

Synthesis, Characterization, Molecular Docking and Enzyme Inhibition Studies of Some Novel Enaminone Derivatives and Their Complexes with Cu(II), Cd(II) and Co(II) Ions

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Two new enaminone ligands, 3-chloro-4-(4-chlorophenyl)amino}pent-3-en-2-one (Ac-PCA), 4-(benzylamino)-3-chloropent-3-en-2-one (Ac-BA) and their metal complexes with transition metal ions [Cu(II), Cd(II) and Co(II)] were prepared and subsequently characterized by FTIR, ICP-AES, UV-Vis, TGA, ¹H NMR, ¹³C NMR and FAB-MS. These newly synthesized compounds were further investigated for anti-acetylcholinesterase (AChE) and anti-urease activities. The (Ac-BA)Cu(II) complex exhibited good anti-AChE while (Ac-BA)₂Co(II) complex was potent against anti-urease activities. Other ligands and complexes showed poor to no enzyme inhibitory activities. The synthesized compounds were docked inside acetylcholinesterase enzymes to determine their putative binding mode.

Keywords: Antiureas activity, enaminone, metal complexes, acetylcholinesterase inhibition studies

One of the main goals for chemists and pharmacologists is the synthesis of novel biologically active compounds that can act as better drugs to fight against diseases. Coordination chemistry, therefore, has developed very rapidly since many ligands of low biological activity became more active when they were converted to metal complexes and some drugs show increased activity when reacted with metal ions to form metal complexes [1, 2]. The literature search has shown several publications on the chemistry of enaminones and their derivatives, their physicochemical properties and biological activities [3-5]. On the other hand, enaminones have been extensively used as key intermediates in organic synthesis and the chemistry of these compounds has been reviewed [6]. The presences of carbonyl group connected by carbon=carbon double bond and an amino group or *N*-substituted amines in enaminones make them bidentate ligands [7]. Enaminones have become more important because they can be used as good chelating ligands for transition metals and the anions generated from them give isoelectronic replacements to cyclopentadienyl-based anions. Therefore, enaminone metal complexes can act as alternative catalysts for olefin polymerization [8, 9]. Complexes of different metal ions with enaminones have also been reported [10-12]. The structure and areas of practical application of fluoroalkyl containing enaminoketone chelates with *d*-metals have been reviewed [13].

Such complexes have wide applications in different areas of industry and engineering [14, 15].

In view of these facts, the present work involves the synthesis of enaminones and their complexes with Cu(II), Co(II) and Cd(II) ions. The newly synthesized enaminones and their metal complexes were characterized by FT-IR, NMR, Mass spectrometry, TGA and elemental analysis. All the synthesized compounds were screened for urease and acetylcholinesterase inhibition activity. The enzyme inhibitory action was explained by the docking studies and

the binding mode of best active compound was also predicted.

Experimental part

Materials

The starting materials and solvents used for the synthesis of ligands and complexes were purchased from commercial dealers and were used without purification. Metal(II) chlorides and acetates were used without any refining because of their analytical ranking.

Physical measurements

The IR spectrum was recorded on Agilent Technologies FT-IR instrument (in range of 4000-400 cm⁻¹). The ¹H-NMR (300 MHz) spectrum was recorded on a Bruker Ascend NMR spectrometer using TMS as an internal standard. The (FAB) mass spectrum was recorded on a JEOL JMS-600H Mass Spectrometer.

Synthesis of Ligands

Ligands (Ac-PCA and Ac-BA) were synthesized in accordance with the reported method [16]. 3-chloro-2,4-pentanedione (0.02 mol, 2.25 mL), *p*-chloroaniline (0.02 mol, 2.5g) / benzylamine (0.02 mol, 2.18 mL), and toluene (150 mL) were refluxed on a heating mantle for 12 h. Dean-Stark apparatus is used to remove the water produced in reaction mixture. The reaction was monitored by TLC (hexane : ethyl acetate; 8:1) and the spots were detected by ultraviolet light. Solvent was removed under reduced pressure and the viscous oily liquid was dissolved in chloroform and stirred with concentrated aqueous sodium bicarbonate. The organic layer was separated, dried over anhydrous sodium sulphate and solvent evaporated. Pure product obtained as shiny crystals were found to be stable in air and light (Scheme 1 and 2).

