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Cytotoxicity and Molecular Docking Studies of a Novel Enaminone and its Cadmium (II) Complex

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Authors' Contribution
TM conceived and designed the experiments. RH executed the experiments and wrote the article. SA helped in performing cytotoxic activities. RM and MTI helped in the characterization of data. KI helped in Molecular docking studies.

Key words
Enaminone, Molecular docking, IC_{50} value, Cytotoxicity, Spectroscopy.

ABSTRACT

The anti-cancerous potential of novel Enaminone and its Cadmium complex was estimated by measuring its cytotoxicity for human breast cancer cell line (MCF-7). Both compounds PFA and PFA-Cd were prepared and subsequently characterized by FTIR, ICP-AES, UV-Vis, TGA, ^{1}H NMR, ^{13}C NMR and FAB-MS. The results were in great consistency with the structures of the both compounds. Furthermore, the ligand and its metal complex were screened for their cytotoxicity against human cancer cell lines. It was found that the compound PFA was more active anti-cancerous agent as compared to PFA-Cd. Molecular Docking studies were carried out to found the predominate binding mode of synthesized compound in the vicinity of double helical DNA structure.

INTRODUCTION

Cancer is a general term used for a huge group of diseases characterized by the growth of abnormal cells that can invade adjacent parts of the body and can spread to other organs. In 2015, 8.8 million deaths occurred due to cancer and it is the second leading cause of death worldwide. According to National Center for Health Statistics (based on Mortality data available through 2015) 1, 735,350 new cancer cases and 609,640 cancer deaths are expected to occur in the United States in 2018 (Madani *et al*., 2011; Rebecca *et al*., 2018). It is therefore vital to produce new drugs to counter the uncontrolled proliferation of cancer cells (Gupta *et al*., 2013; Gulzar *et al*., 2018).

Human body required several metals are in trace amount as these metal ions directly effect on the activity of various enzymes. If the quantity of trace metals increases in the body it may causes some toxic effects. One of the toxic effects is that these metals may act as pro-carcinogene. These metal ions become activated in the body and bind to the electron rich sites in DNA, which results in mutagenesis and finally causes cancer. Chelating agents can be used to remove controlled amount of undesirable metal ions from the body. Moreover, a large number of complexes are well-known to have antitumor activity. It is assumed that they deactivate either the carcinogenic metal or the enzymes essential for the fast growth of both healthy and malignant cells (Tripati *et al*., 2007). Enaminones is a class of compounds having the conjugated system (N-C=C-C=O) (Greenhill, 1997). Enaminone have great potential as reaction intermediates and medicinal compounds due to the presence of nucleophilic and electrophilic sites (Edafiogho *et al*., 1992). A literature survey reveals that enaminones and its derivatives shows a wide range of antimicrobial (Cindric *et al*., 2018), anticonvulsant (Jackson *et al*., 2012), anticancer (Shawali, 2010) and anti-inflammatory activities (Ahmed, 2015). On the other hand, enaminones have been commonly used as intermediates in the preparation of a large number of pharmacologically important compounds (Kalaria *et al*., 2014; El-Azab *et al*., 2016).

In view of these facts, the present work includes the synthesis of a novel enaminone and its Cd complex. The synthesized products were characterized by FTIR, NMR, Mass spectrometry, TGA and elemental analysis and screened for cytotoxicity against human cancer cell lines. The study was extended by the docking studies and the binding mode of best active compound was also predicted.

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MATERIALS AND METHODS

The starting materials and solvents used for the synthesis of ligands and complexes were purchased from market and were used as such.

Synthesis of 3-chloro-4-(florophenyl) amino) pent-3-en-2-one

3-chloro-2,4-pentanedione (0.02 mole L^{-1},2.5 mL), *p*-floroaniline (0.02 mole L^{-1}, 1.89mL), *p*-toluene sulfonic acid (1.0 g) and toluene (150 mL) were heated on heating mantle for12 h. The water produced as a result of condensation in reaction mixture was separated using a Dean–Stark apparatus. The reaction was monitored by TLC. Crystalline product was collected after solvent extraction using chloroform and sodium bicarbonate.

