Synthesis, Characterization and Biological Activities of a Novel Mannich Base 2-[(3,4-dimethoxyphenyl)(pyrrolidinyl) methyl]cyclohexanone and its Complexes with Cu(II), Ni(II), Co(II) and Fe(II) Ions

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The titled Mannich base 2-[(3,4-dimethoxyphenyl)(pyrrolidin-1-yl)methyl]cyclohexanone (DPC) was synthesized by condensing 3,4-dimethoxybenzaldehyde, pyrrolidine and cyclohexanone. The synthesis was carried out by using ethanol as solvent. The development of the reaction was monitored on TLC. The complexation of synthesized Mannich base was carried out with Cu(II) chloride, Co(II) chloride, Ni(II) chloride and Fe(II) chloride. The structures of the synthesized Mannich base and its complexes were confirmed by FT-IR, UV-Vis, ^{1}H-NMR, ^{13}C-NMR, MS and TGA techniques. The proposed geometries of the metal complexes were established on the basis of metal/ligand ratio through AAS/ICP and electronic spectra. The synthesized compounds were evaluated for their antiurease and antibacterial activities. The complex with Co(II) show potent antiurease and antibacterial activity. The nature of SAR of Co(II) has been demonstrated using docking studies.

Keywords: Metal complexes, antiurease, antibacterial activity

Mannich reactions are key tools for chemist to construct carbon-carbon relationship in the field of present synthetic organic chemistry [1-3]. The condensation product of aldehyde, amine and an active hydrogen compound is called Mannich base. In recent years Mannich reactions have got a dominating place in pharmaceutical and chemical fields [4, 5]. Nitrogen containing heterocycles play an important role in the production of variety of medicinal [6-8] biological [9] and natural products [10]. Mannich reactions are simple designs for heterocyclization rather complicated heterocyclic systems. The results of Mannich reactions are extremely valuable biologically active molecules and intermediates for the manufacture of β-Aminoalcohols, β-aminoacids and β-lactams [11]. Mannich bases exist at the same time as building blocks within a numeral natural products which are biologically active [12-15]. β-aminocarbonyl compounds in broad spectrum include as valuable healing agents used extensively for various diseases. These compounds contain anticonvulsant [16], anticancer, cytotoxic [17], anti-HIV [18], analgesic as well as antiinflammatory character [19].

The sensitivity of Mannich bases have a key role in the development of coordination chemistry [20]. Recently Mannich bases and their complexes are extensively studied products owing to the unique behavior of ligand in the direction of a variety of transition metal ions [21, 22]. The transformation of Mannich bases into complexes with metal ions can multiply their medicinal value. Mannich base complexes have key role in better understanding of complicated biological processes. Numerous drugs reflect enhanced biological activity as metal complex [23]. Urease can cause peptic ulcer and stomach cancer [24]. It is important to find effective and cheap antiurease inhibitor to control the diseases cause by urease. In present scenario a novel Mannich base of cyclohexanone and its metal complexes with Cu(II), Co(II), Ni(II) and Fe(II) chlorides are prepared and characterized by various spectroscopic, thermal and chemical techniques. The synthesized scaffolds were investigated for their antiurease activities. The inhibitory action is also explained by the docking. The binding mode of best active compound was also predicted using molecular docking studies.

Experimental part

Materials

All reagents such as 3,4-dimethoxybenzaldehyde, pyrrolidine, cyclohexanone and various Metal(II) chlorides were used without any refining because of their analytical ranking.

Physical measurements

Gallon Camp apparatus was used for the determination of melting points. Shimadzu apparatus was used for Infra-Red spectroscopy. Avance 400 spectrometer was used for ^{1}H and ^{13}C NMR and JEOL JMS-600 for MS.

