Research Article

Synthesis, Characterization, and DNA- Binding Interaction Studies of Ni(II) and Cu (II) Complexes Containing Succinimide Moiety

Sivakami Sudhasankar*
*Department of Chemistry, SRMIST, Kattankulathur - 603203, Tamil Nadu, India.
***Corresponding author's E-mail:** sivakamisudhasankar@gmail.com

Received: 20-01-2019; **Revised:** 25-02-2019; **Accepted:** 05-03-2019.

ABSTRACT

Succinimide (pyrolidine 2,5-dione) is a synthetically versatile substrate used for the synthesis of heterocyclic compounds and as a raw material for drug synthesis. Derivatives of succinimide are of important biological and pharmaceutical interest. A new series of nickel (II) and copper(II) complexes of a new mannichbase1-((2,5-dioxopyrrolidin-1-yl)(4-Methoxyphenyl) Methyl) Thiourea (DMT) have been synthesized. The structures of newly synthesized compounds have been elucidated based on elemental analysis, molar conductance, IR, UV-Visible, NMR and Magnetic measurements. The binding of the copper sulphate complex of the ligand with calf thymus DNA has been investigated using absorption spectroscopy, fluorescence spectroscopy and viscosity measurements.

Keywords: Mannich base, DNA binding, Fluorescence and viscosity measurements.

INTRODUCTION

Mannich reaction consists of amino alkylation of an acidic proton placed next to a carbonyl group with formaldehyde and ammonia or any primary or secondary amine. The final product is a β-amino carbonyl compound. Reactions between imides and aromatic aldehydes have also been considered as Mannich reactions. A review of literature regarding Mannich reactions shows extensive volume on chemical, biological and toxicological feature of Mannich bases.[1-3] Transition metals are essential for normal functioning of living organisms and are, therefore, of great interest as potential drugs.[4-7] The coordination chemistry of nitrogen donor ligands is an interesting area of research. A great deal of attention in this area has been focused on the complexes formed by 3d metals with bidentate ligands using both the nitrogen atoms of the substrates. Several drugs showed increased activity as metal chelates rather than as organic compounds.[8-13] To the best of our knowledge no work has been done on this class of metal complexes with the Mannich base ligand. In the continuation of our research work, herein, we report the synthesis of a new Mannich base derived from succinimide, methoxybenzaldehyde and thiourea (DMT) and the metal complexes with Ni(II) and Cu(II). The characterization studies of all the metal complexes have been done with appropriate methods. All the metal complexes were screened for anti-bacterial activities. The DNA binding and cleavage study of the copper complex containing the ligand is reported.

MATERIALS AND METHODS

All the reagents and solvents used for the synthesis of ligand and the metal complexes were analar grade of highest available purity and used as such without further purification.

Physical measurements

Elemental analysis was performed using Carlo Erba 1108 analyzer and Coleman N analyzer and was found within ± 0.5%. The molar conductivities of the metal complexes were measured in approximately 10^{-3}mol 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}H &^{13}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

Preparation of Mannich base 1-((2,5-dioxopyrrolidin-1-yl)(4-Methoxyphenyl) Methyl) Thiourea (DMT)

A known weight of succinimide (1g, 0.01mol) and a known weight of thiourea (0.7g, 0.01mol) were dissolved in a small amount of distilled water and taken in a round bottomed flask and fitted with a reflux condenser and a dropping funnel. The flask was heated in a water bath. A known weight of methoxybenzaldehyde (1.4g, 0.01 mol) was allowed to fall in drops from the dropping funnel into the flask and the contents are heated for about 30 min. The mixture is cooled, and the contents are transferred into the beaker and stirred for 8-10 hrs. After a week, a solid product formed was filtered, washed with distilled water and dried in an air oven at 60°C and recrystallized using ethanol and chloroform in 1:1 ratio (Reaction scheme – Fig.1).

