Indian Journal of Chemistry
Vol. 63, October 2024, pp. 1044-1052
DOI: 10.56042/ijc.v63i10.12807

Spectral, anticancer and molecular docking studies of paddle-wheel complex tetrakis(μ-acetato) bis(2-pyridone)dicopper(II) against MCF-7 cell line

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Received 28 July 2024; accepted (revised) 23 September 2024

Tetrakis(μ-acetato)bis(2-pyridone)dicopper(II) (Cu_2TAP) has been obtained unexpectedly during refluxing of HPMP, 2-methyl pyridine and $CuCl_2$ in ethanol in 1:1:1mole ratio. The metal complex has been characterized by FT-IR, Raman, UV-Vis and EPR spectroscopy. The complex shows characteristic NH and CO stretching in IR and Raman. UV-Vis shows bands at 293 and 242 nm due to $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ transitions. The compound emits strong fluorescence bands at 726 and 765 nm when excited at 360 and 380 nm respectively. EPR indicates a strong anti-ferro magnetic interaction between the two Cu (II) centers as bridged by the acetate ligands. The cytotoxicity activity has been performed by the MTT assay against MCF-7 breast cancer cells. The binding modes of Cu_2TAPin the active pocket of the target protein Human estrogen receptor alpha (PDB ID: HERT) has been found to be the H-bonding and weak metal-protein interaction. The complex-HERT interaction energy is –8.40 kcal/mol compared to its interaction with doxorubicin (–7.90kcal/mol). Thus Cu_2TAP can be considered as an anticancer agent and may have OLED applications.

Keywords: Tetrakis(μ-acetato)bis(2-pyridone)dicopper(II) (Cu_2TAP), Spectral studies, Anticancer activity, Molecular docking

The syntheses of binuclear Schiff base metal complexes are increasing, due to their applications in areas such as catalysis, enzymatic reaction, magnetism, EPR spectral and bio-inorganic modeling studies. The Schiff base metal complexes showed promising biological activities[1-6]. The drugs showed increasing activity when administrated as metal complexes rather than organic compounds. The first copper complex reported to show efficient DNA cleavage activity is bis(1,10-phenanthroline) copper(I) and then the chemistry of metal complexes of 1,10-phenanthroline or modified ligands were developed as therapeutic agents[7]. Coordination compounds combining Schiff base ligands are designed and synthesized extensively since the metal-ligand interaction controls and enhances the biological, chemical, and electrochemical activities of the metal centers[8]. Schiff base complexes of copper, nickel, cobalt, and zinc are of significant importance in the field of coordination chemistry. Copper and Cobalt complexes can mimic the active sites of metallo enzymes, playing a crucial role in bioinorganic chemistry[9]. Copper is a biologically relevant element and many enzymes that depend on copper for their activity have been identified. Binuclear copper (II) complexes have shown a greater cleaving efficiency or DNA interaction than the mononuclear complexes[10,11].

In general copper complex have biological activities[12]. This present article investigated the anticancer activity of the paddle–wheel binuclear Cu(II) complex. In molecular docking, bioinformatics plays a key role in creating useful information from raw biological data. Drug discovery is a complex and costly effort, where few drugs that reach the clinical testing phase make it to market. High throughput screening

(HTS) is the primary method used by the pharmaceutical industry to identify initial lead compounds. These shortcomings of HTS have encouraged the development of alternative methods to drive the drug discovery process but it is also time and resource consuming. The computational approach consumes less time to screen the drugs on the basis of biological structures[13]. The molecular docking study is considered a reliable tool to identify the active sites of the target receptors and to analyze binding interactions of compounds with the proteins along with the prediction of drug-like characteristics of the molecules[14].

A docking method must have the ability to predict the useful binding strength between the ligand and the receptor complex[15]. The hyperproduction of estrogen is one of the leading causes for the development of breast cancer. Naturally human population contains ER-α and ER-β estrogen receptors (ER)-φ,where ER-α is mainly expressed in the mammary gland and uterus. Estrogen receptor alpha (HERT) is a transcription factor that regulates the gene expression events that culminate in cell division and contributes to its critical role in mammary gland development. The hyperactivity of estrogen hormone may potentially lead to the multiplication of the ER-α in the mammalian cells which lead to the maintenance and growth of types of breast cancers[16], ER-α promotes breast cancer initiation and proliferation as well as oncogenic protein expression[17] and also holds various molecular targets for the investigation of cancer drug. Therefore, this study aims to determine the interaction of tetrakis(μ-acetato)bis(2-pyridone) dicopper(II) (Cu$_2$TAP) with human estrogen receptor alpha (3ERT) as an anticancer drug and compared with the standard drug Doxorubicin.