Synthesis of Ligand

The synthesis was carried out according to established protocol [25] with short discretion as follow; Equimolar solutions of cyclohexanone (0.01 mol, 0.147 mL), 3,4-dimethoxybenzaldehyde (0.01 mol, 0.191 mL) and pyrrolidine (0.01 mol, 0.117 mL) in ethanol were mixed in 50 mL round bottom flask. Anhydrous calcium chloride was added at the same time as a catalyst in one equivalent ratio. The resulting combination was stirred in ice cold conditions for about 20 min. The resulting mixture was heated at 70–90°C for about one and half hour along with

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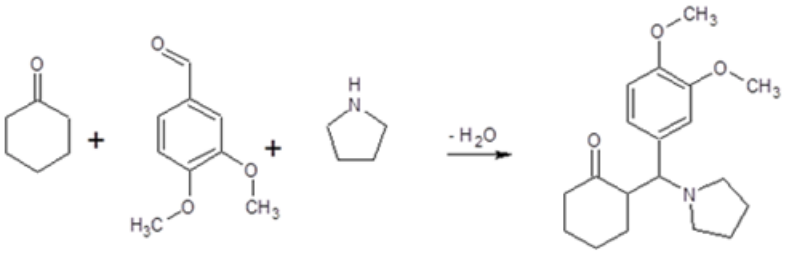
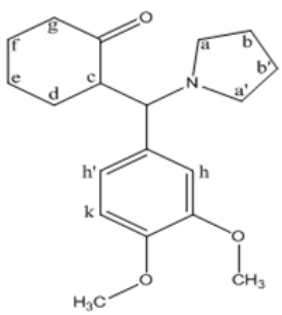


Scheme 1. Synthesis of Mannich base (DPC)



Scheme 2. Labeling of Mannich base (DPC) for $^{1}H/^{13}C$ NMR spectral interpretation

constant stirring at room temperature for one hour. The development of reaction was checked with TLC. After that 5 % $NaHCO_3$ was added that resulted in precipitation of the product. The precipitate was obtained through filtration followed by washing with distilled water. Ethanol was used for final washing. The products were washed thoroughly three times by ethanol (20 mL) and then by diethyl ether (10 mL). The products were finally re-crystallized from hot ethanolic solution, dried at room temperature and stored at dry place.

General method for the synthesis of metal complexes

The Mannich base and Metal(II) chlorides were mixed in ethanol in 1:1 molar ratio. The mixture was gently heated at 50°C for about 10 min. The stirring of contents was continued for about 3 h at room temperature. Resulting solid complex was obtained by evaporating the solvent in air, washed by distilled water, filtered and finally dried out.

MANNICH BASE (DPC): Molecular formula; $C_{19}H_{27}NO_3$, Yellow solid, Yield: 67%, m.p: 136–138°C, Molecular weight: 317.43 g/mole,

FTIR (cm^{-1}): 1150 (C-N-C stretching), 1579 (C=C stretching), 1714 (C=O stretching),

^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.797–1.82 (7H, *m*, H_b , $H_{b'}$ H_c and H_g), 2.91–2.94 (10H, *m*, H_a, $H_{a'}$ H_d, H_e and H_f), 3.90 (6H, *s*, OCH_3), 6.88–6.90 (1H, *d*, *J* = 8.4 Hz, $H_{h'}$), 7.00–7.10 (1H, *d*, *J* = 2.0 Hz, CH), 7.10–7.08 (1H, *dd* , *J* = 8.4 Hz, H_k), 7.73 (1H, *s*, H_h), ^{13}C NMR (400 MHz, $CDCl_3$, δ): 23.03-28.51 (All–CH_2), 55.92 (-OCH_3), 76.68–77.31 (-CH), 110.90–136.80 (phenyl), 148.67–149.62 (-C-O), 190.03 (C=O); EI–MS (*m/z)* (%) = 394.1 observed for $[M(C_{19}H_{27}NO_3)$-H+2K]$^+$ (100), 379.1 $[C_{18}H_{24}NO_3$-+2K]$^+$ (98), 363.1 $[C_{18}H_{24}NO_2$-H+2K]$^+$ (98).

