucleic Acids: Routes Initiated by Hydrogen Abstraction from the Sugar Moiety, *Chem. Rev.*, **98**: 1089-1108 (1998).

[38] Netto L.E.S., Stadtma E.R., The Iron-Catalyzed Oxidation of Dithiothreitol Is a Biphasic Process: Hydrogen Peroxide Is Involved in the Initiation of a Free Radical Chain of Reactions, *Arch. Biochem. Biophy.*, **333**: 233-242 (1996).