Results and Discussion

UV-Vis Spectrum

The peaks at 293.5 and 224.2 nm are due to $n\rightarrow\pi^*$ and $\pi\rightarrow\pi^*$ transitions of 2-pyridone ring[18]. A weak peak at 1050 nm is due to the d-d transition arising from the five coordinated Cu^{2+} centre, with distorted square pyramid geometry. The band at 620 nm is usually observed in square planar mononuclear Cu (II) complexes. Five coordinate structures and antiferromagnetic interaction are responsible for observing the band at 1050 nm in the crystal as well as in the ethanolic solution. The complex is stable in ethanolic solution having the same structure as shown in the crystalline state (Fig. 1).

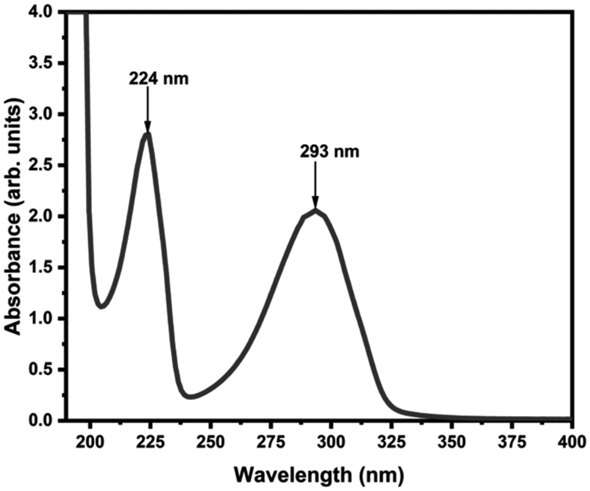


Fig. 1 — UV-Vis spectrum of Cu$_2$TAP

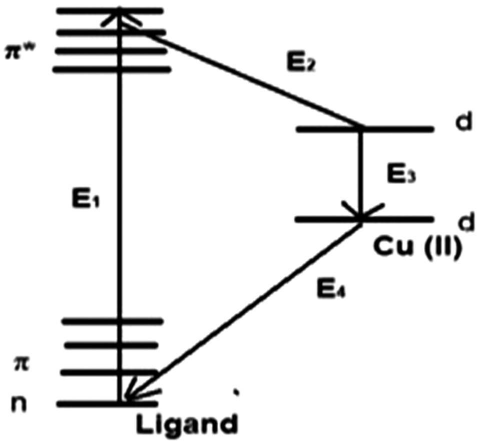


Fig. 2 — Flu emission mechanism of Cu$_2$TAP

Fluorescence Spectrum

When excited at 360 nm, the compound showed a strong emission at 726 nm. When excited at 380 nm, the compound showed a strong emission at 765 nm. The crystals can be used for suitable optoelectronic applications. The complex absorbs in the UV region through 2-pyridone moiety relaxes vibrationally and transfers into d-orbitals like charge transfer transition and relaxes *via* d-orbitals. The intra ligand charge transfer transition from the excited state of the ligand has been relayed into the higher energy d-orbitals of copper. This deexcites to the ground state thereby giving a strong emission in the visible region and showing a higher wavelength (Fig. 2 and Fig. 3) as $E_4 << E_1$.

IR Spectrum

The peaks at 3450 cm^{-1} is due to ν(N-H)[18]. The peaks at 3129, 3018 cm^{-1} are due to ν(C-H) of CH$_3$ and C-H group of 2-pyridone. The peaks at 1654.8

and 1605 cm^{-1} are due to ν(C=O) pyridone and ν(COO) of CH_3COO^- ion. The other peaks are due to 2-pyridone ring and $-CH_3$ groups[20].

