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## DNA- BINDING AND CLEAVAGE STUDIES OF Mn(II) AND Co(II) COMPLEXES OF 1-(2,5-DIOXOPYRROLIDIN-1-YL)(4-HYDROXY PHENYL)METHYL THIOUREA

Sivakami Sudhasankar<sup>\*1</sup>, Muthusamy Vijayachandrasekar<sup>1</sup>, Abirami Natarajan<sup>1</sup> and Sureshbabu Ram Kumar Pandian<sup>2</sup>

Department of Chemistry<sup>1</sup>, SRM University, Kattankulathur - 603203, Tamil Nadu, India.

Department of Biotechnology<sup>2</sup>, Kalasalingam University, Virudhunagar - 626126, Tamil Nadu, India.

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Mannich base,  
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### Correspondence to Author:

**Dr. Sivakami Sudhasankar**

Assistant Professor,  
Department of Chemistry,  
Faculty of Science and Humanities,  
SRM University, Kattankulathur -  
603203, Tamil Nadu, India.

**E-mail:** sivakamisudhasankar@gmail.com

**ABSTRACT:** A novel Mannich base ligand 1-(2,5-dioxopyrrolidin-1-yl)(4-hydroxy phenyl)methylthiourea (PSTU) and its Manganese (II) and Cobalt (II) complexes have been synthesized and characterized by elemental analysis, molar conductance measurements, magnetic susceptibility measurements, FT-IR, <sup>1</sup>H NMR and <sup>13</sup>C NMR spectroscopic studies. The antimicrobial activities and antioxidant activities of the free ligand and its metal complexes were also performed. The results revealed enhanced antimicrobial activities against *Bacillus subtilis* (gram+ive) and *Escherichia coli* (gram-ive bacteria) and also significant antioxidant activity. In addition, DNA binding properties of the ligand and its metal complexes have been investigated by UV-Visible absorption spectroscopy, viscosity measurements, emission spectra and gel electrophoresis, which suggest that the metal complexes act as efficient DNA binders. The results shown that the nuclease activity Co(II) complex was effective which could induce scission of pBr322 super coiled DNA effectively to linear form in presence of H<sub>2</sub>O<sub>2</sub> as oxidizing agent.

**INTRODUCTION:** Metals have been used in treatments since ancient times. The Ebers Papyrus from 1500 BC is the first written account of the use of metals for treatment and describes the use of Copper to reduce inflammation and the use of iron to treat anaemia. Sodium vanadate has been used since the early 20<sup>th</sup> century to treat rheumatoid arthritis. Recently metals have been used to treat cancer, by specifically attacking cancer cells and interacting directly with DNA and in chemotherapeutics<sup>1-12</sup>.

DNA is usually referred as molecule of heredity for genetic propagation of all qualities. The positive charge on most metals can interact with the negative charge of the phosphate backbone of DNA. Some drugs developed that include metals interact directly with other metals already present in protein active sites, while other drugs can use metals to interact with amino acids with the highest reduction potential. Chelation causes drastic change in the structural features and enhances the biological activities of the ligand. Many reports are available in the literature on the synthesis and characterization of metal complexes of Mannich bases<sup>13-17</sup>. Phthalimides possess an imide ring which facilitate them to be biologically active and pharmaceutically useful due to their antibacterial, antifungal, analgesic<sup>18, 19</sup>, antitumor<sup>20, 21</sup>, anxiolytic<sup>22</sup> and anti HIV-1 activities.

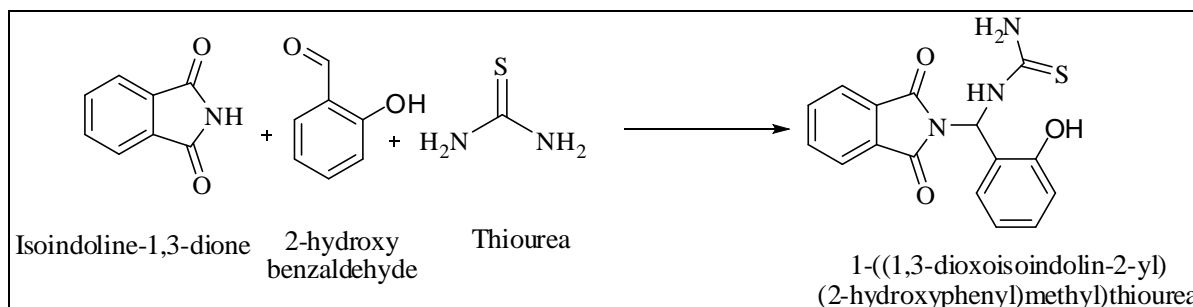
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When phthalimides is subjected to Mannich condensation, it yields Mannich bases<sup>23</sup> which may display very effective biological activities. Current paper describes about the synthesis, characterization of novel Mannich base ligand PSTU and its Mn(II) and Co(II) complexes. Their anti-microbial, anti-oxidant activities, DNA binding and cleavage studies have also been discussed in detail.