Reaction scheme

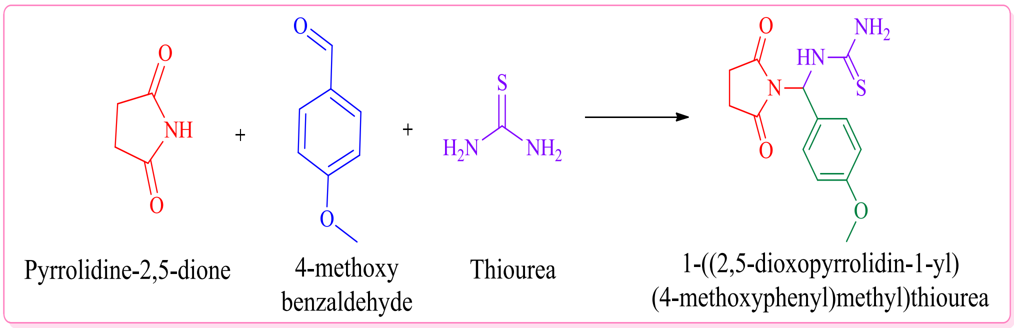


Figure 1: Preparation of Mannich base 1-((2,5-dioxopyrrolidin-1-yl)(4-Methoxyphenyl) Methyl) Thiourea (DMT)

Synthesis of Nickel Chloride Complex of DMT

To a hot methanolic solution of Nickel chloride (0.08g, 3mmol), hot ethanolic solution of the ligand DMT (0.1g, 3mmol) was added dropwise under constant stirring at 60°C for 24hrs. Then the solution was kept aside for evaporation of the solvent. After 10 days, dull green solid mass obtained was filtered, washed with methanol and dried in vacuum (Fig.2).

Synthesis of Nickel Sulphate Complex of DMT

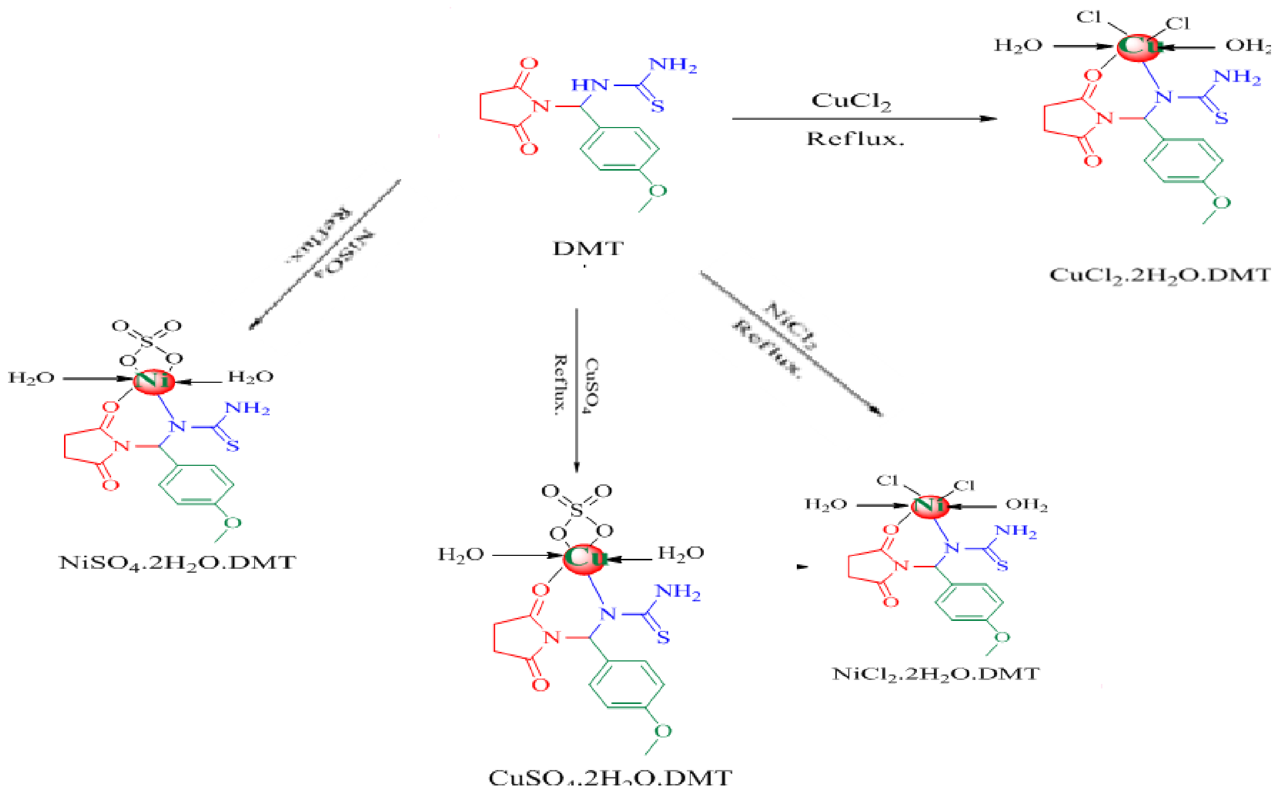
The Nickel sulphate complex of DMT was prepared by refluxing a suspension of a hot methanolic solution of Nickel sulphate (0.09g, 3mmol) with hot ethanolic solution of the ligand DMT (0.1g, 3mmol) for 1hr to obtain a clear solution. Green crystals of the metal complex were separated on evaporating the solution at room temperature for 2 weeks. The compound was filtered and washed with methanol and dried in vacuum (Fig.2).

