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Synthesis, Characterization and Biological Activities of Manganese(II) Complex: Molecular Modeling of DNA Interactions

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ABSTRACT

Mn(II) complex with orthohydroxypropiophenoneisonicotinoylhydrazone has been synthesized. The structural features and other properties were deduced from the IR, Electronic, EPR spectra and powder X-ray diffraction (XRD) techniques. The Electronic spectrum exhibits various bands which were characteristic of Mn(II) in distorted octahedral site symmetry. The Powder X-ray diffraction patterns of the complex have indexed to monoclinic system. The ligand and Mn(II) complex have been tested for in vitro antibacterial and antioxidant activities. Molecular docking experiments were conducted to evaluate the inhibitory activities of ligand and its Mn(II) complex against DNA. The results reveal that the Mn(II) complex shows more activity than the free ligand and the binding energy of docked ligand and Mn(II) complex were found to be -6.9 and -7.4Kcal mol^{-1}, respectively. The more negative relative binding energy of Mn(II) complex suggests greater interaction with DNA than the ligand.

Keywords: Mn(II) complex, biological activities, molecular docking studies, EPR spectra.

INTRODUCTION

There has been growing interest in the study of hydrazones because of their physiological activity, coordination capability and applications in analytical chemistry[1,2]. A number of hydrazone derivatives have interesting bioactivity towards antibacterial, antifungal[3], anticonvulsant[4], anti-inflammatory[5], antimalarial[6], analgesic[7], antiplatelets[8], antituberculosis[9] and anticancer activities[10].

Hydrazones also act as herbicides, insecticides, nematocides, rodenticides and plant growth regulations and were used as plasticizers, stabilizers and antioxidant initiators for polymerization[11]. Transition metals have varying utility and interesting chemistry. Coordination compounds were important due to their role in biological and chemical systems in various ways. Manganese and its compounds find very historical importance in medicine and play a significant role in enzyme activation. It was well known that Mn plays an important role in many biological redox process, including disproportionation of H_2O^2[12] (catalase activity) in microorganisms, decomposition of O_2^- radicals catalysed by superoxide dismutases (SODS) and water oxidation by photosynthetic enzymes (Photosystem II)[13,14]. Moreover, metal complexes with hydrazones show antimicrobial, DNA-binding and cytotoxic activities. It has also been shown that metal complexes with hydrazones can be potent inhibitors of cell growth and DNA synthesis [15-28]. It has thought worthwhile that the metal complexes by the combination of transition metal ion

with a potent hydrazone Schiff base ligand should be more biologically active than the metal salts or the ligand individually.

In view of this we here in describe the synthesis, characterization and bioassay of Mn(II) complex with Orthohydroxypropiophenoneisonicotinoylhydrazone.

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals used were of analytical grade. Organic chemicals such as α-tocopherol, 1,1- diphenyl-2-picrylhydrazyl (DPPH), orthohydroxypropiophenone, isonicotinicacidhydrazide and dimethylformamide (DMF) were procured from Sigma Aldrich and all metal salts were procured from E. Merck.