(Ac-PCA)

Molecular formula: C₁₁H₁₁Cl₂NO; Light yellow solid; Yield: 71 %; m.p.: 77°C; Molecular weight: 244.12 g/mol;

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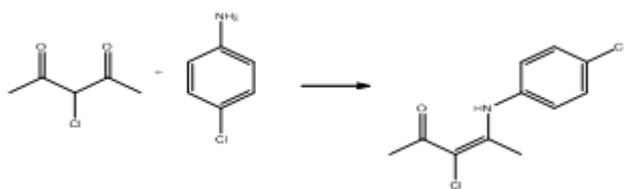
IR (cm⁻¹): 3125 (NH), 1655 (C=O), 1548 (C=C); ¹H NMR (300 MHz, CDCl₃, δ ppm): 12.71 (s, 1H, -NH), 7.35 (d, 2H, -Ar-H), 7.03 (d, 2H, -Ar-H), 2.38 (s, 3H), 2.18 (s, 3H); ¹³C NMR (75 MHz, CDCl₃, δ ppm): 194.97, 157.74, 137.05, 131.73, 129.37, 126.51, 104.93, 28.67, 18.15; FAB-MS: *m/z* = 244.1[M+H]⁺; Anal.Calc., (%) for

C₁₁H₁₁Cl₂NO: C, 54.12; H, 4.54; N, 5.74; Found: C, 52.58; H, 3.92; N, 5.82.

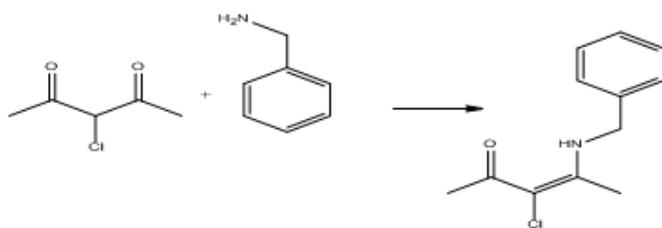
(Ac-BA)

Molecular formula: C₁₂H₁₄ClNO; Pale yellow solid; Yield: 60 %; m.p.: 70°C; Molecular weight: 223.70 g/mol; IR (cm⁻¹): 3132 (NH), 1593 (C=O), 1561 (C=C); ¹H NMR (300 MHz, CDCl₃, δ ppm): 11.68 (s, 1H, -NH), 7.40-7.25 (m, 5H, -Ar-H), 4.52 (d, 2H, -CH₂-), 2.32 (s, 3H), 2.17 (s, 3H); ¹³C NMR (75 MHz, CDCl₃, δ ppm): 193.60, 161.19, 137.50, 128.93,

127.66, 126.77, 102.61, 47.69, 28.66, 16.68; FAB-MS: *m/z* = 224.1[M+H]⁺; Anal.Calc., (%) for C₁₂H₁₄ClNO: C, 64.43; H, 6.31; N, 6.26; Found: C, 65.35; H, 5.24; N, 5.18.



Scheme 1. Synthesis of 3-chloro-4-((4-chlorophenyl)amino)pent-3-en-2-one (Ac-PCA)

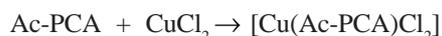


Scheme 2. Synthesis of 4-(benzylamino)-3-chloropent-3-en-2-one (Ac-BA)

Synthesis of metal complexes

Cu(II) complex of 3-chloro-4-((4-chlorophenyl)amino)pent-3-en-2-one (Ac-PCA)₂Cu(II)

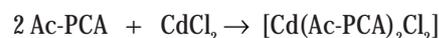
A solution of (Ac-PCA) (0.01 mol) in 10 mL DCM (Dichloromethane) was added drop wise with continuous stirring to a 10 mL of filtered ethanolic solution of the CuCl₂ (0.01 mol) and refluxed for 5 h. The complex was obtained by filtration and evaporated slowly at room temperature. The synthesis of coordination compound had good yield. Molecular formula: [C₁₁H₁₁Cl₄CuNO]; Green solid; Yield: 43 %; m.p.: decomposed above 280°C; Molecular weight: 378.57 g/mol; IR (cm⁻¹): 3337 (NH), 1640 (C=O conjugated), 1562 (C=C stretching), 443 (Cu-N stretching), 547 (Cu-O stretching); Anal.Calc., (%) for C₁₁H₁₁Cl₄CuNO: C, 34.90; H, 2.93; N, 3.70; Found: C, 34.41; H, 2.65; N, 3.50; (%) Cu for [MLCl₂]: theoretical/experimental (16.79/17.48).



