

Physicochemical, computational and DNA cleavage studies of *N'*-[(1*E*)-1-(2,4-dihydroxyphenyl)ethylidene]benzenesulfonohydrazide and its metal complexes

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Abstract : Benzenesulfonohydrazides and their metal complexes are important compounds in medicinal chemistry. In the present investigation synthesis of *N'*-[(1*E*)-1-(2,4-dihydroxyphenyl)ethylidene]benzenesulfonohydrazide, its metal complexes and their structural properties have been investigated by various spectro-analytical and computational studies. Molecular orbital and Quantitative Structure Activity Relationship (QSAR) properties were computed by employing HyperChem 7.5 tools. The pH-metric studies indicated dibasic nature of compound with two corresponding pKa values. The pKa values were further compared with computed values derived by using ChemAxon tools. The interaction of metal ions with candidate compound was monitored by Job's method of continuous variation and mole-ratio methods in ethanol-water medium. The solid metal complexes synthesized were investigated by various techniques viz. IR, UV-Visible, mass, ESR, SEM, TG, DTA and elemental analysis. The DNA cleavage studies carried out on the title compound and its metal complexes indicated that only copper complex has the property to cleave the super coiled DNA to linear form.

Keywords : Sulfonohydrazide [(*N*-dihpebsh)-H₂], computational studies, equilibrium studies, DNA cleavage.

Introduction

Sulphonamides and sulfonyl hydrazones are important in medicinal and polymeric fields. These compounds are widely used chemotherapeutic agents with large spectrum of activity¹ and serve as suitable pharmacophoric equivalent to replace functional groups in drug design². They are known to possess activity against Alzheimer's disease^{3,4}, antibacterial, antitumor, diuretic, hepatitis C virus⁵⁻⁷, specific enzyme inhibition such as carbonic anhydrase, γ -secretase HIV protease, metalloproteinase and hormone regulation^{8,9}. Sulfonyl hydrazones also show DNA interaction ability and cytostatic effects in cancer therapy¹⁰⁻¹³. The review of literature reveals that sulfonyl hydrazones have potential binding sites which can bind with metal ions to form corresponding metal complexes. The biological studies reported earlier on metal complexes revealed their potential to act as drugs in chemotherapy¹⁴.

In the polymeric field sulfonyl hydrazones are used in the manufacture of rubber, epoxy resins, leather, flame resistant fibres and colour developers. They play important role in analytical field as a sensitive fluorescent labelling reagent for determination of aromatic and aliphatic aldehydes¹⁵. In view of the above applications cited in literature and limited work in this area, the present study focuses on the synthesis of *N'*-[(1*E*)-1-(2,4-dihydroxyphenyl)ethylidene] benzene sulfono hydrazide [(*N*-dihpebsh)-H₂] and their metal complexes. The structural properties were explored using various spectro-analytical techniques viz. mass, IR, UV-Visible, ¹H NMR, ¹³C NMR, DEPT, SEM, ESR, TG, DTA studies and elemental analysis. The computational methods employing HyperChem 7.5 and ChemAxon tools were applied to elucidate structural, molecular, QSAR properties and pKa values of title compound. The potential binding sites of title compound with metals ions such as Cu^{II}, Ni^{II} and

Co^{II} were evaluated by equilibrium studies applying the methods of pH-metry and spectrophotometry. DNA interaction studies were carried out on (*N*-dihpebsh)-H₂ and its metal complexes using SC PBR 322 DNA at pH 7.5.

Materials and methodology :

All the chemicals used in the present investigation were of analytical reagent (AR) grade. Benzene sulfonyl hydrazide was procured from Merck.

Synthesis of (*N*-dihpebsh)-H₂ :

The title compound (*N*-dihpebsh)-H₂ was synthesized from 1,3-dihydroxy benzene by two step procedure.

(i) *Preparation of 2,4-dihydroxy acetophenone* : It was prepared from 1,3-dihydroxy benzene following the literature methods¹⁶.

