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비타민 B_{12} 모델 착물: O-주개 리간드인 Thiocyanato Cobaloximes 및 Thiocyanato로 연결된 Dicobaloximes의 합성 및 특성규명: DNA 결합 및 향균 활성

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Vitamin B_{12} Model Complexes: Synthesis and Characterization of Thiocyanato Cobaloximes and Thiocyanato Bridged Dicobaloximes of O-donor Ligands: DNA Binding and Antimicrobial Activity

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요약. **L**이 urea, acetamide, semicrabazide, formamide 인 thiocyanato (L) Cobaloxime 착물을 합성하고 특성을 규명하였다. Thiocyanato(L)cobaloximes ($SCNCo(DH)_2(L)$)과 benzyl (aquo) cobaloxime $PhCH_2Co(DH)_2(OH_2)$를 반응시켜 일반 분자식 $PhCH_2Co(DH)_2SCNCo(DH_2)(L)$를 갖는 일련의 thiocyanato로 연결된 dicobaloximes 생성물을 얻었다. Thiocyanato 리간드의 다리결합을 포함하고 있는 dicobaloximes의 조성에 대한 증거로 말단의 thiocyanocobaloxime ($SCNCo(DH)_2(L)$)으로부터 dicobaloxime이 형성되면서 νCN이 20 - 45 cm^{-1} 증가하게 되는 적외선 데이터를 들 수 있다. 이러한 두 일련의 물질에 대한 더 많은 특성을 (^1H, ^{13}C) NMR, LCMS 및 원소분석을 통하여 확인하였다. Thiocyanato (L) cobaloximes 및 thiocyanato로 연결된 dicobaloxime의 항균 활성은 *E. Coli*에 의해 조사하였다. 두 단량체와 이합체 모두의 DNA-결합 행동은 분광학적 방법 및 점성도 측정을 통하여 조사하였다. 그 결과 이합체 착물은 calf-thymus DNA와 DNA의 염기쌍에 말단의 벤질 고리를 통해 사이에 끼인 형태로 결합되어 있음을 나타내었다. 단량체 착물은 DNA와 상호작용하지 않는 것으로 관찰되었다.

주제어: Thiocyanato 리간드 cobaloximes, Thiocyanato 로 연결된 dicobaloximes, 항균 활성, DNA 결합

ABSTRACT. Complexes of thiocyanato(L)cobaloximes where L is urea, acetamide, semicrabazide and formamide were synthesized and characterized. The reaction of thiocyanato (L) cobaloximes ($SCNCo(DH)_2(L)$) with benzyl (aquo) cobaloxime $PhCH_2Co(DH)_2(OH_2)$ was found to produce a series of thiocyanato bridged dicobaloximes of a general formula of $PhCH_2Co(DH)_2SCNCo(DH_2)(L)$. Evidence for formulation as dicobaloximes containing thiocyanato ligand bridges was obtained from infrared data which show 20 - 45 cm^{-1} increase in νCN upon formation of the dicobaloxime from the corresponding terminal thiocyanocobaloxime ($SCNCo(DH)_2(L)$). Further characterization of these two series was done on the basis of (^1H,^{13}C)NMR, LCMS and elemental analysis. Anti-microbial activity of thiocyanato(L)cobaloximes and thiocyanato bridged dicobaloximes were screened against *E. Coli*. The DNA-binding behaviors of both monomers and dimers were investigated by spectroscopic methods and viscosity measurements. The results indicated that the dimer complexes bind with calf-thymus DNA in an intercalative mode via the terminal benzyl ring into the base pairs of DNA. It was observed that the monomer complexes did not interact with DNA. Fluorescence spectra for the interaction between thiocyanato bridged dicobaloximes and DNA were also studied.

Keywords: Thiocyano ligand cobaloximes, Thiocyanato bridged dicobaloximes, Antimicrobial activity, DNA binding

INTRODUCTION

Cobaloximes are complexes containing the bis(dimethylglyoximato)cobal(III) moiety, $Co(DH)_2^+$. These produce the fundamental reactions of cobalamins and are important in the study of the mechanism of vitamin-B_{12} catalyzed biochemical process.[1] Schrauzer suggested the method for making alkylcobaloximes.[2-3] Varieties of Organocobalt (III) complexes with stable Co-C σ bond were synthesized as model complexes of coenzyme-B_{12}.[4-6] The mechanism of the action of the coenzyme-B_{12} dependent enzymes were shown to involve a net substrate rearrangement in which

