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Original Article

SYNTHESIS, BIOLOGICAL STUDY, DNA INTERACTION OF MANNICH BASE METAL COMPLEXES DERIVED FROM BENZAMIDE

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ABSTRACT

Objective: Coordination compounds occur widely in nature and they comprise a large share of current inorganic research. The Mannich reaction is a classic method for the preparation of Mannich bases, namely, β amino compounds, which are heterocyclic.

Methods: A novel Mannich base of N-((3,4-dimethoxyphenyl)(2,5-dioxopyrrolidin-1-yl) methyl)benzamide and its coordination complexes with transition metals Mn and Co have been synthesized and characterized by elemental analysis, molar conductance, magnetic susceptibility measurements, UV-Visible, IR, NMR, and Mass spectral studies.

Results: Based on the magnetic moment and UV-Visible spectral data, octahedral geometries were assigned for the metal complexes. The metal complexes were screened for antifungal activity.

Conclusion: the metal complexes have shown good activity than the ligand. The binding of selected metal complexes with calf thymus DNA was investigated. It is found that the cobalt (II) metal complex of the ligand showed efficient DNA binding ability.

Keywords: Coordination compounds, Mannich base, UV-Visible, Antifungal activity, DNA binding

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INTRODUCTION

Transition metals have an important role in medicinal biochemistry. Research has shown significant progress in the utilization of transition metal complexes as drugs to treat several diseases like carcinomas, lymphomas, infection control, anti-inflammatory, diabetes, and neurological disorders [1-4]. Manganese is an essential element involved in many chemical processes in the body, including the processing of cholesterol, carbohydrates, and protein. Iron compounds include hemoglobin which keeps our blood red. Iron atoms also help to join organic molecules, forming bactericides or bacteriostatic agents [5]. Cobalt contained in Vitamin B12 is important in protein formation and DNA regulation. Cobalt-60, a radioactive isotope, is used as a commercial source of high-energy radiation in medicine to destroy cancerous tissue. Cobalt-containing drugs are used as cyanide antidotes. Mannich bases of benzamide and succinimide are known to play a prominent role in medicinal chemistry. Despite the assets of available paper, it is found that they possess various pharmacological properties as hypnotic and tranquilizer activity, CNS depressant and cardiac stimulant, tumor-inhibiting properties, and antibacterial activity [6-8]. Moreover, the presence of Mannich side chain increases the solubility and bioavailability of the drug molecule [9-11]. This work focuses on the synthesis of Mn(II) and Co(II) complexes with a new series of Mannich bases derived from succinimide, benzaldehyde, and benzamide.

MATERIALS AND METHODS

All the reagents and solvents used for the synthesis of ligand and the metal complexes were AR grade quality and have been utilized with no purification. Elemental analysis was performed using Carbo Erba 1108 analyzer and Coleman N analyzer and was found within±0.5%. The molar conductivities of 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 the UV-Visible region was recorded in DMF solution using a UV-Visible spectrometer. The 1H and 13CNMR of the ligand were recorded in the Bruker instrument employing TMS as internal standard and DMSO-DMF as internal solvent. 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 the Agar-well diffusion method.

Preparation of N-((3,4-dimethoxyphenyl)(2,5-dioxopyrrolidin-1-yl) methyl)benzamide (NDB)

The new Mannich base N-((3,4-dimethoxyphenyl)(2,5-dioxopyrrolidin-1-yl) methyl)benzamide (NDB)has been synthesized by the condensation of equimolar quantities of succinimide, dimethoxy benzaldehyde, and benzamide by the following method. 1g (0.01 mol) of succinimide and 0.48g (0.01 mol) of benzamide was dissolved in a small amount of distilled water and taken in a 100 ml beaker. 1.67g (0.01 mol) of dimethoxy benzaldehyde dissolved in acetone was added dropwise to the beaker solution and stirred with a magnetic stirrer for one day. Then it is kept aside with occasional stirring for few days. After a week a solid product formed was filtered, washed with distilled water and dried in an air oven at 60 ^{0}C, and recrystallized using ethanol and $CHCl_3$ in 1:1 ratio (fig. 1).