Cd(II) complex of 3-chloro-4-((4-chlorophenyl) amino)pent-3-en-2-one (Ac-PCA)₂Cd(II)

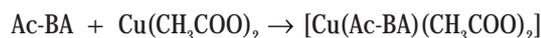
A solution of (Ac-PCA) (0.02 mol) in 10 mL DCM was added drop wise with continuous stirring to a 10 mL of filtered ethanolic solution of the CdCl₂ (0.01 mol) and refluxed for 2 h. The complex was obtained by filtration and evaporated slowly at room temperature. The synthesis of coordination compound had good yield. Molecular

formula: [C₂₂H₂₂Cl₆CdN₂O₂]; Light yellow solid; Yield: 41 %; m.p.: decomposed at 240-244°C; Molecular weight: 671.55 g/mol; IR (cm⁻¹): 3405 (NH), 1645 (C=O conjugated), 1541 (C=C stretching), 454 (Cd-N stretching), 525 (Cd-O stretching); ¹H NMR (300 MHz, CDCl₃, δ ppm): 12.66 (s, 1H, -NH), 7.46 (d, 2H, -Ar-H), 7.27 (d, 2H, -Ar-H), 2.27 (s, 3H), 2.16 (s, 3H); Anal.Calc., (%) for C₂₂H₂₂Cl₆CdN₂O₂: C, 39.35; H, 3.30; N, 4.17; Found: C, 38.10; H, 2.95; N, 4.05; (%) Cd for [ML₂Cl₂]: theoretical/experimental (16.74/14.56).



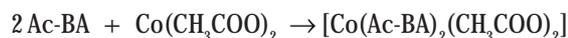
Cu(II) complex of 4-(benzylamino)-3-chloropent-3-en-2-one (Ac-BA)₂Cu(II)

A solution of (Ac-BA) (0.01 mol) in 10 mL DCM was added drop wise with continuous stirring to a 10 mL of filtered ethanol solution of the Copper acetate monohydrate (0.01 mol) and refluxed for 4 h. The complex was obtained by filtration and evaporated slowly at room temperature. The synthesis of coordination compound had good yield. Molecular formula: [C₁₆H₂₀ClCuNO₅]; Light green solid; Yield: 49 %; m.p.: decomposed above 280°C; Molecular weight: 405.33 g/mol; IR (cm⁻¹): 3420 (NH), 1633 (C=O conjugated), 1558 (C=C stretching), 453 (Cu-N stretching), 542 (Cu-O stretching); Anal.Calc., (%) for C₁₆H₂₀ClCuNO₅: C, 47.41; H, 4.97; N, 3.46; Found: C, 46.33; H, 3.99; N, 3.30; (%) Cu for [ML(CH₃COO)₂]: theoretical/experimental (15.68/14.46).



Co(II) complex of 4-(benzylamino)-3-chloropent-3-en-2-one (Ac-BA)₂Co(II)

A solution of (Ac-BA) (0.02 mol) in 10 mL DCM was added drop wise with continuous stirring to a 10 mL of filtered ethanolic solution of the Co(CH₃COO)₂·4H₂O (0.01 mol) and refluxed for 6 h. The complex was obtained by filtration and evaporated slowly at room temperature. The synthesis of coordination compound had good yield. Molecular formula: [C₂₈H₃₄Cl₂CoN₂O₆]; Light pink solid; Yield: 55 %; m.p.: decomposed above 280°C; Molecular weight: 624.42 g/mol; IR (cm⁻¹): 3405 (NH), 1605 (C=O conjugated), 1560 (C=C stretching), 440 (Co-N stretching), 545 (Co-O stretching); Anal.Calc., (%) for C₂₈H₃₄Cl₂CoN₂O₆: C, 53.86; H, 5.49; N, 4.49; Found: C, 53.71; H, 4.75; N, 3.57; (%) Co for [ML₂(CH₃COO)₂]: theoretical/experimental (9.44/8.05).



