Evaluation of DNA Binding and Cytotoxic activity of a new Mannich base and its Metal Complexes

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Abstract— **Succinimide 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. Here the novel Mannich base 1-((2,5-dioxopyrrolidin-1-yl)(4-methoxyphenyl)methyl)thiourea (SMBTU) has been synthesized in good yield by condensation of equimolar quantities of succinimide, methoxy benzaldehyde and thiourea. Manganese (II), Cobalt(II), Nickel(II) and Copper(II) complexes of the above ligand have also been synthesized . Structures of newly synthesized compounds were confirmed by elemental analysis, IR,UV-VIS & NMR. All the complexes adopt octahedral geometry around the metal ions. All the newly synthesized compounds were screened for their anti microbial activity against *E.coli* and *B. subtilis* bacteria by MIC technique. The DNA binding of the cobalt chloride complex of the ligand with calf thymus DNA has been investigated using absorption spectroscopy and fluorescence spectroscopy.**

Keywords- **Mannich base, Metal complexes, Anti bacterial, DNA binding.**

I. Introduction

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. The study of structural and binding features of various Mannich base complexes can play an important role in better understanding of the complex biological processes. Several drugs showed increased activity as metal chelates rather than as organic compounds [1-3]. Cobalt complexes possess antitumor, antiproliferative, antimicrobial and antifungal activity[4-11]. To the best of our knowledge no work has been done on this class of metal complexes with the Mannich base ligand SMBTU. In the continuation of our research work, Herein, We report the synthesis of a new Mannich base derived from succinimide , methoxy benzaldehyde and thiourea (SMBTU) and the metal complexes with Mn(II), Co(II), 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 antibacterial activities. The DNA binding studies of the cobalt complex containing the ligand SMBTU is reported.

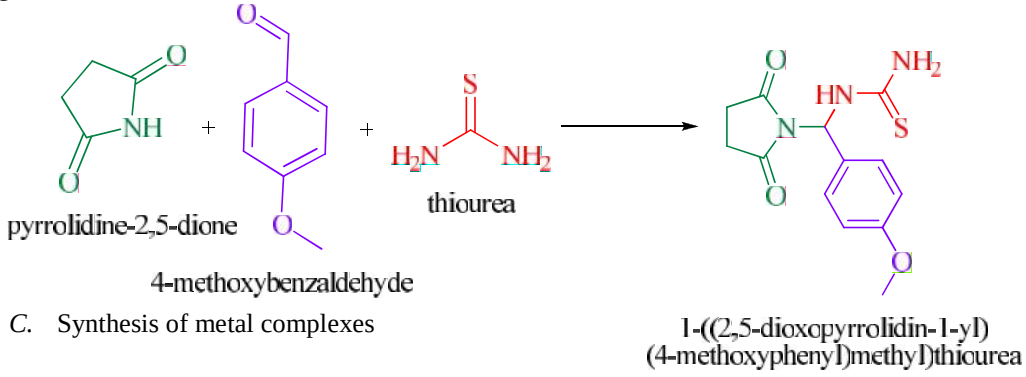
II. Experimental detail

A. 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.

B. Preparation of Mannich base 1-((2,5-dioxopyrrolidin-1-yl)(4-methoxyphenyl)methyl) thiourea (SMBTU)

Succinimide, methoxy benzaldehyde and thiourea were taken in 1:1:1 molar ratio. In aqueous solution of succinimide and thiourea. Methoxy benzaldehyde was added drop wise and the mixture was stirred in a magnetic stirrer at room temperature for 8-10 hours. 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. Fig 1.



C. Synthesis of metal complexes

All the metal complexes of SMBTU 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. The insoluble metal complexes were formed after 2 weeks .It was washed with methanol and ethanol to remove unreacted metal salt and ligand. The products were then dried in an air oven at 60°C. The proposed structures of the metal complexes are shown in Fig 2.