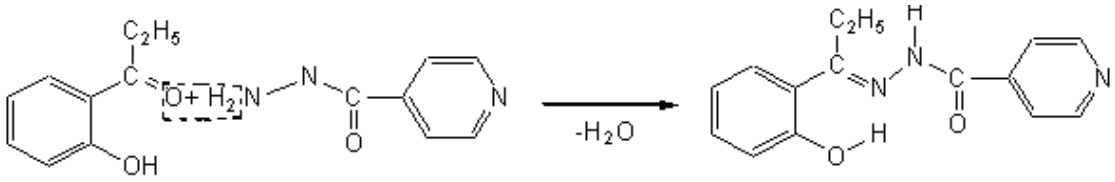
Instrumentation

The IR spectra of the compounds were recorded on a Nicolet FT-IR 560 Magna spectrometer using KBr(Pellet). Mass spectrum was recorded in a Quattro LC, Micro Mass spectrometry. The electronic spectrum of the complex was recorded on a Perkin Elmer UV/VIS Lambda 950. EPR spectrum was recorded on an EPR spectrometer (JEOL FE-1X) operating in the X-band frequencies with a modulation frequency of 100KHz. 100mg of Mn(II) complex sample was taken in a quartz tube for EPR measurement. The magnetic field was scanned from 2200 to 4200G, with a scan speed of 250G min^{-1}. The Absorbances of the samples for DPPH studies were measured using Systronics UV-VIS spectrometer-117. Centrifugation was done using REMI centrifuge. A digital pH meter (Model L1-10 Elico, India) was used for measuring pH. X-ray diffractometer (PHILIPSPW3710) using CuK_{α} (1.5418Å) radiation operated at 45kV and 25mA was used in X-ray investigations.

Experimental section

Synthesis of ligand

Approximately 15ml (0.1mol) of orthohydroxypropiophenone was dissolved in 150ml of methanol and 13.7g (0.1mol) of isonicotinicacidhydrazide was dissolved in 150 ml of water. The two solutions were taken in a 500ml round bottomed flask, two pellets of sodium hydroxide was added and refluxed for two hours on a water bath. The resultant product Orthohydroxypropiophenoneisonicotinoylhydrazone(L) was filtered, washed with water and methanol. It was recrystallised using aqueous methanol and dried. The synthesis and structure of ligand was given in scheme 1.



Orthohydroxypropiophenone Isonicotinicacid- hydrazide Orthohydroxypropiophenone-isonicotinoylhydrazone(L)

Scheme.1. Synthesis and structure of ligand

Synthesis of manganese(II) complex

An aqueous methanolic solution of manganese(II) chloride (0.001mol) was added to hot methanolic solution of free ligand(0.002mol). The reaction mixture was refluxed on water bath for 2-3h at 70^0C. On cooling the contents to room temperature, the resulting dark black colored complex precipitate was filtered, washed with 50% ethanol and dried.

Biological studies

Antibacterial activity

In vitro antibacterial screening was performed by the agar disc diffusion method[29-30]. The bacterial species used in the screening were gram-negative bacteria such as *Klebsiella pneumoniae* and *Escherichia coli* and gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*. Stock cultures of the test bacterial species were maintained on nutrient agar media (Hi-media laboratories, Mumbai) by sub culturing in Petri dishes. The media

were prepared by adding the components as per manufacturer's instructions and sterilized in the autoclave at 121^0C and 15lb pressure for 15min. Each medium was cooled to 45 - 60^0C and 20 ml of it was poured into a Petri dish and allowed to solidify. After solidification, the Petri plates with media were spread with 1.0 ml of bacterial suspension prepared in sterile distilled water. The wells were bored with cork borer and the agar plugs were removed. To each agar well, 100µl of the compound reconstituted in DMF of concentration 1.0mg/ml was added. DMF was used as a negative control and in similar way, antibiotics such as ampicillin and tetracycline were used as positive control standards. All the plates were incubated at 37^0C for 24 h and they were observed for the growth inhibition zones. The presence of clear zones around the wells indicate that both the ligand and Mn(II) complex were active. The diameter of zone of inhibition was calculated in millimeters. The well diameter was deducted from the zone diameter to get the actual zone of inhibition diameter and the values were tabulated.

DPPH scavenging activity

The principle for the reduction in DPPH free radicals was that the antioxidant reacts with stable free radical DPPH and converts it to 1,1- diphenyl-2-picrylhydrazine. The ability to scavenge the stable free radical DPPH was measured by decrease in the absorbance at 517nm. Solutions of the ligand and Mn(II) complex at 100µM concentration were added to 100µM DPPH and kept in ethanol tubes. The tubes were kept at ambient temperature for 20min and absorbances were measured at 517nm. For positive control, α-tocopherol was used [31]. These measurements were run in triplicate. The percentage of scavenging activity was calculated as follows:

Scavenging activity (%) = $[(A_{DPPH}-A_{TEST}) / A_{DPPH}] \times 100$

Where, A_{DPPH} was the absorbance of DPPH without test sample (control) and A_{TEST} was the absorbance of DPPH in the presence of test sample.