**MATERIALS AND METHODS:** All the reagents and solvents used for the synthesis of ligand and the metal complexes were AR grade of highest available purity and used as such without further purification. Elemental analysis was performed using Carlo Erba 1108 analyzer and Coleman N analyzer and was found within  $\pm 0.5\%$ . The molar conductivities of the metal complexes were measured in approximately  $10^{-3}$  M Ethanol solution using a Systronics direct reading digital conductivity meter -304 with dip type conductivity cell. The IR spectra were recorded as KBr pellets on Perkin- Elmer 1000 unit instrument. Absorbance in UV-Visible region was recorded in DMF

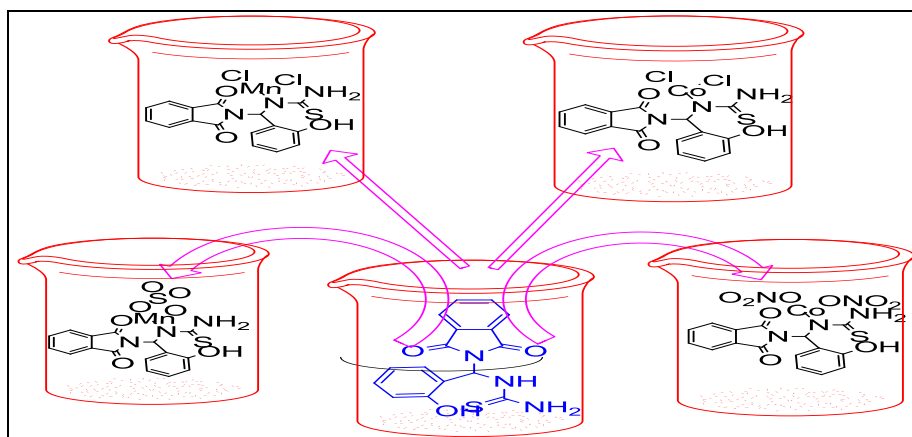
solution using UV-Visible spectrometer. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR of the ligand was recorded on a Bruker instrument employing TMS as internal reference and DMSO – DMF as solvent. The mass spectral study of the ligand was carried out using LC mass spectrometer. Magnetic susceptibility measurements at room temperature were made by using a Guoy magnetic balance. Anti-microbial screening of the test compounds was carried out using Agar-well diffusion method.

**Preparation of 1-(2, 5-dioxopyrrolidin-1-yl) (4-hydroxy phenyl) methyl) thiourea (PSTU):** Phthalimide (1g, 0.1mol), salicylaldehyde (0.3ml, 0.1mol) and thiourea (0.5g, 0.1mol) were taken in equimolar ratio. To an aqueous solution of thiourea and phthalimide taken in the reflux condensor, salicylaldehyde is added in drops and refluxed for 3hrs and cooled in an ice bath. A white solid formed is filtered, washed with distilled water and dried in vacuum. Then the product is recrystallized from hot solution of ethanol. The percentage yield of the compound was 82%.



**Synthesis of Metal Complexes:** The metal complexes of PSTU were prepared by slow addition of hot methanolic solution of the metal salt with hot ethanolic solution of the ligand in 1:1

molar ratio (Fig. 1). The insoluble metal complexes were formed after 2 weeks. It was washed with methanol and ethanol to remove unreacted metal salt or ligand and then dried in an air oven at 60 °C.



**FIG. 1: SCHEMATIC REPRESENTATION OF SYNTHESIS OF METAL COMPLEXES**

**RESULTS AND DISCUSSION:****Elemental Analysis:****TABLE 1: PHYSICAL PROPERTIES AND ELEMENTAL ANALYSIS DATA OF THE LIGAND PSTU AND ITS METAL COMPLEXES**