Synthesis of manganese sulphate complex of NDB

To a salt solution of Manganese sulphate (0.05g), ethanolic solution of the ligand NDB (0.1g) was added slowly with stirring. The solution is stirred well in a hot late with a magnetic stirrer to get a clear solution. It is covered with aluminum foil and kept aside for 2 w. After slow evaporation of the solvent at 28 ^{0}C, a dull white colored compound was obtained. It was filtered and dried in a vacuum (fig. 2).

Synthesis of manganese chloride complex of NDB

To a solution of Manganese chloride (0.061g), ethanolic solution of the ligand NDB (0.1g) was added slowly with stirring. After stirring for 4 h, a clear solution was obtained. It is covered with aluminum foil and kept for a few days. A resulting dull-white solid was separated on evaporating the solution at room temperature. It is filtered and dried in a vacuum (fig. 2).

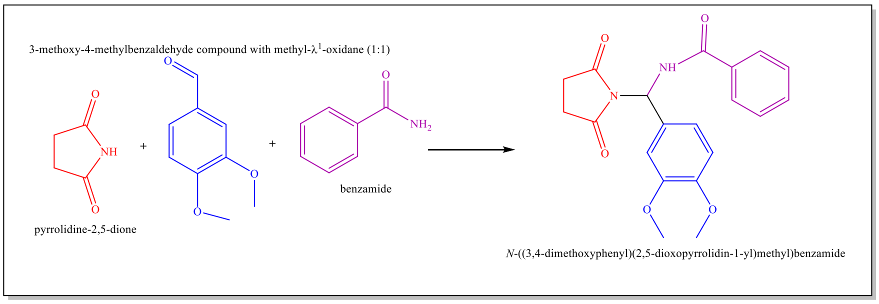

Fig. 1: Preparation of N-((3,4-dimethoxyphenyl) (2,5-dioxopyrrolidin-1-yl) methyl)benzamide (NDB)

Synthesis of cobalt sulphate complex of NDB

The cobalt sulphate complex of NDB was prepared by refluxing a solution of Cobalt sulphate (0.08g) with an ethanolic solution of the ligand NDB (0.1g) for 1 hour to obtain a clear solution. After keeping aside the resultant solution for 2 w, blue crystals of the metal complex were separated on evaporating the solution at room temperature. It is filtered and dried in a vacuum (fig. 2).

Synthesis of cobalt chloride complex of NDB

The cobalt chloride complex of NDB was prepared by refluxing a hot suspension of cobalt chloride (0.735g) with an ethanolic solution of the ligand (0.1g) for an hour to obtain a clear solution. Light blue crystals of the metal complex were formed by the slow evaporation of solvent after 2 w. The compound was filtered and dried in a vacuum (fig. 2).

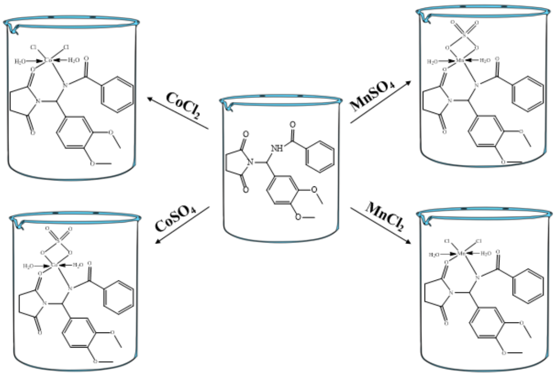

Fig. 2: Synthesis of Co and Mn (II) metal complexes of NDB

RESULTS AND DISCUSSION

Physical measurements

The physical properties and elemental analysis of the prepared ligand and its metal complexes are described in table 1 and 2. The structures of the metal complexes were further confirmed by conductivity measurements and magnetic moment determinations. The molar conductance studies reveal that all the complexes are non-electrolytes. The CHNO values are also in agreement with the calculated values.