Synthesis of Copper Chloride Complex of DMT

To a hot methanolic solution of Copper chloride (0.05g, 3mmol), hot ethanolic solution of the ligand DMT (0.1g, 3mmol) was added dropwise under constant stirring for 24hrs. Then the resulting mixture was kept aside for evaporation of the solvent. After 10 days, light green needle shaped crystals were obtained. It was filtered, washed with methanol and dried in vacuum (Fig.2).

Synthesis of Copper Sulphate Complex of DMT

The Copper sulphate complex of DMT was prepared by stirring a suspension of a hot methanolic solution of Copper sulphate (0.08g, 3mmol) with hot ethanolic solution of the ligand DMT (0.1g, 3mmol) for 1hr in a magnetic stirrer at 60°C to obtain a clear solution. Blue crystals of the metal complex were separated on evaporating the solution at room temperature for 2 weeks. The compound was filtered and washed with methanol and dried in vacuum (Fig.2).



RESULTS AND DISCUSSION

Physical Measurements

The physical properties and elemental analysis of the prepared ligand and their metal complexes are described in Table 1. The structures of metal complexes were further confirmed by conductivity measurements and magnetic moment determinations. (Table 1).

UV-Vis Spectroscopic Studies

The Nickel chloride complex shows absorption bands at 10525 cm^{-1}, 15780cm^{-1} and 24890 cm^{-1} and 35235 $cm^{-1}$for the transitions $^1A_{1g}\rightarrow{}^3T_{1g}$, $^1A_{1g}\rightarrow{}^3T_{2g}$, $^1A_{1g}\rightarrow{}^3T_{1g}$ and charge transfer transitions respectively. The μ_{eff} value was found to be 3.56 B.M suggestive of octahedral geometry.[14]

The Nickel sulphate complex exhibits absorption bands at 10642 cm^{-1}, 16670 cm^{-1}, 23522 cm^{-1} and 35714 cm^{-1} due to $^1A_{1g}\rightarrow 3T_{1g}$, $^1A_{1g}\rightarrow 3T_{2g}$, $^1A_{1g}\rightarrow 3T_{1g}$ transitions respectively. The μ_{eff} value was found to be 1.48 B.M suggesting octahedral geometry.[15]

The copper chloride complex registers absorption bands at 927 5cm^{-1}, 10374 cm^{-1}, 12357cm^{-1} due to $^2B_{1g}\rightarrow{}^2A_{1g}$, $^2B_{1g}\rightarrow{}^2B_{2g}$, $^2E_g\rightarrow{}^2T_{2g}$ transitions respectively. The charge transfer transition bands occur at 24830 and 28327 cm^{-1}. The μ_{eff} value was found at 2.09 B.M suggesting octahedral geometry.[16]

For the copper sulphate complex appears at 8240 cm^{-1}, 11325 cm^{-1},14653 cm^{-1},26391 cm^{-1} and 35670 cm^{-1}due to $^2B_{1g}\rightarrow{}^2$ A_{1g}, $^2B_{1g}\rightarrow{}^2B_{2g}$, $^3E_g\rightarrow{}^2$ T_{2g} and CT transitions respectively. The μ_{eff} value was found to be 1.8 B.M in agreement with distorted octahedral geometry.[17] (Table 1)

Table 1: Analytical data & UV spectral data of the ligand DMT & its metal complexes.