hydrogen atom interchanges with substitutents on an adjacent carbon atom.[7-11] The longstanding hypothesis and most widely accepted explanation for the enzymic process falls under the umbrella term mechanochemical trigger. It was felt that enzyme-induced conformational change in the enzyme leads to a conformation with greatly weakened Co-C bond.[12] The ligand-bridged complexes are of interest in view of their role as reaction intermediates in inner-sphere electron-transfer process[13] and this synthetic strategy was often utilized to provide a general route to synthesize cationic dinuclear cyano-bridged complexes.[14] It is therefore, interesting and useful to study the thiocyanato bridged binuclear inorganic cobaloximes of the type $PhCH_2Co(DH)_2SCNCo(DH_2)(L)$ where (L = urea, acetamide, formamide and semicarbazide) and that should be useful to compare with the corresponding mononuclear cobaloximes $Co(DH)_2SCN(L)$. The present work is concerned with the synthesis and characterization of a new series of thiocyanato bridged dicobaloximes of the general formula $PhCH_2Co(DH)_2SCNCo(DH_2)(L)$. These complexes are of interest because they contain thiocyanato ligand that bridges two cobalt(III) metal centers by simultaneous coordination of the thiocyanato sulphur on cobaloxime and the other thiocyanato nitrogen to another cobaloxime, resulting in an overall neutral dimer. In this paper we wish to report on the preparation, properties and the reactions of benzyl(aquo)cobaloximes with the above mentioned O-donor ligands and complexes. The overall evidence from the literature strongly establishes that many of the chemical properties related to the axial fragment, such as the geometry and spectroscopic behavior, are significantly affected by a change in the equatorial ligand (cis effect and cis influence). Hence, there has been a sustained interest in the synthesis of new organocobaloximes with new or modified equatorial ligands. We have, therefore, undertaken the study of cobaloximes of the type $LCo(DH)_2SCN$ and $PhCH_2Co(DH)_2SCNCo(DH)_2L$. Most of the complexes are new and have been synthesized for the first time. The cis and trans influence was studied by UV-visible spectra. IR, ^1H & ^{13}C-NMR, elemental analysis and LC/MS.

Metal ion coordination to nucleic acids is not only required for charge neutralization, it is also essential for the biological function of nucleic acids. Canpolat *et al.*[15] reported that vic-dioxime of cobalt(III) complexes were the most active and may be promising candidates for the development of new antibiotics. The precise understanding of the DNA binding properties of metal complexes gains importance because of therapeutic approaches. Binuclear transition metal complexes can bind to DNA by non-covalent interactions such as external surface binding, groove binding for large molecules and for compounds containing a ring system. In this paper, we report the synthesis and characterization of the monomer $SCNCo(DH)_2L$ and dimers of the complexes $PhCH_2Co(DH)_2SCNCo(DH_2)L$ and their ability to bind with CT-DNA. The binding properties of DNA were studied by electronic absorption and luminescence spectra.

EXPERIMENTAL METHODS

Materials

$Co(NO_3)_2\ 6H_2O$, KSCN and $PhCH_2Br$ were purchased from Aldrich Chemicals, solutions of calf thymus DNA in 5 mM Tris-HCl buffer (pH 7.2), 50 mM NaCl showed a ratio of UV absorbance at 260 and 280 nm of about 1.9 indicating that the DNA was free from protein.[16] The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 $M^{-1}cm^{-1}$) at 260 nm.[17] All other Chemicals used were of analytical reagent grade and were used without further purification unless otherwise noted. The complexes of the type $Co(DH)_2SCN(L)$ and $PhCH_2Co(DH)_2SCNCo(DH_2)L$ were prepared by using the following procedures.

Synthesis of thiocyanato bridged dicobaloxime

To prepare thiocyanato bridged dicobaloximes of the type $PhCH_2Co(DH)_2SCNCo(DH)_2(L)$, first thiocyano ligand cobaloximes[18] of the type $SCNCo(DH)_2(L)$ were synthesized by bubbling air (direct air oxidation method)[19] for 2 to 6 hours through an ethanolic solution of cobalt(II)thiocyanate $Co(SCN)_2$ which was in turn synthesized by metathetic reaction of hydrated $Co(NO_3)_2$ & KSCN in methanolic medium. The precipitated KNO_3 was filtered off and the solution was used as the source of $Co(SCN)_2$ which was mixed with dimethylglyoxime and the ligand taken in 1:2:2 proportions. This solution was allowed to stand and the resulting crystalline solid was washed with ethanol and ether, finally dried in vacuo. The reaction presumably proceeded in the following manner:

$$2KSCN + Co(NO_3)_2 \rightarrow 2KNO_3 + Co(SCN)_2 \quad (1)$$

$$2Co(SCN)_2 + 4DH_2 + 2L + 1\backslash 2O_2 \rightarrow 2[SCNCo(DH)_2L] + 2HSCN + H_2O \quad (2)$$

The thiocyanato bridged dicobaloximes was synthesized as follows:[20] 2.45×10^{-4} moles of $PhCH_2Co(DH)_2(OH_2)$ was dissolved in a minimum amount of chloroform at 40 °C to give an orange solution, to this an equimolar concentration

of the complex $SCNCo(DH)_2L$ which was dissolved separately in a minimum amount of chloroform at 40 °C was added. The two solutions were mixed and stirred constantly at 40 - 50 °C for 1 hour. The solvent was removed under reduced pressure to give a yellow powder, which was washed with water and 90% of methanol and ether then dried in vacuo.