Table 1: Elemental analysis of the ligand (NDB) and its metal complexes

Compound	Found (calculated %)			
	C	H	N	O
NDB $C_{20}H_{20}N_2O_5$	65.16(65.21)	5.42(5.47)	7.59(7.60)	21.69(21.72)
$MnSO_4.2H_2O$. NDB $C_{21}H_{26}MnN_2O_{11}S$	44.23(44.29)	4.1(4.6)	4.88(4.92)	30.89(30.91)
$MnCl_2.2H_2O$. NDB $C_{21}H_{26}Cl_2MnN_2O_7$	46.31(46.34)	4.80(4.81)	5.13(5.15)	20.56(20.58)
$CoSO_4.2H_2O$. NDB $C_{21}H_{26}CoN_2O_{11}S$	43.94(43.98)	4.76(4.78)	4.88(4.89)	30.65(30.69)
$CoCl_2.2H_2O$. NDB $C_{21}H_{26}Cl_2CoN_2O_7$	45.97(46.00)	4.74(4.78)	5.09(5.11)	20.40(20.43)

Table 2: Molar conductance and magnetic susceptibility

Compound	Molar conductance Λm (mhocm²/mol)	Magnetic susceptibility μeff (B. M)
NDB	-	-
$MnSO_4.2H_2O$. NDB	26	4.73
$MnCl_2.2H_2O$. NDB	30	5.20
$CoSO_4.2H_2O$. NDB	28	4.28
$CoCl_2.2H_2O$. NDB	31	4.90

UV-visible spectroscopic measurements

Absorbance in the UV-Visible region was recorded in DMF solution using the UV-Visible spectrometer (table 3).

Manganous sulphate complex has given the sharp absorption peaks at 17981 cm^{-1}, 24865 cm^{-1}, and 28000 cm^{-1} due to $^6A_{1g}\rightarrow{}^4T_{1g}$, $^6A_{1g}\rightarrow{}^4E_g+{}^4A_{1g}$ and $^6A_{1g}\rightarrow{}^4T_{2g}$ transitions, respectively. This type of absorption confirms the octahedral geometry, which is further evident by the magnetic moment value at 4.73B. M [12].

Manganous chloride complex has shown four absorption bands at 18100 cm^{-1}, 24860 cm^{-1}, 29156 cm^{-1}, and 31196 cm^{-1} for the transitions $^6A_{1g}\rightarrow{}^4T_{1g}$,$^6A_{1g}\rightarrow{}^4E_{2g}$,$^6A_{1g}\rightarrow{}^4E_{1g}$, and charge transfer. The magnetic moment data was found to be 5.20, suggestive of high spin octahedral geometry [13].

For Cobalt sulphate complex, the spectra appeared at 6910 cm^{-1}, 15010 cm^{-1}, 18523 cm^{-1} and 24100 cm^{-1} due to $^4A_2\rightarrow{}^4T_2$,$^4A_2\rightarrow{}^4T_1$,$^4T_{1g}\rightarrow{}^4T_{2g}$, and CT respectively. The magnetic moment data was found as 4.28 in agreement with octahedral geometry [14].

Cobalt chloride complex registered four peaks corresponding to $^4T_{1g}\rightarrow{}^4T_{2g}$, $^4T_{1g}\rightarrow{}^4A_{2g}$, $^4A_1$g$\rightarrow{}^4T_{1g}$, and charge transfer transition at 6698 cm^{-1}, 14300 cm^{-1}, 18736 cm^{-1} and 29075 cm^{-1}. The magnetic moment value was obtained as 4.90 supporting octahedral geometry [15].

Table 3: UV-Visible spectral analysis

Compound	λ_{max} (cm^{-1})	Transition assignment	Geometry
$MnSO_4.2H_2O$. NDB	17981	$^6A_{1g}\rightarrow{}^4T_{1g}$	Octahedral
	24865	$^6A_{1g}\rightarrow{}^4E_g+{}^4A_{1g}$	
	28000	$^6A_{1g}\rightarrow{}^4T_{2g}$	
$MnCl_2.2H_2O$. NDB	18100	$^6A_{1g}\rightarrow{}^4T_{1g}$	High spin Octahedral
	24860	$^6A_{1g}\rightarrow{}^4E_{2g}$	
	29156	$^6A_{1g}\rightarrow{}^4E_{1g}$	
	31196	CT	
$CoSO_4.2H_2O$. NDB	6910	$^4A_2\rightarrow{}^4T_2$	Octahedral
	15010	$^4A_2\rightarrow{}^4T_1$	
	18523	$^4T_{1g}\rightarrow{}^4T_{2g}$	
	24100	CT	
$CoCl_2.2H_2O$. NDB	6998	$^4T_{1g}\rightarrow{}^4T_{2g}$	Octahedral
	14300	$^4T_{1g}\rightarrow{}^4A_{2g}$	
	18736	$^4A_1$g$\rightarrow{}^4T_{1g}$	
	29075	CT	