Thermal study (TGA/DTA)

The metal complexes were exposed to TGA to test their stability and decomposition pattern on heating. Thermal studies were done by using SDT-Q 600 V20.9 Build 20 in an inert atmosphere containing nitrogen gas from ambient to 800°C.

Biological evaluation

Acetylcholinesterase assay

Anti-AChE activity was done according to the Ellman method with some changes [17]. 100 μL of reaction mixture contained 60 μL - 50 mM phosphate buffer, pH 7.7, 10 μL sample (0.5 mM per well) was added to it, followed by the addition of 10 μL (0.005 unit per well) electric eel enzyme (Sigma Inc.). The reaction mixture were pre-read at 405 nm using Synergy HT, BioTek, USA. Then contents were pre-incubated for 10 min at 37°C and reaction was started by the addition of 10 μL of 0.5 mM per well

acetylthiocholine iodide substrate, followed by the adding 10 μL 0.5 mM per well DTNB. Absorbance was measured at 37°C, after 15 min of incubation. All experiments were performed in triplicate with their respective controls. For positive control Eserine was used. The % inhibition was calculated as mentioned below for urease assay.

Urease assay

In vitro urease inhibition activity was measured by the phenol hypochlorite method [18]. Reaction mixture of 85 μL contained 10 μL of phosphate buffer, pH 7.0 in each well in the 96-well plate followed by the addition of 10 μL of test solution (0.25 mM) and 25 μL of 0.015 units of jack beans urease (Sigma Inc.). At 37°C, the contents were pre-incubated for 10 min and 40 μL of 20 mM urea solution was added to each well of plate and incubation continued for further 10 min and pre-read at 625 nm using Synergy HT BioTek, USA reader. Phenol hypochlorite reagent (115 μL) was added in each well (freshly prepared by mixing 45 μL phenol reagent with 70 μL of alkali reagent) and incubation is continued for another 10 min. Absorbance (after-read) was read. The percentage enzyme inhibition was calculated by the following formula:

$$\text{Inhibition (\%)} = 100 - \left[\frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \right]$$

IC_{50} values (concentration at which 50 % enzyme catalyzed reaction occurs) of active compounds were found by measuring activities at lower concentrations (0.5, 0.25, 0.125, 0.06, 0.03 mM) of test compounds and data was computed by using EZ-Fit Enzyme software, Perrella Scientific Inc. Amherst USA.

Molecular Docking studies

The synthesized series of ligands and their metal complexes were docked inside acetylcholinesterase enzymes to determine their putative binding mode. Auto Dock v4.2 and MGL Tools v1.5.6 [19] was used to carry out docking as previously reported [20]. The protein structures of acetylcholinesterase i.e. PDB ID 4BDT was downloaded from RCSB protein data bank. Preliminary steps of protein preparation such as removal of co-crystallized solvents; addition of hydrogen atoms and assigning appropriate charges to the residues was performed. The ligand molecules and their respective metal complexes were sketched and 3D optimized using Marvin Sketch.

The docking protocol was first reassured by redocking the reference ligand huprine inside acetylcholinesterase. After successfully reproducing similar binding pose for huprine, docking of ligands and their complexes was performed. Lamarckian genetic algorithm was used to generate different poses of the test ligands and complexes. The top ranking poses with respect to their binding affinity were then selected and carefully visualized in Discovery Studio Visualizer v4.0 and the binding pose was then selected [21].

Results and discussions

The analytical data of enaminone ligands and complexes are given in the previous section. Ligands are soluble in ethanol, methanol, dichloromethane and chloroform. Complexes are soluble in DMSO and DMF. Molar conductance of the complexes (0.001 M) was determined in DMSO. The conductance values (1-20 $\text{ohm}^{-1} \text{cm}^2 \text{mol}^{-1}$) indicate that the complexes were non-electrolyte in nature [22]. The C, H, and N analysis of enaminone ligands and their metal complexes were found

in good agreement with the expected values. λ_{max} (nm) of the complexes were determined by UV-Visible spectroscopy.