Molecular formula: $C_{11}H_{11}ClFNO$, off white crystalline solid, Yield: 60%, m.p: 59°C, Molecular weight: 227.66 g/mole, IR (cm-[1]): cm^{-1} 3113(NH), 1591 (C=O), 1511 (C=C)), ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 12.67 (s, 1H, -NH), 7.07(d,J=6.3 Hz,4H), 2.37(s, 3H), 2.14(s, 3H).^{13}C NMR (75 MHz, $CDCl_3$) δ: 194.74, 162.56, 159.29, 134.45, 127.39, 116.28, 104.36, 28.67, 18.15, FAB-MS: *m/z* = 228.1[M+H] $^+$, Anal. Calcd. for $C_{11}H_{11}ClFNO$: C, 58.03; H, 4.87; N, 6.15%. Found: C, 56.58; H, 5.92; N, 5.82%.

Synthesis of cadmium (II) complex of 3-chloro-4-(florophenyl) amino) pent-3-en-2-one (PFA-Cd)

A solution of (PFA) (0.02 mole) in 10 mL DCM was added drop wise with continuous stirring to a 10 mL of filtered ethanolic solution of the $CdCl_2$ (0.01 mol) and refluxed for 2 h. The complex was obtained by filtration and evaporated slowly at room temperature. The synthesis of coordination compounds had good yields

Molecular formula: $[C_{22}H_{22}Cl_4CdF_2N_2O_2]$, Light yellow Solid, Yield: 47%, M.p: Decomposed 240-244°C, Molecular weight: 638.64 g/mole, IR (cm-[1]): 3130 (NH), 1581 (C=O conjugated), 1533 (C=C stretching); 450(Cd-N stretching), 527(Cd-O stretching),^1H NMR (300 MHz, $CDCl_3$, δ ppm): 12.64 (s, 1H, -NH), 7.32-7.21 (m, 4H, - Ar-H), 2.26(s, 3H), 2.12(s, 3H).Anal. Calcd. for $[C_{22}H_{22}Cl_4CdF_2N_2O_2]$,: C, 41.37; H, 3.47; N, 4.39%. Found: C, 40.20; H, 2.75; N, 3.95%. % metal for $[ML_2Cl_2]$: Theoretical/experimental (17.60/16.56).

Elecro-analytical characterization

The IR spectrum was recorded on Agilent Technologies FT-IR instrument (in range of 4000-400 cm^{-1}).The ^1H-NMR spectrum was recorded on a Bruker Ascend NMR spectrometer. The (FAB) mass spectrum was recorded on a JEOL JMS-600H mass Spectrometer. Conductivity were measured with the inoLab Cond 720 conductometer using DMSO. Cytotoxicity were carried out and their IC_{50} values were compared by using standard drug.

Cultivation of cell lines

Cell lines were obtained from laboratory culture established in The University of Lahore. Cells were plated in the culturing flask in High glucose Dulbacco Modified Eagle medium (DMEM-HG) (GIBCO, USA) including 10 % serum, 100 U of penicillin and 100µg/ml streptomycin. When cell lines (MCF7) achieved 70-80% confluence, these were trypsinized by using 0.5ml of 0.05% trypsin and 0.53 EDTA (Thermo Scientific, USA). Medium of the cultured cells was changed after every three days. MCF-7 cell lines were cultured in 96-well plates (~2x104 per well) for cell proliferation assay. The treatments were given as 0.0 µg/ml, 5 µg/ml, 10 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml and 500 µg/ml of the PFA, PFA-Cd and Cis-platin in DMEM for 24 h.

