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SPECTRAL PROPERTIES, DNA STUDIES OF IRON(II) COMPLEXES OF ISONICOTINOYL HYDRAZONES

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ABSTRACT

A series of novel iron (II) complexes of general formula of $Fe(L)_2$ [L=isonicotinoyl hydrazones) have been synthesized and characterized based on elemental analysis, molar conductivity, magnetic susceptibility measurements, infrared and electronic spectroscopy. Electrochemical behaviour of these complexes is investigated by cyclic voltammetry. The DNA binding constants K_b of the complexes are determined systematically with spectrophotometric titrations by using Calf Thymus DNA (CT-DNA). The changes in absorption spectra complexes upon addition of DNA suggest strong π- stacking interaction between the complexes and DNA base pairs. Cleavage activities of these complexes have been investigated on double stranded pBR322 plasmid DNA by gel electrophoresis in the absence and in presence of oxidant. The complexes behave as efficient chemical nucleases with hydrogen peroxide activation in the presence of reductant (DTT).

KEYWORDS: iron (II) complexes, hydrazones, DNA binding, Nuclease activity.

INTRODUCTION

Transition metal ions play an important role in a number of chemical and biological reactions. The reactivity of metal complex can be modulated at high levels by simply changing the metal ions and their oxidation states. The successful synthesis and application of metal complexes can have a great impact on all areas of chemistry viz., organic, medicinal and biological chemistry.[1-3] Hydrazones and their metal complexes are found to have potential application in biology and medicine. Metal complexes of hydrazones are used as model compounds to mimic biological processes.[4-6]

Isonicotinoyl hydrazones play an important role in medicinal chemistry.[7] They are used as their complexes with metal ions for the treatment of number of diseases. As these organic compounds contain nitrogen as well as oxygen, they have the ability to form complexes easily with many metal ions. The hetero atoms, nitrogen as well as oxygen can form coordinate bonds with many metal ions and thus, form stable complexes. Number of reports is available on the medicinal importance of these compounds. Isonicotinoyl hydrazones have been used as anti-tuberculosis[8, 9] and anticancer drugs.[10]

In the light of the above and in continuation of our ongoing research work, here in, we report synthesis, spectral characterization and DNA binding and cleavage activity of Transition metal complexes with a series of three INH ligands. Three ligands viz. 2,4-dihydroxy benzaldehyde isonicotinoyl hydrazone (DBINH), 2,4-dihydroxy acetophenone isonicotinoylhydrazone (DAPINH) and 2, 4-dihydroxy benzophenone isonicotinoyl hydrazone(DBPINH) are synthesized and characterized.

EXPERIMENTAL

Materials and methods

Isoniazid, 2,4-dihydroxybenzaldehyde, 2,4-dihydroxyacetophenone, 2,4-dihydroxy benzophenone 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. 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 methanolic solutions of isonicotinylhydrazide (5mmol), carbonyl compound (5mmol) 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 and dried in vacuum. General structure of ligands is shown in Fig. 1.

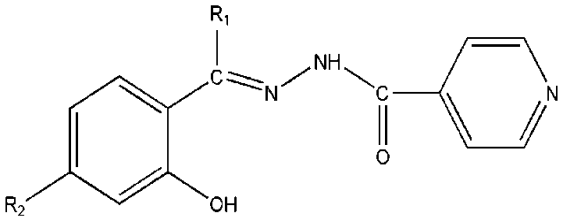

Fig 1: A general structure of ligands

R_1	R_2	Ligand
H	OH	DBINH
CH_3	OH	DAPINH
C_6H_5	OH	DBPINH

2,4-Dihydroxy benzaldehyde isonicotinoyl hydrazone (DBINH)

Yield 73% M.Pt.235-237^{0}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. δ(12.40) (singlet 1H) & δ(10.09) (singlet 1H), δ(11.32) (singlet 1H), δ(8.62) (singlet 1H), δ (7.8)(multiplet 4H) δ(6.6) (multiplet 3H), assigned to 2-OH, -NH, and =CH-, pyridine H, aromatic ring protons respectively. Mass spectra of DBINH shows molecular ion peak at 257.