Raman Spectrum

The peak at 3350 cm^{-1} are due to ν(N-H)[18]. The peaks at 3233 and 3040 cm^{-1} are due to ν(C-H) of 2-pyridone ring and CH_3 of CH_3COO^- group. The peaks at 1690 cm^{-1} and 1605 cm^{-1} are due to ν(C=O) of 2-pyridone and acetate ion[19]. The infrared and Raman spectra of the complex was shown in Fig. 4.

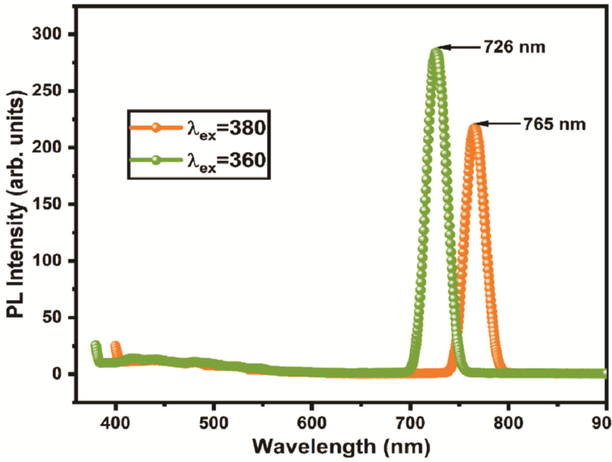


Fig. 3 — Fluorescence spectra of Cu_2TAP

EPR Spectrum

The X-band EPR spectrum of the crystal is shown in Fig. 5. The spectrum is typical of isolated dinuclear units. The spectrum contains a classical triplet state (S=1) for dinucler copper(II)[19-22]. A usual conformation of the Cu^{2+}centre has been observed with distorted square pyramid geometry. The parallel and perpendicular values are found at 2.2115 and 2.089 showing that $g_{\parallel} > g_{\perp}$. The $g_{\parallel}$ component has shown hyperfine splitting as Cu has a spin of I = 3/2 with $A_{\parallel}$ value of 58.2 Gauss. The $g_{\perp}$ component did not show extensive splitting and small splitting with $A_{\perp}$ as 8.6 Gauss. The broadness of EPR spectral peaks is due to the spin–pair and the antiferro magnetic interaction between the two Cu (II) centres. The bridging acetate ligands bring the paramagnetic centres closer. The studies are in accordance with the reported $[Cu_2(CH_3COO)_4(C_5H_5NO)_2]$[22]. Further, the EPR