FT-IR spectra

In the infrared spectra of the ligands (Ac-PCA and Ac-BA), band at (3125 cm^{-1} and 3132 cm^{-1}) respectively is due to $\nu(\text{N-H})$ vibration. There are two strong bands at (1655 cm^{-1} , 1593 cm^{-1}) and (1548 cm^{-1} , 1561 cm^{-1}) are assignable to $\nu(\text{C=O}, \text{conjugated})$ and $\nu(\text{C=C})$, respectively. The IR spectra of the synthesized complexes exhibited that ligands (Ac-PCA and Ac-BA) coordinate in the neutral bidentate mode. Bands at 1605 cm^{-1} to 1645 cm^{-1} assigned to carbonyl group and that at 1541 cm^{-1} to 1570 cm^{-1} assigned to C=C . The presence of $\nu(\text{NH})$ band at 3405-3450 cm^{-1} in the spectra of all the complexes suggested that the hydrogen atom is not removed and the (NH) group is involved in the coordination [23]. Moreover, new bands in the spectra of all metal complexes appeared in the low frequency regions at 443-463 cm^{-1} and 525-560 cm^{-1} are assignable to $\nu(\text{M-N})$ and $\nu(\text{M-O})$ bonds, respectively [24].

^1H NMR spectra

$^1\text{H-NMR}$ spectrum of synthesized compounds were recorded. The indication for connecting mode of ligand was also provided by the $^1\text{H-NMR}$ spectrum of ligand along with the complex of Cd(II). $^1\text{H-NMR}$ of ligand (Ac-PCA) revealed the presence of a single proton at 12.71 ppm due to the enamine N-H group [25]. Aromatic protons were found as two doublets of triplet at 7.35 ppm and 7.03 ppm. Moreover, methyl protons also show two singlets at 2.38 ppm and 2.18 ppm. These chemical shift values corresponded well with the proposed structure of the ligand. $^1\text{H NMR}$ of ligand (Ac-BA) showed the presence of a single proton at 11.68 ppm due to the enamine N-H group. Aromatic protons merged with each other and emerged as multiplet in the range 7.40-7.25 ppm. The $-\text{CH}_2$ group exhibits a doublet at 4.52 ppm. Moreover, methyl protons also show two singlets at 2.32 ppm and 2.17 ppm. These chemical shift values corresponded well with the suggested structure of the ligand.

In the complex of Cd(II), the slight shifting of chemical shift values of all protons confirms the formation of metal-ligand bonds. In addition, the presence of NH signal in the spectrum of complex indicated that N forms coordinate covalent bond with metal ion while its hydrogen remains intact. So enaminone ligand (Ac-PCA) acts as a bidentate ligand by bonding through nitrogen and oxygen [11]. These observations are in consistent with the interpretation of IR spectra.

^{13}C NMR Spectra

The number of signals in ^{13}C NMR spectrum support well with the proposed structure of ligands along with the number of carbon atoms which are chemically different. ^{13}C NMR of ligand (Ac-PCA) showed peaks at 194.97, 157.74, 137.05, 131.73, 129.37, 126.51, 104.93, 28.67, 18.15 ppm. Similarly, ^{13}C NMR of ligand (Ac-BA) showed peaks at 193.60, 161.19, 137.50, 128.93, 127.66, 126.77, 102.61, 47.69, 28.66, 16.68 ppm. These signals are in consistent with the proposed structures.

Mass spectrum

FAB-MS of ligands were recorded in which Ac-PCA showed peak at 244.1 as $[\text{M}+\text{H}]^+$ while in the spectrum of Ac-BA signal was observed at 224.1 as $[\text{M}+\text{H}]^+$.

Thermogravimetry (TGA)

TGA of metal complexes were done to check their stabilities and decomposition pattern on heating. TGA data of the complexes are given in (table 1). The correlations between the weight losses and decomposition steps of the complexes are discussed in terms of the suggested formulae of the complexes.

