Synthesis, Characterization and Biological Study of a New Mannich Base, 2-[(4-fluorophenyl)(phenylamino)methyl] cyclopentanone (FPC) and its Transition Metal Complexes with Cu(II), Ni(II), Co(II), Fe(II) and Zn(II)

MUHAMMAD LIAQAT[1], TARIQ MAHMUD[1], MUHAMMAD IMRAN[1], MAZHAR IQBAL[2], MUHAMMAD MUDDASSAR[3], TAUQEER AHMAD[4], LIVIU MITU[5]*

[1]Institute of chemistry, University of the Punjab, Lahore-Pakistan
[2]National Institute of Biotechnology and Genetic Engineering (NIBGE), Faisalabad-Pakistan
[3]Department of Biosciences, COMSATS Institute of Information Technology, Park Road, Islamabad-Pakistan
[4]Department of Chemistry, University of the Sargodha Subcampus, Mianwali-Pakistan
[5]University of Pitesti, Department of Chemistry, 1 Targu. din Vale Str., 110040, Pitesti, Romania

One pot Mannich reaction involving three components (4-fluorobenzaldehyde, aniline, cyclopentanone) was performed using ethanol as a solvent. The resulting Mannich base (FPC) was isolated and further reacted with chloride salts of Cu(II), Ni(II), Co(II), Fe(II) and Zn(II) ions to afford respective metal complexes. The structure of synthesized ligand and transition metal complexes were elucidated on the basis of IR, ^{1}H NMR, ^{13}C NMR, mass spectroscopy and elemental analysis. The geometries of the resulting complexes were proposed on the basis of electronic spectroscopic data and magnetic moment. The anti-enzymatic activity of the ligand and its metal complexes were carried out against urease. FPC shows potent antiurease activity with IC_{50} value (0.83± 0.002 μM) which is greater than standard. The Cu-complex shows excellent inhibitory action with IC_{50} value (16.87± 0.03 μM) while other complexes i.e Co-complex (35.59± 0.04 μM) and Ni-complex (49.93± 0.01 μM) exhibit good to moderate IC_{50} values as compared with control thiourea (IC_{50} value, 21.25± 0.15 μM). Molecular docking studies were also done on the antiurease activities of FPC and its complexes.

Keywords: Mannich base, metal complex, biological activities

Mannich reactions are most fascinating routes for the production of nitrogen containing organic molecules [1-3]. The Mannich base is the end product of Mannich reaction. A typical Mannich reaction consists of three components including an active hydrogen compound (ketone), aldehyde and an amine. Mannich bases have vital importance as intermediate in the production of various natural, medicinal products and catalysts [4-9]. Chemists are trying to develop environment friendly protocols using harmless catalyst. Calcium chloride has been used to catalyze the Mannich reaction and is a non-volatile, low price and eco-friendly solid [10].

The fluorinated Mannich bases have prime importance in the pharmaceutical chemistry due to their therapeutic and pharmacological values [11]. The special character of fluorine (size, electro negativity) has crucial effect in the biological activity for the development of medicinal products. The Mannich bases are considered to be the best candidates with regards to their versatility for complexation with different transition metal ions [12-14]. Keeping in the view, various characteristics of these Mannich bases, present study was performed to synthesize fluorinated Mannich base and its transition metal complexes which are likely to be highly potent biological active agents. Therefore, a novel Mannich base, 2-[(4-fluorophenyl) (phenylamino)methyl]cyclopentanone (FPC) and its transition metal complexes have been synthesized, characterized and tested for their antibacterial as well as anti-urease activity.

*email: ktm7ro@yahoo.com; Phone: 0040/725160304

Experimental part

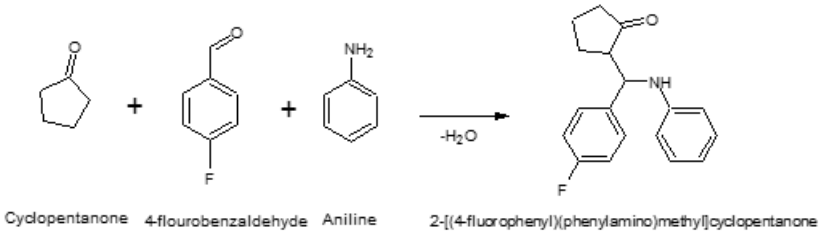
Aldehydes, ketones, amines, metal salts and solvents used were of analytical grade and were used without further purification. Melting points were determined on Gallon Kamp apparatus and are uncorrected. FT-IR spectra were measured on Perkin Elmer IR Spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded by Brucker Avance 400. Mass spectra were measured on JEOL JMS-600. Elemental analyses were performed using a EuroEA Elemental Analyser**.**

Synthesis of ligand

According to a typical reported procedure [15], an ethanolic solution of 4-fluorobenzaldehyde, aniline and cyclopentanone were taken in 1:1:1 mole ratio followed by one equivalent of calcium chloride. The contents were mixed under ice cold conditions for ten minutes. Resulting mixture was heated at 70-90°C for 90 min. After that, the reaction mixture was stirred at room temperature for about 24 h. The progress of reaction was monitored by TLC. 5 % $NaHCO_3$ solution was added in the mixture. Yellow precipitates were obtained which were then filtered, washed with distilled water, ethanol and dried.