(ii) *Preparation of (*N*-dihpebsh)-H₂* :

To a solution of phenyl sulfonyl hydrazide 1.72 g (0.01 mole) in 20 ml of methanol, 1.52 g (0.01 mole) of 2,4-dihydroxy acetophenone in 25 ml of methanol was added and the reaction mixture was refluxed with 5–6 drops of glacial acetic acid for 5 h (Fig. 1). The completion of reaction was monitored by TLC, then excess methanol was distilled off and the residue is treated with dilute ethanol. Yellowish orange product thus obtained was recrystallised from aqueous ethanol. The purity of the compound was checked by TLC and LC-MS. The melting point of the compound is 165 °C and yield of the product is 80%. The scheme for the synthesis is given in Fig. 1.

Synthesis of Cu^{II}, Ni^{II} and Co^{II} complexes : A solution of respective metal chloride was added to the hot methanol solution of (*N*-dihpebsh)-H₂ in 1 : 2 molar ratio. The reaction mixture was refluxed for about 10 h. The corresponding metal complex separated was filtered,

washed with hot methanol, followed by washings with petroleum ether and finally dried over CaCl₂ in a desiccator. The metal complexes were characterized by mass, IR, UV-Visible, SEM, EDX, ESR, TG and DTA studies.

Physical measurements : Mass spectral data were recorded on Shimadzu LCMS-2010A using atmospheric pressure chemical ionization in negative mode. IR spectra (KBr) were recorded on a Perkin-Elmer 337 spectrophotometer and UV-Visible spectra on Shimadzu UV spectrophotometer. ¹H NMR and ¹³C NMR spectra were recorded on Bruker Biospin Avance-111400 MHz spectrometer. Particle size and morphology were recorded on Zeiss Scanning electron microscope and INCA EDX instrument was used for elemental analysis. ESR spectrum was recorded on Bruker-EMX, ER-073. Thermal studies were carried out using Shimadzu TGA-50H in nitrogen atmosphere. Spectrophotometric studies¹⁷ were carried out on SL-177 scanning mini spec spectrophotometer. The equilibrium studies were carried out on a digital Digisun 707 pH-meter with an assembly of combined glass electrode maintaining constant temperature. The dissociation constants of the title compound were calculated using Irving-Rossotti titration technique¹⁸.

Computational studies :

The computational studies were carried out by employing ChemAxon¹⁹ and HyperChem 7.5 software tools²⁰. The pK_a values, energies, dipole moments, eigen values of HOMO, LUMO and QSAR properties were computed from these studies.

Results and discussion

(A) *Spectral and equilibrium studies of title compound* :

The title compound (*N*-dihpebsh)-H₂ was synthesized and characterized by spectral analysis. As the compound

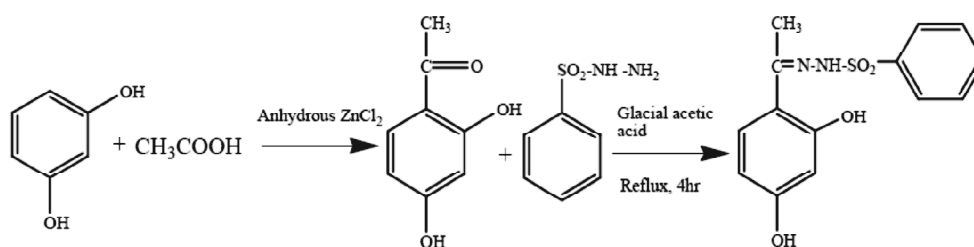


Fig. 1. Scheme showing synthesis of *N'*-[(1*E*)-1-(2,4-dihydroxyphenyl)ethylidene]benzenesulfonylhydrazide.

has potential donor sites its binding properties with metal ions is explored employing pH-metry and spectrophotometry.

LC-MS :

LC chromatogram of (*N*-dihpebsh)-H₂ showed single peak at 0.558 min (negative mode). The mass spectrum of (*N*-dihpebsh)-H₂ shows a dominant peak at *m/z* 305, which is in accordance with the expected [M-1]⁺ peak, analyzed under negative conditions. This shows that the measured mass is in good agreement with the theoretical value (*m/z* 306).

IR :

The IR spectrum of (*N*-dihpebsh)-H₂ exhibited peaks at 3500 cm⁻¹ and 3155 cm⁻¹ which can be assigned to OH and NH stretching vibrations respectively. The bands at 1631 cm⁻¹ and 1521 cm⁻¹ indicate the stretching frequencies corresponding to C=N and aromatic C=C. The peaks at 1327 cm⁻¹ and 1076 cm⁻¹ can be assigned to asymmetric stretching vibrations of SO₂ and aromatic-S stretching. The peak at 1220 cm⁻¹ corresponds to C-O stretching of OH. Characteristic bending vibrations were observed at 1450 cm⁻¹, 1400 cm⁻¹, 983-741 cm⁻¹ and 594-565 cm⁻¹ which can be assigned for CH₃, OH, NH and SO₂ respectively.