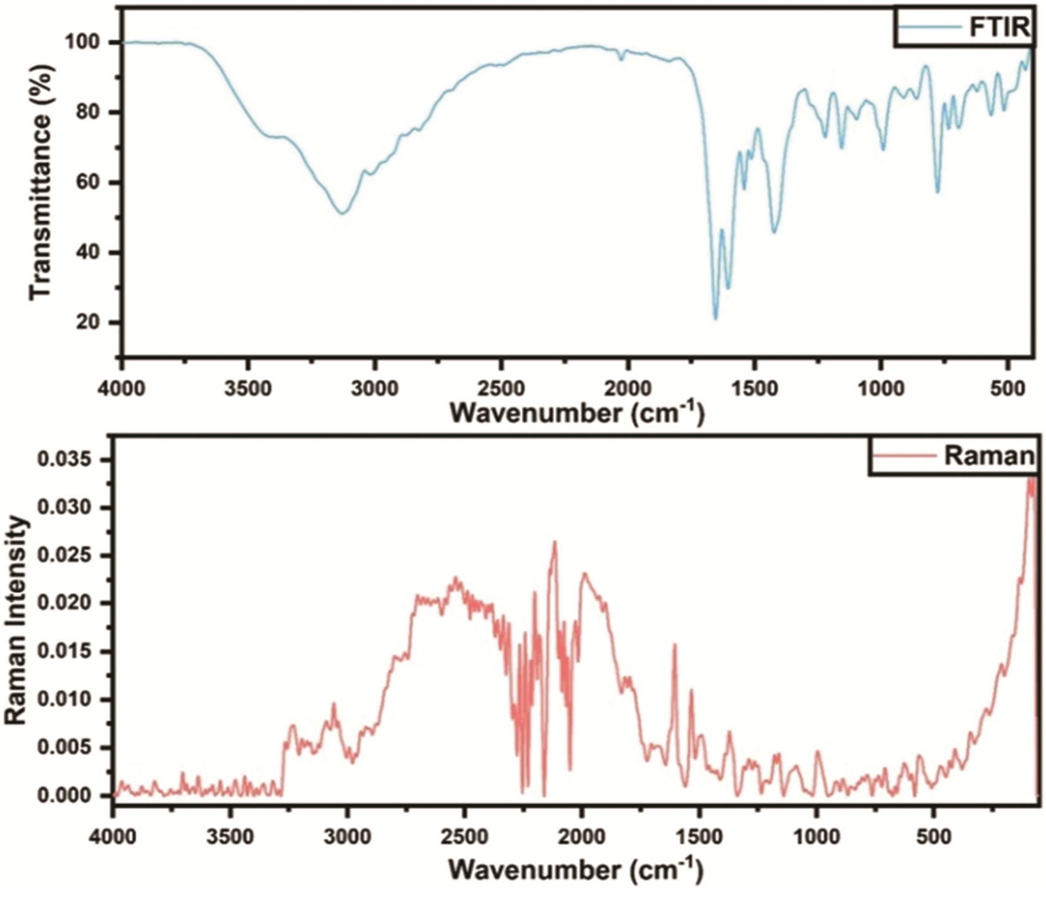


Fig. 4 — FT-IR and Raman spectra of Cu_2TAP

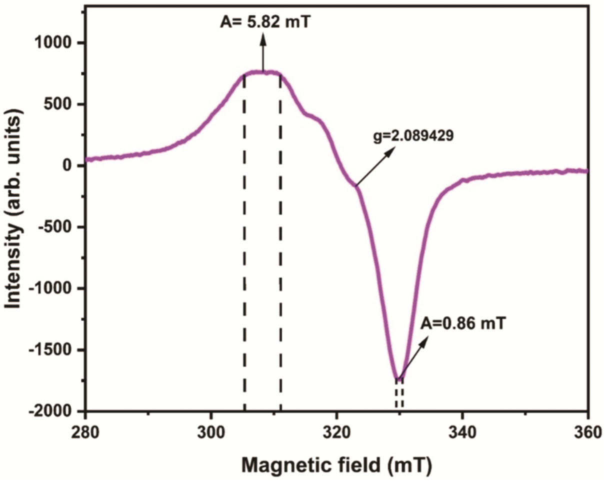


Fig. 5 — EPR spectrum of Cu_2TAP

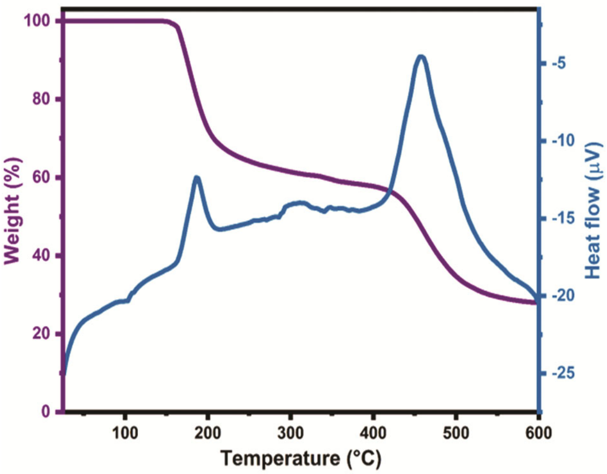


Fig. 6 — TG/DTA spectrum of Cu_2TAP using heating rate of 10°C min^{-1}

spectrum is similar to the reported five coordinated complex $[Cu(SNS)Cl_2]$ (SNS = ligand)[22]. The values are characteristic of the binuclear copper (II) complex. The binding carboxylate acetate ions increase the anti-ferromagnetic interaction between the two Cu(II) center. A five coordination through SNS to Cu^{2+} also showed a similar anisotropic behaviour[21].

TG-DTA

The TG-DTA showed (Fig. 6) three step weight loss. In the first stage, the two 2-pyridonemoieties are lost showing a weight loss of 34.18% (Theoretical: 34.08%). The second stage involves the formation of $2Cu(CH_3COO)_2$ with a weight loss of 55.92% (Theoretical: 55.80%). The third stage involves the formation of 2CuO showing a weight loss of 72.18% (Theoretical: 71.99%). DTA has shown the simultaneous exothermic peaks for the combustion of the ligands along with the TG data.