The benzyl(aquo) cobaloxime was prepared by using the procedure of Brown *et al*.[21] Eq (3-5)

$$Co(CH_3COO)_2{\cdot}4H_2O + 2DH_2 \rightarrow [Co^{11}(DH)_2(H_2O)] + 2CH_3COOH + 3H_2O \quad (3)$$

$$[Co^{11}(DH)_2(H_2O)] + 1/2H^+ + 1/2H^- \rightarrow H[Co(DH)_2H_2O] \quad (4)$$

$$H[Co(DH)_2H_2O] + PhCH_2Br \rightarrow [PhCH_2Co(DH)_2(H_2O) + HBr \quad (5)$$

All manipulations were performed under minimal illuminations due to photolability of benzyl(aquo)cobaloxime bond and the solutions were covered with aluminum foil. *Fig*. 1.

Physical measurement

Transition metal complexes often have absorption bands in the visible region leading to their interesting coloration. Ligand field spectra of these thiocyano ligand cobaloxime complexes in ethanol show a peak of weak to moderate intensity[22] at around 19800 cm^1.

This is the spin allowed $^1A_{1g} \rightarrow {}^1T_{1g}$ transition. The $^1A_{1g} \rightarrow {}^1T_{2g}$ of the trans $SCNCo(DH)_2L$ complexes is masked by the intense charge-transfer bands. IR spectra were recorded in KBr discs on a Perkin-Elmer FTIR 1600 spectrometer, ^1H and ^{13}C NMR were measured on a varian XL-300 MHz spectrometer, with DMSO-d_6 solution as the solvent at room temperature by using tetramethylsilane (TMS) as the internal standard, Chemical shifts (δ) were given in ppm. For the absorption spectra an equal amount of DNA was added to both the complex solution and the reference solution to eliminate the absorption of the DNA itself.

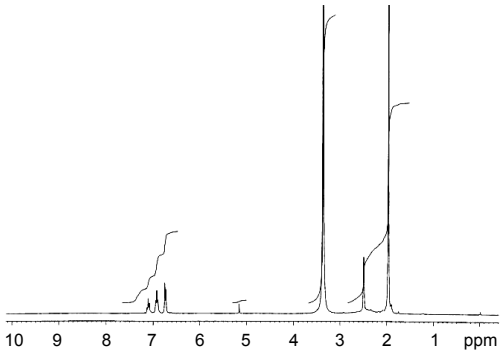


***Fig.* 1.** ^1H NMR spectra of benzyl (aquo) cobaloxime in DMSO-d_6.

DNA binding

Concentrated stock solutions of metal complex were prepared by dissolving the complex in acetone: water (1:100) and suitably diluted with buffer to required concentrations for all experiments. All the experiments involving the interaction of Co dibridged complexes with DNA were conducted in a pH 7.2. Tris buffer containing 5 mM tris (hydroxymethyl) aminomethane (Tris), 50 mM NaCl in doubly distilled water. Solutions of CT-DNA (calf-thymus DNA) showed a ratio of UV absorption at 260 and 280 nm, of about 1.91 indicating that DNA was sufficiently free of protein contamination.[23] DNA concentration per nucleotide was determined (ε = 6600 $M^{-1}cm^{-1}$) at 260 nm.

Antimicrobial activity

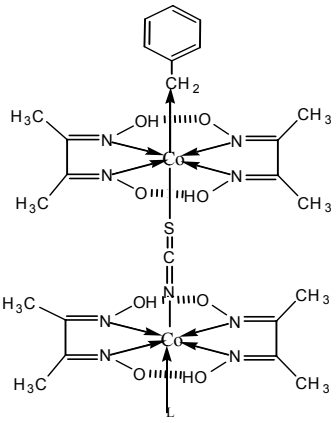
For the determination of antimicrobial activity, well diffusion method was carried out using Mueller-Hinton agar. The complexes were dissolved in acetone and the medium was sterilized in an autoclave at 135 °C for 5 minutes. Twenty mL of the sterilized medium was poured into pre-sterilized Petri-plates and allowed to solidify. After the solidification, the wells were made on agar using cork borer using L-shape glass rod, 50 µL of *E. coli* culture at a density of about 10^6 cells/mL was derived from a single colony grown overnight in LB broth plated under aseptic conditions. Complexes in acetone were then added into the agar wells at the concentration of 30 µL from 10^{-3} M solutions and controls were made with acetone alone (blank). The plates were incubated at 37 °C for 18 - 24 hours. The diameter of the zone of inhibition formed around each well was noted.