S. No	Compound	Mol. Weight	C	H	N	M
1.	PSTU C <sub>16</sub> H <sub>13</sub> N <sub>3</sub> O <sub>3</sub> S	327.36	58.70 (58.62)	4.00 (3.98)	12.84 (12.75)	-
2.	MnSO <sub>4</sub> .2H <sub>2</sub> O.PSTU C <sub>17</sub> H <sub>15</sub> MnN <sub>3</sub> O <sub>7</sub> S <sub>2</sub>	492.38	41.47 (41.40)	3.07 (3.00)	8.53 (8.48)	11.16 (11.09)
3.	MnCl <sub>2</sub> .2H <sub>2</sub> O.PSTU C <sub>17</sub> H <sub>15</sub> Cl <sub>2</sub> MnN <sub>3</sub> O <sub>3</sub> S	467.23	43.70 (43.62)	3.24 (3.19)	8.99 (8.91)	11.76 (11.68)
4.	Co(NO <sub>3</sub> ) <sub>2</sub> .PSTU C <sub>17</sub> H <sub>15</sub> CoN <sub>5</sub> O <sub>9</sub> S	524.33	38.94 (38.89)	2.88 (2.82)	13.36 (13.30)	11.24 (11.22)
5.	CoCl <sub>2</sub> .PSTU C <sub>17</sub> H <sub>15</sub> Cl <sub>2</sub> CoN <sub>3</sub> O <sub>3</sub> S	471.22	43.33 (43.27)	3.22 (3.18)	8.92 (8.87)	12.51 (12.46)

**UV-Vis Spectroscopic Studies:** The electronic spectra of the metal complexes were recorded for their solution in DMSO in the range of 180-1800nm (Table 2). The UV-Vis spectrum of Manganese sulphate metal complex shows absorption bands at 18250 cm<sup>-1</sup>, 22965 cm<sup>-1</sup>, 30280 cm<sup>-1</sup> for <sup>6</sup>A<sub>1g</sub>→<sup>4</sup>T<sub>1g</sub>, <sup>6</sup>A<sub>2g</sub>→<sup>4</sup>E<sub>2g</sub> and CT transitions respectively. The μ<sub>eff</sub> value was found to be 4.97 B.M which suggests octahedral geometry<sup>24-28</sup>.

The electronic spectrum of Manganese chloride complex exhibits three absorption bands at 17950 cm<sup>-1</sup>, 24785 cm<sup>-1</sup>, 27236 cm<sup>-1</sup> and 31565 cm<sup>-1</sup> for <sup>6</sup>A<sub>1g</sub>→<sup>4</sup>T<sub>1g</sub>, <sup>6</sup>A<sub>1g</sub>→<sup>4</sup>E<sub>2g</sub> and CT transitions

respectively. The μ<sub>eff</sub> value was found to be 4.85 B.M points which proposes high spin octahedral geometry<sup>29</sup>.

The Cobalt nitrate complex shows four absorption bands at 3790 cm<sup>-1</sup>, 5125 cm<sup>-1</sup>, 6892 cm<sup>-1</sup>, 27992 cm<sup>-1</sup> assigned for <sup>4</sup>A<sub>2</sub>→<sup>4</sup>T<sub>2</sub>, <sup>4</sup>A<sub>2</sub>→<sup>4</sup>T<sub>1</sub>, <sup>4</sup>A<sub>2</sub>→<sup>4</sup>T<sub>1</sub> and charge transfer transition. The μ<sub>eff</sub> value was found to be 4.53 B.M which agree with tetrahedral geometry<sup>30</sup>. The Cobalt chloride complex shows four absorption bands at 6965 cm<sup>-1</sup>, 14560 cm<sup>-1</sup>, 37000 cm<sup>-1</sup> assigned for <sup>4</sup>A<sub>2</sub>→<sup>4</sup>T<sub>2</sub> transitions. The μ<sub>eff</sub> value was found to be 4.61 B.M which agree with square planar geometry.

**TABLE 2: MOLAR CONDUCTANCE (IN DMF), MAGNETIC MOMENT, ASSIGNED TRANSITIONS WITH λ<sub>max</sub> AND GEOMETRY OF THE METAL COMPLEXES**

Name of the Complex	Λm (ohm <sup>-1</sup> cm <sup>2</sup> mol <sup>-1</sup> )	μ <sub>eff</sub> (B.M)	λ <sub>max</sub> (cm <sup>-1</sup> )	Transition Assignment	Geometry
MnSO <sub>4</sub> .2H <sub>2</sub> O.PSTU	70	4.97	18362 22457 31293	<sup>6</sup> A <sub>1g</sub> → <sup>4</sup> T <sub>1g</sub> <sup>6</sup> A <sub>1g</sub> → <sup>4</sup> E <sub>2g</sub> CT	High spin Octahedral
MnCl <sub>2</sub> .2H <sub>2</sub> O.PSTU	62	4.85	18050 24985 31272	<sup>6</sup> A <sub>1g</sub> → <sup>4</sup> T <sub>1g</sub> <sup>6</sup> A <sub>1g</sub> → <sup>4</sup> E <sub>2g</sub> CT	High spin Octahedral
Co(NO <sub>3</sub> ) <sub>2</sub> .PSTU	67	4.53	3820 5085 6732 28548	<sup>4</sup> A <sub>2</sub> → <sup>4</sup> T <sub>2</sub> <sup>4</sup> A <sub>2</sub> → <sup>4</sup> T <sub>1</sub> <sup>4</sup> A <sub>2</sub> → <sup>4</sup> T <sub>1</sub> CT	Tetrahedral
CoCl <sub>2</sub> .PSTU	35.2	4.61	6703 14365 36990	<sup>4</sup> A <sub>2</sub> → <sup>4</sup> T <sub>1</sub> <sup>4</sup> A <sub>2</sub> → <sup>4</sup> T <sub>1</sub> <sup>4</sup> A <sub>2</sub> → <sup>4</sup> T <sub>2</sub>	Square planar