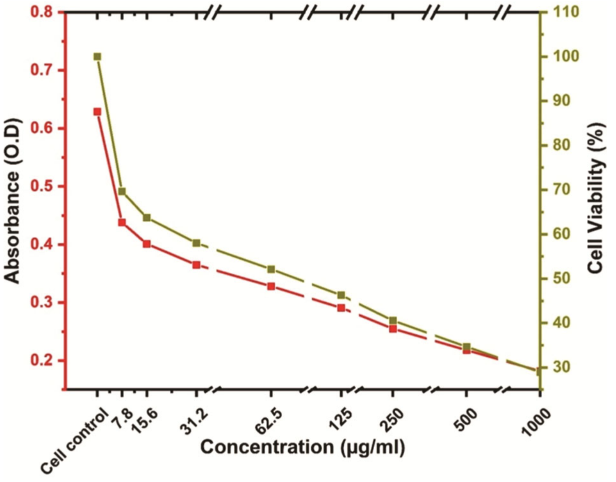


Fig. 7 — Anticancer activity of Cu_2TAP

Table 1 — Anticancer effect of Cu_2TAP *on* MCF-7 cell line

S. No.	Concentration (µg/mL)	Absorbance (O.D.)	Cell viability (%)
1	1000	0.182	28.93
2	500	0.218	34.65
3	250	0.255	40.54
4	125	0.291	46.26
5	62.5	0.32	52.14
6	31.2	0.365	58.02
7	15.6	0.401	63.75
8	7.8	0.438	69.63
9	Cell control	0.629	100

Anticancer Activity

The anticancer effect of the dinuclear copper (II) complex was studied against MCF-7 breast cell line by MTT assay method[14-16]. The complex with molecular weight of 557.5 g/mol showed a IC-50 value of 62.5 µg/mL (113 µM). The IC 50 value even though not lower than that of cisplatin and the compound showed a satisfactory cytotoxic effect. The action interestingly due to hydrogen bonding between the 2-pyridone and acetate ligand. The copper(II) centers are five coordinated and give room for the other biochemical moieties to coordinate at sixth position either strongly or weakly. The NH and oxo group of 2-pyridone can play a vital role in bringing the anticancer activity. The plot of the % of Cell Viability *vs* Cu_2TAP concentration was obtained. The cell control and sample control were included in each assay to compare the full cell viability assessments. The obtained anticancer effect of Cu_2TAP *on* MCF-7 cell line was presented in Table 1. The activity plot and the corresponding images are shown in Fig. 7 and Fig. 8.

Table 2— Molecular docking results of the complex Tetrakis (μ -acetato) bis (2-pyridone) dicopper (II) with the target Human estrogen receptor alpha (PDB ID: 3ERT)

Chemotherauptic agent (CID)	H-bond donors/acceptors	Binding affinity (kcal/mol)	Ligand binding site of target (Protein ID: 3ERT) Amino acids
Cu_2TAP	2/10	–8.40	**Leu 539, Leu 536, Val 534, Val 533,** Asn 532, **Leu 525, Lys 531,** Cys 530, **Pro 535, Lys 529, Ala 350,** ,**Asp 351,** Tyr 526, Thr 347, **Leu 354,** Trp 383, **Leu 387**
*Doxorubicin	5/11	–7.90	**Lys 529, Leu 525, Leu 387,** Trp 383, **Leu 354, Ala 350, Asp 351, Leu 539, Val 534, Val 533, Leu 536, Pro 535, Lys 531**

* Sandard drug Doxorubicin; Amino acid **black bold** residues indicate the binding site of the anticancer drug Doxorubicin, whose response to the present selected Cu_2TAP compounds exhibits anticancer property.

