

Synthesis, structural characterization and DNA studies of trivalent cobalt complexes of (2*E*)-⁴*N*-substituted-2-[4-(propan-2-yl)benzylidene]hydrazinecarbothioamide

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Abstract: This paper describes trivalent cobalt complexes of hydrazinecarbothioamides derived from 4-(propan-2-yl) benzaldehyde and substituted thiosemicarbazides NH₂NHC(S)NHR, where R = H (**1**), Me (**2**), Et (**3**) or Ph (**4**) have been synthesized and characterized. The prepared ligands and complexes were characterized using various physicochemical techniques viz. elemental analysis, molar conductance, magnetic susceptibility measurements, IR, electronic absorption spectral studies and cyclic voltammetry. The electronic spectra in DMSO solvent and magnetic susceptibility data of complexes reveal that the complexes are diamagnetic with low spin octahedral cobalt(III) complexes. The absorption titration studies revealed that each of these complexes is an avid binder to calf thymus-DNA. The apparent binding constants are in the order of 10⁷–10⁸ M⁻¹. The nucleolytic cleavage activities of the ligands and their complexes were assayed on pUC18 plasmid DNA using gel electrophoresis in the presence and absence of H₂O₂. The ligands showed increased nuclease activity when administered as cobalt complexes. All the complexes behave as efficient chemical nucleases with hydrogen peroxide activation. These studies revealed that the complexes exhibit both oxidative and hydrolytic chemistry in DNA cleavage.

Keywords: 4-(Propan-2-yl)benzaldehyde; thiosemicarbazones; Co(III) complexes; DNA studies.

Introduction

Thiosemicarbazones (TSCs) are a class of compounds obtained by condensation of thiosemicarbazide with suitable aldehydes or ketones. Designing of novel thiosemicarbazone ligands have been growing interest due to their diverse chelating properties and pharmacological activities viz. antibacterial ¹, antifungal ² antihypertensive, antineoplastic, antiproliferative activity ³⁻⁶, anticancer activity ^{7,8} etc. The biological activity of the ligands is due to the ability to form chelates with transition metal ions bonding with azomethine nitrogen and sulphur. The presence of additional groups makes them potential polydentate ligands. In most complexes thiosemicarbazones behave as bidentate ligands because they can bond to metals through sulphur and the hydrazinic nitrogen atoms ⁹, although in a few cases they behave as unidentate ligands and bond through only sulphur atom ¹⁰. In some cases, thiosemicarbazones act as a C, N, S donor, forming cyclometallated complexes^{11, 12}. The metal complexes of thiosemicarbazones are not only the bioinorganic relevance but also the chemistry of transition metal

complexes of the thiosemicarbazones is receiving significant current attention as potent Analytical agents ¹³⁻¹⁵, Photocatalysts ^{16,17}, intermediates for the synthesis of pharmaceutical, dyes, photographic films, plastic and in textile industry.

It is well established that the transition metal complexes of TSCs are more biologically active than the free ligands, probably due to the increased lipophilicity (which controls the rate of entry into the cell) of the complexes. The presence of metal ions does not only improve upon their biological activities, selectivity, chemical stability, and their usually low water solubility, but also mitigates their side effects ¹⁸.

Recently, Pd(II), Pt(II) ^{19,20}, Zn(II), Cd(II) ²¹ and Cu(II) ⁹, Cu(I)¹⁰, Ni(II) ²² complexes of (2*E*)-⁴*N*-substituted-2-[4-(propan-2-yl)benzylidene]hydrazinecarbothio-amide have been synthesized, characterized, and found to exhibit strong to moderate biological activities. In the view of these finding and continuation of our work on thiosemicarbazones ^{9,10,16,17,24-28}, we report the

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use of ⁴N-substituted 2-[4-(propan-2-yl)benzylidene]hydrazinecarbothioamides.

Experimental

Materials and methods

Thiosemicarbazide, 4-methyl-3-thiosemicarbazide, 4-ethyl-3-thiosemicarbazide, 4-phenyl-3-thiosemicarbazide and cuminaldehyde (p-isopropyl benzaldehyde) were of reagent grade purchased from Sigma-Aldrich. All other chemicals were of AR grade and used as supplied. The solvents were distilled before use. Calf thymus DNA was purchased from Genie Bio labs, Bangalore, India. The plasmid pUC18 DNA was isolated from *E. coli* DH5a strains in Lusbria Broth (LB) medium supplemented by ampicillin cells from 5 ml culture by Qiagen column following the manufacturer's protocol.