RESULTS AND DISCUSSION

The synthetic route used in this work for the synthesis of thiocyano bridged dicobaloxime takes advantage of the lability of the coordinated water in the benzyl aquocobaloximes which allows for substitution by the nitrogen of the coordinated thiocyanide as shown in Eq. (6)

$$PhCH_2Co(DH)_2(OH_2) + Co(DH)_2SCN(L) \rightarrow PhCH_2Co(DH)_2SCNCo(DH)_2(L) + H_2O \quad (6)$$

This analogous to the method made by Haim *et al*.[24] in synthesis of different cyano bridged compounds. The reaction



***Structure* 1.** Thiocyano bridged (benzyl ligand) dicobaloximes

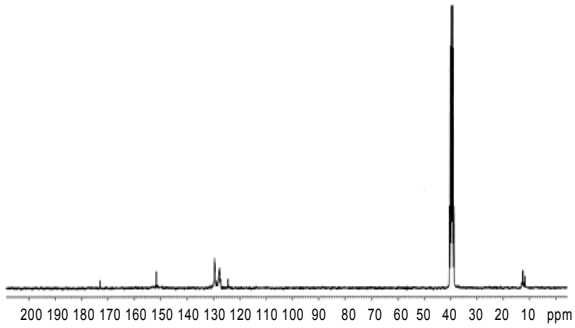


***Fig*. 2.** ^{13}C [^{1}H] NMR spectra of $PhCH_2Co(DH)_2SCNCo(DH)_2SC$ complex in DMSO-d_6.

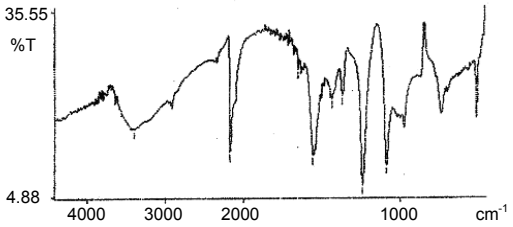


***Fig*. 3.** IR spectra of $[PhCH_2Co(DH)_2SCNCo(DH)_2AC]$.

represented by Eqn. 6 was performed in methanol; the displaced water was removed in vacuo to form the crystalline product (*Structure* 1).

IR absorption frequencies show the main bands due to the coordinated dimethyl glyoxime, the base ligands and vibrations of the ambidentate thiocyanide ligands for the monomers and dimers which are presented in *Table* 1. The comparison of IR spectra of these thiocyanato ligand cobaloximes was made with those of the thiocyano bridged binuclear cobaloximes. The infrared spectra of products of Eq. (6) show no absorptions attributable to coordinated H_2O, this proves that it is the H_2O ligand in $PhCH_2Co(DH)_2(OH_2)$ substituted in this reaction. The only other major difference in the infrared spectra of the monomers and the dimers for this reaction is that the CN stretching frequency of the SCN ligand is at higher energy (20 - 45 cm^{-1}) in the dicobaloximes (2155 cm^{-1}) than in the monothiocyano cobaloxime (2109 cm^{-1}) The ν(C=N) band shift to higher energy is explained[25] in terms of removal of electron density from the lowest filled C=N σ*(*s*) orbital on the coordinating nitrogen of the cyanide group. Moreover, by bridge formation there is a simple mechanical constraint on the CN motion imposed by the presence of the second metal center which shifts ν(C=N) to higher frequency. This shift to higher frequency upon bridging is explained on the basis of force field arguments.[26] This type of increase in the C=N stretching frequency upon forming complexes containing bridging cyanide ligands from terminal thiocyanide complexes was documented by Wilmarth *et al*.[27] Since, then it has found to be a general phenomenon. This behavior is taken as a justification for the formulation of these dimers as thiocyano bridged dicobaloximes. The decrease in σ donor strength of the trans ligands in these complexes results in a regular increase in stretching frequency of C=N of the dimers. IR spectra of $PhCH_2Co(DH)_2SCNCo(DH)_2$

***Table* 1.** IR spectral data of $Co(DH)_2SCN(L)$ and the ν(CN) of dibridged cobaloxime complexes

S. No	Complex	ν(CN)*	(CS)	(OH)	(CH_3)	(C=N)	(N-O)	(Co-N)	(NH)
1	$SCNCo(DH)_2U$	2143.1 (2174.2)**	738.4	1775	1364.2	1557.5	1229.6 1090.0	511.9	3205.3
2	$SCNCo(DH)_2AC$	2108.0 (2165.2)**	749.0	1684.1	1364.0	1558.3	1232.5 1114.4	513.8	3206.6
3	$SCNCo(DH)_2SC$	2108.3 (2138.2)**	764.2	1693.8	1374.4	1555.9	12837 1104.2	551.0	3222.2
4	$SCNCo(DH)_2FA$	2113.8 (2154.2)**	737.5	1773	1384.7	1557.4	1230.8 1088.6	513.0	3193.8