NMR :

The ¹H NMR spectrum of (*N*-dihpebsh)-H₂ in DMSO-*d*₆ recorded signals at δ 2.27 (s, 3H) for methyl protons and δ 6-6.6 (m, 3Aromatic H) for the aromatic ring with hydroxyl groups. Another multiplet was observed in the region of δ 7.2-7.9 which can be attributed to five aromatic protons present on the phenyl ring attached to sulphur atom. The presence of OH and NH signals were observed at δ 9.8 (OH), δ 11.8 (OH) and δ 11.9 (NH). The presence of OH and NH peaks were corroborated by deuterium exchange studies as they readily exchanged with deuterium and the corresponding signals were absent in the D₂O spectrum.

The ¹³C NMR spectrum in DMSO-*d*₆ recorded signals at 15 ppm for methyl carbon and from 100-150 ppm for aromatic carbons. The quaternary carbons were recorded from 150-175 ppm.

The azomethine carbon signal was observed at 205 ppm. The DEPT spectrum at 135 recorded positive peak

for methyl carbon at 15 ppm and CH carbons from 100-135 ppm. The quaternary carbons were absent in the DEPT spectrum.

pH-Metry :

The potentiometric titrations were carried out with (*N*-dihpebsh)-H₂ in 70% v/v 1,4-dioxane-water medium at 303 K and 0.1 M (KNO₃) ionic strength. The proton-ligand dissociation constants of the ligand have been evaluated by analyzing pH titration curves using Irving-Rossotti technique¹⁸. These studies revealed that the title compound has two dissociable protons with pK_a values of 8.7 and 11.7 (Fig. 2). The lower pK_a value corresponds to dissociation of hydroxyl group present on the phenyl group and the higher pK_a value for dissociation of NH proton present in sulfonyl hydrazone moiety of compound.

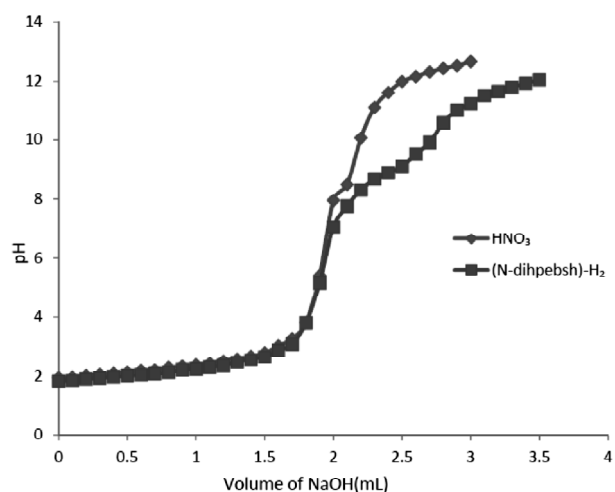


Fig. 2. pH titration curve of [(*N*-dihpebsh)-H₂] in 70% v/v 1,4-dioxane-water medium at 303 K and 0.1 M KNO₃.

Spectrophotometry :

An attempt is made to establish the stoichiometric metal to ligand ratio of the complex formed from Cu^{II} and (*N*-dihpebsh)-H₂ spectrophotometrically by using Job's and mole-ratio methods¹⁷.

In Job's continuous variation method, the solutions of Cu^{II} and (*N*-dihpebsh)-H₂ with equal concentrations (0.001 M) are mixed in different volume ratios, keeping total volume of the mixture constant (25 mL)¹⁷. The pH of the solution was maintained at 6.04 by addition of sodium acetate buffer and absorbance of each solution was measured at a wavelength of 420 nm (λ_{\max}). The graph plot-

ted between absorbance (Y-axis) and mole fraction of the ligand (X-axis) showed a maximum absorbance value at 0.5 mole fraction of the ligand indicating formation of 1 : 1 metal-ligand complex.