Compound	C	H	N	O	Λm (ohm-1 cm2 mol-1) & μeff (B.M)	λ_{max} (cm^{-1})	Transition Assignment	Geome-try
DMT ($C_{13}H_{15}N_3O_3S$)	53.20 (53.17)	5.10 (5.08)	14.27 (14.26)	16.32 (16.31)	-			
$NiCl_2.2H_2O$.DMT ($C_{14}H_{20}Cl_2N_3NiO_5S$)	35.61 (35.58)	4.21 (4.20)	8.88 (8.86)	16.91 (16.89)	41 (3.56)	10525 15780 24890 35235	$^1A_{1g}\rightarrow{}^3T_{1g}$ $^1A_{1g}\rightarrow{}^3T_{2g}$ $^1A_{1g}\rightarrow{}^1T_{1g}$ CT	Octahedral
$NiSO_4.2H_2O$.DMT ($C_{14}H_{20}N_3NiO_9S_2$)	36.17 (36.15)	3.12 (3.12)	8.99 (8.97)	24.27 (23.26)	44 (3.53)	10642 16670 23522 35714	$^1A_{1g}\rightarrow{}^3T_{1g}$ $^1A_{1g}\rightarrow{}^3T_{2g}$ $^1A_{1g}\rightarrow{}^1T_{1g}$ CT	Octahedral
$CuCl_2.2H_2O$.DMT ($C_{14}H_{20}Cl_2CuN_3O_5S$)	35.22 (35.21)	4.19 (4.18)	8.80 (8.79)	16.73 (16.72)	40 (2.09)	9275 10374 12557 24330 & 28327	$^2B_{1g}\rightarrow{}^2A_{1g}$ $^2B_{1g}\rightarrow{}^2B_{2g}$ $^2E_g\rightarrow{}^2T_{2g}$ CT	Octahedral
$CuSO_4.2H_2O$.DMT ($C_{14}H_{16}CuN_3O_7S_2$)	36.00 (35.98)	3.16 (3.15)	8.99 (8.97)	23.91 (23.89)	43 (2.26)	8240 11325 14653 26391 & 35670	$^2B_{1g}\rightarrow{}^2A_{1g}$ $^2B_{1g}\rightarrow{}^2B_{2g}$ $^2E_g\rightarrow{}^2T_{2g}$ CT	Distorted Octahedral

IR Spectral Analysis of DMT and its Metal Complexes

In order to study the binding mode of the ligand to metal in the complexes, the IR spectrum of the free ligand was compared with the corresponding metal complexes. Selected vibrational bands of the ligand and its metal complexes and their assignments are listed in Table 2. The IR spectrum the free ligand exhibited a strong band at 1690cm^{-1} which could be assigned to $\nu_{C=O}$ of the succinimide ring. A band around 3297cm^{-1} could be attributed to stretching vibration of $\nu_{N=H}$ bond.[18] A strong band observed around 1392cm^{-1} can be assignable to $\nu_{C=S}$ vibration mode. In the metal complexes, the band corresponding to $\nu_{C=O}$ of succinimide ring was shifted to lower frequency range suggesting the coordination of carbonyl group with the metal ion. There is no shifting of bands at 1400 cm^{-1} and 750 cm^{-1} indicating the absence of coordination of C=S group with the metal ion. The N-C-N stretching frequency of the ligand at 1472cm^{-1} was shifted towards lower values in all the complexes, indicating the involvement of the nitrogen of thiourea in coordination to the central metal ion. The participation of oxygen and nitrogen in coordination with the metal ion is further supported by the new band appearance of $ν_{M-N}$ around 420-425cm^{-1} in the far infrared region.

Table 2: Characteristic IR Spectral Data (cm^{-1}) of DMT and its Metal Complexes

Compound	$ν_{NH}$	$ν_{C=O}$	$ν_{C=S}$	N-C-N	$-OCH_3$	H_2O Coord	M-X	M-S
DMT	3297	1690	1392	1472	1272	-	-	-
$NiCl_2.2H_2O$.DMT	3290	1683	1396	1463	1271	3747,1590,808	423	-
$NiSO_4.2H_2O$.DMT	3320	1701	1381	1470	1294	3774,1624,817	-	427
$CuCl_2.2H_2O$.DMT	3292	1687	1400	1466	1268	3377,1591,808	424	-
CuSO4. $2H_2O$.DMT	3384	1699	1385	1510	1273	3775,1626,816	-	423

^{1}H NMR Data of DMT

(DMSO/TMS, 500 MHz): A The1H NMR spectrum of the ligand shows a singlet at δ 2.56 due to $–CH_2$ proton. The singlet for one proton at δ 3.7 is assigned to $-OCH_3$ proton. A multiplet in the range δ 6.41-7.78 is assigned for aromatic protons. The singlet for one proton at δ 10.10 is assigned to$–NH_2$proton. Another singlet at δ 11.062 is assigned to -NH proton.