General procedure for the synthesis of metal complexes

Equimolar hot ethanolic solution of ligand (FPC) and metal chlorides were stirred separately and then mixed. The resulting mixture was further stirred at room temperature for about one hour. The resulting solid formed was then filtered, washed with distilled water, ethanol and finally dried under vacuum.



Cyclopentanone 4-flourobenzaldehyde Aniline 2-[(4-fluorophenyl)(phenylamino)methyl]cyclopentanone

Scheme 1. Synthetic route for the synthesis of ligand

Characterization of ligand and metal-complexes

Mannich base (FPC): Molecular formula: $C_{18}H_{18}FNO$, Off weight solid, Yield: 73 %, m.p: 150-152°C, Molecular weight: 283.34, λ_{max}(nm): 290.

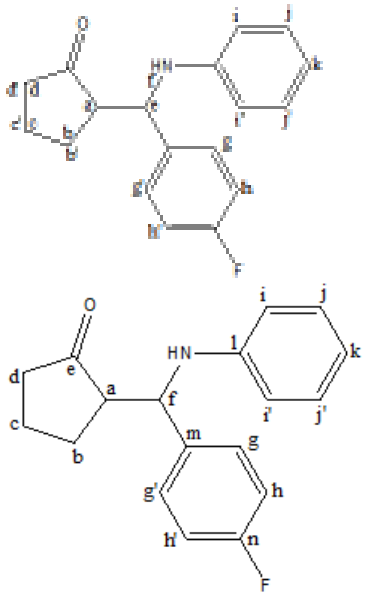
IR (cm^{-1}): 1161 (C-N-C stretching), 1504 (C=C stretching), 1726 (C=O stretching), 3377 (N-H stretching)

^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.362–1.436 (1H, p, H_c), 1.637–1.695 (2H, m, H_b, $H_{c'}$), 1.773-1.866 (1H, m, $H_{b'}$), 2.015–2.026 (1H, m , H_d), 2.159–2.205 (1H, br m, $H_{d'}$), 2.640–2.699 (1H, m, H_a), 4.708-4.722 (1H, t, H_e, J = 5.6 Hz), 6.161- 6.176 (1H, d, H_f, J = 6.0 Hz), 6.473- 6.494 (3H, d, H_i, $H_{i'}$, H_k J = 8.0 Hz), 6.949- 6.969 (2H, t, H_g, $H_{g'}$ J= 8.0 Hz), 7.070 – 7.091(2H, t, H_h, $H_{h'}$, J = 8.4 Hz), 7.271 – 7.306 (2H, m, H_j, $H_{j'}$)

^{13}C NMR (400 MHz, $CDCl_3$, δ): 20.43(C_b), 26.67(C_c), 39.16(C_d), 53.96(C_a), 58.76(C_f), 114.55(C_i,$C_{i'}$), 115.46($C_{j'}$, C_j), 115.60(C_k), 118.47(C_l), 128.77(C_g, $C_{g'}$), 128.82(C_h, $C_{h'}$), 129.04(C_m), 161.29(C_n), 162.92(C_e)

EI–MS (*m/z*) (%) = 306 observed for $[M + Na]^+$ (100), 284 $[M+H]^+$ (28), 323 $[M+K]^+$ (20)

Anal. Calc. for $C_{18}H_{18}FNO$: C, 76.30; H, 6.40; N, 4.94; O, 5.65. Found: C, 76.04; H, 6.31; N, 4.82.



Scheme 2. Labeling scheme of FPC for ^{1}H NMR and ^{13}C NMR data

FPC-Cu(II): Molecular formula: $C_{18}H_{18}Cl_2CuFNO$, Light green solid, Yield: 47%,

m.p: Decomposed above 280°C, Molecular weight: 417.79 g/mole, λ_{max}(nm): 248.