Molecular docking studies

The 3D co-ordinates of the crystal structure of the DNA duplex receptor structure (PDB ID: 423D) was downloaded from the Protein Data Bank (http://www.rcsb.org/pdb/home.do) with 12 base pairs sequence was $d(ACCGACGTCGGT)_2$. Before docking, all water molecules were removed from DNA file 423D. After removing the water molecules, H-atoms were added to DNA for correct ionization. The structures of the free ligand and Mn(II) complex were constructed using ChemSketch. This can be used for generating chemical structure of bioactive compounds, 2D structure cleaning and 3D optimization[32]. MGL tools 1.5.4 with Autodock vina were used to setup and perform blind docking calculations between the bioactive compounds and DNA sequence[33]. The DNA was enclosed in a box with number of grid points in x×y×z directions, 80×60×64 and grid spacing of 0.375Å. The default settings were used for all other parameters.

RESULTS AND DISCUSSION

Characterization of ligand

The ligand, Orthohydroxypropiophenoneisonicotinoylhydrazone(L), was obtained by condensation reaction with equimolar solutions of orthohydroxypropiophenone and isonicotinicacidhydrazide. The free ligand was a pale yellow crystalline. The yield was about 86%. The ligand was analyzed by elemental analysis, IR and mass spectroscopy. The mass spectrum of ligand (Figure 1) shows a molecular ion (M^+) peak at m/z value of 269, corresponding to the species $C_{15}H_{15}N_3O_2$. These spectral data confirms the proposed formula of the ligand $C_{15}H_{15}N_3O_2$.

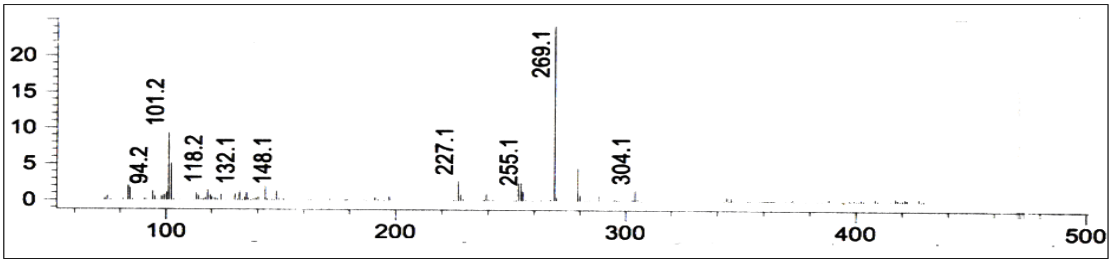


Figure 1 Mass spectrum of the ligand

Characterization of Mn(II) complex

The newly formed Mn(II) complex was characterized by FT-IR, electronic, EPR spectra and powder X-ray diffraction (XRD) techniques.

IR studies

The IR spectra of ligand and Mn(II) complex were shown in figures 2 & 3, respectively. The IR spectrum of the ligand shows broad bands at 3448, 3280, 1680, 1600cm^{-1}, which can be attributed to the phenolic *ν*(OH), *ν*(NH) *ν*(C=O) and *ν*(C=N) group stretching frequencies respectively. The phenolic OH band was absent in the complex, indicating the coordination through the phenolic OH group and also a considerable negative shift in *ν*(C=O), *ν*(C=N) was observed indicating a consequence of coordination through the carbonyl-oxygen atom and azomethine nitrogen atom of the free ligand. The –NH stretching absorption in free ligand occurs at 3280cm^{-1} which remains unaffected after complexation. This precludes the possibility of coordination through phenolic OH, carbonyl–oxygen atom and imine nitrogen atom. However, some new bands with medium to weak intensities appear in the regions 425-513cm^{-1} in the complex under study, which were tentatively assigned to *ν*(M−O)/*ν*(M−N) modes[23].