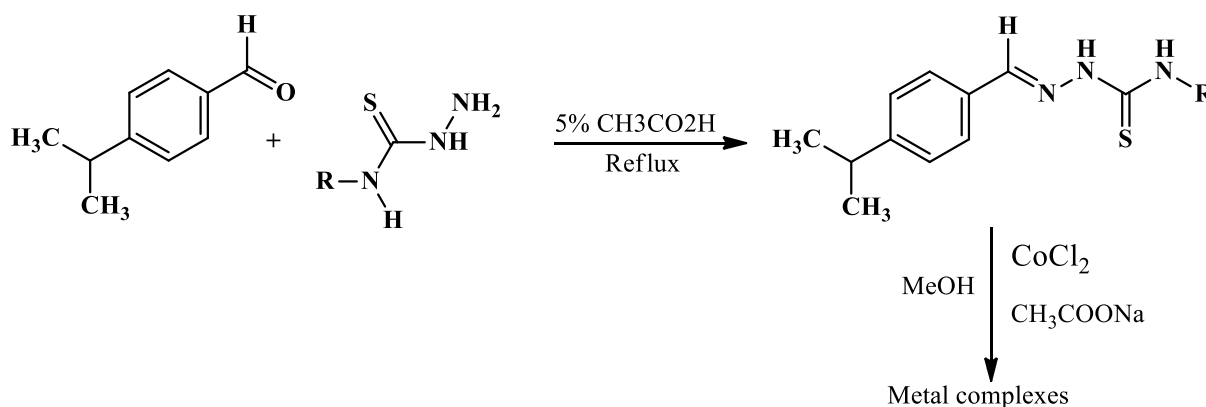
Physical measurements

Elemental analysis was carried out on a Perkin-Elmer 2400 CHNS elemental analyzer. Magnetic susceptibility measurements were carried out on a magnetic susceptibility balance (Sherwood Scientific,

Cambridge, England), high purity CuSO₄·5H₂O was used as a standard. Molar conductance (10⁻³M) in DMF at 30±2°C was measured with a CC180 model (ELICO) direct reading conductivity bridge. The electronic spectra were recorded in DMSO with a Shimadzu UV-160A spectrophotometer. FT-IR spectra were recorded in the range 4,000–270 cm⁻¹ in KBr discs on a Nicolet protégé 460 IR Spectrometer. The cyclic voltammetric measurements were performed on a Bio Analytical System (BAS) CV-27 assembly equipped with an X-Y recorder. Measurements were made on degassed (N₂ bubbling for 5 min) ligand/complex solutions (10⁻³ M) in DMF and ethanol containing tetrabutylammonium perchlorate (0.1 M) as a supporting electrolyte. The three-electrode system consisted of a glassy carbon (working), platinum wire (auxiliary) and Ag/AgCl (reference). The ¹H- and ¹³C{¹H}-NMR spectra were recorded on a Bruker Spectrospin DPX-300 NMR spectrometer at 300.13 and 75.47 MHz, respectively.

Preparation of the thiosemicarbazones

The ligands were prepared (Scheme 1) according to published procedure ⁹.



Scheme 1. Preparation of the ligands and their metal complexes

Preparation of the complexes

To a methanolic solution of appropriate ligand (2mol) added 1 gm of sodium acetate to maintain pH (8-9) of the solution. Then added a solution of cobalt(II) chloride (1mol) in methanol. The reaction mixture was refluxed for about 1 hr., during which time a solid complex formed was cooled to room temperature, the resulting product was washed with hot water and finally with diethyl ether and dried in vacuum desiccators over anhydrous CaCl₂.

DNA binding experiments

A solution of CT-DNA in 0.5mM NaCl/5mM Tris-HCl (pH 7.0) gave a ratio of UV absorbance at 260 and 280 nm (A₂₆₀/A₂₈₀) of 1.8–1.9, indicating that the DNA was sufficiently free of proteins ²⁹. A concentrated stock solution of DNA was prepared in 5 mM Tris-HCl/50 mM NaCl in water at pH 7.0 and the concentration of

CT-DNA was determined per nucleotide by taking the absorption coefficient (6,600 dm³mol⁻¹cm⁻¹) at 260 nm ³⁰. Stock solutions were stored at 4°C and were used after no more than 4 days. Doubly distilled water was used to prepare buffer solutions. Solutions were prepared by mixing the complex and CT-DNA in DMF medium. After equilibrium was reached (ca. 5 min) the spectra were recorded against an analogous blank solution containing the same concentration of DNA.