IR (cm-^{1}): 1190 (C-N-C stretching), 1502 (C=C stretching), 1726 (C=O stretching), 3375 (N"H stretching), 445 (Cu-N stretching), 526 (Cu-O stretching)

^{1}H NMR ($CDCl_3$, 400MHz): δ 1.260-2.353 (6H, br-m, H_b, $H_{b'}$, H_c $H_{c'}$, H_d, and $H_{d'}$), 2.596-2.864 (1H, br-q, H_a), 5.296-5.614 (1H, br-d, H_e), 6.503-6.650 (1H, br-s, H_f), 7.011-7.361 (9H, br-m, H_g ,$H_{g'}$,H_h, $H_{h'}$ H_i, $H_{i'}$, H_j $H_{j'}$, H_k)

^{13}C NMR (400 MHz, $CDCl_3$, δ): 20.43 (C_b), 26.67 (C_c), 39.16 (C_d), 53.96 (C_a), 58.76 (C_f), 114.55 (C_i, $C_{i'}$), 115.46 (C_j, $C_{j'}$), 115.60 (C_k), 118.47 (C_l), 128.77 (C_g, $C_{g'}$), 128.82 (C_h, $C_{h'}$), 129.04 (C_m), 161.29 (C_n), 165.12 (C_e)

Anal. Calc. for $C_{18}H_{18}Cl_2CuFNO$: C, 51.75; H, 4.34; N, 3.35; Cu, 15.21. Found: C, 51.11; H, 4.31; N, 3.16; Cu, 15.09.

FPC-Ni(II): Molecular formula: $C_{18}H_{18}Cl_2NiFNO$, Greenish yellow solid, Yield: 58 %, m.p.: Decomposed above 280°C, Molecular weight: 412.94 g/mole, λ_{max}(nm) 246.

IR (cm^{-1}): 1161 (C-N-C stretching), 1438 (C=C stretching), 1722 (C=O stretching), 3350 (N-H stretching), 468 (Ni-N stretching), 547 (Ni-O stretching)

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.983-2.838 (6H, br-m, H_b, $H_{b'}$, H_c $H_{c'}$, H_d $H_{d'}$), 3.061-3.599 (1H, br-q, H_a), 5.253 (1H, s, H_e), 7.261-7.313 (1H, s, H_f), 7.728-7.802 (3H, s, H_i, $H_{i'}$, H_k), 7.988-8.110 (6H, br-t, H_g, $H_{g'}$ H_h $H_{h'}$, H_j, $H_{j'}$)

^{13}C NMR (400 MHz, $CDCl_3$, δ): 20.42 (C_b), 26.65 (C_c), 39.14 (C_d), 53.86 (C_a), 58.77 (C_f), 114.51 (C_i, $C_{i'}$), 118.51 (C_j, $C_{j'}$), 127.34 (C_k), 128.64 (C_l), 128.84 (C_g, $C_{g'}$), 129.06 (C_h, $C_{h'}$), 133.18 (C_m), 147.21 (C_n), 164.50 (C_e)

Anal. Calc. for $C_{18}H_{18}Cl_2NiFNO$: C, 52.35; H, 4.39; N, 3.39; Ni, 14.21. Found: C, 51.97; H, 4.27; N, 2.99; Ni, 14.32.

FPC-Co(II): Molecular formula: $C_{18}H_{18}Cl_2CoFNO$, Off wight solid, Yield: 56%, m.p: Decomposed above 280°C, Molecular weight: 413.18 g/mole, λ_{max}(nm) 246

IR (cm^{-1}): 1190 (C-N-C stretching), 1440 (C=C stretching), 1726 (C=O stretching), 3466 (N-H stretching), 447 (Co-N stretching), 538 (Co-O stretching)

^{1}H NMR ($CDCl_3$, 400MHz): δ 1.545-1.919 (6H, br-m, H_b, $H_{b'}$ H_c, $H_{c'}$ H_d, $H_{d'}$), 2.108-2.561 (1H,br-q,H_a), 4.529 (1H,s,H_e), 6.323-6.432 (1H, br-d, H_f), 6.556-6.711 (3H, br-s, H_i, $H_{i'}$, H_k), 6.996-7.346 (6H, br-dd, H_g, $H_{g'}$ H_h, $H_{h'}$, H_j, $H_{j'}$)

^{13}C NMR (400 MHz, $CDCl_3$, δ): 20.43 (C_b), 26.67 (C_c), 39.16 (C_d), 53.96 (C_a), 58.76 (C_f), 114.55 (C_i, $C_{i'}$), 115.46 (C_j , $C_{j'}$), 115.60 (C_k), 118.47 (C_l), 128.77 (C_g, $C_{g'}$), 128.82 (C_h, $C_{h'}$), 129.04 (C_m), 161.29 (C_n), 164.82 (C_e)

Anal. Calc. for $C_{18}H_{18}Cl_2CoFNO$: C, 52.32; H, 4.39; N, 3.39; Co, 14.26. Found: C, 51.43; H, 4.21; N, 3.17; Co, 14.33.