FT-IR spectral analysis of the ligand NDB and its metal complexes

For finding out the possible coordination sites, the IR spectra of the ligand and metal complexes were recorded in FT-IR spectrometer in the KBr phase. Characteristic vibrational bands of the ligand and its metal complexes and their assignments are given in table 4. The IR spectrum of the free ligand exhibited a strong band at 1680 cm-1 for ν(C=O) stretching of the succinimide ring [16]. A band at 3302 cm-1 corresponds to the stretching vibration of the (N-H) bond. In metal complexes, there is a shift of ν(C=O) vibration to lower frequency range suggesting the coordination of carbonyl group of succinimide ring with the metal atoms in the complexes. The N-C-N stretching frequency of the ligand at 1400 cm-1 was lowered in the metal complexes confirming the involvement of the nitrogen atom of benzamide with the metal atoms. This is further supported by the appearance of new bands around 400-420 cm^{-1} in the far IR region for the coordination of metal-nitrogen and metal-oxygen [17].

Table 4: FT-IR frequency bands of the ligand NDB and its metal complexes

Compound	ν_{NH}	$\nu_{C=O}$	N-C-N	M-N	M-O
NDB	3302	1680	1400	-	-
$MnSO_4.2H_2O$. NDB	3284	1674	1411	420	413
$MnCl_2.2H_2O$. NDB	3297	1668	1395	401	425
$CoSO_4.2H_2O$. NDB	3185	1673	1407	415	412
$CoCl_2.2H_2O$. NDB	3267	1672	1410	405	418

^{1}H NMR spectral data of NDB

The ^{1}H NMR spectra of NDB shows a singlet at δ 2.57 due to methylene proton. The doublet at δ 3.35 δ is assigned to methoxy proton. A multiplet between 6.26-6.88 δ is assigned for aromatic protons. The singlet for one proton at δ 8.48 is assigned to-NH proton.

^{13}C NMR spectral data of NDB

The number of signals of sharp peaks represents the number of carbons of the ligand, which are not chemically equivalent. ^{13}C NMR–40.0, 60.36, 116.00, 119.23, 120.32, 126.73, 131.04, 139.62, 159.62, 172.66, 179.35.

Mass spectral data of NDB

The molecular formula of NDB based on elemental analysis is $C_{20}H_{20}N_2O_5$. Observed molecular mass by LC-MS spectrometer is 367.25, which is in closer value with the calculated value 368.14.

Anti-fungal activity

Mannich bases and their metal complexes have potent antimicrobial activities [19]. The anti-fungal property of the synthesized Mannich base and its metal complexes were examined against A. niger and C. albicans. The anti-fungal activity of each compound was compared with the standard drug Fluconazole. The inhibition zones were measured at the concentration of 100µg/ml and 400µg/ml. at the concentration of 400µg/ml, all the metal complexes have shown better anti-fungal activity against the selected microorganisms. The percentage inhibition for fungi is calculated after five days using the formula.

Percentage of inhibition = 100(X-Y)/X (X-Area of the colony in control plate; Y= Area of the colony in test plate).

DNA binding and cleavage activities

The interaction of the synthesized Co (II)sulphate complex with DNA is monitored with the help of absorption titrations. At fixed concentrations of the complex, the interaction with DNA (pBR322 and calf thymus) was investigated using UV absorption spectra. There is a redshift (hypochromic effect) in the absorption spectra for the metal complex, which is usually characteristic for non-covalent intercalative binding of the compound to DNA helix, which is because of effective stacking interaction of aromatic chromophore of the compound with the base pairs of DNA [20]. Fig. 3 represents the absorption spectra of the complex in the absence and presence of increasing amounts of DNA. In the UV region, a strong absorption peak was observed due to the metal complex. The gradual addition of DNA resulted in hypochromism and bathochromic shift in UV spectra [21]. The bathochromism result is due to the decrease in the energy of π-π* transition. Here, the π orbital of the DNA base pair might have been coupled with π-π* orbital of the intercalated ligand.