Molecular Docking analysis

From the molecular docking analysis of the prepared complex, the lower negative docking score represented a high binding affinity between the receptor and the complex molecule which showed the higher efficiency. The docked ligand molecules were selected based on docking energy and good interaction with the active site residues and the results are shown in Table 2 (Fig. 9, Fig. 10 and Fig. 11).

represent the structures of the complex, drug and protein employed in this study. Similarly, Fig. 12 and Fig. 13 illustrate the 3D view of complete and docks of complex with proteins. The docking energy of Tetrakis(μ-acetato)bis(2-pyridone)dicopper(II) (–8.40kcal/mol) and Doxorubicin(Std.) (–7.40kcal/mol). The binding interactions of the compound showed hydrogen bonding and hydrophobic interactions with the target protein. The docking studies confirmed the anticancer activity of tetrakis(μ-acetato)bis(2-pyridone)dicopper(II) and there by inhibition of target protein as Human estrogen receptor alpha (3ERT) through the binding interactions. The fluorescence microscope analysis also indicated a gradual increase in cell death (apoptosis) as the concentration of the titled compound is increased when compared with the control. There is a satisfactory cell tumour cell damage at IC_{50} value of 62.50 g/mL (113nm).

Experimental Section

Materials and Methods

The ligand 2[hydrazinylidene(phenyl)methyl] pyridine [HPMP] has been prepared from 2-benzoylpyridine and hydrazine. India. 2-Picoline, acetone, ethanol and DMSO are of the analytical grade were purchased from Merck and used as received without further purification.

Synthesis of ligand

The ligand 2-[hydrazinylidene(phenyl)methyl] pyridine (HPM)[19-21] has been prepared by the interaction of phenyl 2-benzoylpyridine with hydrazine hydrate. The HPMP was reacted with $Cu(CH_3COO)_2.2H_2O$ in ethanol-water mixture in the presence of 2-picoline and refluxed for 3h. Then the obtained product was oxidized in atmosphere in ethanol medium gave pyridine 2-one. This reaction is illustrated in Scheme 1.

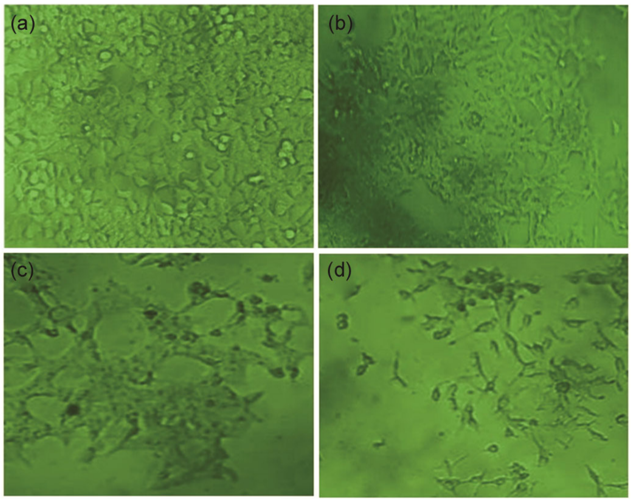


Fig. 8 — Anticancer activity image of Cu_2TAP (a) Control cells (b) 7.8 µg/mL (c) 62.5 µg/mL (d) 1000 µg/mL