FPC-Zn(II): Molecular formula: $C_{18}H_{18}Cl_2ZnFNO$, Light brown solid, Yield: 57 %, m.p.: Decomposed above 280UC, Molecular weight: 419.65 g/mole, λ_{max}(nm) 246

IR (cm^{-1}): 1163 (C-N-C stretching), 1504 (C=C stretching), 1726 (C=O stretching), 3377 (N-H stretching), 458 (Zn-N stretching), 526 (Zn-O stretching)

^{1}H NMR ($CDCl_3$, 400MHz): δ 1.921–1.989 (1H, m, H_c), 2.152–2.188 (2H, m, H_b, $H_{c'}$), 2.317-2.365 (2H, m, $H_{b'}$, H_d), 2.573–2.576 (1H, m, $H_{d'}$), 2.726–2.741 (1H, q, H_a), 4.796-4.808 (1H, d, H_e J = 4.8 Hz), 5.362 (1H, s, H_f), 6.766-6.782 (3H, d, H_i, $H_{i'}$, H_k J = 6.4 Hz), 6.917-6.941 (2H, t, H_g, $H_{g'}$), 7.247-7.276 (2H, t, H_h, $H_{h'}$,), 7.315–7.623 (2H, m, H_j, $H_{j'}$)

^{13}C NMR (400 MHz, $CDCl_3$, δ): 20.44 (C_b), 26.62 (C_c), 39.20 (C_d), 54.12 (C_a), 58.37 (C_f), 114.19 (C_i, $C_{i'}$), 115.43 (C_j, $C_{j'}$), 115.57 (C_k), 118.06 (C_l), 128.65 (C_g, $C_{g'}$), 128.70 (C_h, $C_{h'}$), 129.06 (C_m), 147.29 (C_n), 163.97 (C_e)

Anal. Calc. for $C_{18}H_{18}Cl_2ZnFNO$: C, 51.52; H, 4.32; N, 3.34; Zn, 15.59. Found: C, 51.44; H, 3.99; N, 3.19; Zn, 15.13.

FPC-Fe(II): Molecular formula: $C_{18}H_{18}Cl_2FeFNO$, Light brown solid, Yield: 57%, m.p.: Decomposed above 280UC, Molecular weight: 410.09 g/mole, λ_{max}(nm) 242

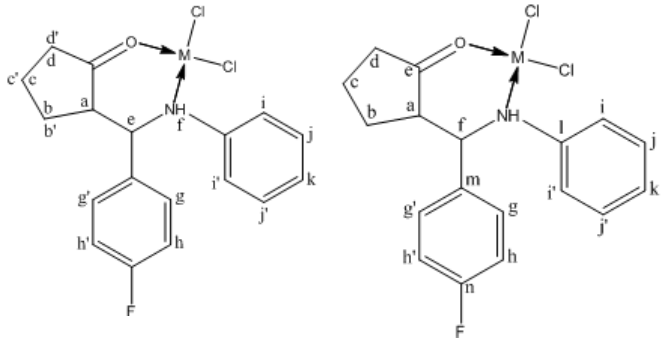
IR (cm^{-1}): 1162 (C-N-C stretching), 1508 (C=C stretching), 1723 (C=O stretching), 3378 (N-H stretching), 456 (Fe-N stretching), 545 (Fe-O stretching)

^{1}H NMR ($CDCl_3$, 400MHz): δ 1.449"2.106 (6H, br-m, H_b, $H_{b'}$ H_c, $H_{c'}$ H_d, $H_{d'}$), 2.282-2.665 (1H, br-q, H_a), 4.738-5.297

(1H, br-d, H_e), 6.712-6.864 (1H, br-s, H_f), 7.197-7.546 (9H, dd, H_i, $H_{i'}$, H_k, H_g, $H_{g'}$, H_h, $H_{h'}$, H_j, $H_{j'}$)

^{13}C NMR (400 MHz, $CDCl_3$, δ): 20.45 (C_b), 26.62 (C_c), 39.20 (C_d), 54.12 (C_a), 58.37 (C_f), 114.20 (C_i,$C_{i'}$), 115.43 (C_j, $C_{j'}$), 115.57 (C_k), 118.06 (C_l), 128.65 (C_g, $C_{g'}$), 128.70 (C_h, $C_{h'}$), 129.01 (C_m), 147.29 (C_n), 163.11 (C_e)

Anal. Calc. for $C_{18}H_{18}Cl_2FeFNO$: C, 52.72; H, 4.42; N, 3.42; Fe, 13.62. Found: C, 52.11; H, 4.33; N, 3.21; Fe, 13.41.



Scheme 3. Labeling scheme of metal complexes for ^{1}H NMR and ^{13}C NMR data

Biological evaluation

Antiurease assay

This assay was carried out according to Berthelot assay with slight modification [16]. A total reaction volume (85 µL) contained 10 µL of 50 mM phosphate buffer (*p*H 7.0), 10 µL of test compound and 25 µL of jack beans urease (0.015 units). The contents were pre-incubated at 37°C for 10 min. Then, 40 µL of 20 mM urea was added to each well and incubation continued at 37°C for further 10 min followed by pre-read at 625 nm using the 96-well plate reader Synergy HT (Biotek Inc.). Freshly prepared phenol hypochlorite (115µL) reagent was added in each well (by mixing 45 µL phenol reagent with 70 µL of alkali reagent). Incubation was again continued for another 10 min followed by measurement of absorbance at 625 nm. The percentage enzyme inhibition was calculated by the following formula:

$$\text{Inhibition (\%)} = 100 - [(\text{Abs. of test sample} / \text{Abs. of control}) \times 100].$$

IC_{50} values of active compounds were determined by measuring activities at further dilutions and the data was computed by using EZ-Fit Enzyme software (PerrellaInc, USA).