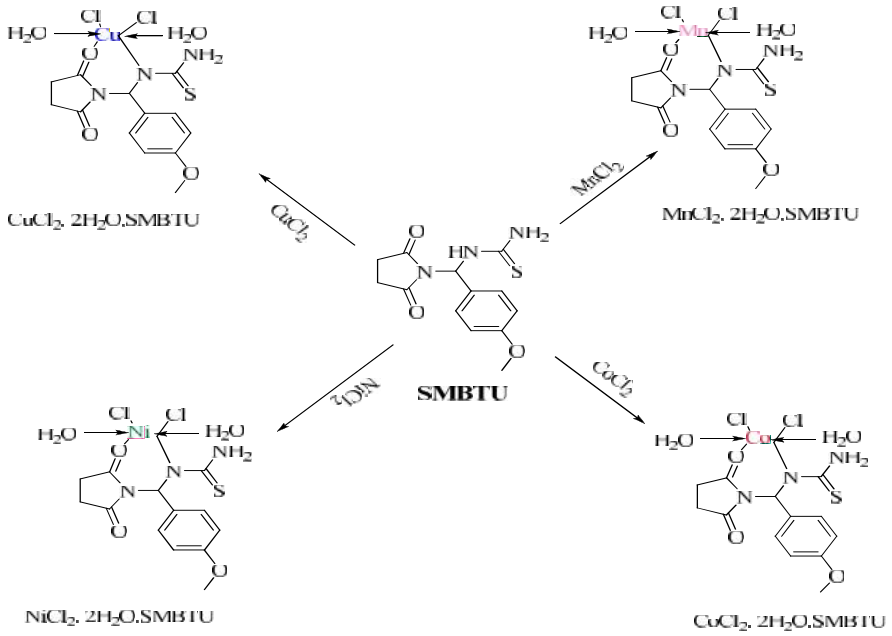


Figure 2: Proposed structures of the metal complexes

III. Results and Discussion

A. UV-Vis Spectral studies

Table 1: Molar Conductance (in DMF), magnetic moment, assigned transitions with λ_{max} and geometry of the metal complexes

Complex	Λm (ohm^{-1} cm^2 mol^{-1})	μ_{eff} (B.M)	λ_{max} (cm^{-1})	Transition Assignment	Geometry
$MnCl_2$. $2H_2O$.SMBTU	62	4.85	18050 24985 29125 31272	$^6A_{1g} \rightarrow {}^4T_{1g}$ $^6A_{1g} \rightarrow {}^4E_{2g}$ $^6A_{1g} \rightarrow {}^4E_{1g}$ CT	High spin Octahedral
$CoCl_2$. $2H_2O$.SMBTU	55.5	4.48	6703 14365 18742 29066	$^4T_{1g} \rightarrow {}^4T_{2g}$ $^4T_{1g} \rightarrow {}^4A_{2g}$ $^4A_{1g} \rightarrow {}^4T_{1g}$ CT	Octahedral
$NiCl_2$. $2H_2O$.SMBTU	61	3.56	10525 15780 24890 35235	$^1A_{1g} \rightarrow {}^3T_{1g}$ $^1A_{1g} \rightarrow {}^3T_{2g}$ $^1A_{1g} \rightarrow {}^1T_{1g}$ CT	Octahedral
$CuCl_2$. $2H_2O$.SMBTU	89	2.09	9275 10374 12557 24330,28327	$^2B_{1g} \rightarrow {}^2A_{1g}$ $^2B_{1g} \rightarrow {}^2B_{2g}$ $^2E_g \rightarrow {}^2T_{2g}$ CT	Octahedral

B. Important IR spectral data

Table 2: Characteristic IR Absorption Frequencies (cm^{-1}) of SMBTU and its Metal Complexes

Compound	ν_{NH}	$\nu_{C=O}$	$\nu_{C=S}$	$\nu_{CH(st)}$	$\nu_{CH(b)}$	N-C-N	$-OCH_3$	H_2O Coord	M-X	M-S
SMBTU	3297	1690	1392	3174	814	1472	1272	-	-	
$MnCl_2.2H_2O$. SMBTU	3289	1699	1394	3170	811	1468	1271	3746,1592, 728	422	-
$CoCl_2.2H_2O$. SMBTU	3291	1684	1398	3170	808	1467	1271	3778,1591, 728	425	-
$NiCl_2.2H_2O$. SMBTU	3290	1683	1396	3170	808	1463	1271	3747,1590, 728	423	-
$CuCl_2.2H_2O$. SMBTU	3292	1687	1400	3169	808	1466	1268	3747,1591, 727	424	-

C. ^{1}H NMR Data (DMSO/TMS, 500.3MHz):

δ2.57 (s, 4H), 3.61 (s, 3H), 6.92 to 7.20 (m, 4H,), 10.62 (s, 1H), 11.06 (s, 2H), ppm.

D. ^{13}C NMR Data (DMSO/TMS, 125.7 MHz):

δ179.4, 156.4, 147.8, 133.9, 129.6, 124.3, 40.0, 29.5 ppm.