The (Ac-PCA)Cu(II) complex with molecular formula $[C_{11}H_{11}Cl_4CuNO]$ displayed three stages of mass loss between (180–550°C). The first stage is at 180–220°C and showed a mass loss of 19 %, resulted in the loss of two chlorides (calc., 18.7 %). The second step occurs at 220–280°C, with a mass loss of 60 %, leading to the loss of $C_{11}H_{11}Cl_2N$ (calc., 60.23 %). The last stage of decomposition takes place between 280–550 °C, with a mass loss of 21 %, leading to the loss of CuO (calc., 21.08 %).

The (Ac-PCA)₂Cd(II) complex with molecular formula $[C_{22}H_{22}Cl_6CdN_2O_2]$ exhibited three steps of mass loss between 90–530°C. The first step occurs at 90–120°C, with a mass loss of 5%, leading to the loss of one chloride (calc., 5.28 %). The second step of decomposition occurs at 120–250°C and is due to the loss of five chlorides with a mass loss 26.0 % (calc., 26.40 %). The third step of decomposition takes place between 250–530°C, with a mass loss of 69 %, leading to the loss of $C_{22}H_{22}CdN_2O_2$ (calc., 68.20 %).

The (Ac-BA)Cu(II) complex with molecular formula $[C_{16}H_{16}ClCuNO_5]$ displayed four steps of mass loss between 50–550°C. The first step is between 50–190°C, and is due to a mass loss of 27 %, leading to the loss of two acetates (calc., 28.8 %). The second step takes place at 190–275°C, with a mass loss of 29 %, leading to the loss of C_5H_7ClN (calc., 28.6 %). The third step of decomposition takes place at 275–345°C, with a mass loss of 23 %, leading to the loss of C_7H_7 (calc., 22.4 %). The fourth step of decomposition take place between 345–550°C, with a mass loss of 20 %, leading to the loss of CuO (calc., 19.6 %).

The (Ac-BA)₂Co(II) complex with molecular formula $[C_{28}H_{34}Cl_2CoN_2O_5]$ displayed four steps of mass loss between 180–450°C. The first step is at 50–170°C, and showed a mass loss of 18 %, leading to the loss of two acetates (calc., 18.9 %). The second step takes place at 170–260°C, with a mass loss of 12 %, leading to the loss of two chlorides (calc., 11.3 %). The third step of decomposition takes place between 260–325°C, with a

mass loss of 31 %, leading to the loss of $C_{12}H_{14}NO$ (calc., 30.1 %). The fourth step of decomposition take place between the temperature range 325–450°C, with a mass loss of 39 %, leading to the loss of $C_{12}H_{14}CoNO$ (calc., 39.5 %). The decomposition patterns of the newly synthesized complexes are in consistent with their suggested structures.

UV-Vis data and Magnetic moment

The electronic absorption spectrum of the complexes in DMSO was recorded in UV-visible region. It was noted that all complexes show characteristic metal-ligand charge transfer absorption bands between 265–385 nm, which are due to metal ($d\pi-M$) to ligand ($d\pi^*$) charge transfer [26]. The electronic spectrum of (Ac-PCA)Cu(II) and (Ac-BA)Cu(II) complexes exhibited absorption bands at 745 nm and 730 nm respectively that can be due to the ${}^2T_2 \rightarrow {}^2E$ transition of a tetrahedral geometry. (Ac-PCA)Cu(II) and (Ac-BA)Cu(II) complexes showed magnetic moment value (2.15 B.M.) and (2.10 B.M.) respectively, that also support 4-coordinate tetrahedral geometry [27]. The (Ac-BA)₂Co(II) complex showed a band of moderate intensity at 550 nm which is allocated to the transition ${}^4T_1(F) \rightarrow {}^4T_1(P)$ (i_3). The magnetic moment value of (Ac-BA)₂Co(II) complex was found to be (4.19 B.M.) which also reveals that it has typically octahedral geometry [26]. The (Ac-PCA)₂Cd(II) complex did not show any $d-d$ transition and its spectrum showed only charge transfer band. As expected it is diamagnetic; the complex is suggested to be octahedral geometry based on analytical, IR and conductance data [28, 29].