In vitro growth inhibition test

Cytotoxic activity of the enaminone and its cadmium complex was determined by using 96 well level bottomed micro plates using the standard MTT colorimetric protocol (Mossman, 1983). Cell culture with the convergence of 6×104 cells/mL was arranged and presented (100 µL/well) into 96-well plates. Medium was removed after the incubation of one night, and 200 micro liter of new medium was incorporated with the different concentrations (0-500 µg/ml) of sample. After 24 h, 25µL MTT (5 mg/mL) was added to each well and further incubated for next 4 h. Accordingly, 100 µL of dimethyl sulphoxide was introduced into each well. The degree of MTT diminishment to formazan inside cells was computed by absorbance measurement at 570 nm, utilizing a smaller scale plate peruser (Spectra Max in addition, Molecular Devices, CA, USA). Cytotoxic results were obtained as their concentration responsible for 50% growth retardation (IC_{50}). The % inhibition was computed by using the accompanying equation:

$$\% \text{ Cell viability} = \frac{\text{Experimental (OD570)}}{\text{Control (OD570)}} \times 100$$

Molecular docking studies

To govern the predominate binding mode of synthesized compound in the vicinity of double helical DNA structure Molecular Docking studies was executed using Auto Dock v4.2 and Auto Dock tools v1.5.6 (Morris *et al.*, 1998). The best possible conformation and the interaction of the most probable conformation were

investigated. Crystal structure of DNA was taken from RCSB Protein Data Bank with PDB ID1BNA. Structures of both compounds were drawn and 3D optimized using Marvin sketch. Prior to docking DNA structure was prepared by adding hydrogen and a grid box of 80×80×120 dimensions was created. Additionally, Lamarckian genetic algorithm which is modified for flexible ligand-receptor docking was used and it consents to grip greater degrees of freedom. A total of 100 different confirmations were secured the utmost pose was then subjected to visualization for analysis of interactions between the DNA and the synthesized compound.

Table I.- Cytotoxicity of the new compounds against MCF-7 cell line.

Compound	Cell viability at concentrations (µg/ml)								
	0	**5**	**10**	**25**	**50**	**100**	**200**	**500**	**IC_{50}**
PFA	100	67	66	64	63	57	50	39	200
PFA-cd	100	191	104	76.3	69.7	65.3	57.3	44.0	334.5
Cis-platin	100	89	68	40	11	5.8	4.3	2.1	20

RESULTS AND DISCUSSION

The newly prepared enaminone and its cadmium complex were characterized by NMR, IR and elemental analysis. The molecular mass of enaminone was confirmed by MS (FAB). PFA showed peak at 228.1 as $[M+H]^+$. ^{13}C NMR of enaminone PFA showed peaks at: 194.74, 162.56, 159.29, 134.45, 127.39, 116.28, 104.36, 28.67, 18.15. These signals are in consistent with the proposed structure of enaminone PFA. Both compounds showed very low conductance values indicating their non-electrolyte nature.

FTIR

In the infrared spectra of the enaminone (PFA), band at 3113 cm^{-1} was allocated to NH stretching vibration. Two strong bands were observed at 1591 cm^{-1} and 1557 cm^{-1} that are assignable to ν (C=O, conjugated) and ν (C=C), respectively. The presence of ν (NH) band at 3130 cm–1 in the spectra of the PFA-Cd suggested that the hydrogen atom is not removed and the (NH) group is involved in the coordination (Souad *et al*., 2014). The (C=O) stretching vibration of conjugated carbonyl group of free ligand shifted to a Lower side 1581 cm_1 in metal complex, indicating the coordination of the ligand through the oxygen of Carbonyl group (Tyagi *et al*.,2015). Moreover new bands in the spectra of PFA-Cd appeared below 600 cm–1 assignable to (M-N) and (M-O) bonds (Kavitha *et al*., 2017).