**FT-IR Studies:** The coordination mode or bonding sites of the ligand and the metal complexes were investigated with the characteristic absorption bands of the free ligand and the metal complexes. (Table 3). The IR spectrum of the ligand PSTU show a broad band in the region of 3192cm<sup>-1</sup> due to

ν<sub>NH</sub> stretching and aromatic C-H stretching vibrations. The C=O stretching frequency was observed as sharp band at 1747 cm<sup>-1</sup>. The characteristic C=S stretching frequency of thiourea for the ligand was appeared in the region of 1386 cm<sup>-1</sup>. In the spectra of the metal complexes, the ν<sub>NH</sub>

stretching frequency was found to be decreased thus showing the coordination of nitrogen atom of thiourea with the metal ion. The carbonyl stretching

frequency of phthalimide has also decreased gradually, thus suggesting the coordination with the metal ion which is illustrated in **Table 3**.

**TABLE 3: CHARACTERISTIC IR ABSORPTION FREQUENCIES (cm<sup>-1</sup>) OF PSTU AND ITS METAL COMPLEXES**

Compound	$\nu_{\text{NH}}$	$\nu_{\text{C=O}}$	$\nu_{\text{C-S}}$	N-C-N	H <sub>2</sub> O Coordination	M-X	M-S
PSTU	3192	1747	1386	1305	-	-	-
MnSO <sub>4</sub> .PSTU	3190	1746	1388	1304	3383,1601,799,641	-	528
MnCl <sub>2</sub> .PSTU	3204	1741	1381	1301	3394,1627,714,641	532	-
Co(NO <sub>3</sub> ) <sub>2</sub> .PSTU	3203	1744	1385	1305	-	-	-
CoCl <sub>2</sub> .PSTU	3124	1732	1399	1302	-	535	-

**NMR and Mass Spectral Studies of PSTU:** The <sup>1</sup>H NMR spectra of the ligand shows a singlet at 2.561 $\delta$  due to -CH proton of aromatic aldehyde. The multiplet between 6.394-8.138 $\delta$  corresponds to aromatic protons. Two singlets at 10.009 and 10.199 $\delta$  are assigned for -NH proton. The singlet at 11.154 $\delta$  is assigned for -NH<sub>2</sub> proton. The <sup>13</sup>CNMR spectra reveals sharp peaks at  $\delta$  177.3, 177.4, 177.3, 156.4, 139.6, 131.0, 126.7, 120.3, 119.2, 116.0, 60.4 represents the number of carbons of the ligand which are not chemically equivalent. The *m/z* from mass spectrum for PSTU is 310.15 and calculated is 311.36.

**Anti-Bacterial Activity:** The ligand PSTU and the synthesized metal complexes were dissolved in DMSO and the working concentrations of the above were taken in Milli-Q water for treatment. Gram positive (*Bacillus subtilis*) and Gram Negative (*Escherichia coli*) bacteria were taken to analyze the anti-bacterial activity of metal complexes. Primarily, Minimal Inhibitory Concentration (MIC) was determined by spectrophotometer method. For that purpose, equal number of colonies (1x10<sup>10</sup> CFU/ml) were taken in 0.7% of sterile saline and the final concentrations of metal complexes were varied from 50 $\mu$ g to 400 $\mu$ g. 12 hour incubation was given and absorbance was taken at 600nm. 50% of reduction was calculated as MIC. Afterward, the activity of the drug was visualized by well-diffusion assay and the zone of inhibition was calculated and is shown in the **Table 4**. It is seen that all the metal complexes of PSTU exhibit higher activity than the free ligand against both *Bacillus subtilis* and *Escherichia coli*. The enhanced activity of the metal complexes can be explained on the basis of chelation theory<sup>31</sup>. Chelation reduces the polarity of the metal ion and increases lipophilic and hydrophobic character of the metal chelates

favouring the diffusion of the metal chelates through the cell membrane. In other words, the chelation of the metal ion leads to the breakdown of the permeability barrier of the cell wall. Hence, the permeation mechanism through the lipid layer of the microorganism becomes more effective, causing an increase in the antibacterial activity of the complexes<sup>32</sup>.