Recorded as KBr discs and values in cm^{-1}, where $(DH)_2$ dimethylglyoxime, U = urea, AC = acetamide, SC = semicabazide, FA= formamide. ν(CN)* of monomer, ν(CN)** values of dimer complexes in parenthesis

acetamide is given in *Fig*. 3. It is possible to make structural prediction of the studied complexes by using LC/MS analysis. These complexes are typically dominated by a single ion peak that corresponds to the molecular weight of the complex which is de-protonated in the positive ion mode $(M+H)^+$. Thiocyano bridged dicobaloxime of urea with a molecular weight of 787 *m*/*z* in the positive ion mode will result in a spectrum with a base peak at 788 *m*/*z*. Similarly $PhCH_2Co(DH)_2SCNCo(DH)_2TU$ with a molecular weight of 803 *m*/*z* in the positive ion mode will result in a spectrum with a base peak at 804 *m*/*z* (*Fig*. 5). In the elemental analysis of these complexes, it exhibited that all the complexes were in fairly good agreement with the calculated values while conforming to the formation of the monomer and dimer complexes. Here, it should be kept in mind that the elemental analysis is not perfectly accurate at all times because the experimental error will generally produces atom ratios that are not perfect integers but are close to integers (*Table* 2).

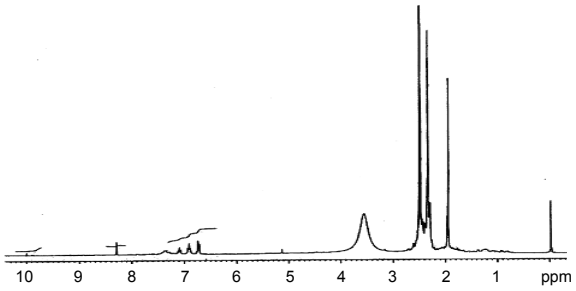


***Fig*. 4.** ^{1}H NMR spectra of $[PhCH_2Co(DH)_2SCNCo(DH)_2U]$ complex in DMSO-d_6.

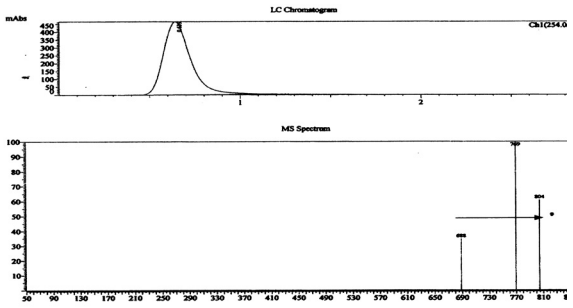


***Fig*. 5.** LC/MS spectra of $[PhCH_2Co(DH)_2SCNCo(DH)_2TU]$ complex.

^{1}H NMR spectra of these monomer complexes are easily assigned on the basis of the chemical shifts. The signals are assigned according to their relative intensities with the literature values of the related cobaloxime complexes.[28] The spectra of $SCNCo(DH)_2L$ complexes contain well resolved absorptions corresponding to the ligands and equatorial methyl groups of dimethyl-glyoximes. Four equatorial methyl groups appear at about 2.3 ppm, ∂ due to OH proton in glyoxime appears at 11.5 ppm. In urea and acetamide complexes the signal at 7.89 and 8.36 ppm corresponds to the NH_2 which are shifted to down field as compared to the free ligand 5.7 and 5.6 ppm respectively, where NH_2 of formamide observed at 8.16 ppm and NH_2 of semicarbazide which is bonded to NH_2-C=O as it appears at 7.6. This down field shift is due to resonance of the lone pair on NH_2 with C=O. ^{13}C NMR

***Table* 2.** Analytical data of thiocyano (ligand) cobaloximes and thiocyano bridged dicobaloxime

S. No	Complex	Formula (Mol. wt)	Found (cal) %		
			C	H	N
1	SCN $Co(DH)_2$ U	$CoC_{10}H_{18}N_7O_5S$ 407	29.36 (29.48)	4.26 (4.42)	24.01 (24.07)
2	$SCNCo(DH)_2AC$	$CoC_{11}H_{19}N_6O_5S$ 406	32.43 (32.51)	4.53 (4.67)	20.54 (20.68)
3	$SCNCo(DH)_2SC$	$CoC_{10}H_{19}N_8O_5S$ 422	28.12 (28.43)	4.32 (4.50)	26.41 (26.54)
4	$SCNCo(DH)_2FA$	$CoC_{10}H_{17}N_6O_5S$ 392	30.40 (30.91)	4.26 (4.33)	21.09 (21.42)
5	$PhCH_2Co(DH)_2SCNCo(DH)_2U$	$Co_2C_{25}H_{39}N_{11}O_9S$ 787	37.96 (38.11)	4.26 (4.95)	19.21 (19.56)
6	$PhCH_2Co(DH)_2SCNCo(DH)_2AC$	$Co_2C_{26}H_{40}N_{10}O_9S$ 786	39.43 (39.69)	4.74 (5.08)	17.54 (17.81)
7	$PhCH_2Co(DH)_2SCNCo(DH)_2SC$	$Co_2C_{25}H_{40}N_{12}O_9S$ 802	38.12 (37.40)	4.32 (4.98)	20.41 (20.94)
8	$PhCH_2Co(DH)_2SCNCo(DH)_2FA$	$Co_2C_{25}H_{38}N_{10}O_9S$ 772	30.40 (38.86)	4.26 (4.92)	21.09 (18.13)