The data were then fitted into Eq. 1 to obtain the intrinsic binding constant (K_b) ³¹:

$$\frac{[DNA]}{\epsilon_A - \epsilon_B} = \frac{[DNA]}{\epsilon_B - \epsilon_F} + \frac{1}{K_b(\epsilon_B - \epsilon_F)} \quad \text{---- (1)}$$

Where [DNA] is the concentration of DNA in base pair, ϵ_A , ϵ_B , ϵ_F corresponds to the molar extinction coefficients of apparent, bound and free metal complexes respectively. A plot of

$[\text{DNA}]/(\epsilon_A - \epsilon_F)$ Vs $[\text{DNA}]$, gave a slope $1/(\epsilon_B - \epsilon_F)$ and a Y-intercept equal to $1 / K_b (\epsilon_B - \epsilon_F)$; K_b is the ratio of slope to the intercept.

Assay of nuclease activity

DMF solutions of the complexes were placed in clean Eppendorf tubes and 1 μg of pUC18 DNA was added. The contents were incubated for 30 min at 37°C and loaded on 0.8% Agarose gel after mixing 5 μl of loading buffer (0.25% bromophenol blue + 0.25% Xylene cyanol + 30% glycerol sterilized distilled water). Electrophoresis was performed at constant voltage (100 V) until the bromophenol blue reached to the 3/4th of the gel. The gel was stained for 10 min by immersing in an ethidium bromide solution. The gel was then destained for 10 min by keeping in sterilized distilled water and the plasmid bands visualized by photographing the gel under a UV Transilluminator. The efficiency of DNA cleavage was measured by determining the ability of the complex to form open circular (OC) or nicked circular (NC) DNA from its super coiled (SC) form. The reactions were carried out under oxidative and/or hydrolytic conditions. Control experiments were done in the presence of hydroxyl scavenger, DMSO.

Results and discussion

Characterization of the free thiosemicarbazones and their metal complexes

The detailed characterization of the ligands was reported from the same group^{9, 10, 22}. The analytical data of the thiosemicarbazones are given in Table 1.

A Brown colored cobalt(III) complexes of thiosemicarbazones (**1-4**) are stable at room temperature, non-hygroscopic, sparingly soluble in methanol, ethanol, and readily soluble in chloroform, pyridine, dimethylformamide (DMF) and dimethylsulphoxide (DMSO). The analytical data (Table 1) suggest 1: 3 (M: L) composition for the complexes.

Conductivity and Magnetic susceptibility measurements:

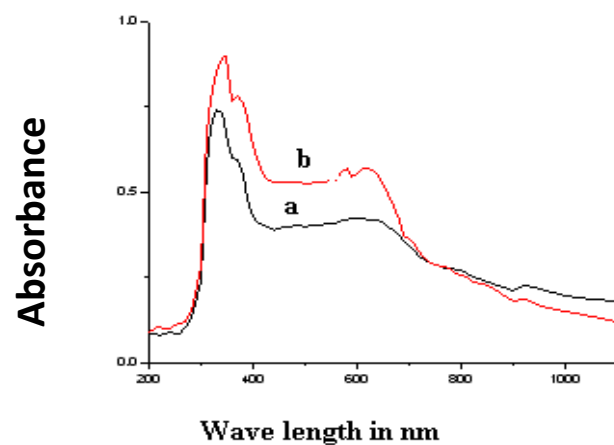
All cobalt complexes are highly soluble in DMF. Therefore, the metal complexes were dissolved in DMF to perform conductivity measurements. The molar conductivities of cobalt complexes in DMF at room temperature are found in the range of 26-46 $\text{Ohm}^{-1}\text{cm}^2\text{mol}^{-1}$ suggest the non-electrolytic nature³² of complexes. The Magnetic susceptibility of cobalt complexes are presented in Table 1. The data reveal that the complexes are diamagnetic in favour of formation of low spin octahedral cobalt(III) complexes.

Electronic spectra

Typical electronic spectra of complexes **1a** and **2a** are given in Fig. 1. The electronic spectra of complexes were recorded in DMSO solvent. Spectral data and their assignments are given in Table 2. Two d-d bands are observed in the electronic spectra present cobalt(III) complexes. High energy bands overlap considerably with CT band.

Table 1. The physical and analytical data of the ligand and its metal complexes.