**TABLE 4: DIAMETER OF INHIBITION AGAINST BACTERIA IN MILLIMETER (mm) OF THE LIGAND AND THE METAL COMPLEXES**

Compound	Diameter of Inhibition	
	<i>E. coli</i> (300 $\mu$ g/l)	<i>B. subtilis</i> (300 $\mu$ g/l)
PSTU	19	18
MnSO <sub>4</sub> .2H <sub>2</sub> O. PSTU	21	16
MnCl <sub>2</sub> .2H <sub>2</sub> O. PSTU	14	17
CoSO <sub>4</sub> .2H <sub>2</sub> O. PSTU	17	20
Co(NO <sub>3</sub> ) <sub>2</sub> .2H <sub>2</sub> O. PSTU	20	15
Tetracyclin	25	25

**Anti-oxidant Activity:** The metal complexes exhibited anti-oxidant activity as, measured by DPPH method. These assays prove that metal complexes have the ability to scavenge free radicals generated *in vitro* by donating hydrogen atom<sup>33</sup>. The metal complexes, at a concentration of 250 $\mu$ g/ml demonstrated equal or higher activity than the standard anti-oxidants analyzed as illustrated in **Table 5**. Observing the outcomes from DPPH assay, it confirms that the metal complexes act as anti-oxidant agents. PSTU, coordinated with MnCl<sub>2</sub>, had shown greater anti-oxidant effect compared with other compounds.

**TABLE 5: ANTI-OXIDANT ACTIVITY OF SELECTED METAL COMPLEXES OF PSTU**

Compound	Anti-oxidant Activity
MnCl <sub>2</sub> .2H <sub>2</sub> O. PSTU	+
MnSO <sub>4</sub> . PSTU	+
CoCl <sub>2</sub> . PSTU	+
Co(NO <sub>3</sub> ) <sub>2</sub> .2H <sub>2</sub> O. PSTU	+



**DNA Binding Activity:**

**Absorption Spectral Studies:** The binding ability of the nickel complexes in DMF solutions (10%) with calf thymus (CT) DNA are studied by measuring their effects on the UV spectroscopy. Absorption titration experiments of  $\text{MnCl}_2\cdot\text{PSTU}$  (1),  $\text{MnSO}_4\cdot\text{PSTU}$  (2),  $\text{CoCl}_2\cdot\text{PSTU}$  (3) and  $\text{Co}(\text{NO}_3)_2\cdot\text{PSTU}$  (4) complexes in buffer were performed by using fixed concentration of the metal complexes to which increments of the DNA stock solution were added.

To compare quantitatively the affinity of the two complexes toward DNA, the binding constants  $K$  of the complexes to CT DNA were determined by monitoring the changes of absorbance around 260 nm with increasing concentration of DNA (Fig. 2 and 3). The appreciable decrease in absorption intensity and significant red shift of the  $\pi\text{-}\pi^*$  band of the complexes are similar to that observed for its interaction with CT DNA in DMF solution, suggesting that the complex binds to DNA strongly.

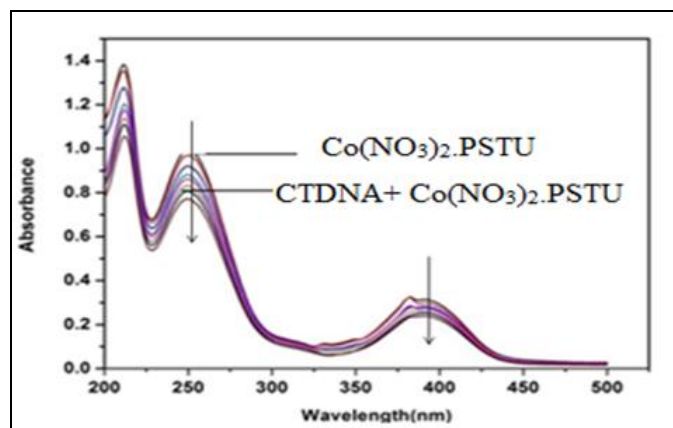


FIG. 2: ABSORPTION SPECTRA OF THE COMPLEX  $\text{Co}(\text{NO}_3)_2\cdot\text{PSTU}$  IN THE ABSENCE AND PRESENCE OF INCREASING AMOUNTS OF CT-DNA (0-250 $\mu\text{m}$ ) IN TRIS-HCl BUFFER

These spectral characteristics obviously suggest that the complexes in this work interact with DNA most likely through a mode that involves a stacking interaction between the aromatic chromophore and the base pairs of DNA. Structurally, the ligand should provide aromatic moiety to overlap with the stacking base pairs of the DNA helix by intercalation which results in hypochromism and bathochromism. The aromatic moiety in the ligand of the complexes facilitates its potential intercalative and / or DNA major groove binding.