Biological activity

Anti-AChE activity

Compound (Ac-BA)Cu(II) exhibited significant AChE inhibition of about 80 % at 0.5 mM with IC_{50} value of $22.43 \pm 0.12 \mu M$ as compared to the less active compound (Ac-PCA)Cu(II) which had IC_{50} value of $186.82 \pm 0.14 \mu M$. Ligands and Co(II) or Cd(II) complexes did not show enzyme inhibition. There seems an involvement of Cu(II) complex in the inhibition of the enzyme (table 2).

Anti-urease activity

Table 1
THERMOGRAVIMETRIC ANALYSIS DATA FOR THE COMPLEXES

Sample code	Temperature range (°C)	(%) Weight loss calc./found	Assignments
(Ac-PCA)Cu(II)	180-220	18.70(19)	Two chlorides
	220-280	60.23 (60)	$C_{11}H_{11}Cl_2N$
	280-550	21.08(21)	CuO
(Ac-PCA) ₂ Cd(II)	90-120	5.28(5)	Cl
	120-250	26.40(26)	Five chlorides
	250-530	68.20(69)	$C_{22}H_{22}CdN_2O_2$
(Ac-BA)Cu(II)	50-190	28.8(27)	Two acetate groups
	190-275	28.6(29)	Partial decomposition of the complex (C_5H_7ClN)
	275-345	22.4(23)	Complete decomposition of the complex (C_7H_7)
	345-550	19.6(20)	CuO
(Ac-BA) ₂ Co	50-170	18.9(18)	Two acetate groups
	170-260	11.3(12)	Two chlorides
	260-325	30.1(31)	Partial decomposition of the complex ($C_{12}H_{14}NO$)
	325-450	39.5(39)	Complete decomposition of the complex ($C_{12}H_{14}CoNO$)

Table 2
ACHE AND UREASE INHIBITION STUDIES OF LIGANDS AND THEIR COMPLEXES (mean \pm s.e.m., n = 3)

Sr. No.	Compound	AChE		Urease	
		Inhibition (%) at 0.5 mM	IC ₅₀ (μ M)	Inhibition (%) at 0.25 mM	IC ₅₀ (μ M)
1.	Ac-PCA	3.96 \pm 0.15	-	41.27 \pm 0.16	-
2.	(Ac-PCA) ₂ Cd(II)	8.35 \pm 0.13	-	49.25 \pm 0.18	-
3.	(Ac-PCA)Cu(II)	81.64 \pm 0.18	186.82 \pm 0.14	65.32 \pm 0.19	133.81 \pm 0.16
4.	Ac-BA	24.25 \pm 0.13	-	58.71 \pm 0.18	172.46 \pm 0.15
5.	(Ac-BA)Cu(II)	79.87 \pm 0.19	22.43 \pm 0.12	63.95 \pm 0.19	152.71 \pm 0.16
6.	(Ac-BA) ₂ Co(II)	31.43 \pm 0.23	-	91.24 \pm 0.17	26.54 \pm 0.13
7.	Thiourea	-	-	98.21 \pm 0.18	21.25 \pm 0.15
8.	Eserine	91.27 \pm 1.17	0.04 \pm 0.0001	-	-

Table 3
BINDING FREE ENERGY OF LIGANDS AND METAL COMPLEXES INSIDE ACHE ENZYME

Sr. No.	Code	AChE
		Binding Free Energy (Kcal mol ⁻¹)
1.	Ac-PCA	-6.55
2.	(Ac-PCA) ₂ Cd(II)	+3.82
3.	(Ac-PCA)Cu(II)	-7.03
4.	Ac-BA	-7.24
5.	(Ac-BA)Cu(II)	-8.52
6.	(Ac-BA) ₂ Co(II)	+9.46

complex several non-bonding interactions were found with residues of esteric site of binding pocket. The metal complex does not form any significant interaction with any residue. However, it may be responsible for maintaining the pose in its lowest energy state inside the active pocket and thus yielding highest binding affinity. (Fig. 1) shows the plausible binding poses of 3(Ac-PCA)Cu(II) (a) and 3(Ac-BA)Cu(II) complex (b).