MANNICH BASE (DPC)-Cu(II) complex: Molecular formula; $C_{19}H_{27}Cl_2CuNO_3$,

Light green solid, Yield: 47%, m.p: Decomposed above 280°C, Molecular weight: 451.93 g/mole, % metal for $MLCl_2$: Theoretical/experimental (14.06/13.46), FTIR (cm^{-1}): 1147 (C-N-C stretching), 1581 (C=C stretching), 1710 (C=O stretching), 445 (Cu–N stretching), 526 (Cu–O stretching), ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.80–1.91 (3H, *m*, H_c, H_g, H_b and $H_{b'}$), 2.91–2.94 (10H, *m*, H_a , $H_{a'}$, H_d, H_e and H_f),3.82–3.84(4H,*m*,H_a,$H_{a'}$), 3.88 (6H, *s*, OCH_3), 6.882–6.903 (1H, *d* , *J* = 8.4 Hz, $H_{h'}$), 7.00–6.99 (1H, *d*, *J* = 5.2 Hz, CH), 7.10–7.08 (1H, *d*, *J* = 8.4 Hz, H_k), 7.73 (1H, *s*, H_h).

MANNICH BASE (DPC)-Ni(II) complex:

Molecular formula; $C_{19}H_{27}Cl_2NiNO_3$, Greenish yellow solid, Yield: 58%, m.p: Decomposed above 280°C, Molecular weight: 447.12 g/mole,% metal for $MLCl_2$: Theoretical/experimental (13.13/12.16),

FTIR (cm^{-1}): 1149 (C-N-C stretching), 1583 (C=C stretching), 1703 (C=O stretching), 468 (Ni–N stretching), 547 (Ni–O stretching), ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.81 (7H, *s*, H_b, $H_{b'}$ H_c and H_g), 2.93 (10H, *s*, H_a, $H_{a'}$, H_d, H_e and H_f), 3.89 (6H, *s*, OCH_3), 6.90–6.88 (1H, *d*, J = 6.8 Hz, $H_{h'}$), 7.00 (1H, br, *s* , H_h), 7.10–7.08 (1H, *d*, *J* = 6.4 Hz, CH), 7.73 (1H, *s*, H_h).

MANNICH BASE (DPC)-Co(II) complex:

Molecular formula; $C_{19}H_{27}Cl_2CoNO_3$, off white solid, Yield: 56%, m.p: Decomposed at 280°C, Molecular weight: 447.36 g/mole, % metal for $MLCl_2$: Theoretical/experimental (13.18/11.86),

FTIR (cm^{-1}): 1149 (C-N-C stretching), 1583 (C=C stretching), 1700 (C=O stretching), 447 (Co–N stretching), 538 (Co–O stretching), ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.81 (7H, br *s*, H_b, $H_{b'}$ H_c and H_g), 2.93 (10H, br *s*, H_a, $H_{a'}$ H_d, H_e and H_f), 3.90 (6H, *s*, OCH_3), 6.90 (1H, br-s, $H_{h'}$), 7.00 (1H, br, *s*, H_k), 7.10–7.08 (1H, *d*, *J* = 6.0 Hz, CH), 7.73 (1H, *s*, H_h).

MANNICH BASE (DPC)-Fe(II) complex:

Molecular formula; $C_{19}H_{27}Cl_2FeNO_3$, light brown solid, Yield: 57%, m.p: Decomposed at 280°C, Molecular weight: 444.43 g/mole, % metal for $MLCl_2$: Theoretical/experimental (12.57/10.96),

FTIR (cm^{-1}): 1147 (C-N-C stretching), 1585 (C=C stretching), 1693 (C=O stretching), 466 (Fe–N stretching), 551 (Fe–O stretching), ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.81 (7H, *s*, H_b, $H_{b'}$ H_c and H_g), 2.93 (10H, *s*, H_a, $H_{a'}$ H_d, H_e and H_f), 3.90 (6H, *s*, OCH_3), 6.90 (1H, br-*s*, $H_{h'}$), 7.00 (1H, br, *s*, H_k), 7.08 (1H, *s*, CH), 7.73 (1H, *s*, H_h).