E. LC Mass Data: Calculated for SMBTU $C_{15}H_{15}N_3OS$ *m/z*=293.08; Found 294.15 (M+1).

F. Anti-bacterial activity of Mannich base

The minimal inhibitory concentration of ligand SMBTU was found to be 300 µg for E.coli and B. subtilis. The activity was higher rate when the growth inhibition was observed at 600 nm. But at low concentrations survival of bacteria was observed.

G. DNA Binding:

One of the most important approaches in the development of drugs and chemotherapy against some cancers, viral and parasitic diseases involve drugs which interact reversibly with DNA. Hence, synthesis of new metal complexes which can bind with specificity to DNA and bring about its cleavage are of importance in the development of new antitumor agents.12-13.

H. Electronic Absorption Spectra

Absorption titration experiments were carried out by varying the DNA concentration (0-100μM) and maintaining themetal complex concentration constant. Absorption spectra were recorded after successive addition of DNA and equilibration (approximately 10 minutes). Absorption titration experiments with CT-DNA show intense absorption peaks at 230 and 280 nm in the UV region of the complex due to inter ligand π- π* transition of the coordinated groups in the complex. On addition of increasing amounts of DNA to the complex, both of the two characteristic peaks decreased gradually with the maximum hypochromicity of 15% & 20 % respectively, suggesting the strong interaction between the complex and DNA (Fig 3).

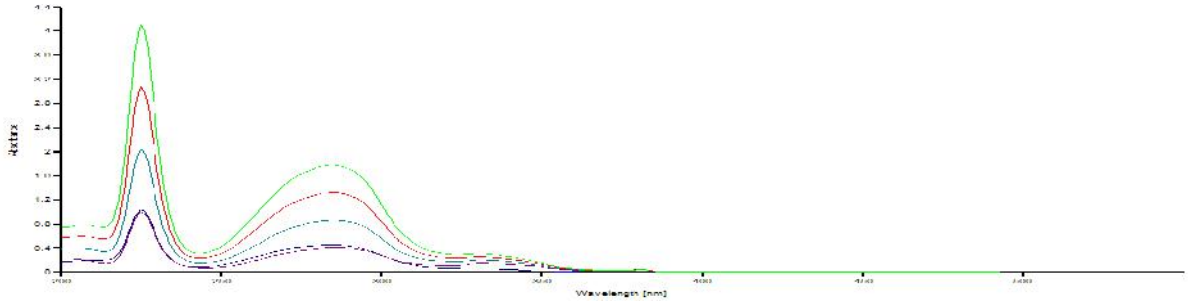


Figure 3

I. Fluorescence spectra

Fluorescence quenching experiments were performed with ethidium bromide bound DNA with increasing concentrations of metal complex to determine the extent of binding between the molecule and DNA. The fluorescence quenching curves of EB bound to DNA in absence and presence of the complex was monitored. The addition of the metal complex to EB bound to DNA has shown a reasonable reduction in emission intensity indicating that the complex is bound to DNA at the sites occupied by EB (Fig 4).

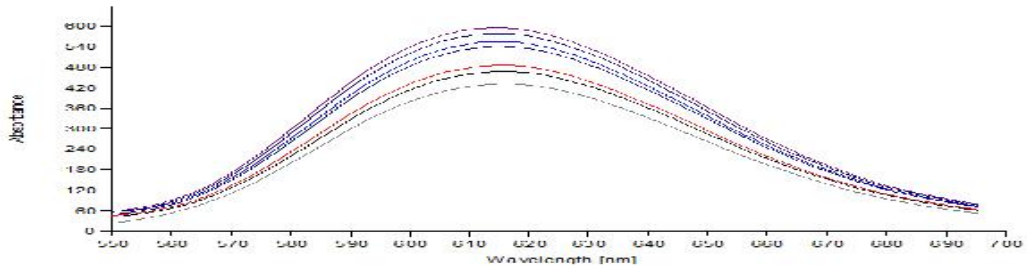


Figure: 4

IV. Conclusion:

In this paper coordination chemistry of a Mannich base ligand obtained from the reaction of succinimide, methoxy benzadehyde and thiourea is described. Mn(II) ,Co(II) , 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 ligand and its metal complexes have shown significant antibacterial activity . The Co(II) metal complex showed efficient DNA binding ability and the binding constant value is consistent with other typical intercalators.

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