Figure 2 IR spectrum of Ligand

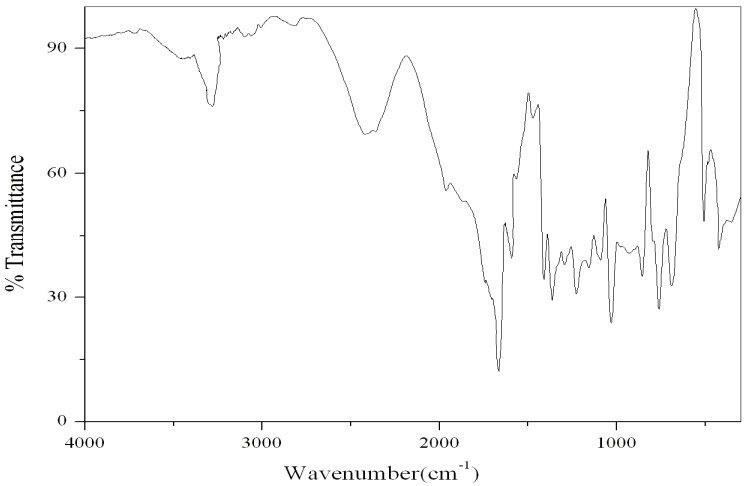


Figure 3 IR spectrum of Mn(II) complex

Electron paramagnetic spectrum of the Mn(II) complex

The X-band EPR spectrum of the Mn(II) complex observed at room temperature was shown in figure 4, has six hyperfine lines centered around g = 2.015, which was characteristic of octahedral site symmetry of Mn(II) complex. These EPR parameters indicate that this Mn(II) species has octahedral coordination, consistent with an extra-framework position[34]. In general, the g-value for the hyperfine splitting was indicative of the nature of the bonding. If the g-value shows a negative shift with respect to the free electron value (2.0023) then the bonding was ionic and conversely, if the shift was positive, then the bonding was more covalent. In the present case, the g-value shows positive shift, indicative of covalent bonding between Mn(II) and ligand.

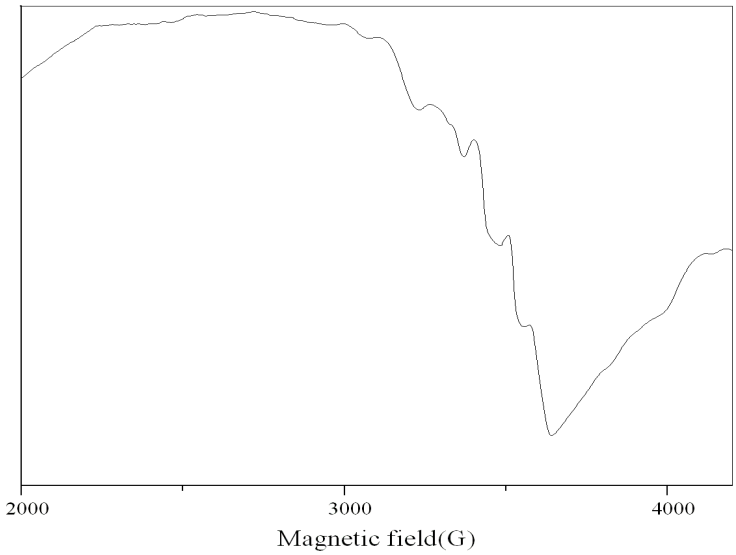


Figure 4 Powder X-band EPR spectrum of Mn(II) at room temperature(v = 9.205GHz)

Electronic spectrum of the Mn(II) complex

Mn^{2+} ions belong to d^5 configuration and will have a ground state of $^6S_{5/2}$. These ions occur in Mn(II) complex in either octahedral or tetrahedral environments. The nature and position of the bands in figure 5 shows Mn(II) complex has an octahedral symmetry. In the presence of octahedral symmetry, one can predict a few absorption bands for Mn(II) complex in the visible region corresponding to the transitions $^6A_{1g}(S) \rightarrow {}^4T_{1g}(G)$, $^6A_{1g}(S) \rightarrow {}^4T_{2g}(G)$, $^6A_{1g}(S) \rightarrow {}^4A_{1g}(G)$, $^4E_{1g}(G)$, $^6A_{1g}(S) \rightarrow {}^4E_{2g}(D)$, etc., In the present work, the observed four spectral bands located at 24,753, 22,124, 20,121 and 17,793cm^{-1} were assigned to spin forbidden transitions $^6A_{1g}(S) \rightarrow {}^4E_g(D)$, $^6A_{1g}(S) \rightarrow {}^4T_{2g}(D)$, $^6A_{1g}(S) \rightarrow {}^4A_{1g}(G)$, $^4E_g(G)$ and $^6A_{1g}(S) \rightarrow {}^4T_{2g}(G)$, respectively. The charge transfer band was obtained at 37,737cm^{-1}. The observed band positions were given in Table 1.