In mole-ratio method, the absorbance was measured for series of solutions prepared by keeping concentration of the metal ion constant and varying the ligand concentration at pH 6.04. The graph plotted between absorbance (Y-axis) and volume of the ligand (X-axis) resulted in two straight lines of different slopes; the intersection of the two lines occurs at a mole ratio corresponding to 1 : 1 composition of metal and ligand.

(B) Computational studies :

The computational studies were carried out using HyperChem 7.5 tools and ChemAxon software to derive data for molecular, binding energies of frontier orbitals,

QSAR properties and pKa values to sought correlation between computed data and experimental results.

HyperChem studies : The molecule of title compound was built using the HyperChem 7.5 software^{20,21}. The geometry optimization was carried out using the semi-empirical method at Parametric method 3 (PM3) level. The computed values for the energy, dipole moment, and energies of HOMO and LUMO were given in Table 1.

The geometry optimized structure, electron density surfaces of highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) and electrostatic potential diagrams for (*N*-dihpebsh)-H₂ and its ionic form are displayed (Fig. 3). The orientation of HOMO and LUMO orbital's of the title compound at sulfonyl hydrazone group is suitable for the coordination with metal ions.

Table 1. Molecular and orbital properties

Molecular properties		Orbital properties					
Total energy kcal/mole	Dipole moment Debye	Neutral			Ionic form		
		B.E _{HOMO}	B.E _{LUMO}	ΔB.E	B.E _{HOMO}	B.E _{LUMO}	ΔB.E
-82037	5.857	+8.377 eV	+0.601 eV	7.77 eV	-7.477 eV	-11.68 eV	4.203 eV

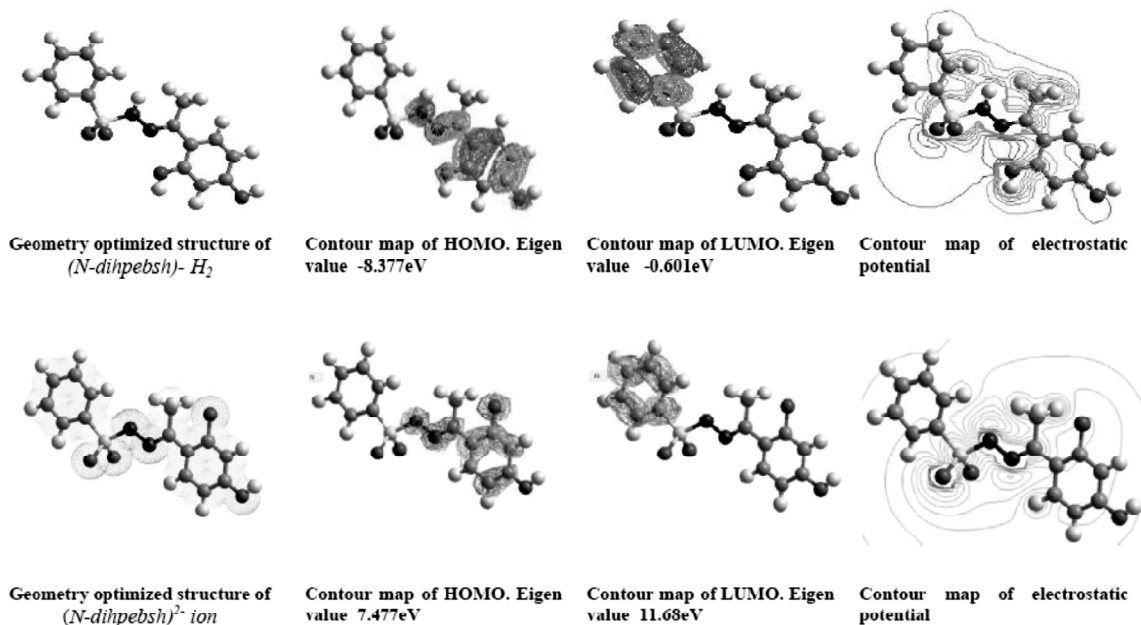


Fig. 3. Geometry optimized structures, contour maps of frontier orbital's and electrostatic potential of (*N*-dihpebsh)-H₂ and (*N*-dihpebsh)²⁻ ion.

The energies for HOMO and LUMO orbital's in ionic form were found to be -7.477 and -11.68 eV and the energy gap between HOMO and LUMO was found to be 4.203 eV. This value is less when compared to the value in non ionic form which indicates that electrons can be donated more easily and thus it will be more reactive in nature.