2,4-Dihydroxy acetophenone isonicotinoyl hydrazone (DAPINH)

Yield 65%, M.Pt.252-254^{0}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. δ(13.39) (singlet 1H) & δ(9.93) (singlet 1H), δ(11.42) (singlet 1H), δ(8.8)(multiplet 4H) δ(7.2) (multiplet 3H), δ(2.50) (singlet 3H), assigned to 2–OH, -NH, pyridine H, aromatic ring, -CH_3 protons respectively. Mass spectrum of DAINH shows molecular ion peak at 271.

2,4-Dihydroxy benzophenone isonicotinoyl hydrazone (DBPINH)

Yield 84%, M.Pt. 269-270^{0}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. δ(10.19) (singlet 1H) & δ(9.78) (singlet 1H), δ(10.04) (singlet 1H), δ (8.5)(multiplet 4H), δ(7.7) (multiplet 5H),δ(6.6) (multiplet 3H), assigned to 2-OH, -NH, pyridine H, aromatic ring protons respectively. Mass spectrum of DBPINH shows molecular ion peak at 333.

Preparation of complexes

To a methanolic solution of ligand (5mmol), the aqueous solution of metal salt ($FeCl_2$. $4H_2O$) was added. The resulting solution was refluxed with stirring for one hour and then kept at room temperature and then filtered washed with methanol and dried in vacuo. The analytical data of all the complexes are given in Table 1. The ES^+I mass spectrum of $Fe(DBINH)_2$ complex are shown in Fig. 2.

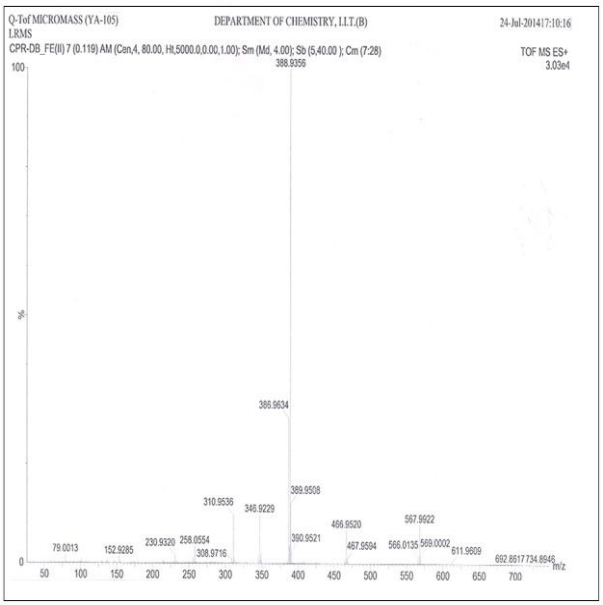

Fig 2 ES^+I Mass spectrum of $Fe(DBINH)_2$

DNA binding experiments

The interaction of the complexes with DNA was studied in tris-buffer medium. 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.[11] The DNA concentration per nucleotide was determined by absorption coefficient (6600 dm^3 mol^{-1} cm^{-1}) at 260 nm.[12] Stock solutions stored at 4ºC 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 P^H 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 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

Physical properties of complexes are given in **Table 1.** All the complexes are stable at room temperature, non-hygroscopic, slightly soluble in water, but more soluble in methanol, ethanol and readily soluble in CH_3CN, DMF and DMSO. The analytical data are consistent with the proposed molecular formulae of complexes. Low molar conductivity values of present complexes suggest non-electrolytic nature of the complexes. The effective magnetic moments (μ_{eff}) of the iron(II) complexes (1-6) lie in the range 4.3-15.9 B.M. at room temperature. The values suggest high spin octahedral geometry for the complexes.[13]

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

Complex	Melting Point °C	Elemental analysis found(cal.)%			μ_{eff}	$\Lambda_M{}^a$
		Carbon	Hydrogen	Nitrogen		
$Fe(HBINH)_2$	295-296	57.54(58.20)	3.25(3.73)	15.01(15.67)	5.21	4.3
$Fe(HAPINH)_2$	268-270	58.31(59.37)	4.46(4.25)	14.27(14.89)	5.15	15.9
$Fe(HBPINH)_2$	>300	67.31(66.27)	4.18(4.06)	12.89(12.20)	5.24	13.4