^{13}C NMR Data of DMT

(DMSO/TMS, 125 MHz): The number of signals of sharp peaks represents the number of carbons of the ligand which are not chemically equivalent. ^{13}C NMR - δ 179.4, 156.9, 149.9, 139.5, 127.6, 120.9, 111.3, 108.2, 55.6, 40.0, 29.5.

LC Mass Data of DMT

DMT Molecular formula: $C_{13}H_{15}N_3O_3S$, Observed *m/z*=294.37, Calculated *m/z*=293.08.

DNA Binding Studies

Absorption spectral studies

The transition metal complex can bind to DNA via both covalent and/or non-covalent interactions. It is well known that electronic absorption spectroscopy is an effective method to examine the binding modes and binding extent of the metal complexes with DNA. In the UV region, the intense absorption bands observed in the region 300-360 nm were attributed to intra ligand π-π* transition. Increase in concentration of CT-DNA resulted in the hypochromism and blue-shift in UV-Vis spectrum of the metal complexes of DMT.

These spectral characteristics suggest that the metal complexes of DMT might bind to DNA by an intercalative mode due to a strong stacking interaction between the aromatic chromophore of the complex and the base pairs of the DNA. After intercalating the base pairs of DNA, the π* orbital of the intercalated ligand could couple with the π orbital of the base pairs, thus decreasing the π-π* transition energy and further resulting in the blue shift. On the other hand, the coupling of the π orbital was partially filled by electrons, thus decreasing the transition probabilities and concomitantly, resulting in the hypochromism.

The spectroscopic methods were used to ascertain the interaction mode of the metal complexes $NiCl_2$.DMT, $NiSO_4$.DMT, $CuCl_2$.DMTand $CuSO_4$.DMT with CT-DNA. The metal complexes exhibited intense absorption bands around 260 nm which are assigned to intraligand charge transfer transition of aromatic chromophore and at 370 nm which are attributed to metal ligand charge transfer bands.[19-22] The absorption spectra of complexes in the absence and presence of CT-DNA are shown in the Fig 3.Addition of increasing amounts of CT-DNA resulted in an appreciable decrease in absorption intensity of complexes and red shift in wavelength. In order to compare quantitatively the affinity of the metal complexes of DMT towards CT-DNA, the intrinsic binding constants K_b of the complexes with CT-DNA were obtained by monitoring the changes in absorbance with increasing concentration of CT-DNA.

The binding constant K_b of the complexes $NiCl_2$.DMT, $NiSO_4$.DMT, $CuCl_2$.DMT and $CuSO_4$.DMT are 1.3x10^4, 1.8 x10^4, 2.6 x10^4, 2.8 x10^4 and 2.4 x 10^4 M^{-1}respectively (Table 3).The hypochromism and red shift are associated with the binding of the metal complexes to the DNA helix, due to the intercalative mode involving a strong stacking interaction between the aromatic chromophore of the complexes and the base pairs of DNA. The values suggest that the copper sulphate complex has stronger binding affinity than the other complexes.

Table 3: Binding Parameters of DMT metal Complexes with DNA

Complexes	DNA		
	K_b x10^4 (M^{-1})	K_{sv} x10^4 (M^{-1})	K_{app}x 10^6 (M^{-1})
$NiCl_2$.DMT	1.3	2.08	1.33
$NiSO_4$.DMT	1.8	2.1	1.42
$CuCl_2$.DMT	2.6	3.15	1.51
$CuSO_4$.DMT	2.8	4.9	2.2

Fluorescence spectral studies

In order to investigate further the interaction modes between these metal complexes and CT-DNA, the ethidiumbromide (EB) fluorescence displacement experiments were further used. In general, the intrinsic fluorescence intensity of DNA is very low, and that of EB in Tris buffer is also not high due to quenching by the solvent molecules. However, on addition of DNA, the fluorescence intensity of EB will be enhanced because of its intercalation into the DNA. Thus, EB can be used to probe the interaction of complexes with DNA. The fluorescence intensity of EB can be quenched by the addition of another molecular due to decreasing of the binding sites of DNA available for EB. Thus fluorescence spectral method is used to study the relative binding of the synthesized complexes and ethidiumbromide (EB) with CT-DNA.