Molecular modeling studies

Crystal structure (4ubp) of urease enzyme was retrieved from the Protein Data Bank [17]. The 3D structure of the downloaded protein was prepared by removing the co-crystallized chains (A and C) water molecules and other heteroatoms and ions from the structure using PDB2RECEPTOR utility implemented in Open Eye Scientific Software. For docking simulations of compound-2 Openeye Fred docking tool [18] was used for docking simulations and its FRED Chemgauss4 score was used for ranking the poses of docked compound. Maestro software was used for the drawing and 3D conversion of the structures of synthesized compound. The compound was energy minimized using the molecular mechanics based force field. The binding modes of the compounds were analyzed using the Pymol visualization program [19].

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 [20]. 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.

Results and discussions

The physico-analytical informations of FPC and its metal-complexes are shown in experimental section. All the compounds are stable and colored solid at room temperature. FPC and its metal-complexes are insoluble in common organic solvents and soluble in chloroform, DMSO and DMF. The complexes reflect non-electrolytic nature as revealed by conductometric measurements [21].

IR spectra

In order to get meaningful information about the connecting modes of FPC to the metal ions in the complexes, IR spectrum of FPC was compared with the spectral data of its metal- complexes. Important peaks at 1116, 1504, 1726 and 3377 cm^{-1} can be attributed to C-N-C, C=C, C=O and N-H correspondingly [22-24]. These signals are quite in agreement with our proposed and designed structure of free ligand. The indication of coordination through oxygen of carbonyl and nitrogen of aniline was provided by IR spectra of all the complexes in which the peaks due to C=O were shifted to lower frequencies (1726-1722) while peaks due to C-N-C were shifted to higher frequencies (1162-1190) [25]. Coordination from these sites is further supported by the appearance of some new bands at 445-468 cm^{-1} and 526-551 cm^{-1} assignable to M-N and M-O bonds respectively [26].

NMR spectra

An additional evidence for coordinatining modes of FPC is also given via the ^{1}H-NMR spectral values of ligand and its complexes. The chemical shifts indicated by ^{1}H-NMR spectrum of ligand (fig. 1) are given in experimental section, which match very well with the designed structure of ligand. In the metal complexes, the change in chemical shift values of protons attached to C=O and C-N-C groups, confirms the coordination of metal ions through oxygen and nitrogen [27a]. This observation is in consistent with the interpretation of IR spectral data. The different kinds of carbon in ligand are indicated through ^{13}C-NMR spectral data. All the chemical shifts of the ^{13}C-spectrum of FPC (fig. 2) and its complexes are given in experimental section and are well in consistent with their proposed structures. The most significant chemical shift is due to carbon of C=O group which is shifted from 162.92 (free ligand) [27b-e] to 164.53 (for example) in case of nickel complex. This shift support the coordination of C=O group to the metal ions.

Mass spectrum (EI)

EI mass spectrum of FPC was recorded. In the said spectrum (fig. 3), the base peak was observed at 306 which can be attributed to $[M + Na]^+$ [28]. Some peaks of other fragments at 284 and 323 were also observed and can be attributed to $[M + H]^+$ and $[M + K]^+$ respectively. The mass spectra of metal complexes revealed complicated fragmentation and hence their formulae were proposed from elemental analysis. Moreover the geometries of these complexes were elucidated from