Compound	Yield %	M.P (°C)	Elemental analysis Cal (Found)%				Magnetic susceptibility(χ_g)# $\times 10^{-6}$ #	Λ_M^*
			C	H	N	S		
CTH (1)	87	144–147	59.50 (59.70)	6.20 (6.80)	18.9 (18.9)	14.40 (14.50)	-	-
Co(CT)₃ (1a)	82	195-198	55.29 (55.06)	5.58 (5.88)	16.94 (17.51)	12.55 (13.33)	- 0.202	47
CMTH (2)	79	145–147	60.90 (61.20)	7.10 (7.30)	17.4 (17.8)	13.60 (13.60)	-	
Co(CMT)₃(2a)	86	208-211	55.02 (56.75)	6.18 (6.35)	15.08 (16.54)	11.89 (12.62)	- 0.553	37
CETH (3)	59	115–118	62.90 (62.60)	7.60 (7.70)	16.9 (16.8)	14.10 (14.10)	-	-
Co(CET)₃ (3a)	54	220-222	57.71 (58.27)	6.48 (6.77)	15.02 (15.68)	12.88 (11.97)	- 0.561	26
CPTH (4)	45	140–142	68.50 (68.60)	6.20 (6.40)	13.90 (14.10)	10.70 (10.80)	-	-
Co(CPT)₃(4a)	61	155-157	64.65 (64.60)	5.55 (5.74)	12.34 (13.29)	10.80 (10.15)	- 0.324	38

#cgs units * $\text{Ohm}^{-1} \text{cm}^{-2}\text{mol}^{-1}$ **Figure 1.** Electronic spectra of (a) complex 1a (b) complex 2a in DMSO

These bands (from low energy to high energy) are assigned to ${}^1A_{1g} \rightarrow {}^1T_{2g}$ and ${}^1A_{1g} \rightarrow {}^1T_{1g}$ transitions in favour of octahedral low spin cobalt(III) complexes.

Table 2. Electronic spectral data of cobalt(III) complexes.

Complex	CT transition	${}^1A_{1g} \rightarrow {}^1T_{2g}$	${}^1A_{1g} \rightarrow {}^1T_{1g}$
1a	30769 (88.4)	24390 (82.9)	15923 (41.9)
2a	30303 (74.9)	23809 (40.8)	16393 (42.1)
3a	28571 (79.8)	22988 (60.1)	16129 (45.9)
4a	27778 (87.8)	25641 (76.3)	16449 (56.9)

Table 3. Selected I.R. bonds (cm^{-1}) of Cobalt (III) complexes with tentative assignment.

Ligand / Complex	$\nu(\text{NH}_2/\text{NHR})$	$\nu(\text{NH})$	$\nu(\text{C}=\text{N})$	$\nu(\text{C}=\text{S})$	$\nu(\text{C}-\text{S})$	$\nu(\text{Co}-\text{S})$	$\nu(\text{Co}-\text{N})$
1	3412(s) 3280(s)	3156(s)	1590(s)	1181(m)	-	-	-
2a	3416 (s) 3261 (br)	-	1554 (s)	-	692 (s)	349 (s)	538 (br)
2	3316(br)	3159(m)	1608(s)	1177(m)	-	-	-
2a	3319 (br)	-	1600 (s)	-	690 (s)	-	544 (br)
3	3300(br)	3143(br)	1607(s)	1178(m)	-	-	-
3a	3315 (s)	-	1533 (s)	-	697 (s)	398(s)	537 (br)
4	3306(s)	3133(br)	1596(s)	1196(s)	-	-	-
4a	3315 (s)	-	1598 (s)	-	664 (s)	368 (s)	542 (s)

s=sharp, m=medium, br=broad

Infrared Spectra

Important IR spectral bands of the complexes are compared with the ligands spectra. Important infrared spectral data and their tentative assignments are presented in Table 3. The IR spectra of the ligands show bands in 3412–3280 cm^{-1} region assigned to terminal NH_2/NHR group vibrations. These bands are not affected in complexes suggesting non-participation of terminal $-\text{NH}_2/\text{NHR}$ group in coordination. In the spectra of the ligands a strong band is observed in 1177–1196 cm^{-1} region due to $\nu(\text{C}=\text{S})$ stretching vibration, no band is observed near 2575 cm^{-1} suggesting that the ligands remain in thione form at least in solid state. In IR spectra of complexes this band is disappeared indicating the bond formation between cobalt and enolic sulphur.