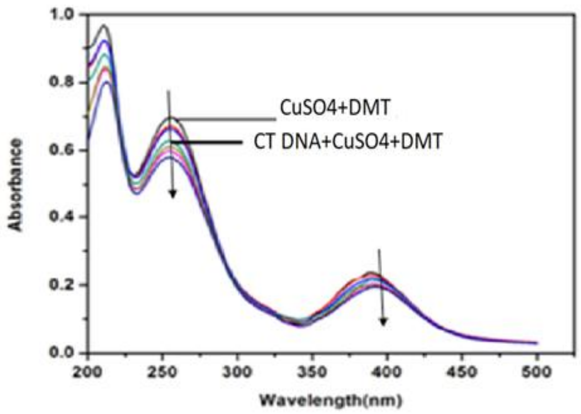


Figure 3: Absorption Spectra of the Copper complex of DMT in the absence and presence of increasing amounts of CT-DNA(0-200µM) in tris-HCl buffer

Complexes are non-emissive both in presence and absence of CT-DNA. EB emit intense fluorescence at about 600 nm in the presence of CT DNA because of its strong interaction between the adjacent DNA base pairs). If the metal complex intercalates in to DNA, it leads to a decrease in the binding sites of DNA available for EB and a decrease in the fluorescence intensity of the EB-DNA system.[23]

The observed quenching of EB-DNA fluorescence intensity upon addition of complex into EB-DNA system suggest that the complex may displace EB from the EB-DNA and complex can interact with CT-DNA probably by the intercalative mode (Fig.5).The Stern-Volmer quenching constant K_{sv} values of the complexes $NiCl_2$.DMT(1), $NiSO_4$.DMT(2), $CuCl_2$.DMT(3) and $CuSO_4$.DMT(4) were calculated as 2.08×10^4, 2.1×10^4, 3.15×10^4 and 4.9×10^4 respectively. On the other hand, it is noteworthy that 50% of EB molecules were replaced from DNA-bound EB at a concentration ratio of [complex]/[EB] = 21and the apparent binding constant (K_{app}) of complexes can be calculated by using the following equation

$$K_{app} = K_{EB}[EB]_{50\%} / [complex]_{50\%}$$

where K_{EB} is the DNA-binding constant of EB, and $[EB]_{50\%}$ and $[complex]_{50\%}$ are EB and complex concentrations at 50% fluorescence. K_{EB} is known as $1.0 \times 10^7\ M^{-1}$. The K_{app} values of the complexes were calculated as 1.33×10^6, 1.42×10^6, 1.51×10^6 and $2.2 \times 10^6 M^{-1}$ respectively. From this spectral data, it is seen that the copper complex binds well through intercalative than the other complexes.

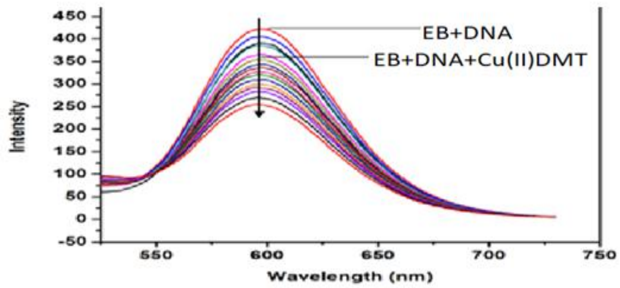


Figure 4: Emission spectra of EB bound to DNA in tris-HCl buffer (pH 7.2) in the absence and presence of the copper complex. [EB]= 4µM, [DNA] = 0µM, Cu (II).DMT] = 0 to 160µM. (λex = 520nm)

Viscosity measurements

Viscosity measurements were carried out to further clarify the mode of interaction of metal complexes to DNA. A classical intercalative mode causes a significant increase in viscosity of DNA solution due to an increase in the separation of base pairs at intercalation sites and hence an increase in the overall DNA length. With increasing [complexes]/[DNA] ratios from 0.02 to 0.12, the complexes $NiCl_2$. DMT (1), $NiSO_4$. DMT (2), $CuCl_2$. DMT (3) and $CuSO_4$. DMT (4) exhibits a tendency of increasing relative viscosity of the [complexes-DNA] system, which strongly suggests the intercalation as the main binding mode of these complexes with CT-DNA.

DNA Cleavage Studies

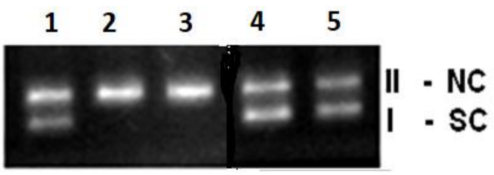
Complexes can cleave DNA via hydrolytic and/or oxidative pathways. In the oxidative pathway, they have been shown to react with molecular oxygen or H_2O_2 to produce a variety of reactive oxidative species (ROS). The cleavage mechanism was studied by using a series of scavengers that could inhibit production of the ROSs. For example, DMSO and t-BuOH can be used as scavenger of $HO^{\cdot}$ radical, while NaN_3 can be used as singlet-oxygen scavenger.