Table 5: Antifungal activity of NDB and its metal complexes

Compound	***A. niger***		***C. albicans***	
	100µg/ml	**400 µg/ml**	**100µg/ml**	**400 µg/ml**
NDB (1AF)	12	20	11	19
$MnSO_4.2H_2O$. NDB (2AF)	14	23	15	24
$MnCl_2.2H_2O$. NDB (3AF)	16	22	14	20
$CoSO_4.2H_2O$. NDB(4AF)	13	19	12	19
$CoCl_2.2H_2O$. NDB(5AF)	18	24	18	23

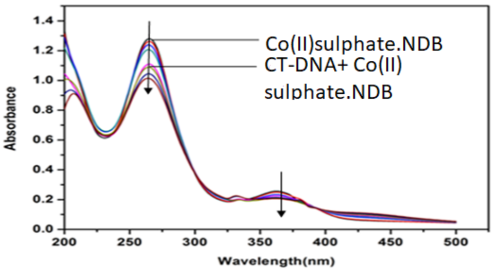

Fig. 3: Absorption spectra of the cobalt (II)sulphate complex of NDB in the absence and presence of increasing amounts of CT-DNA (0-200μM) in Tris-HCl buffer

Agarose gel electrophoresis

The electrophoresis of the samples $MnSO_4.2H_2O$. NDB, $MnCl_2.2H_2O$. NDB, $CoSO_4.2H_2O$. NDB was done according to the following procedure: 200 mg of agarose gel is weighed and dissolved in 25 ml of TAE buffer (4.84g Tris base, pH 8, 0.5M EDTA/1lr) by boiling. When the gel attained approximately 55^0 C, it is poured into the gel cassette fitted with the comb and solidified. The comb was carefully removed, and the gel is placed in the electrophoresis chamber flooded with TAE buffer. DNA sample is then loaded carefully into the wells, along with standard DNA marker and 50V of electricity is passed for around 45 min. Then the gel is removed carefully, and the bands are observed under UV transilluminator. From the photocleavage studies, it is understood that Mn (II) metal complexes have shown complete cleavage of DNA [22]. Co (II) sulphate complex at 30µM concentration has cleaved supercoiled (Form I) DNA, whereas the linear (Form II) is clearly seen (fig. 4). But at 50µM concentration, the complex has cleaved DNA completely [23]. 40µM H_2O_2 alone has not displayed any activity over DNA.

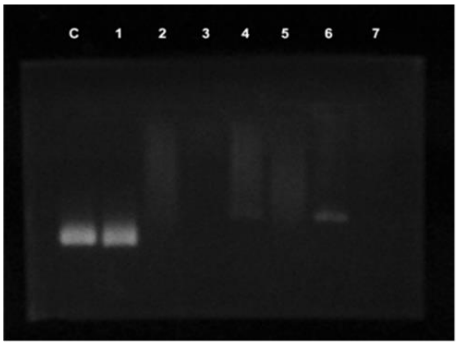

Fig. 4: C-Control DNA (untreated sample); 1-DNA with 40 µM H_2O_2; 2 and3-$MnSO_4.2H_2O$. NDB at 30 and 50 µM; 4 and 5-$MnCl_2.2H_2O$. NDB at 30 and 50 µM; 6 and 7-$CoSO_4.2H_2O$. NDB at 30 and 50 µM, respectively

CONCLUSION

In this article, a new Mannich base and its coordination complexes with transition metals Mn and Co have been synthesized and characterized based on spectral and analytical data. The Mannich base coordinated with the metal atom through the nitrogen of benzamide and oxygen of succinimide. All the complexes exhibit octahedral geometry. The ligand and the metal complexes have shown good antifungal activity. The Co (II) sulphate metal complex has shown marked DNA binding ability. The nuclease activity of the synthesized Co (II) complex could induce the cleavage of supercoiled DNA effectively to linear form in the presence of H_2O_2 as the oxidizing agent.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

The author declares no competing interest exists.

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