The binding constant ( $K_b$ ) values of the complexes 1-5 are given in Table 6.

TABLE 6: BINDING PARAMETERS OF THE METAL COMPLEXES OF PSTU WITH DNA

Complexes	DNA		
	$K_b$ ( $\text{M}^{-1}$ )	$K_{sv} \times 10^4$ ( $\text{M}^{-1}$ )	$K_{app} \times 10^6$ ( $\text{M}^{-1}$ )
$\text{MnCl}_2\cdot\text{PSTU}$	$0.69 \times 10^5$	0.23	2.2
$\text{MnSO}_4\cdot\text{PSTU}$	$0.60 \times 10^5$	0.18	2.1
$\text{CoCl}_2\cdot\text{PSTU}$	$0.22 \times 10^5$	0.14	3.11
$\text{Co}(\text{NO}_3)_2\cdot\text{PSTU}$	$1.82 \times 10^5$	1.48	4.62

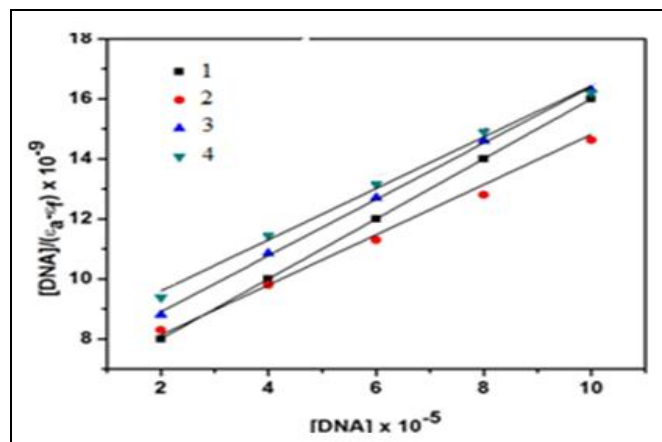


FIG. 3: PLOT OF  $[\text{DNA}]/(\epsilon_A - \epsilon_F)$  vs  $[\text{DNA}]$  FOR ABSORPTION TITRATION OF CT-DNA AND THE COMPLEXES  $\text{MnCl}_2\cdot\text{PSTU}$ (1),  $\text{MnSO}_4\cdot\text{PSTU}$ (2),  $\text{CoCl}_2\cdot\text{PSTU}$ (3) AND  $\text{Co}(\text{NO}_3)_2\cdot\text{PSTU}$ (4)

**Fluorescence Spectral Studies:** The fluorescence spectral method is used to study the relative binding of these complexes to CT-DNA. Ethidiumbromide (EB) emits intense fluorescence at about 600nm in the presence of DNA because of its strong interaction between the adjacent DNA base pairs. Addition of second molecule, which binds to DNA stronger than EB, would quench the EB- DNA by either replacing the EB or by accepting the excited-state electron of the EB through a photoelectron transfer mechanism. The addition of complex to EB bound CT-DNA solution caused obvious reduction in emission intensities (Fig. 4) indicating that complex competitively bound to CT-DNA with EB. It may be due to the complex interacting with DNA through intercalation binding and thus releasing free EB from the EB-DNA complex. The Stern-Volmer quenching constant  $K_{sv}$  value of the complexes  $\text{MnCl}_2\cdot\text{PSTU}$  (1),  $\text{MnSO}_4\cdot\text{PSTU}$  (2),  $\text{CoCl}_2\cdot\text{PSTU}$  (3) and  $\text{Co}(\text{NO}_3)_2\cdot\text{PSTU}$  (4) were calculated and given in Table 6.

The  $K_{app}$  values of the complexes  $MnCl_2.PSTU$  (1),  $MnSO_4.PSTU$  (2),  $CoCl_2.PSTU$  (3) and  $Co(NO_3)_2.PSTU$  (4) were calculated as  $2.2 \times 10^6$ ,  $3.1 \times 10^6$ ,  $3.6 \times 10^6$  and  $2.87 \times 10^6$  respectively. From this spectral data, the complex  $Co(NO_3)_2.PSTU$  binds well through intercalative than the other complexes due to reactive ability and binding mode of the metal with DNA.