This is confirmed by the presence of new band in 664–697 cm^{-1} region assignable to $\nu(\text{C}-\text{S})$. This band is possible when sulphur binds to metal in the thiol form^{33, 34}. A strong band is observed in 1590–1608 cm^{-1} region is due to $\nu(\text{C}=\text{N})$ stretching vibration of azomethine nitrogen atom in ligands. This band is shifted lower/higher frequencies ($\Delta\nu = \pm 8-17 \text{ cm}^{-1}$), suggesting coordination of azomethine nitrogen atom in complex formation^{35, 36}. Thus the ligands act as mono anionic bidentate ligands.

Based on the molar conductance, magnetic moment, electronic and I.R. data, it is suggested that all the cobalt complexes are non-electrolytes and have octahedral structure (Fig.2).

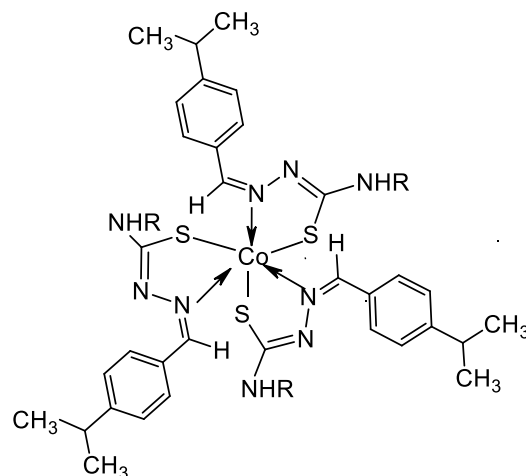


Figure 2. A General and tentative structure for cobalt(III) complexes of ligands.

Electrochemical studies

Electrochemical behavior of cobalt complexes is studied by using cyclic voltammetry. Cyclic voltammograms of the cobalt(III) complexes were recorded in DMF and ethanol in tetra butyl ammonium perchlorate (0.1M) as supporting electrolyte. The electrochemical data of all complexes obtained at the glassy carbon electrode

are given in Table 4. Cyclic voltammograms of cobalt(III) complexes showed two active responds in ethanol and DMF medium. In ethanolic medium, two $E_{1/2}$ values are obtained in the potential regions of -1.235 to -1.385 V and -0.545 to -0.715 V vs Ag/AgCl reference electrode.

Table 4. Cyclic voltometric data of cobalt complexes.

Complex	Redox couple	E_p /V		E_p /V		ΔE_p /mV		$E_{1/2}$		$\log K_c^a$		$-\Delta G^\circ$ ^b	
		EtOH	DMF	EtOH	DMF	EtOH	DMF	EtOH	DMF	EtOH	DMF	EtOH	DMF
1a	III/II	-0.08	-0.23	-1.01	-0.86	930	630	-0.54	-0.54	0.035	0.052	207	306
	II / I	-1.16	-1.08	-1.31	-1.44	150	360	-1.24	-1.26	0.218	0.090	1285	530
2a	III/II	+0.02	-0.20	-1.19	-0.82	1210	620	-0.58	-0.51	0.027	0.052	159	306
	II / I	-1.18	-1.09	-1.32	-1.46	140	370	-1.25	-1.27	0.233	0.088	1374	518
3a	III/II	-0.20	-0.19	-1.23	-0.94	1030	745	-0.71	-0.57	0.032	0.043	188	253
	II / I	-1.28	-1.08	-1.49	-1.45	210	325	-1.38	-1.26	0.155	0.087	974	513
4a	III/II	+0.10	-0.24	-1.23	-0.97	1240	725	-0.61	-0.61	0.026	0.045	152	265
	II / I	-1.30	-1.10	-1.42	-1.46	120	365	-1.36	-1.28	0.272	0.089	1604	524

^a $\log K_c = 0.434ZF/RT\Delta E_p$, ^b $\Delta G^\circ = -2.303RT \log K_c$

These are respectively assigned to Co(II)/ Co(I) and Co(III)/ Co(II) redox couples. Repeated scans as well as various scan rates showed that dissociation does not take place in these complexes. The non-equivalent current intensity of cathodic and anodic peak difference ($\Delta E_p = 120-1240$ mV) indicates quasi reversible behaviour of these complexes. The ΔE_p values are greater than the Nernstian values ($\Delta E_p \approx 59$ mV) for one electron redox system. This indicates a considerable reorganization of the coordination sphere during electron transfer has been observed for a number of other cobalt (III) complexes. From Table 4, $E_{1/2}$ values of the complexes in DMF medium are slightly higher than the values obtained in ethanol.

A comparison of the $E_{1/2}$ values of this redox couple of the present complexes with other analogous nitrogen donor macro cycles reveal that these complexes undergo more facile redox change which seem to be a requirement to the DNA cleavage³⁷.