Electronic spectra

Electronic absorption spectra of iron(II) complexes were recorded in DMF. The important electronic spectral data of iron(II) complexes are presented in **Table 2**. All the complexes show strong intense bands in the region 34527-35319 cm^{-1} attributed due to intraligand and π-π* aromatic ring. Another sharp peak shows at the region of 28872-29153 cm^{-1} is due to n-π* transition. One medium intensity band observed in the range 18268-19230 cm^{-1} is due to metal to ligand charge transfer transition (MLCT). A weak band is observed in the region of 10152-11413 cm^{-1} is due to d-d transition which is assigned to the $^5T_{2g} \rightarrow {}^5E_g$ transition in octahedral field.

Table 2: Electronic Spectral data(cm^{-1}) of Iron(II) complexes

Complex	π-π* transition	n-π* transition	CT transition	d-d transition
$Fe(DBINH)_2$	34527	29411	19230	10152
$Fe(DAPINH)_2$	34615	28872	18523	11413
$Fe(DBPINH)_2$	35319	29153	18268	11297

IR spectra

The important bands in infrared spectra of the ligands and their metal complexes are discussed. Important IR spectral bands of complexes are presented in **Table 3**. An intense band is observed in the region of 3181-3436 cm^{-1} in IR spectra of ligands due to phenolic ν(-OH) group. The band is disappeared in iron complexes. This indicates deprotonation of phenolic group and band formation between phenolic oxygen and iron ion.[14-16] In ligands a strong band is observed in the region of 1629-1658 cm^{-1} which is assigned to ν(C=O) group. In the spectra of iron complexes this peak is shifted (8-25cm^{-1}) to lower wave numbers suggesting the involvement of >C=O group in chelation. The C=N (imine) vibration is observed in 1509-1631 cm^{-1} range in the IR spectra of ligands. This band is shifted to lower wave number in IR spectra of all the complexes suggesting the participation of azomethine nitrogen atom in coordination with iron ion. The non-ligand absorption bands occurring in the regions 545-508 cm^{-1} and 403-412 cm^{-1} are assigned to $\nu_{(M-O)}$ and $\nu_{(M-N)}$ vibrations respectively.[17]

Based on analytical, physicochemical and spectral data, a general structure (**Fig. 5)** is proposed for the iron(II) complexes.

Table 3: IR spectral data of Iron(II) complexes

Compound	ν(O-H) cm^{-1}	ν(NH) cm^{-1}	ν(C=O) cm^{-1}	ν(C=N) cm^{-1}	ν(C-O) cm^{-1}	ν (M-O) cm^{-1}	ν (M-N) cm^{-1}
DBINH	3181	3017	1629	1509	1239	-	-
$Fe(DBINH)_2$	-	3052	1621	1548	1214	508	412
DAPINH	3436	3023	1647	1631	1254	-	-
$Fe(DAPINH)_2$	-	3069	1635	1576	1287	545	406
DBPINH	3435	3002	1658	1602	1226	-	-
$Fe(DBPINH)_2$	-	3031	1633	1569	1269	512	403

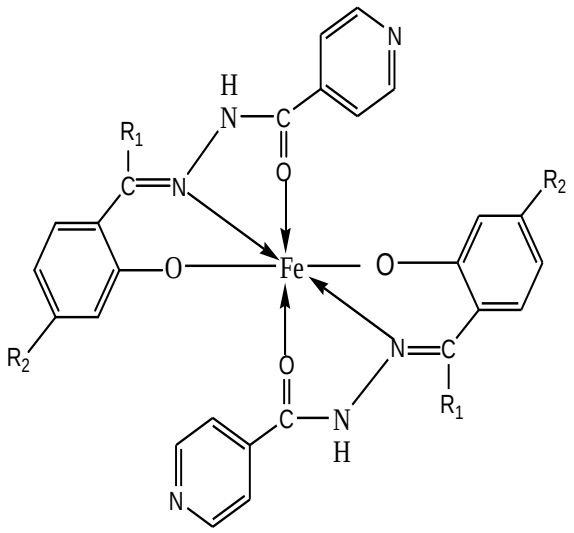

Fig. 3 Structure of metal complexes.