The cleavage of supercoiled 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 metal complexes of DMT showed remarkable cleavage. Fig.5 shows the results of the gel electrophoretic separations of plasmid pBR322 DNA by the complexes in the presence of H_2O_2. Under similar conditions, no cleavage of pBR322 DNA occurred for free H_2O_2 (40 µM) or complex (30 µM). All the complexes showed the cleavage at 30 µM concentrations in presence of H_2O_2 at 40 µM. Hence it is reasonable to suggest that the higher DNA cleavage activity of the complex is due to presence of aromatic group in the ligand, which enhances the binding and cleavage ability of the molecule.

The cleavage mechanism of pBR322 DNA induced by complex was investigated and clarified in the presence of hydroxyl radical scavenger 0.4 M DMSO, SOD (3&4 units) and EDTA as a chelating agent under aerobic conditions. As shown in Fig 5, the DNA cleavage mechanism by complex is shown as follows: both DMSO and SOD are completely ineffective, this rules out the possibility of cleavage by hydroxyl radical and superoxide. The singlet oxygen scavenger NaN_3 did not show inhibition of DNA cleavage suggesting that $^1O_2$ did not take part in the cleavage mechanism. The EDTA can efficiently inhibit the activity of the compounds.[24]

The absence of inhibition of DNA cleavage performed by the complex clearly suggest that the DNA scission mechanism might followed a hydrolytic path way rather than oxidative pathway. The complexes cleave DNA even in the absence of oxygen, approximately to the same extent as under aerobic conditions. It is thus concluded that the complexes are likely to cleave DNA by a hydrolytic mechanism.

(A)



(B)

Figure 5(A): Cleavage of SC pBR322 DNA (0.2 µg, 33 µM) by $NiCl_2$.DMT, $NiSO_4$.DMT, $CuCl_2$.DMT and $CuSO_4$.DMT (30 µM) in presence of H_2O_2 (40 µM) in 50 mMTris-HCl / NaCl buffer (pH 7.2). Lanes 1, DNA control; 2, DNA + H_2O_2 +$NiCl_2$.DMT (30 µM); 3, DNA + H_2O_2 + $NiSO_4$.DMT (30 µM); 4, DNA + H_2O_2 + $CuCl_2$.DMT (30 µM); 5, DNA + H_2O_2 + $CuSO_4$.DMT (50 µM)

(B) Cleavage Mechanism of SC pBR322 DNA (33 µM) by the Metal Complexes of DMT (50 µM) in the Presence of H_2O_2 (40 µM) in 50 mMTris-HCl / NaCl Buffer (pH 7.2). Lanes 1, DNA control; 2, DNA + H_2O_2 + $Nicl_2$complex + SOD (4 units); 3, DNA + H_2O_2 + $NiSO_4$ complex + SOD (4 units); 4, DNA + H_2O_2 + $CuCl_2$complex + DMSO (70 mM) and 5, DNA + $CuSO_4$ complex + NaN_3 (70 mM).

CONCLUSION

In this paper coordination chemistry of a Mannich base ligand obtained from the reaction of succinimide, methoxybenzadehyde and thiourea is described. Ni(II), and Cu(II) complexes have been synthesized using the above Mannich base ligand and characterized on the basis of analytical, magnetic and spectral data. The Mannich base coordinates through its thiourea nitrogen and oxygen of succinimide to the metal ion and acts as a neutral bidentate ligand. All the complexes exhibit octahedral geometry. The Cu(II) metal complex showed efficient DNA binding ability and the binding constant value is consistent with other typical intercalators. The nuclease activity of the synthesized Cu(II) complex was effective which could induce scission of pBr322 super coiled DNA effectively to linear form in presence of H_2O_2 as oxidising agent.

Acknowledgements: The author wish to thank Biogenics, Hubli for the DNA cleavage studies, Madras University for DNA binding studies and viscosity measurements and ISISM, SRMIST for spectral studies.