DNA binding studies

The interaction of cobalt complexes with calf thymus DNA was studied by absorption titrations using spectrophotometer. The absorption titrations were carried out with increasing amount of CT-DNA in 363-367 nm regions. With addition of DNA all cobalt complexes showed hyperchromic shift.

Table 5. Electronic absorption data upon addition of CT-DNA to nickel complex.

Complex	λ_{\max}/nm		$\Delta\lambda/\text{nm}$	H (%)	$K_b (M^{-1})$
	Free	Bound			
1a	365	364	1.0	-25.85	9.58×10^7
2a	365.5	364	1.5	-33.29	2.84×10^7
3a	365	366	1.0	-28.35	4.54×10^7
4a	365	366	1.0	-24.92	1.21×10^8

From Table 5, it is revealed that in the presence of increasing amount of CT-DNA absorption spectra of complexes show either red-shift or blue-shift ($\Delta\lambda_{\max}: 1.0-1.5$ nm) and hyperchromism [hyperchromism: -25.85 % for **1a**, -33.29 % for **2a**, -28.35 % for **3a**, and -24.92 for **4a**. The orders of binding constants of complexes are $2a < 3a < 1a < 4a$.

DNA nuclease activity

Gel electrophoresis experiments using pUC18 plasmid DNA were performed with cobalt complexes in presence/absence of H_2O_2 as oxidant. At micro molar concentration for 30 min incubation time all

complexes show significant cleavage activity in absence and presence of oxidant. Fig. 3 shows the cleavage pattern of cobalt complexes at physiological conditions. (Compare to lanes 1 and 2, lanes 3-10, shows the linear form in addition to super coiled and nicked forms). To know the cleavage mechanism, control experiments were performed in presence of hydroxyl free radical scavenger, DMSO and singlet oxygen quencher, azide ion (Fig. 4). In the presence of DMSO, azide, there is no significant cleavage activity for complexes. This observation suggests that the complexes produce hydroxyl free radicals (in the presence of oxidant) that cleave DNA.

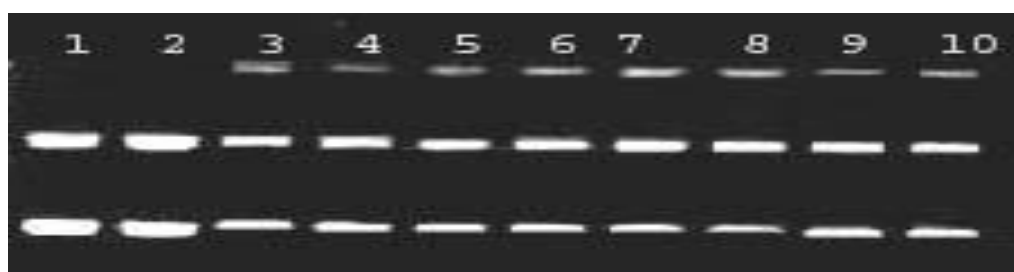


Figure 3. Agarose gel (0.8%) showing results of electrophoresis of 3 μL of pUC18 DNA; 2 μL 0.1M TBE buffer (pH 8); 2 μL complex in DMF (10^{-3} M); 10 μL water, 2 μL H_2O_2 (Total volume 20 μL) were added respectively, incubated at 37 $^\circ\text{C}$ (30 min): Lane 1: DNA control, Lane 2: DNA+ H_2O_2 , Lane 3:**1a**+DNA, Lane 4:**1a**+DNA+ H_2O_2 , Lane 5: **2a**+DNA, Lane 6: **2a**+DNA+ H_2O_2 , Lane 7: **3a**+DNA, Lane 8:**3a**+DNA+ H_2O_2 , Lane 9:**4a**+DNA, 10:**4a**+DNA+ H_2O_2 .



Figure 4. Lane 1: Marker, Lane 2: DNA control; Lane 3: DNA+CoCl₂; Lane 4: 1a+DNA; Lane 5: 1a+DNA+DMSO; Lane 6: 1a+DNA+azide; Lane 7: 2a+DNA; Lane 8: 2a+DNA+DMSO; Lane 9: 2a+DNA+azide; Lane 10: 3a+DNA; lane 11: 3a+DNA+DMSO; Lane 12: 3a+DNA+azide; Lane 13: 4a+DNA; Lane 14: 4a+DNA+DMSO; Lane 15: 4a+DNA+azide.

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