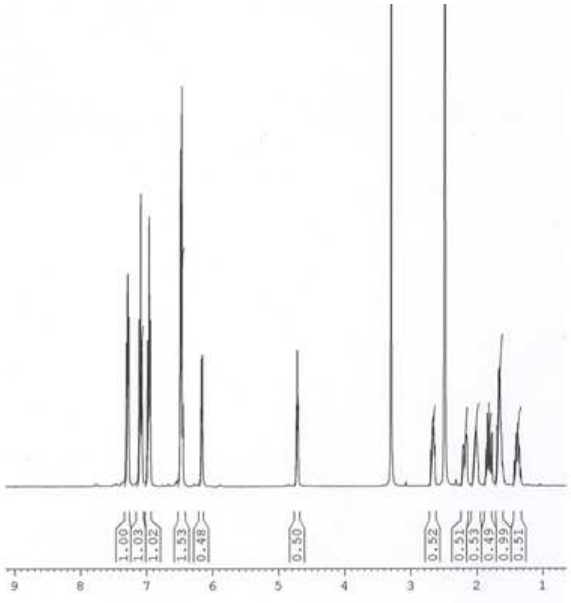


Fig. 1. ^{1}H NMR spectrum of FPC

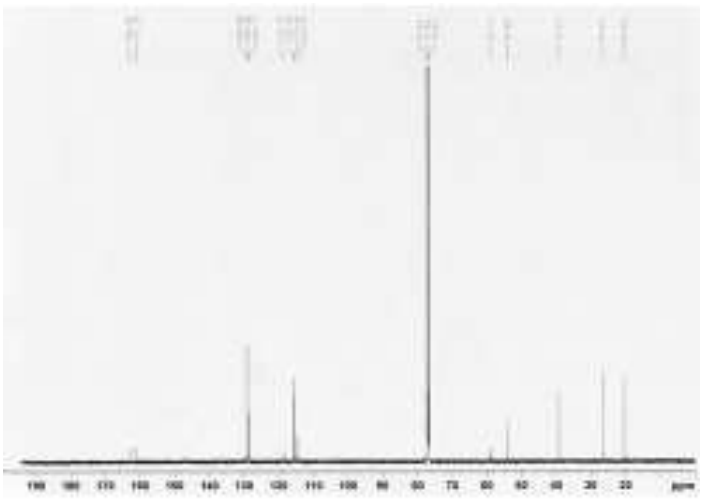


Fig. 2. ^{13}C NMR spectrum of FPC

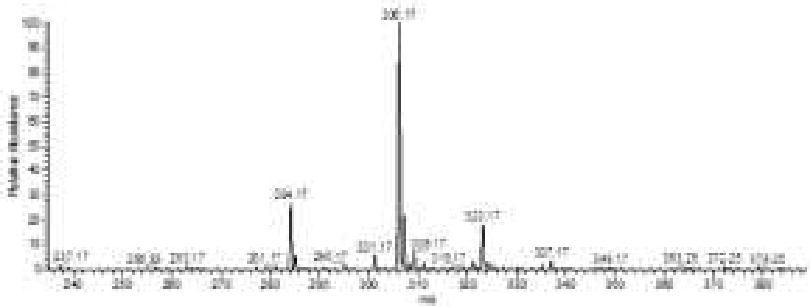
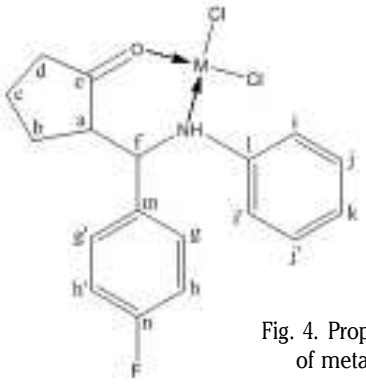


Fig. 3. Mass spectrum of ligand FPC



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

Fig. 4. Proposed structures of metal complexes

magnetic moment and electronic spectral data as discussed above.

UV/Vis data and Magnetic moment

The electronic spectrum of Cu(II)-FPC complex shows an absorption band at 13176 cm^{-1} that can be assigned to $^{2}T_2$ ———> ^{2}E transition of a 4-coordinate tetrahedral geometry. The magnetic moment value (2.16 B.M.) of this complex also support 4-coordinate tetrahedral geometry [29]. The electronic spectrum of Ni(II)-FPC complex exhibits two absorption bands at 11655 cm^{-1}, 15439 cm^{-1} assignable to $^{3}T_1(F)$ ———> $^{3}A_2(F)$, $^{3}T_1(F)$ ———> $^{3}T_1(P)$ transitions of tetrahedral environment around nickel ion. Similarly the magnetic moment value (3.31 B.M.) also supports this environment around nickel ion [30]. Electronic spectrum of the Co(II)-FPC complex depicts two allowed transitions at 11753 cm^{-1}, 17367 cm^{-1} assignable to $^{4}A_2(F)$———>$^{4}T_1(F)$ and $^{4}A_2(F)$ ———> $^{4}T_1(P)$ transitions respectively, which are in good agreement with tetrahedral stereochemistry for Co(II) ion. The magnetic moment value of Co(II) complex is at 4.38 B.M. revealing that the Co(II) complex has typically tetrahedral geometry [30]. The electronic spectrum of Fe(II)-FPC complex shows an absorption band at 8560 cm^{-1} which can be attributed to ^{5}E ———> $^{5}T_2$ transition of a tetrahedral geometry. The room temperature magnetic moment (4.67 B.M.) corresponds with the tetrahedral symmetry [31]. Zinc complex has d^{10} configuration and was found to be diamagnetic. The diamagnetic nature of this complex supports its tetrahedral geometry. Keeping in view the electronic, magnetic and other supportive data as discussed above, following structure for these metal complexes has been proposed.