Conclusions

The current studies provide a simple protocol for the manufacture of enaminones and their metal complexes. The synthesized compounds were investigated for anti-ureas as well as anti-AChE activity. The complex (Ac-

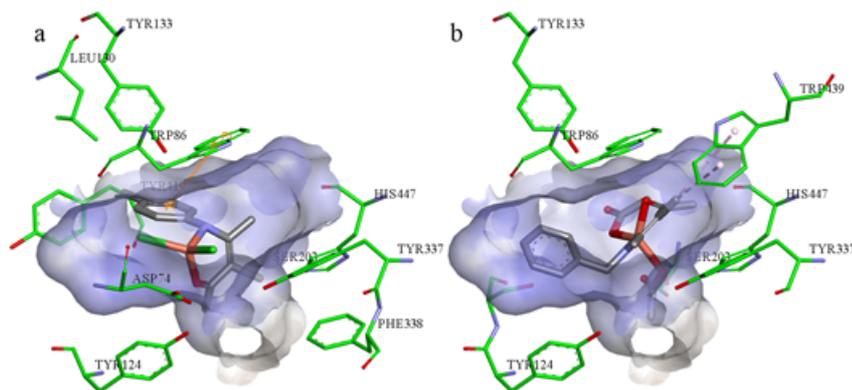


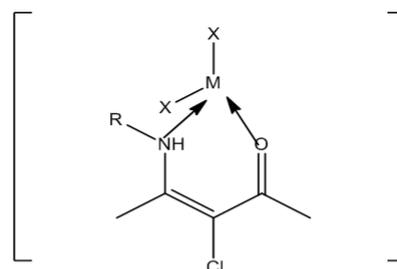
Fig. 1. Putative Binding Pose of 3(Ac-PCA)Cu(II) (a) and 3(Ac-BA)Cu(II) (b); Metal complex inside Human AChE Binding Pocket

When the same compounds were screened for urease inhibition (table 2), the (Ac-BA)₂Co(II)

complex was found a potent inhibitor with IC₅₀ value of 26.54 \pm 0.13 μ M as compared to that of thiourea. Cu(II) and Cd(II) complexes did not show significant urease enzyme inhibition. Urease enzyme contains two Ni atoms as a part of the active site and there seems more interaction of Co(II) with Ni atoms than Cd(II) or Cu(II) ions.

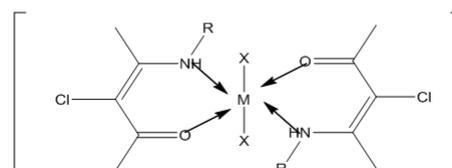
Molecular docking studies

Molecular docking was carried out with an aim to determine the most plausible binding poses of the complexes inside the active pocket of acetylcholinesterase. The Cu(II) complexes of ligands AC-PCA and AC-BA were found to have higher binding affinity as compared to rest of the synthesized complexes (table 3). The highest binding affinity of -8.52 Kcal mol⁻¹ was observed for (Ac-BA)Cu(II) complex while in case of (Ac-PCA)Cu(II) complex, the observed binding affinity was -7.03 Kcal mol⁻¹. Cu(II) complexes of Ac-BA and Ac-PCA were found to reside inside the esteric and anionic binding site of the active site. Chlorophenyl ring of (Ac-PCA)Cu(II) complex forms π - π stacking -interaction with residue Trp-86 of the anionic binding pocket while in case of (Ac-BA)Cu(II)



Where M= Cu(II)
X=Cl/CH₃COO
R=-Ph-Cl/Ph-CH₂

Fig. 2. Proposed structure of 4-coordinated enaminone complexes



Where M= Co(II)/Cd(II)
X=Cl/CH₃COO
R=-Ph-Cl/Ph-CH₂

Fig. 3. Proposed structure of 6-coordinated enaminone complexes

BA)Cu(II) exhibited good anti-AChE activity with IC₅₀ value 22.43 ± 0.12 μM and (Ac-BA)₂Co(II) potent anti-urease activities with IC₅₀ value 26.54 ± 0.13 μM. Molecular docking studies showed Cu(II) complexes of ligands AC-PCA and AC-BA were found to have higher binding affinity as compared to rest of the synthesized complexes.

Spectroscopic techniques were well supported to our designed structures (fig. 2 & 3).

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