Biological evaluation

Antiurease assay

This assay was modified from Berthelot assay [26]. A total reaction volume of 85 µL contained 10 µL of 50 mM phosphate buffer, *p*H 7.0, 10 µL of test compound (0.5 mM) and 25 µL of jack beans urease (0.015 units, from Sigma). The contents were preincubated at

37°C for 10 min. Then, 40µL of 20 mM urea was added as substrate to each well and incubation continued at 37 °C for further 10 min after given time, contents were pre-read at 625 nm using the 96-well plate reader Synergy HT (Biotek Inc.). Freshly prepared phenol hypochlorite (115 µL) reagent was added in each well (by mixing 45 µL phenol reagent with 70 µL of alkali reagent). Incubation was continued for another 10 min followed by measurement of absorbance at 625 nm. The percentage enzyme inhibition was calculated by the following formula: Inhibition (%) = 100 – [(Abs. of test sample/Abs. of control) × 100]. Active compounds were serially diluted (e.g., 0.25, 0.125, 0.0625, 0.03125 mM, etc). A graph of percent inhibition on y-axis and inhibitor concentration on x-axis was plotted by EZ-Fit Enzyme software (Perrella Inc, USA). IC_{50} values of these active compounds were determined by this software.

In vitro antibacterial screening

The synthesized compounds were evaluated for their in vitro antibacterial activity against *Bacillus thuringiensis* and *Escherichia coli* by disc diffusion method [27]. Each compound was used at a concentration of 20 mg/mL in DMSO. The zone of inhibition was measured after 48 h in incubation at 37°C.

Molecular modeling

For molecular docking simulations, Surflex-Dock program [28] implemented in Sybyl-X 2.1 [29] by CARTRA Company was used under Red Hat Linux 5.0 operating system. The structure of compound 4 was drawn in the Sybyl package and proper atom type for each atom in the molecule was assigned. After sketching the compound, it was minimized using the Powell method until the gradient was 0.05 Kcal/mol and the maximum iterations to reach was 1000 with the Tripos force field. The crystal structure of urease (4ubp.pdb) [30] from Protein Data Bank was retrieved. It was refined and prepared using the protein preparation wizard implemented in SYBYL, all heteroatoms were removed except the nickel ions. Similarly hydrogen atoms were added and protonation states were fixed at the termini of the protein. Docking simulations of most active compound into the binding pocket of urease was performed using the Surflex-dock module of SYBYL package. Surflex-dock utilizes a so-called *whole* molecule alignment algorithm based on shape similarity between the ligand and target protein [31]. This method aligns the ligand to a *protomol* or idealized ligand in the active site of the target. Similarly to generate the protomol using Surflex-dock program, two parameters that significantly affect the size and extend of the protomol are the threshold and the bloat values. In this study the both were set to 0.5 and 0, respectively. During whole docking process, the protein was considered to be rigid, and the ligand molecules were flexible. However all other default parameters were used.

The Protomol was generated near the active site residues and Surflex-Dock was operated to generate 20 docked conformers. The C-Score was calculated for each conformer and the best binding mode was chosen based on the score and the fitting of the compound in the active site.

Thermal study (TGA/DTA)

The metal complexes were subjected to TGA/DTA to test their stability and decomposition pattern on heating. Thermal studies were carried out by using SDT-Q 600 V20.9 Build 20 by heating up to 1000°C.

Results and discussions

The analytical data of DPC and its complexes is shown in the previous section. At room

temperature each complex is stable and colored solid. DPC and its complexes are insoluble in most of the organic solvents, except DMSO, chloroform and DMF. The complexes are non-electrolytes [32]. λ_{max}(nm) of the synthesized compounds were determined by UV–Visible spectroscopy.