spectra of $SCNCo(DH)_2L$ complexes showed signals for the equatorial methyl and oxime carbons at about 12.4 and 152 ppm respectively. Signal at about 124 ppm is attributed to the cyanide carbon of the SCN. The broadness of these resonances are generally attributed to the quadrupolar relaxation by the ^{59}Co nucleus (I = 7/2).[29] The dimethyl glyoxime methyl resonance of the benzyl group appears up field by about 0.2 ppm when compared with the values of the dimethylglyoxime monomer of thiocyano group. The up field shift is due to the interaction of the benzyl group with equatorial dimethyl glyoxime methyl through space or the anisotropy of cobalt atom alone can be invoked to explain this behavior. The C=O carbons of the base ligands are absorbed at about 175 ppm. ^1H NMR of Thiocyano bridged dicobaloximes exhibited CH_2 of benzyl at about 5.2 ppm, C-H of aromatic ring between 6.7 - 7.5 ppm and NH_2 of base ligands remained unchanged, ^1H-NMR spectra of $PhCH_2Co(DH)_2SCNCo(DH)_2U$ complex is given in *Fig*. 4 ^{13}C of SCN resonance is observed slightly down field from the corresponding terminal thiocyano cobaloximes at about 126 ppm, benzyl carbons appeared between 128 - 130 ppm and the sharp resonance near to 153 ppm due to the oxime carbons which remained unchanged in the absence of ^1H decoupling. *Fig*. 2. ^{13}C [^1H] NMR of $PhCH_2Co(DH)_2SCNCo(DH)_2SC$. *Table* 3.

Antimicrobial activity

For antimicrobial activity, all the above monomers and dimers were dissolved in a mixture of acetone and ethanol and incubated for 18 - 24 hours at 37 ^oC in agar plates in a humidified CO_2 atmosphere. It was observed that the studied complexes inhibited the growth of *E. coli*. The ranges of inhibited areas were 8 - 13 mm. Since cobalt alone has anti-microbial activity towards many microbes mainly bacteria, the results present in *Table* 4 indicate that the complexes of dimers have higher anti-microbial activity when compared to their monomers. The growth of cells in the plate supplemented with 30 μL of 10^{-3} M solution of complexes continued to decline, while the growth in the control plates (blank without complex) was uninhibited. In general all these complexes were found to show anti-microbial activity and may be a promising candidate for progression of new drugs.

DNA binding studies

The application of electronic absorption spectroscopy in DNA binding studies is one of the most useful techniques.[30,31] The interaction of the complex with DNA was investigated using absorption spectra. The absorption spectra of complex in the absence and presence of DNA (at a constant concentration of the complex) was studied. *Fig*. 6. represents the absorption spectra of $PhCH_2Co(DH)_2SCNCo(DH)_2U$ in the absence and presence of CT- DNA at 442 nm the λ_{max} of the complex. There are several ways that molecules can interact with DNA. Ligands may interact with DNA by covalently binding, electrostatcially binding, or intercalating. In the case of our dimer complexes, intercalation occurs when the benzyl ligands of an appropriate size and chemical nature fit them-

***Table* 3.** ^{13}C [^1H] NMR spectral data[a] of $SCNCo(DH)_2L$ and its dimer complexes

S. No.	Ligand/Complex	Dimethyl glyoxime Carbons		ligand Carbons		
		CH_3	C=N	SCN	C=O	Ring carbons
1	$SCNCo(DH)_2U$ Urea	12.80	151.39	120.65	164	-
2	$SCNCo(DH)_2AC$ Acetamide	12.34	152.43	130	166	
3	$SCNCo(DH)_2SC$ Semicarbazide	12.34	147.4	125.6	174	
4	$SCNCo(DH)_2FA$ Semicarbazide	12.2	148.2	124.1	171	
5	$PhCH_2Co(DH)_2SCNCo(DH)_2U$	12.80 (12.5)	148	125	165	127 - 130
6	$PhCH_2Co(DH)_2SCNCo(DH)_2AC$	12.34 (12.1)	152	130	164	127 - 129
7	$PhCH_2Co(DH)_2SCNCo(DH)_2SC$	12.5 (12.3)	152	125	173	127 - 130
8	$PhCH_2Co(DH)_2SCNCo(DH)_2$ FA	11.9 (12.2)	149	126	174	128 - 131