Quantitative structure activity relationship studies (QSAR) : The surface area, volume, hydration energy, $\log P$, refractivity, polarisability of (*N*-dihpebsh)-H₂ were also determined (Table 2). The QSAR properties are important in the evaluation of biological activity of compound.

Surface area	451.88 Å ²
Surface area grid	507.65 Å ²
Volume	837.84 Å ³
Hydration energy	17.64 kcal/mole
Refractivity	75.63 Å ³
Polarizability	28.46 Å ³
$\log P$	6.61

ChemAxon studies :

The dissociation constant (pKa) values of the title compound have been computed using ChemAxon software¹⁹, which is an advanced java, based chemical editor for drawing chemical structures. The dissociation constant values obtained for hydroxyl proton and NH proton were 8.52 and 9.50 respectively. The comparison of computed pKa values with experimental data reveals that there is close agreement in pKa value of hydroxyl proton (8.7) and variation in pKa of NH proton (11.7). Such deviation may be attributable to factors like solvent effects, keto enol tautomerism, conformational isomerism etc., under experimental conditions.

(C) *Spectroanalytical studies of metal complexes of title compound :*

Mass :

The mass spectrum of Cu^{II} complex exhibited peak at

m/z 475 corresponding to 1 : 1 metal to ligand composition. The other moieties present in complex are coordinated chloride and water as well as lattice water molecules. The mass spectral data of Ni^{II} and Co^{II} complexes also confirmed 1 : 1 metal to ligand composition.

IR :

The most characteristic vibrations are selected for the comparison of IR spectrum of the title compound and their metal complexes. The IR spectra of copper, nickel and cobalt complexes exhibited a broad trough in the range of $3450-3200$ cm⁻¹ indicating the presence of water molecule. The band of $\nu_{C=N}$ in the region of 1631 cm⁻¹ in the title compound is shifted to lower wave number in all the metal complexes indicating the participation of azomethine nitrogen in the coordination. The band at 1276 cm⁻¹ due to phenolic C-O has shifted to lower frequency of 1242 cm⁻¹ substantiating the involvement of hydroxyl group in coordination with metal ion. The new peaks in the region of 584 and 487 cm⁻¹ in the spectrum of the copper complex were assigned to stretching frequencies of M-O and M-N bonds.

UV-Visible :

The significant electronic spectra of the title compound and its metal complexes were recorded in DMSO. The important bands were observed in the region of 260-280 and 300-320 nm. They may be attributed to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions respectively. The spectra of Cu^{II}, Ni^{II} and Co^{II} complexes exhibited bands in the region of 390, 385 and 440 nm which may be assigned to *d-d* transitions.

TGA and DTA :

The thermogram of Cu^{II} complex showed a multistep decomposition. The weight loss in the temperature range 280 to 400 °C may be probably due to loss of coordinated as well as lattice water. Gradual decomposition of the metal complex is observed from 500 to 820 °C.

A further loss in the complex is observed from 1000 to 1200 °C. The residual mass at the end of the TG analysis was found to be 25% which corresponds to ox-

ide form of metal ion.

The physical transformations due to mass changes in the TG curve are recorded in the DTA thermogram. The DTA curve shows exothermic peaks at 220 and 750 °C where, decomposition of the metal complex occurs by release of heat indicating various phase changes. An endothermic peak at 1100 °C indicates total decomposition of complex.

The TGA curve of the cobalt complex showed decomposition in two steps. The mass loss observed from 100 to 300 °C is minimum and it can be assigned to loss of occluded water and water of crystallization. A maximum weight loss in the temperature range 350 to 800 °C corresponds to decomposition of the metal complex. The mass loss observed at the final stage was found to be 87% and the residue left corresponds to the weight of the metal ion.

The DTA curve showed three exothermic peaks at 250, 400 and 520 °C and one endothermic peak at 1120 °C. The exothermic peaks are attributable to various chemical and phase changes during decomposition, while endothermic peak indicates total loss of organic moiety.

SEM :

The SEM and EDX were recorded for title compound and their metal complexes. There is considerable variation in the morphology of title compound and its respective metal complexes. EDX data confirmed the elemental

composition including metal ion.

Physical, analytical and mass spectral data derived from these studies is given in Table 3.