FTIR spectra

In order to get meaningful information about the connecting modes of DPC to the metal ion in the complexes, IR spectra of DPC was compared with the spectral data of its metal complexes. Important peaks at 1150, 1579 and 1714 cm^{-1} can be given to C-N-C, C=C and C=O respectively. These signals are quite in agreement with our designed construction of free ligand. The indication of coordination through oxygen of carbonyl and ring nitrogen of pyrrolidine was provided by IR spectra of all the complexes in which the peaks due to C-N-C and C=O were shifted to lower frequencies (1150–1147) and (1714–1693) respectively [33]. Coordination from these sites was supported by the appearance of some new bands at 445-468 and 526-551 cm^{-1} assignable to í M–N and í M–O bonds respectively [34].

UV-Vis data and Magnetic moment

The electronic spectrum of Cu(II)-DPC complex exhibits an absorption band at 13110 cm^{-1} that can be assigned to $^2T_2 \rightarrow {}^2E$ transition of a 4-coordinate tetrahedral geometry. The magnetic moment value (2.13 B.M.) of this complex also support 4-coordinate tetrahedral geometry [35]. The Ni(II)-DPC complex exhibits two absorption bands at 11680 cm^{-1}-, 15390 cm^{-1} which are attributed to $^3T_1(F) \rightarrow {}^3A_2(F)$, $^3T_1(F) \rightarrow {}^3T_1(P)$ transitions of tetrahedral environment around nickel ion. Similarly, the magnetic moment value (3.35 B.M.) further supports this environment around nickel ion [36]. Electronic spectrum of the Co(II)-DPC complex shows two spin allowed transitions at 11820 cm^{-1}---, 17315 cm^{-1} assignable to $^4A_2(F) \rightarrow {}^4T_1(F)$ and $^4A_2(F) \rightarrow {}^4T_1(P)$ transitions respectively, which are in good agreement with tetrahedral stereochemistry for Co(II) ion. The magnetic moment value of Co(II) complex is at 4.25 BM indicating that the Co(II) complex has typically tetrahedral stereochemistry [36]. The electronic spectrum of Fe(II)-DPC complex contains an absorption band at 8530 cm^{-1} that can be assigned to $^5E \rightarrow {}^5T_2$ transition of a tetrahedral geometry. The room temperature magnetic moment (4.87 B.M.) corresponds with the tetrahedral symmetry [37].

^{1}H NMR spectra

The proof for connecting modes of DPC is furthermore given via the ^{1}H NMR spectral values of ligand recorded. The signals indicated by ^{1}H NMR spectrum of ligand are following; signals at *ä* 1.79–1.82 (7H, *m*, H_b, $H_{b'}$, H_c and H_g), 2.91–2.94 (10H, *m*, H_a, $H_{a'}$, H_d, H_e and H_f), 3.90 (6H, *s*, OCH_3), 6.88–6.90 (1H, *d*, *J* = 8.4 Hz, $H_{b'}$) [38], 7.00-7.10 (1H, *d*, *J* = 2.0 Hz, CH) [39, 40], 7.10–7.08 (1H, *dd* , *J* = 8.4 Hz, H_k), 7.735 (1H, *s* ,H_h).These chemical shifts corresponds well with the designed structure of ligand. In the metal complexes, the very fine information was not observed but the slight shifting of chemical shift values of H_c, H_a and $H_{a'}$ protons in the down field region confirmed the coordination of metal ions through oxygen and nitrogen [41]. This observation was in consistent with the interpretation of IR spectral data.

^{13}C NMR spectra

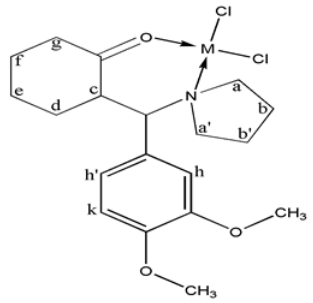
The different kinds of carbon in ligand are indicated through ^{13}C NMR spectral data. The ^{13}C-spectrum of ligand shows following signals in the range: 23.03-28.51 (–CH_2), 55.92 (-OCH_3), 76.68–77.31 (-CH), 110.9–136.8 (Ar-C_6H_4), at 148.67–149.62 (Ar-C-O), 190.03 ppm (C=O). These chemical shifts are well in consistent with the proposed structure of ligand.