Table. 1 Electronic band transitions of Mn(II) complex

Transition	Wave length(nm)	Wave numbers(cm^{-1})
$^6A_{1g}(S) \rightarrow {}^4T_{2g}(G)$	562	17,793
$^6A_{1g}(S) \rightarrow {}^4A_{1g}(G), {}^4E_g(G)$	497	20,121
$^6A_{1g}(S) \rightarrow {}^4T_{2g}(D)$	452	22,124
$^6A_{1g}(S) \rightarrow {}^4E_g(D)$	404	24,753
Charge transfer	265	37,737

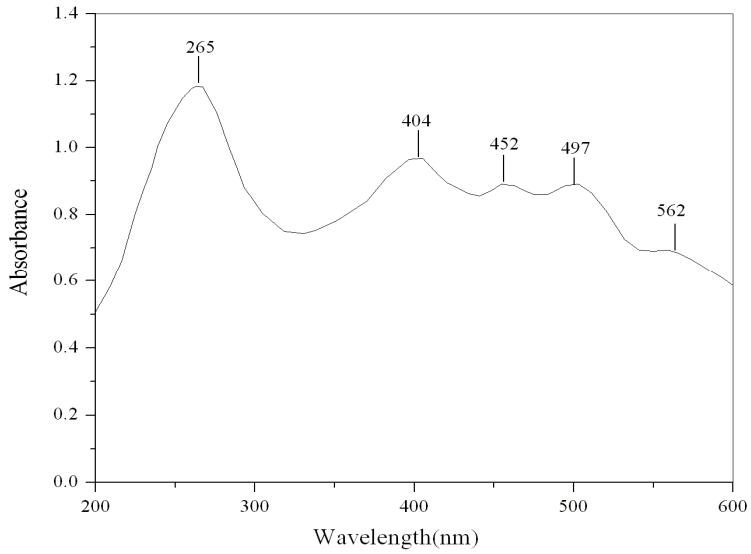


Figure 5 Electronic spectrum of Mn(II) complex

X-ray diffraction studies

The powder X-ray diffractogram of Mn(II) complex was shown in figure 6. The average particle size of the sample was calculated using Scherrer's formula, with full width at half maximum intensity of the plane (-624) pattern. The size of the crystal was found to be 85nm. This pattern can be indexed to a monoclinic unit cell. The unit cell parameters for the prepared crystalline Mn(II) complex were a = 21.4738Å, b = 9.6151Å, c = 10.2617Å, β = 136.340° and unit cell volume V = 1462.76Å^3 . The calculated and observed X-ray diffraction data was given in Table 2.