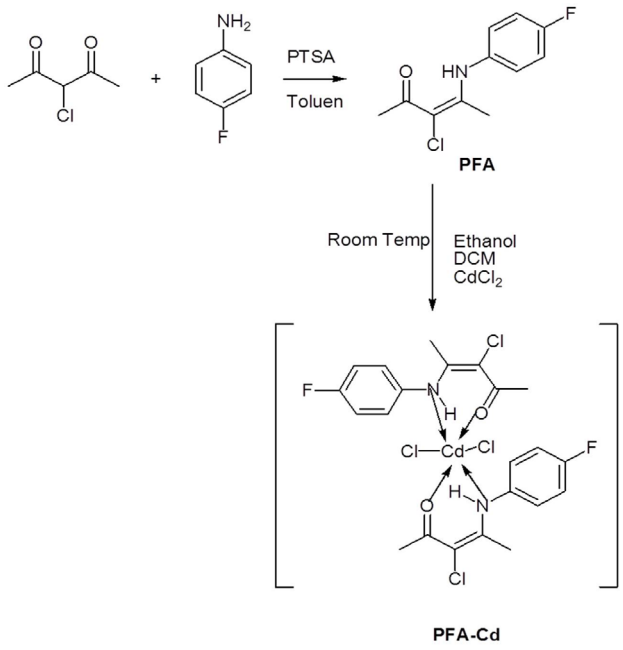

Fig. 1. Scheme for the synthesis of the Enaminone PFA and its complex PFA-Cd.

Proton NMR

^1H-NMR spectrum of synthesized compounds was recorded. ^1H-NMR of ligand (PFA) revealed the presence of a single proton at 12.66 ppm due to the enamine N–H group (Shi *et al*., 2005). Aromatic protons were found as a doublets at 7.07 (d,J=6.3). Moreover methyl protons also show two singlets at 2.36 and 2.14 ppm. In metal complex, the slight shifting of chemical shift values of all protons confirms the formation of metal ligands bonds. In addition the presence of NH signals in the spectra of complexes indicated that N forms coordinate covalent bond with metal ions while its hydrogen remains intact. So enaminone ligand acts as a bidentate ligand by bonding through Nitrogen and oxygen (Jeragh and Abdel-Zaher, 2015). These observations are in consistent with the interpretation of IR spectra.

In vitro *growth inhibition test*

The cytotoxic effects of compounds PFA and PFA-Cd against human breast cell line (MCF-7) was evaluated using Cis-platin as a standard sample with an IC_{50} of 20µg/ml. The analysis of the data obtained indicated that the values of IC50 for PFA and PFA-Cd compounds against MCF-7 cell line are 200 µg/ml (Fig. 2A) and 334.6 µg/ml (Fig. 2B), respectively. The values of IC50 indicated that the tested compounds have cytotoxic activities at higher concentrations (200µg/ml and 334µg/ml) as compared to the reference drug (20µg/ml).

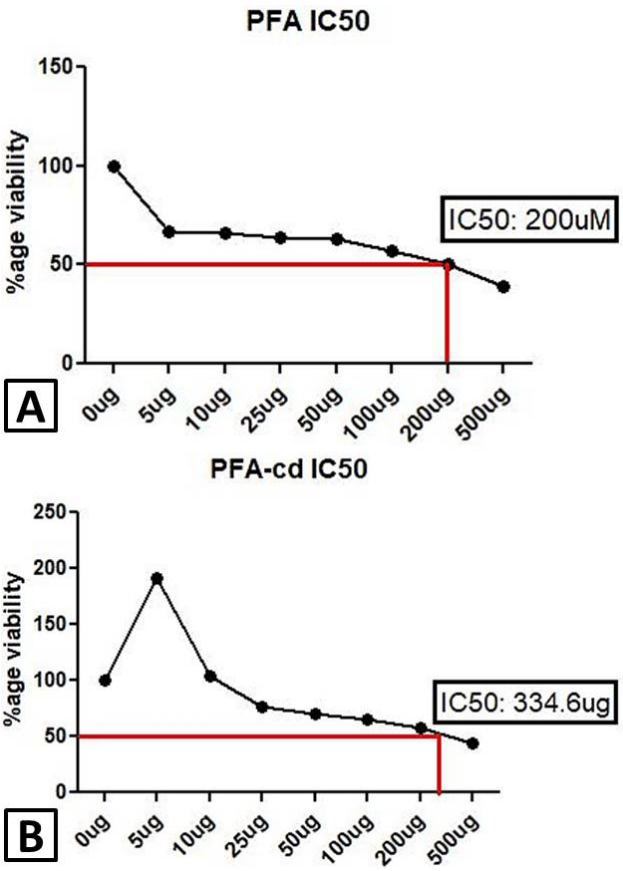

Fig. 2. Effect of concentration of PFA (A) PFA-Cd (B) and on MCF-7 line.