Mass spectrum (EI)

EI mass spectrum of ligand was recorded. In the said spectrum the base peak was observed at 394.1 which can be attributed to $[M\text{-}H^+ + 2K^+]^+$ [42]. Some other fragments at 379.1 and 363.1 were also observed and can be attributed to $[C_{18}H_{24}NO_3\text{-}H^+ + 2K^+]$ and $[C_{18}H_{24}NO_2\text{-}H^+ + 2K^+]$ respectively.

Thermal study (TGA/DTA)

Thermal analysis was carried out to get knowledge about the thermal behavior of ligand and its complexes. The TGA curve of metal complexes showed an endothermic peak at 200°C, which was corresponding to the elimination of chlorine that coordinated to the metal ion. The organic part decomposed around 460°C. Above 600°C there is no change in weight, the plateau observed is corresponds to the oxide of respective metal. The decomposition patterns of the synthesized compounds were in consistent with their proposed structures. From the above structural discussion following the structure formula of metal complexes have been proposed (fig.1).



M = Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}

Fig. 1. Proposed structure of metal complexes

Biological activity

Antiurease / antibacterial activity

The ligand DPC and its metal complexes were screened for their antiurease activities and their IC_{50} values were recorded. The activity was observed in the following order:

DPC-Co(II) > DPC-Cu(II) > DPC-Ni(II) with IC_{50} values 5.82 ± 0.007 > 152.73 ± 0.26 > 361.45 ± 0.93 µM respectively compared with standard thiourea with IC_{50} value 21.25 ± 0.15 µM. The results of antiurease activity are given in table 1 and graphically represented in (fig.2). The synthesized compounds were also evaluated for their antibacterial activity against *Bacillus thuringiensis* and *Escherichia coli* by disc diffusion method. The zone of inhibition in case of synthesized metal complexes as well as that of standard drug (Gentamicin) has been tabulated in table 2 and graphically represented in (fig.3). It was found that when compared with standard drug, only copper and cobalt complexes exhibited appreciable activity against *Bacillus thuringiensis.*

Molecular docking simulations

In order to identify the plausible binding mode of the synthesized compounds, the most active compound-4 was docked in urease X-rays crystal structure of *Bacillus pasteurii* as shown in (fig.4). The compound is gorged well in the binding site of the urease enzyme with good C-score value. Further we observed that methoxy oxygens of tail group phenyl ring have weak ionic interactions with nickel atoms available in the active site of the urease enzyme. We believe this interaction might be one of the reasons for these complexes to show biological activities against this enzyme. Similarly phenyl ring of the compound-4 is positioned towards the His-323 residue and making the T-type Pi stacking which might also be responsible for higher activity of this compound towards urease. Our studies are in conjunctions with previous reported molecular docking