REFERENCES

1. Martell AE, Hancoc RD and Motekaitis RJ, Factors affecting stabilities of chelate, macrocyclic and macrobicyclic complexes in solution, Coordination Chemistry Reviews, 133, 1994, 39-65.
2. Orvig C and Abrams MJ, Medicinal inorganic chemistry: Introduction, Chemical Reviews,99(9) 1999, 2201-2204.
3. Lev E, Medicinal exploitation of inorganic substances in the Levent in the Medieval and early Ottoman periods, Adler Museum Bulletin, 28(2), 2002, 11-16.
4. Seklon BS and Gandhi L, Medicinal uses of inorganic compounds- 1, Resonance 11(4)**,** 2006, 75-89.
5. Evenas J, Malmendal A and Forsen S, Calcium, Current opinion in chemical biology, 2(2), 1998, 293-302.
6. Raman N, Ravichandran S, Synthesis and Characterization of a New Schiff base and its metal complexes derived from the Mannich base, N-(1-Piperidino benzyl) acetamide, Synth. React. Inorg. Met. Org. Nano-Metal Chem., 35, 2005, 439. Misra, V.K., and Draper, D.E., "On the role of Magnesium ions in RNA stability," Biopolymers, 48 (2-3), 1998, pp.113-135.
7. Frerickson CJ, Koh JY and Bush AI, The neuro biology of Zinc in health and disease, Nature Reviews. Neuroscience, 6(6), 2005, 449-462.
8. Thomas DD, Ridnour LA, Isenberg JS, Flores-Santana W, Switzer, CH, Donzelli S, Hussain P, Vecoli C, Paolocci, N, Ambs S, Colton CA, Harris CC, Roberts DD and Wink DA, The chemical biology of nitric oxide : implications in cellular signaling, Free Radical Biology and Medicine, 45 (1), 2008, 18-31.
9. Chen PR and He C, Selective recognition of metal ions by metallo regulatory proteins, Current Opinion in Chemical Biology, 12(2), 2008, 214-221.
10. Pennella MA and Giedroc DP, Structural determinations of metal selectivity in prokaryotic metal responsive transcriptional regulators, Biometals, 18(4), 2005, 413-428.
11. Cowan JA, Bertini I, Gray HB, Stiefel EI and Valentine JS, Editors, *Structure and Reactivity: Biological Inorganic Chemistry*,3, University Science Books, Sausalito, 2007, 175.

12. Lippard SJ and Berg JM, Principles of Bioinorganic Chemistry: University Science Books, Mill Valley California, 7, 1994,115.

13. Holm RH, Kennepohl P and Solomon E, Structural and functional aspects of metal sites in biology, Chemical Reviews, 96 (7), 1996, 2239-2344.

14. Lv J, Liu T, Cai S, Wang X, Liu L, Wang Y, Synthesis, structure and biological activity of cobalt(II) and copper(II) complexes of valinederived Schiff bases, J. Inorg. Biochem., 100, 2006, 1888–1896.

15. Sawyer TK, Chemical Biology and Drug Design: three dimensional, dynamic and mechanistic nature of two multi disciplinary fields, Chemical Biology and Drug Design, 67(3), 2006, 196-200.

16. Stockwell BR, Exploring biology with small organic molecules, Nature, 43**,** 2007, 504-507.

17. Fricker SP, Metal based drugs from serendipity to design, Dalton Transactions, 43**,** 2007, 4903-4917.

18. Schreiber SL, Small molecules: the missing link in the central dogma, Nature Chemical Biology, 1, 2005, 64-66.

19. Lippard SJ, The Inorganic side of Chemical Biology, Nature Chemical Biology, 10, 2006, 504-507.

20. David SS and Meggers E, Inorganic Chemical Biology: from small molecules in biological systems to metalloproteins, Current Opinion in Chemical Biology, 12(2), 2008, 194-196.

21. Doudna JA, Chemical biology at the cross roads of molecular structure and mechanism, Nature Chemical Biology, 1 (6), 2005, 300-303.

22. Cohen SM, New approaches for medicinal applications of bioinorganic chemistry, Current Opinion in Chemical Biology, 11(2), 2007, 115-120.

23. Ochiai, E, Bio inorganic chemistry: a survey, medical applications of inorganic compounds, Academic press, Chapter 12, 2008, 311-326.

24. Negm NA, Morsy SMI and Said MM, Biocidal activity of some Mannich base cationic derivatives, Bioorganic Medicinal Chemistry, 13(21), 2005, 5921-5926.

Source of Support: Nil, **Conflict of Interest:** None.