Cyclic voltammetry

The redox behavior of the complexes has been investigated by cyclic voltammetry in DMF using 0.1 M tetrabutylammonium hexafluorophosphate as supporting electrolyte. The cyclic voltammogram of $Fe(DAPINH)_2$complex is given in **Fig 4.** The electrochemical data of iron(II) complexes are presented in **Table 4.** The data reveal that Iron(II) complexes have single cathodic wave, corresponding to one electron reduction Fe(II)→Fe(I). The reduction is reversible which occurs in the range -0.876 to -0.948 vs Ag/AgCl reference electrode. The separation between cathodic and anodic peaks (ΔE = 174-235 mV) indicates quasi-reversible character. The potential difference $\Delta Ep = Ep_c - Ep_a$ in all the complexes exceeds the Nerstian requirement of 59/n mV (n = number of electrons involved in oxidation-reduction) which further suggests quasi-reversible character of the electron transfer reaction.

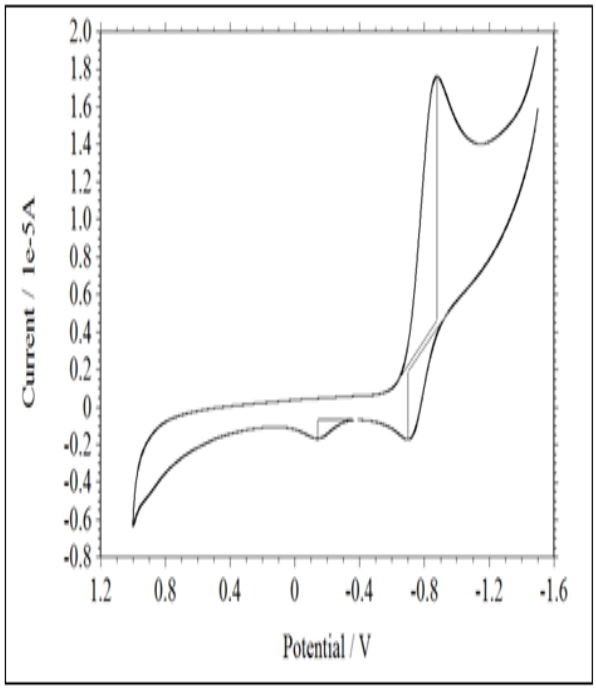

Fig 4 Cyclic voltammogram of $Fe(DAPINH)_2$

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

Complex	Redox couple	$E_{pc}V$	$E_{pa}V$	ΔE (mV)	$E_{1/2}$	$logK_c^a$	$-\Delta G^0$
$Fe(DBINH)_2$	II/I	-0.948	-0.713	235	-0.830	0.142	815
$Fe(DAPINH)_2$	II/I	-0.876	-0.698	178	-0.787	0.188	1083
$Fe(DBPINH)_2$	II/I	-0.924	-0.750	174	-0.837	0.193	1108

DNA binding studies

The binding interactions of the complexes with DNA were monitored by comparing their absorption spectra with without CT-DNA. It has been observed that for each addition of CT-DNA to all the complexes shows a decrease in molar absorptivity (hypochromism, Δε, +32.13 to +41.57%, **Table 5**) of the π-π^* absorption band as well as a bathochromic shift of a few nm(3-5nm). The intrinsic binding constant(K_b), was determined by using the equation. The intrinsic binding constants of iron complexes are given in **Table 5.**

$$\frac{[DNA]}{\varepsilon_a - \varepsilon_f} = \frac{[DNA]}{\varepsilon_a - \varepsilon_f} + \frac{1}{K_b} (\varepsilon_a - \varepsilon_f) \quad \text{----------(1)}$$

Hypochromism results from the contraction of DNA in the helix axis as well as from the change in conformation

on DNA. Spectral changes suggest due to intercalative mode of binding of iron complex involving strong stacking interactions of complex between nitrogenous base pairs of DNA.[18]