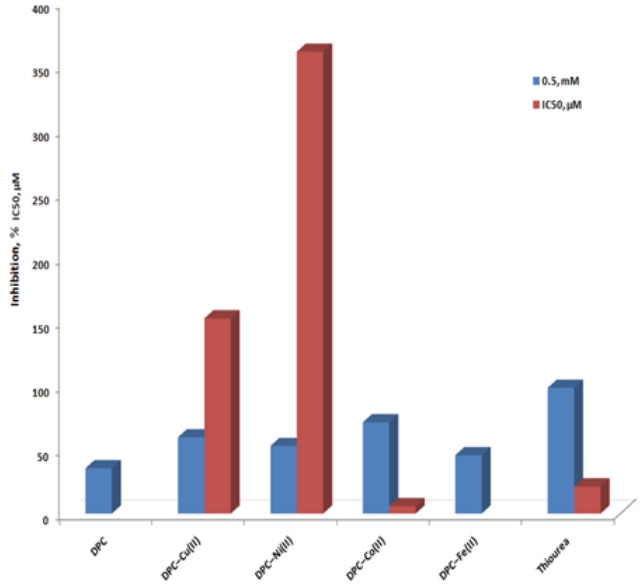


Fig. 2. Antiurease activity of DPC and its complexes

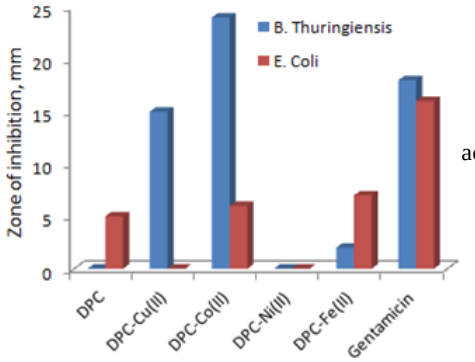


Fig. 3. Antibacterial activity of DPC and its complexes

S. No.	Sample code	Inhibition, % at 0.5 mM	IC_{50}, µM
1	DPC	35.58 ± 0.22	-
2	DPC–Cu	59.72 ± 0.65	152.73 ± 0.26
3	DPC –Ni	52.88 ± 1.24	361.45 ± 0.93
4	DPC–Co	71.37 ± 0.15	5.82 ± 0.007
5	DPC–Fe	45.67 ± 0.15	-
Standard	Thiourea	98.45 ± 0.87	21.25 ± 0.15

Table 1

ANTIUREASE ACTIVITY OF DPC AND ITS COMPLEXES [DATA IS MEAN OF THREE INDEPENDENT EXPERIMENTS (MEAN ± S.E.M., N=3)]

Table 2

ANTIBACTERIAL ACTIVITY DATA OF METAL COMPLEXES (ZONE OF INHIBITION DETERMINED IN mm AND IS MEAN OF THREE INDEPENDENT EXPERIMENTS)

Test organisms	DPC mm	DPC-Cu mm	DPC-Co mm	DPC-Ni mm	DPC-Fe mm	Gentamicin Mm
Bacillus thuringiensis	-	15	24	-	2	18
Escherichia coli	5	-	6	-	7	16

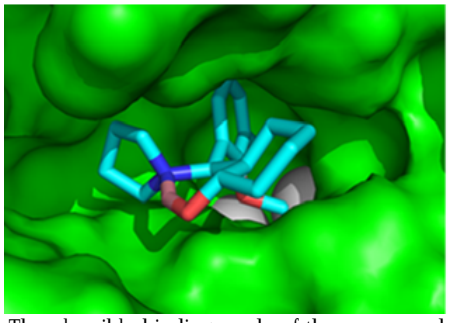


Fig. 4. The plausible binding mode of the compound-4 (cyan) in binding site of the urease enzyme (green); white color balls are representing the Ni(II) ions. Figure was generated using pymol software

studies of metal complexes in urease enzymes [43]. Where it was observed that coordinate covalent bonded metals ions were exposed towards the solvent side. We have also observed similar docking poses for our synthesized most active compound.

Conclusions

The current studies provide a simple protocol for the manufacture of β-aminoketones. The synthesized compounds were investigated for antiurease as well as antibacterial activities. Most of the compounds reflect inhibitory activity against *Bacillus thuringiensis*, *Escherichia coli* and Jack bean urease. The complex with Co(II) exhibit potent antiurease activity with IC_{50} value 5.82 ± 0.007 µM as compared to standard thiourea with IC_{50} value 21.25 ± 0.15 µM. The docking analysis also proved this potential. This complex also possesses potent antibacterial potential as well. The complex with Cu(II) exhibits moderate activity while least activities were possessed by DPC and its complexes with Ni(II) and Fe(II). Spectroscopic techniques were well supported to our designed structures.

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