Table II.- Binding free energy of compounds.

S. No	Compound	Binding free energy (Kcal mol^{-1})
1.	Standard drug Cis-platin	-4.17
2.	PFA	-6.03
3.	PFA-Cd	-5.86

Molecular docking simulations

Enaminone PFA and its cadmium complex (PFA-Cd) has been synthesized and evaluated for cytotoxicity. Standard drug Cis-platin was found most effective. It basically interferes with DNA replication. Generally anticancer agents intermingle with the DNA by four bonding modes such as interaction with minor groove or major groove, surface binding and intercalation. There are different targets for anticancer activity so this era was of great interest for medicinal chemists.

To evaluate anticancer potential of both compounds, molecular docking studies was performed. Both compounds were found in major and minor grove. No surface binding and intercalation was observed. Least binding energy values are given in Table II. The Compound PFA was found to have the highest binding affinity with a value of -6.03 Kcal mol^{-1}as compared with the complex of cadmium as well as with reference Cis-platin.

Visualization of this compound using Discovery Studio Visualizer v4.0 cleared that it binds to the minor groove of DNA helix. Several interactions were observed with the DNA such as hydrogen acceptor interaction with DA20 nucleotide and hydrogen donor with DA18 nucleotide. Moreover electrostatic interactions were also observed between ligand PFA and nucleotide of DNA such as DC9. Molecular docking also supports the IC_{50} values obtained from anticancer activity performed that PFA binds with highest affinity to DNA double helix. Carefully selected view of PFA inside DNA helix is shown in Figure 3.

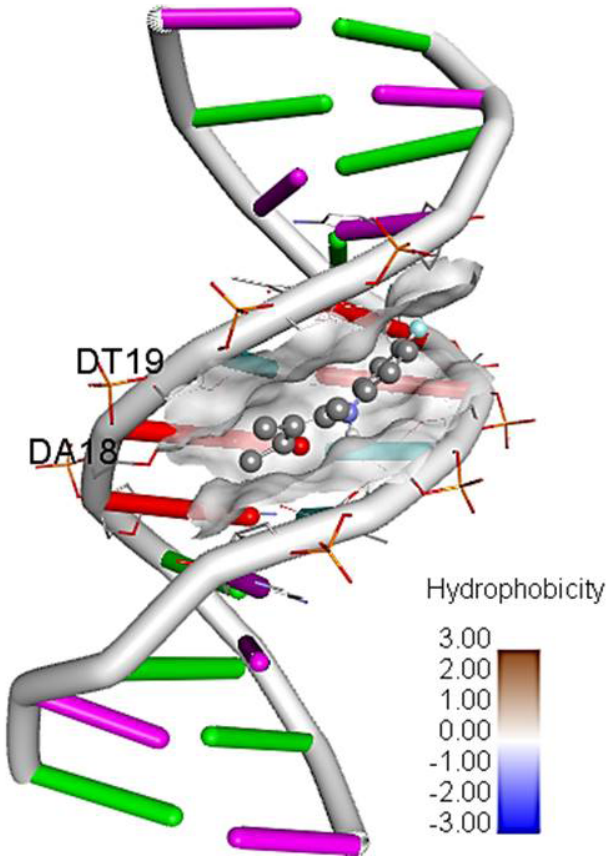

Fig. 3. Selected binding pose of PFA with DNA double helix.

CONCLUSION

Two novel compounds were synthesized, characterized electro-analytically and evaluated for their cytotoxic

activities. All the spectroscopic results confirmed the formation of the targeted compounds. From the cytotoxic results of the synthesized compounds, it is concluded that the compound PFA having electron withdrawing 'Group at its para position, was proved to be moderate cytotoxic agent as compared to the cadmium complex PFA-Cd. Molecular Docking studies showed that PFA binds with highest affinity to DNA double helix.

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Statement of conflict of interest

The authors declare no conflict of interest.

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