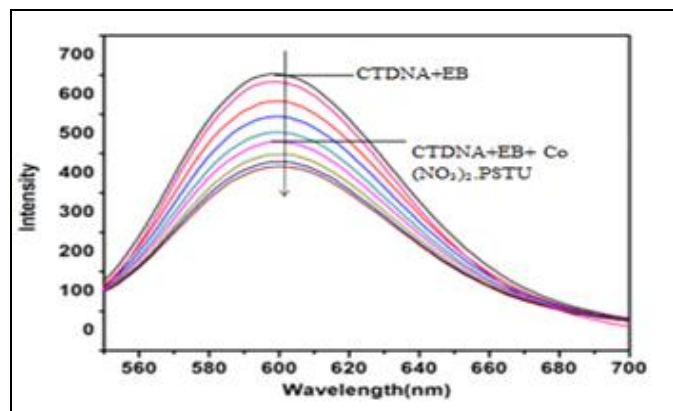


FIG. 4: PLOT OF  $I_0/I$  vs  $[COMPLEX]/[DNA]$  FOR FLUORESCENCE QUENCHING CURVES OF EB-DNA BY THE COMPLEXES  $MnCl_2.PSTU$ (1),  $MnSO_4.PSTU$ (2),  $CoCl_2.PSTU$ (3) AND  $Co(NO_3)_2.PSTU$  (4)

**Viscosity Measurements:** Viscosity measurement, which is sensitive to the changes in the length of DNA molecule, is regarded as the least ambiguous and the most critical test of evaluating the binding mode of metal complexes with DNA in solution, and provides stronger arguments for intercalative binding mode. A classical intercalation model results in lengthening the DNA helix, as base pairs are separated to accommodate the bound ligand, leading to the increase of CT-DNA viscosity. In contrast, a semi-intercalation of ligand could bend (or kink) DNA helix, reduce its effective length, and concomitantly its viscosity. To further clarify, the intercalation mode of the metal complexes 1-5 of PSTU with CT-DNA, viscosity measurements were carried out on the DNA by varying the concentration of the added complexes.

The results of the effect of the metal complexes 1-5 on the viscosities of CT-DNA are shown in Fig. 5. As illustrated in the figure, on increasing the amount of the metal complexes, the relative viscosity of CT-DNA increased steadily, showing that these complexes bind to CT-DNA in the mode of intercalation. Thus, the results obtained from viscosity studies are consistent with our foregoing conclusions.

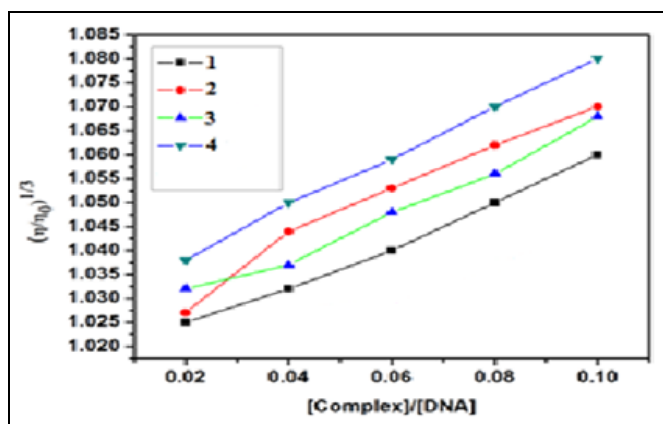


FIG. 5: EFFECT OF THE INCREASING AMOUNT OF THE METAL COMPLEXES  $MnCl_2.PSTU$ (1),  $MnSO_4.PSTU$ (2),  $CoCl_2.PSTU$ (3) AND  $Co(NO_3)_2.PSTU$ (4) ON THE RELATIVE VISCOSITY OF CT-DNA AT 289 ( $\pm 0.1$ ) K,  $[DNA] = 0.1$  mM

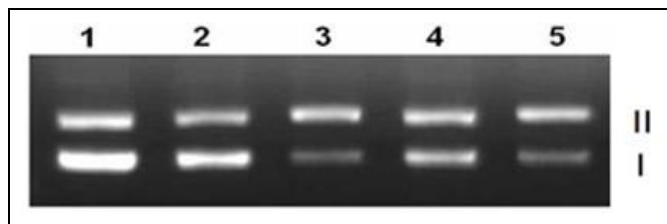
**DNA Cleavage Studies:** The cleavage of super coiled pBR322 DNA was studied in a medium of 50 mM Tris-HCl / NaCl Buffer (pH = 7.2) in the presence of  $H_2O_2$ . All the complexes  $MnCl_2.PSTU$  (1),  $MnSO_4.PSTU$  (2),  $CoCl_2.PSTU$  (3) and  $Co(NO_3)_2.PSTU$  (4) showed remarkable cleavage. Fig. 6 shows the results of the gel electrophoretic separations of plasmid pBR322 DNA by the complexes  $MnCl_2.PSTU$  (1),  $MnSO_4.PSTU$  (2),  $CoCl_2.PSTU$  (3) and  $Co(NO_3)_2.PSTU$  (4) in the presence of  $H_2O_2$ . Under similar conditions, no cleavage of pBR322 DNA occurred for free  $H_2O_2$  (40  $\mu M$ ) or complexes (30  $\mu M$ ) (Lane 1–5). At a slightly higher concentration of the complexes (50  $\mu M$ ) the cleavage is found to be much more efficient, as seen from the formation of nicked circular (form II) and linear form (form III).