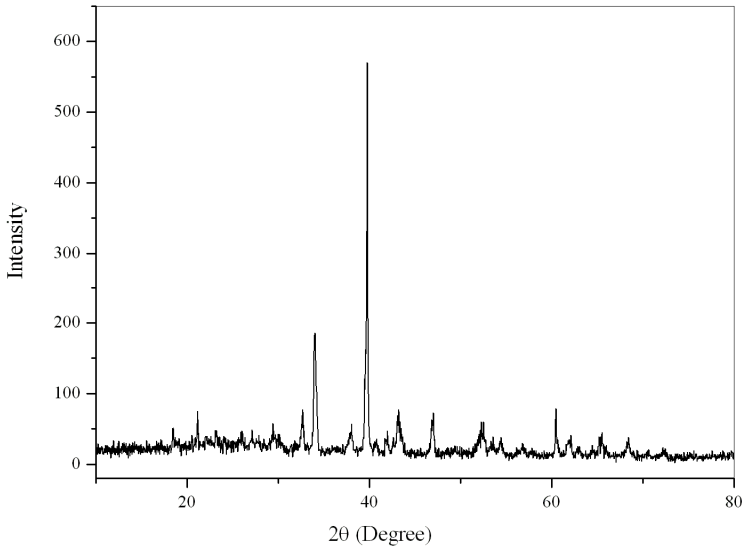


Figure 6 Powder X-ray diffraction spectrum of Mn(II) complex

Table.2 Powder X-ray diffraction data of Mn(II) complex

d-spacing (Å)		2θ values		Δ 2θ	(hkl)
Observed	Calculated	Observed	Calculated		
9.6683	9.6683	9.14	9.14	0.000	(-101)
7.4126	7.4126	11.93	11.93	0.000	(200)
4.8076	4.8076	18.44	18.44	0.000	(020)
4.1998	4.1998	21.14	21.14	0.000	(-102)
3.4197	3.4189	26.03	21.04	-0.006	(-422)
3.0341	3.0297	29.14	29.46	-0.043	(221)
2.5781	2.5779	34.77	34.77	-0.003	(-802)
2.2647	2.2633	39.77	39.79	-0.027	(-624)
2.0920	2.0908	43.21	43.23	-0.025	(-542)
1.9309	1.9308	47.02	47.02	-0.001	(-104)
1.7480	1.7483	52.29	52.28	0.010	(-1102)
1.5295	1.5296	60.47	60.47	0.001	-554)
1.4227	1.4237	65.56	65.51	0.052	(750)
1.3696	1.3696	68.44	68.44	-0.002	(452)
1.3069	1.3067	72.22	72.24	-0.016	(-572)
1.1422	1.1426	84.81	84.77	0.041	(752)

Based on all spectral studies, the proposed structure of the Mn(II) complex was shown in figure 7.

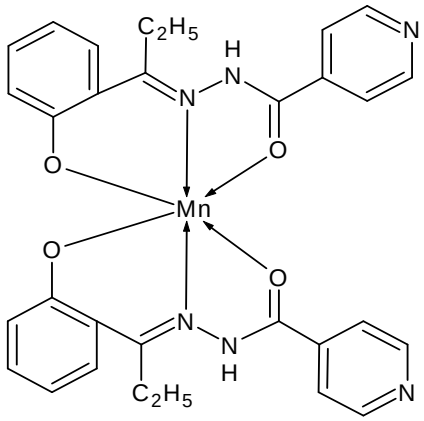


Figure 7 The proposed structure of Mn(II) complex

Antibacterial activity

The *in vitro* Antibacterial activity of the ligand and its Mn(II) complex was tested against different micro-organisms. The activities of the ligand and its Mn(II) complex were compared to the standard antibiotics such as ampicillin and tetracycline was given in Table 3. The Mn(II) complex shows higher antibacterial activity than that of free ligand.

Table 3. Antibacterial screening data of the ligand and Mn(II) complex (Zone of inhibition in mm)

Compound	*K. Pnuemoniae*	*E. Coli*	*B. Subtilis*	*S. aureus*
Ligand(L)	06	14	08	-
Mn(II) complex	12	22	14	09
Ampicillin	43	40	43	42
Tetracycline	32	33	30	32

Antioxidant activity

The synthesized ligand and its Mn(II) complex have been screened for reduction in DPPH free radicals at 100µM concentration. The Mn(II) complex shows good activity in DPPH scavenging (40%) than the free ligand (22%) but has less activity than standard antioxidant α-tocopherol (53%).