[a]In ppm relative to TMS - solvent DMSO-d_6 and acetone. [b]Equatorial methyl groups of dimethylglyoxime of benzyl in parenthesis

***Table* 4.** Antimicrobial activity of $Co(DH)_2SCN(L)$ and thiocyano bridged dicobaloxime complexes on *E. coli*.

SCN $Co(DH)_2L$	Zone of inhibited area in mm	$PhCH_2Co(DH)_2SCNCo(DH)_2L$	Zone of inhibited area in mm
SCN $Co(DH)_2U$	8	$PhCH_2Co(DH)_2SCNCo(DH)_2U$	12
SCN $Co(DH)_2AC$	9	$PhCH_2Co(DH)_2SCNCo(DH)_2AC$	13
SCN $Co(DH)_2SC$	9	$PhCH_2Co(DH)_2SCNCo(DH)_2SC$	12
SCN $Co(DH)_2FA$	8	$PhCH_2Co(DH)_2SCNCo(DH)_2FA$	11
tetracycline	16	Tetracycline	16

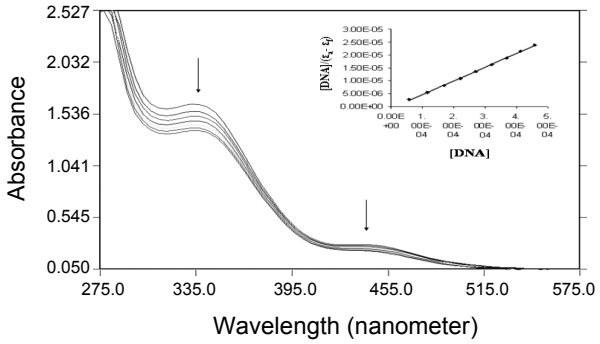


***Fig*. 6.** Absorption spectra of $PhCH_2Co(DH)_2SCNCo(DH)_2U$ (top) in the absence of CT DNA, the absorbance changing upon increasing CT DNA concentrations. The arrow shows the intensity chanage upon increasing DNA concentration

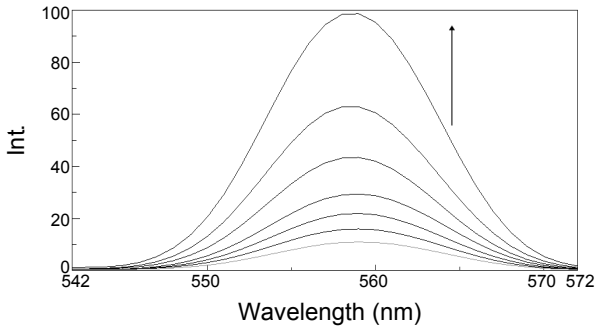


***Fig*. 7.** Emission spectra of $PhCH_2Co(DH)_2SCNCo(DH)_2U$ complex in aqueous buffer at ph 7.2 in the presence of CT-DNA. Arrow shows the intensity chanage upon increasing DNA concentrations

selves in between base pairs of DNA. The electronic spectra of the $PhCH_2Co(DH)_2SCNCo(DH)_2U$ complex in buffer A is characterized by an intense ligand centered (π-π^*) transition in the uv region at 335 nm and metal ligand charge transfer (MLCT) in the visible region at 442 nm. When CT-DNA is added to the complex, hypochromism and red shift is observed. Hypocromism and red shifting indicate strong interaction between the DNA bases and the complex; such facts are consistent with the intercalative binding. This interaction between the dimer complex and DNA involves the insertion of a planar fused aromatic ring system between the DNA base pairs, leading to significant π-electron overlap. This mode of binding is usually favored by the presence of an extended fused aromatic ligand.[32] There is no interaction seen between the complex and the DNA base pairs, in the case of neutral monomer complexes.The intrinsic binding constant *K*, with CT-DNA was determined according to Eqn. 7 through a plot of $[DNA]/(\varepsilon_a-\varepsilon_f)$Vs [DNA], where [DNA] is the concentration per nucleotide, the apparent absorption co-efficients ε_a, ε_f and ε_b correspond to A_{obsd}/[Co(III)], the extinction co-efficients for the free cobalt complex, extinction co-efficients of complex in presence of DNA and the extinction co-efficients for the cobalt complex in the fully bound form, respectively. Intrinsic binding constant *K* of $PhCH_2Co(DH)_2SCNCo(DH)_2U$ complex is $4.5 \pm 0.3 \times 10^4$ M^{-1} was obtained from the decay of absorbance. The binding constant indicates that the complex binds moderately with the DNA.