ESR :

ESR spectral data provides information of metal ion geometry and ligand bond covalency. The ESR spectrum of Cu^{II} complex showed two g values indicating anisotropy. The $g_{||}$ value at 2.4328 and g_{\perp} value at 2.0907 indicate tetragonal geometry around copper.

(D) DNA cleavage studies :

DNA cleavage studies were carried out on the title compound and its metal complexes. Agar-gel electrophoresis technique was used with SC PBR 322 DNA at pH 7.5 in Tris-HCl buffer solution. Hydrolytic DNA cleavage experiment was monitored by the addition of the complexes (0–250 μ M) to 2 μ L of PBR 322 DNA, the total volume was increased to 16 μ L by adding 5 mM Tris-HCl/5 mM NaCl buffer. After mixing, DNA solutions were incubated for 3 h at 37 °C. The samples were electrophoresed in TAE (Tris acetic acid EDTA buffer) for 2 h at 60 V. The gel was stained using 0.5 μ g/ml EB and photographed under UV light.

The cleavage activity of the samples in present investigation was depicted in Fig. 4. Only copper complex cleaved super coiled DNA into linear DNA in hydrolytic conditions.

Table 3. Physical, analytical and mass spectral data of (*N*-dihpebsh)-H₂ and its Cu^{II}, Ni^{II} and Co^{II} complexes

Sl. No.	Ligand/Metal complex	Colour	Analysis (%) : Calcd. (Found)				Mass (m/z) [M-1] ⁺
			C	H	N	S	
1.	(N-dihpebsh)-H ₂	Yellowish orange	54.90	4.57	9.15	10.45	305
			(54.62)	(4.24)	(9.01)	(10.32)	
2.	Cu[(N-dihpebsh).Cl.H ₂ O].x.H ₂ O	Green	35.25	2.93	5.87	6.71	475
			(35.34)	(2.86)	(5.72)	(6.82)	
3.	Ni[(N-dihpebsh).Cl.H ₂ O].x.H ₂ O	Green	30.89	2.57	5.14	5.88	543
			(30.72)	(2.42)	(5.21)	(5.92)	
4.	Co[(N-dihpebsh).Cl.H ₂ O].x.H ₂ O	Brown	30.88	2.57	5.14	5.88	544
			(30.62)	(2.63)	(5.02)	(5.79)	

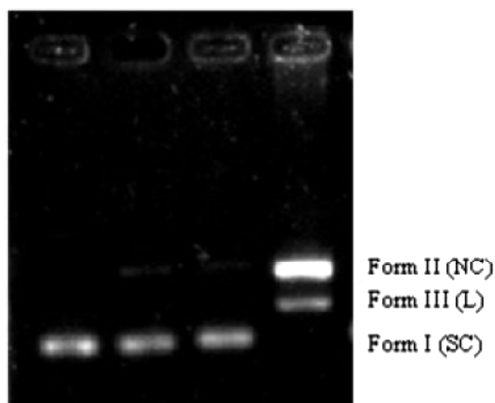


Fig. 4. DNA cleavage activity of [(*N*-dihpebsh)-H₂] and its Co^{II} and Cu^{II} complexes; Lane 1 : DNA marker, Lane 2 : (*N*-dihpebsh)-H₂, Lane 3 : [Co-(*N*-dihpebsh)], Lane 4 : [Cu-(*N*-dihpebsh)].

Conclusions

The title compound (*N*-dihpebsh)-H₂ was synthesized and characterized employing spectro analytical techniques. As the title compound has ligational properties, the equilibrium studies were made applying pH-metry and spectrophotometry techniques to explore the number of donor sites suitable for bonding with specific metal ion. The dibasic acid nature of compound revealed from pH metric studies was further corroborated by ChemAxon software calculations. The molecular properties, molecular orbital and QSAR parameters were determined by computational methods and correlated with experimental results. The electron density figures of HOMO computed for both molecular and ionic forms of compound under study support the involvement of molecular orbital's oriented along sulfonyl hydrazone moiety in bonding with metal ions. The metal complexes of Cu^{II}, Ni^{II} and Co^{II} have been synthesized and characterized by spectro-analytical data. The DNA cleavage studies with the title compound and its complexes under study inferred the active role of copper complex. The Cu^{II} complex cleaved hydrolytically super coiled form of DNA to linear form thus emphasizing the role of copper metal.

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