S. No.	Compounds	Inhibition (%) at 0.5 mM	IC_{50}(μM)
1	FPC	69.68 ± 0.07	0.83 ± 0.002
2	FPC–Cu	67.78 ± 0.08	16.87 ± 0.03
3	FPC–Co	63.61 ± 0.27	35.59 ± 0.04
4	FPC–Ni	73.28 ± 0.07	49.93 ± 0.01
5	FPC–Fe	32.89 ± 0.34	-
6	FPC-Zn	48.34 ± 0.13	-
Standard	Thiourea	98.45 ± 0.87	21.25± 0.15

Table 1
ANTIUREASE ACTIVITY OF FPC AND ITS METAL COMPLEXES

Test Organism	FPC mm	FPC-Cu mm	FPC-Co mm	FPC-Ni mm	FPC-Fe mm	FPC-Zn mm	Gentamicin mm
Bacillus thuringiensis	03	13	09	04	05	04	18
Escherichia coli	02	03	22	12	03	03	16

Table 2
ANTIBACTERIAL ACTIVITY OF FPC AND ITS METAL COMPLEXES

Biological activity

Antiurease activity

The ligand FPC and its metal complexes were screened for their antiurease activities and their IC_{50} values were recorded. The best activity IC_{50} value 0.83 ± 0.002 was found for free ligand FPC followed by FPC"Cu(II) complex (16.87 ± 0.03). The IC_{50} value for standard thiourea was noted to be 21.25 ± 0.15 µM which is even higher than free ligand and copper complex. The other complexes exhibit higher IC_{50} values and are given in the table 1 and graphically represented in (fig. 5).

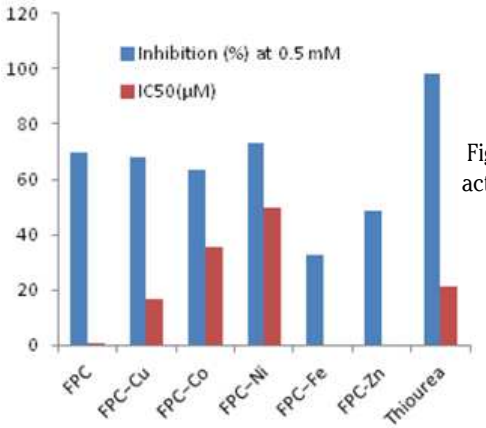


Fig. 5. Antiurease activity of FPC and its metal-complexes

Antibacterial activity

The synthesized compounds were also evaluated for their antibacterial activity against *Bacillus thuringiensis* and *Escherichia coli* by disc diffusion method. The zone of inhibition has been tabulated in table 2. The antibacterial activity of all metal complexes was lower than the standard drug, however to some extend enhanced when compared with free ligand.

Molecular docking simulations

The plausible binding mode of the most active metal complex molecule (2) was identified using molecular docking simulations in urease X-rays crystal structure of bacillus pasteurii as shown in (fig. 6). The compound docked well at the binding site of the enzyme and yielded high FRED Chemgauss-4 score (-5.964). The Chemgauss-4 scoring function uses the gaussian smoothed potentials to measure the complementarily of ligand poses within the active site. It recognizes the shape, hydrogen bonding between ligand and protein, hydrogen bonding interactions with implicit solvent and metal-chelator interactions. It has been observed that fluoro benzene ring of molecules-2 is making *p-p* stacking with HIS323 residue of urease along with other hydrophobic interactions with side chains of the residues. Similarly the Cu^{2+} in complex with O and N atoms of FPC is directed towards the solvent side, which is in agreement with previously published docking results [32].

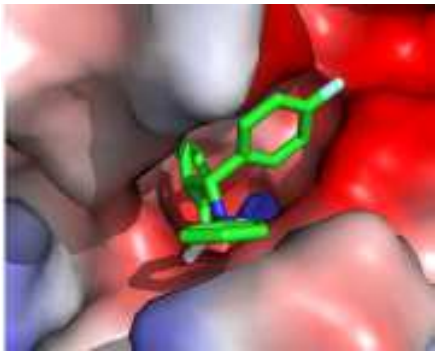


Fig. 6. The predicted binding mode of the compound-2 (green) in binding site of the urease enzyme (blue and red surface)
Figure was created using pymol software

Conclusions

The synthesized scaffolds were screened for their antiurease as well as antibacterial activities. Most of the compounds show inhibitory activity against *Bacillus thuringiensis, Escherichia coli* and Jack bean urease. It is noteworthy that FPC and FPC-Cu exhibits potent activity to inhibit Jack bean urease with IC_{50} values 0.83 ± 0.002 µM and 16.87 ± 0.03 µM respectively which are superior to the standard thiourea with IC_{50} value 21.25 ± 0.15 µM. The docking analysis also proved this potential. Additionally, the mild conditions and convenient operation as well as straightforward route made it believable that our method would play an important role in the synthesis of similar scaffolds. It might be helpful in drug designing in future to control the diseases caused by Jack bean urease.

Acknowledgement: The authors are grateful to institute of chemistry university of Punjab Lahore and HEC Pakistan for providing financial and laboratory facilities.