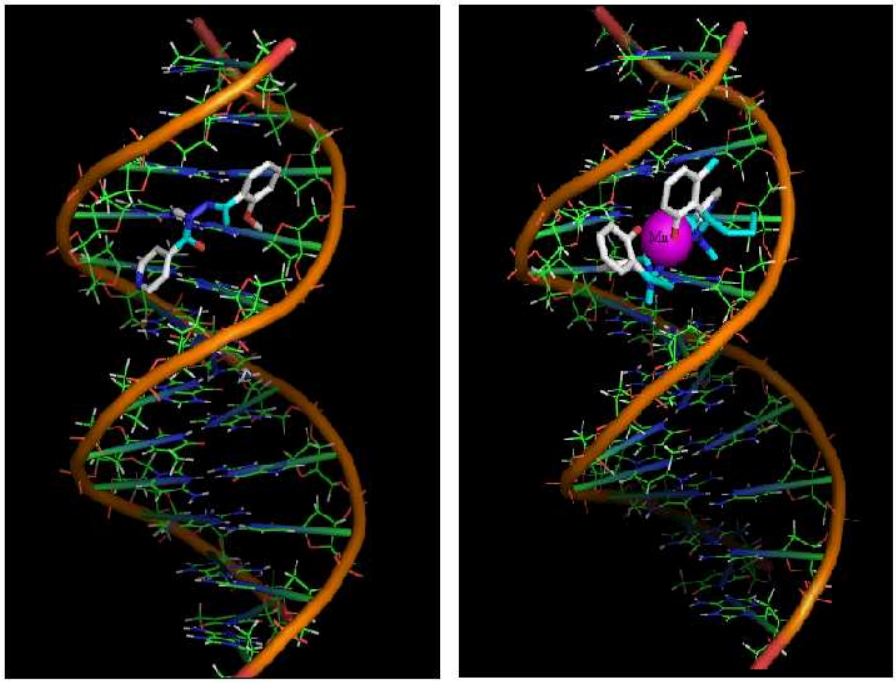
Molecular docking studies

The binding energy was obtained from the Schiff base ligand and its Mn(II) complex with DNA as receptor. The output binding energy values (Kcal/mol) of the ligand and Mn(II) complex were shown in Table 4. According to this docking experiment, complex reasonably bind with DNA sequence $d(ACCGACGTCGGT)_2$. The less binding

energy was obtained for Mn(II) complex (-7.4Kcal/mol) when compared to the free ligand(-6.9Kcal/mol) and their binding models in major groove of DNA were depicted in figure 8. It was observed that two kinds of hydrogen bonds were present in the binding model of Mn(II) complex. One kind of bond was formed by the phenolic oxygen atom of complex and the amino hydrogen atom of C18 (length of hydrogen bond: C18 N-H…$O_{complex}$ = 2.3Å) and other was composed of amino hydrogen of G7 and the nitrogen atom of Mn (II) complex (length of hydrogen bond: G7 N-H…$N_{complex}$ = 2.3Å). In contrast, there were two kinds of hydrogen bonds in the binding model of free ligand. One kind of bond was formed by the phenolic oxygen atom of ligand and the amino hydrogen atom of A5 (length of hydrogen bond: A5 N-H…O_{ligand} = 2.6Å) and other was composed of amino hydrogen of G4 and the oxygen atom of ligand (length of hydrogen bond: G4 N-H…O_{ligand} = 2.3Å). From the results of molecular docking study we concluded that Mn(II) complex shows the better interaction with DNA than free ligand via its major groove. These studies suggested that Mn(II) complex cleaves DNA following the oxidative pathway[35].

Table 4. Docking results and consensus scores of synthesized free ligand and Mn(II) complex

Compound	Binding energy (Kcal/mol)	No. of hydrogen bonds	Acceptor group (Y-H)	Donor group Z	Distance (Å)
Ligand	-6.9	2	H(N3)(A5)(DNA-chain A) H(N3)(G4)(DNA-chain A)	O8(ligand) O20(ligand)	2.6 2.3
Mn(II)complex	-7.4	2	H(N4)(C18)(DNA-chain A) H(N7)(G7)(DNA-chain A)	O20(complex) N1(complex)	2.3 2.3



(a) **(b)**

Figure 8 Interaction of (a) ligand and (b) Mn(II) complex with d(CGCGAATTCGCG)$_2$ strands of DNA by minor groove binding approach.

CONCLUSION

In the light of above discussions we have proposed octahedral geometry for Mn(II) complex. The XRD study suggested monoclinic crystal system for Mn(II) complex. The Mn(II) complex was biologically active and has enhanced antibacterial and antioxidant activities compared to its ligand. The Mn(II) complex showed moderate activity against four types of bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Escherichia coli*). The docking results revealed that binding energy of docked ligand and Mn(II) complex was found to be -6.9 and -7.4Kcal mol^{-1}, respectively. The more negative relative binding energy of Mn(II) complex suggested greater interaction with DNA than the ligand.

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