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			
$Fe(DBINH)_2$	360	364	4	+36.94	$2.83x10^6$
$Fe(DAPINH)_2$	412	417	5	+41.57	$1.57x10^5$
$Fe(DBPINH)_2$	276	279	3	+32.13	$4.43x10^6$

DNA cleavage studies

Nuclease activity of iron(II) complexes 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. **Fig 5** show the cleavage activity of iron (II) complexes. Iron complexes of shows high nuclease activity. In the presence of H_2O_2, the complexes cleave supercoiled DNA(Form I) into linear DNA (Form II) and nicked (Form III) [Figures 5]. Cleavage activity increases in the presence of H_2O_2 to metal complex. It is due to the reaction of iron complex 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 the (2)Haber–Weiss mechanisms.[19,20] The necessity for a reductant for the cleavage of DNA by iron complexes indicates that Fe (II) ions are being reduced to Fe(I) ions, which are susceptible to oxidation.[21,22] These OH^- free radicals participate in the oxidation of the deoxyribose moiety.[23] The mechanistic pathway for the above reaction as follows:

$Fe^{2+} + H_2O_2 \rightarrow Fe^{+} + HOO\bullet + H^{+}$ …….(1)

$Fe^{+} + H_2O_2 \rightarrow Fe^{2+} + OH^- + \bullet OH$ …….(2)

On comparision of lanes 4,7 and 10 with lanes 5,8 and 11, it is clear that the cleavage rate is enhanced in the presence reductant(DTT). The Fe(II) formed in the second step is reduced to Fe(I) by DTT, and reduced Fe(I) ion react with H_2O_2 and produces more hydroxyl radicals.[24]

The oxidative cleavage becomes catalytic in the presence of reductant as indicated below. This observation provides an evidence for the oxidative cleavage of DNA.

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

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

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

Finally we conclude that nuclease activity of complexes become catalytic in the presence of H_2O_2 & DTT reagents.

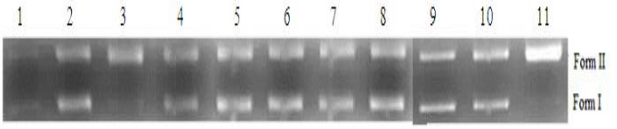

Fig 5 Agarose gel (0.8%) showing results of electrophoresis of 1 µl of pBR 322 Plasmid DNA; 4 µl of Tris–HCl/NaCl (50 mM/5 mM) buffer (pH-7); 2 µl of complex in DMF($1x10^{-3}$ M); 11 µl of sterilized water; 2 µl of H_2O_2 (total volume 20 µl) were added, respectively, incubated at 37°C (30 min); Lane 1:DNAcontrol; Lane 2: DNA control + H_2O_2; Lane 3: $Fe(DBINH)_2$ + DNA; Lane 4: $Fe(DBINH)_2$ + DNA + H_2O_2; Lane 5: $Fe(DBINH)_2$ + DNA +H_2O_2+ DTT; Lane 6: $Fe(DAPINH)_2$ + DNA; Lane 7: $Fe(DAPINH)_2$ + DNA+H_2O_2; Lane 8: $Fe(DAPINH)_2$ + DNA+H_2O_2+DTT; Lane 9: $Fe(DBPINH)_2$+ H_2O_2 + DNA; Lane 10: $Fe(DBPINH)_2$ + DNA + H_2O_2; Lane 11: $Fe(DBPINH)_2$ + DNA + H_2O_2+ DTT.

CONCLUSIONS

Iron(II) complexes of a new series of isonicotinoylhydrazones bearing phenolic group have been synthesized and characterized based on physico-chemical and spectral studies. Physico-chemical data revealed that the complexes have general formula FeL_2 (where L = hydrazone). The hydrazones act as uni-negative tridentate ligand. Electronic spectral data suggest that the complexes have octahedral geometry. Absorption titrations suggest that the complexes bind DNA through intercalation involving a strong π -stacking interaction of the aromatic chromophore of complex between base pairs of DNA. In 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 effectively suggesting that the complexes cleave DNA by oxidative path.

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