$$[DNA]/(\varepsilon_a-\varepsilon_f) = [DNA]/(\varepsilon_b-\varepsilon_f) + 1/K_b(\varepsilon_b-\varepsilon_f) \quad (7)$$

Fluorescence spectroscopic studies

Fluorescence spectroscopy is one of the most common and at the same time most sensitive ways to analyze metal complex-DNA interactions. The complex $PhCH_2Co(DH)_2SCNCo(DH)_2U$ luminescence in tris buffer at room temperature, with maxima at *ca*. 558 nm. Its interaction with CT-DNA was monitored with luminescence. The results of emission titrations for $PhCH_2Co(DH)_2SCNCo(DH)_2U$ with CT DNA are illustrated in *Fig*. 7. Upon addition of CT DNA, the emission intensity of the dimer complex increases steadily. This observation is further supported by the emission quenching experiments with $[Fe(CN)_6]^{4-}$ as quencher. This ion has been shown to be able to distinguish differently bound Co(III) species and positively charged free complex ions should be readily quenched by $[Fe(CN)_6]^{4-}$. The complex bound with DNA can be protected from the quencher because the highly negatively charged $[Fe(CN)_6]^{4-}$ ions would be repelled by the negatively charged DNA phosphate backbone. Thus hindering the emission quenching of the bound complex. The method essentially consists of titrating a given amount of DNA- metal complex by increasing the concentra-

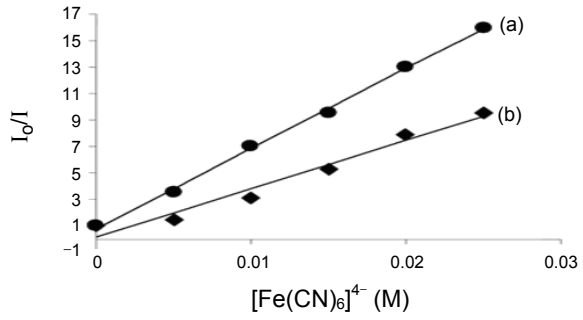


***Fig*. 8.** Emission quenching curves of complex in absence of DNA (a) presence of DNA (b).

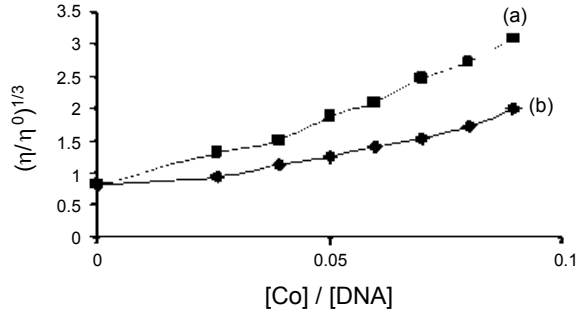


***Fig*. 9.** Effect of increasing amounts of ethedium bromide (a) and complex (b) $PhCH_2Co(DH)_2SCNCo(DH)_2U$ on the relative viscosity of CT DNA at 25 ± 0.1 °C.

tion of $[Fe(CN)_6]^{4-}$ and measuring the change in fluorescence intensity in *Fig*. 7. The Ferro cyanide quenching curves for this complex in presence and absence of CT DNA are in *Fig*. 8. The absorption and fluorescence spectroscopy studies determine the binding of the complex with DNA.

Viscosity measurements

To further clarify the interaction between the complexes and DNA, viscosity measurements were performed. Optical photochemical probes provide necessary but not sufficient clues to support a binding model. Hydrodynamic measurements that are sensitive to the length change (i.e. viscosity and sedimentation) are regarded as the least ambiguous and the most critical test of a binding model in solution in the absence of crystallographic structural data.[33,34] A classical intercalation model requires that the DNA helix lengths are separated to accommodate the binding ligand leading to an increase in DNA viscosity. A classical intercalation model demands that the DNA helix must lengthen as base pairs are separated to accommodate the binding ligand, leading to the increase of DNA viscosity. *Fig*. 9 shows the changes in viscosity upon addition of the complex (b) as well as the known DNA intercalator ethidium bromide. On increasing the amounts of (b) $PhCH_2Co(DH)_2SCNCo(DH)_2U$, the relative specific viscosity of DNA increases steadily. The result suggests that the complex (b)$PhCH_2Co(DH)_2SCNCo(DH)_2U$ intercalates between the base pairs of DNA, which is consistent with our hypothesis.

CONCLUSION

Thiocyanato (ligand) cobaloximes and Thiocyanato bridged dicobaloximes are synthesized and characterized. The binding of bridged complex $PhCH_2Co(DH)_2SCNCo(DH)_2U$ with DNA are studied. The absorption and viscosity studies support the intercalative binding. The phenyl ring of benzyl cobaloxime intercalates between the base pairs of DNA. CN stretching frequencies of dimers shifts to 40 - 50 cm^{-1} higher compared to monomers, which supports the formation of dimers.

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