References

1.KLEINNMANN, I.F., In Trost BM (ed.), Comprehensive organic synthesis, Pergamon press, New York, Review, 1991, Chp.4.1
2.AREND, M., WESTERMAN, B., RISH, N., Angew.Chem.Int.Ed.,37, Review, 1998, p. 1044
3. BLICKE, F.F., Org.react.1, Review, 1942, p. 303
4.DAVIS, F.A., ZHANG,Y., ANIL KUMAR, G., J.Org.Chem., **68**, 2003, p. 8061
5.EVAN, G.B., FURNEAUX, R.H., TYLER, P.C., Org.Lett., **5**, 2003, p. 3639
6.RIVERA, A., QUEVEDO, R., Tetrahedron Lett., **47**, 2006, p. 9045
7. PRUKALA, D., Tetrahedron Lett., **47**, 2006, p. 9045
8. LIST, B., J.Am.Chem.Soc., **122**, 2000, p. 9336
9.IBRAHEM, I., ZOU, W., ENGQVIST, M., XU, Y., CORDOVA, A., Chem.Eur.J., **11**, 2005, p. 7024
10. *** Monatsh.Chem, **143**, 2012, p. 625
11. CHALUVARAJU, K.C., Int.J.Pharm.Tech.Res., **3**, 2014, p. 1904
12. RAMAN, N., RAVICHANDRAN, S., Polish J.Chem., **78**, 2004, p. 2005
13. POUL, R.C., KAPILA, P.A., BEDI, S., Vasisht, K.K., J.Indian Chem.Soc., **53**, 1976,p. 768
14. RAMAN, N., RAVICHANDRAN, S., Asian J.Chem., **15**, 2003, p. 1848
15. KULKARNI, P., TOTAWAR, B., Monatsh.Chem., **143**, 2012, p. 625
16. WEATHERBURN, M.W., Anal.Chem., **39**, 1967, p. 971
17. BENINI, S., RYPNIEWSKI, W.R., WILSON, K.S., MILETTI, S., CIURLI, S., MANGANI, S., J.Biol.Inorg.Chem., **5**, 2000, p. 108
18. FRED, P., J.Chem.Information and Modeling, **51**, 2011, p. 578
19. The PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC
20. BAUER, A.W., KIRBY, W.M., SHERRIS, J.C., Turck.Am.Clin.Pathol., **9**, 1966, p. 493
21. GEARY, W.J., Coord.Chem.Rev., **7**, 1971, p. 81

22.SIMPY, M., BALGAR, S., MOITHA, S., SHEIKH, H.N., KALSOTRA, B.L., Chin.J.Chem., **29**, 2011, p. 53
23. SABASTIYAN, A., SUVAIKIN, M.Y., Adv.Appl.Sci.Res., **3**, 2012, p. 45
24. VENDAN, D.T., RAJESWARI, S., ILAVENIL, S., PRABHU, G.V., Orbital Elec.J.Chem., **2**, 2012, p. 201
25.ANAD, S., Transition Metal.Chem., **32**, 2007, p. 816
26.ABDUL-GHANI, A.J., AL-JABOORI, M.J., AL-KARAWIS, A.M., J.Coord.Chem., **62**, 2009, p. 2736
27. (a) SATHYA, D., SENTHIL, J., PRIA, S., Inter.J.Chem.Tech.Res., **3**, 2011, p. 248 (b) JAVID, A., HERAVI, M.M., BAMOHARRAM, F.F., Monatshefte für Chemie, **143**, 2012, p. 831 (c) THIRUGNANAM, T., TAMILVENDAN, D., AMALADASAN, M., World J.Pharmacy and Pharmaceutical Sciences, **2**, 2013, p. 5863 (d) TAMIL, D., PRABHU, V., Orbital - The Electronic Journal of Chemistry, **2**, 2010, p. 201 (e) NEVES, A.P., BARBOSA, C.C., GRECO, S.J., VARGAS, M.D., .Braz.Chem.Soc., **20**, 2009, p. 712
28.GREWAL, R.N., ARIT, H.E., SMITH, J.C., CHRISTOPHER, F., Int.J.Mass Spect., **12066**, 2002, p. 1
29. BRUNDIC, A.H., KAITNER, B., KAMENAR, B., LEOVAC, V.M., Inorg.Chim.Acta, **188**, 1991, p. 151
30.SIVASANKARAN, M.A., JOSEYPHUS, R.S., Spectrochimica Acta-A: Molecular and Biomolecular Spectroscopy, **70**, 2008, p. 749
31. CHEN, M.Z., SUN, H.M., LI, W.F., J.Organomet.Chem., **691**, 2006, p. 2489
32. CHEN, W., CUI, Y., ZHANG, X., ZHU, H.L., ZENG, Q., European Journal of Medicinal Chemistry, **45**, 2010, p. 4473

Manuscript received: 12.04.2017