Interestingly, the DNA cleavage ability of the Mn(II) and Co(II) complexes show better cleavage activity than the other complexes. It is reasonable to suggest that the noticeable DNA cleavage activity of the complexes are due to binding mode of the ligand with the metal, which enhances the binding and cleavage ability of the molecule. The cleavage mechanism of pBR322 DNA induced by the complexes Mn(II) and Co(II) were investigated and clarified in the presence of hydroxyl radical scavenger 0.4 M DMSO (Lanes 1-5), SOD (4 units) and EDTA as a chelating agent under aerobic conditions as shown in Fig. 6. Both DMSO and SOD and are completely ineffective, these rule out the possibility of cleavage by hydroxyl radical and superoxide.

The EDTA can efficiently inhibit the activity of the complexes. In order to understand the cleavage mechanism, the cleavage studies were carried out under anaerobic conditions as shown in Fig. 7. Under anaerobic conditions, the complex display considerable cleavage (lanes 1-5) in the presence of hydrogen peroxide. This fact implies that DNA cleavage reaction by the binuclear metal / H<sub>2</sub>O<sub>2</sub> system should be due to hydrolytic mechanism.



**FIG. 6: CLEAVAGE OF SC PBR322 DNA BY THE COMPLEXES (75  $\mu$ m) INTRIS-HCl BUFFER (pH 7.2) AT 37 °C FOR 3H. LANE 1, DNA CONTROL, LANE 2, DNA + H<sub>2</sub>O<sub>2</sub> + MnCl<sub>2</sub>.PSTU, LANE 3, DNA + H<sub>2</sub>O<sub>2</sub> + MnSO<sub>4</sub>.PSTU, LANE 4, DNA + H<sub>2</sub>O<sub>2</sub> + CoCl<sub>2</sub>.PSTU, LANE 5, DNA + H<sub>2</sub>O<sub>2</sub> + Co(NO<sub>3</sub>)<sub>2</sub>.PSTU**



**FIG. 7: AGAROSE GEL SHOWING CLEAVAGE OF PBR 322 DNA INCUBATED WITH COMPLEX Cu(NO<sub>3</sub>)<sub>2</sub>. PSTU (100  $\mu$ m) IN TRIS-HCl/NaCl BUFFER (pH = 7.2) AT 37 °C FOR 3H. LANE 1, DNA CONTROL, LANE 2, DNA+ H<sub>2</sub>O<sub>2</sub>+ MnCl<sub>2</sub>. PSTU (100  $\mu$ m), LANE 3, DNA + H<sub>2</sub>O<sub>2</sub>+ MnSO<sub>4</sub>( 100  $\mu$ m) ) + NaN<sub>3</sub>(5 mm), LANE 4, DNA+ H<sub>2</sub>O<sub>2</sub> + CoCl<sub>2</sub>(NO<sub>3</sub>)<sub>2</sub>.PSTU ( 100  $\mu$ m)+ L-HISITIDINE (5 mm), (4 UNITS). LANE 5, DNA + H<sub>2</sub>O<sub>2</sub> + Co(NO<sub>3</sub>)<sub>2</sub>.PSTU (100  $\mu$ m) + 4 SOD UNITS**

**CONCLUSION:** In conclusion, Mannich base ligand PSTU and its Mn(II) and Co(II) complexes were synthesized and characterized on the basis of analytical, magnetic and spectral data. The Mannich base coordinates through its thiourea nitrogen and oxygen of phthalimide to the metal ion and acts as a neutral bidentate ligand. Mn(II) complexes exhibit octahedral geometry and Co(II) complexes exhibit tetrahedral geometry. The ligand and its metal complexes have shown significant antibacterial and antioxidant activity. The Co(II) metal complex showed efficient DNA binding ability and the binding constant value is consistent with other typical intercalates. The nuclease activity of the synthesized Co(II) complex was

effective which could induce scission of pBr322 super coiled DNA effectively to linear form in presence of H<sub>2</sub>O<sub>2</sub> as oxidizing agent.

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