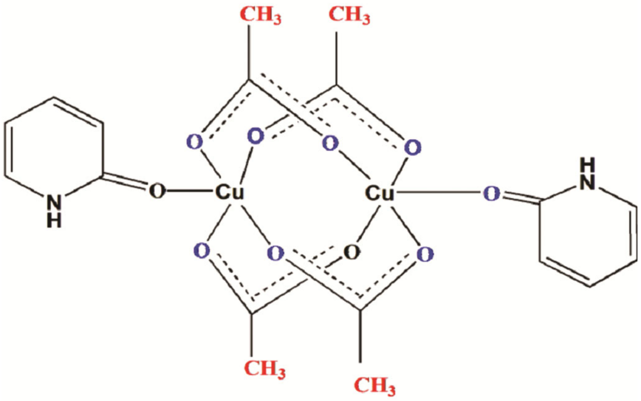


Fig. 9 — The 2D view of the complex, Cu_2TAP

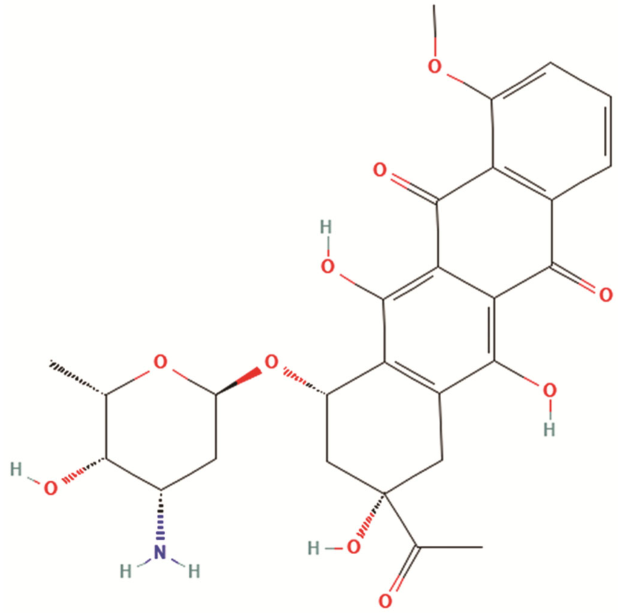


Fig. 10 — The 2D view of Doxorubicin (Standard drug)

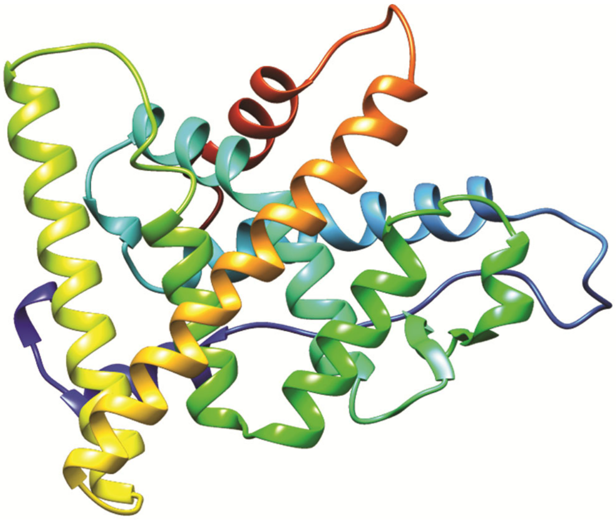


Fig. 11 — The 3D ribbon view of target protein Human estrogen receptor alpha (PDB ID: 3ERT)

Preparation of tetrakis(μ-acetato)bis(2-pyridone) dicopper(II)(Cu_2TAP)complex

About 1.2 g of 2-picoline, [hydrazinylidene(phenyl) methyl]pyridine (HPMP) (1.98 g), $Cu(CH_3COO)_2.2H_2O$ (2.1 g) in ethanol were refluxed for 4 h (Scheme 2). Then the solution was cooled and dropped into a separatory funnel. Add 100 cm^3 of DCM and 50 cm^3 of distilled water and shake well for homogenous mixing. The organic layer was separated and dried over anhydrous $MgSO_4$ and filtered when orange colour crystals were obtained after three days of evaporation.

The physicochemical properties of the prepared tetrakis(μ-acetato)bis(2-pyridone)dicopper(II)(Cu_2TAP) complex is presented in Table 3.

In vitro assay for Anticancer activity (MTT assay)

Cell line and culture

The MCF 7 Cell line was obtained from the National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 μg/mL) in a humid atmosphere of 50 μg/mL CO_2 at 37 °C.

In vitro assay for anticancer activity

The MCF-7 (1×10^5/well) was taken in 24 wells and incubated at 37°C in 5% CO_2 atmosphere[14-17]. After the cell reaches the confluence, the various concentrations of the Cu_2TAP were added and incubated for 24h. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (*p*H 7.4) or DMEM without serum. 100μl/mol (5mg/mL) of 0.5% of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was added per well and left for 4 h. After incubation, 1 mL of DMSO was added in all the wells. The absorbance at 570nm was measured using a UV visible spectrophotometer with DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC_{50}) was determined graphically. The % cell viability was calculated using the following formula:

$$\% \text{ Cell viability} = [\text{A570 of treated cells} / \text{A570 of control cells}] \times 100$$

Ligand preparation for docking analysis

ACD Chem Sketch can draw and convert SMILES notations for the tetrakis(μ-acetato)bis(2-pyridone) dicopper(II) structures. The SMILES for each structure were generated by the structure file generator using Swiss ADME. The structural analogue based drug design has been performed using Swiss ADME tool.

Protein preparation

The three-dimensional structure of the Human estrogen receptor alpha was retrieved from the Protein Data Bank (PDB ID: 3ERT). Human estrogen receptor alpha (PDB ID: 3ERT) preparation was generally to remove all water molecules and any other Ligand molecules prior to docking using Pymol

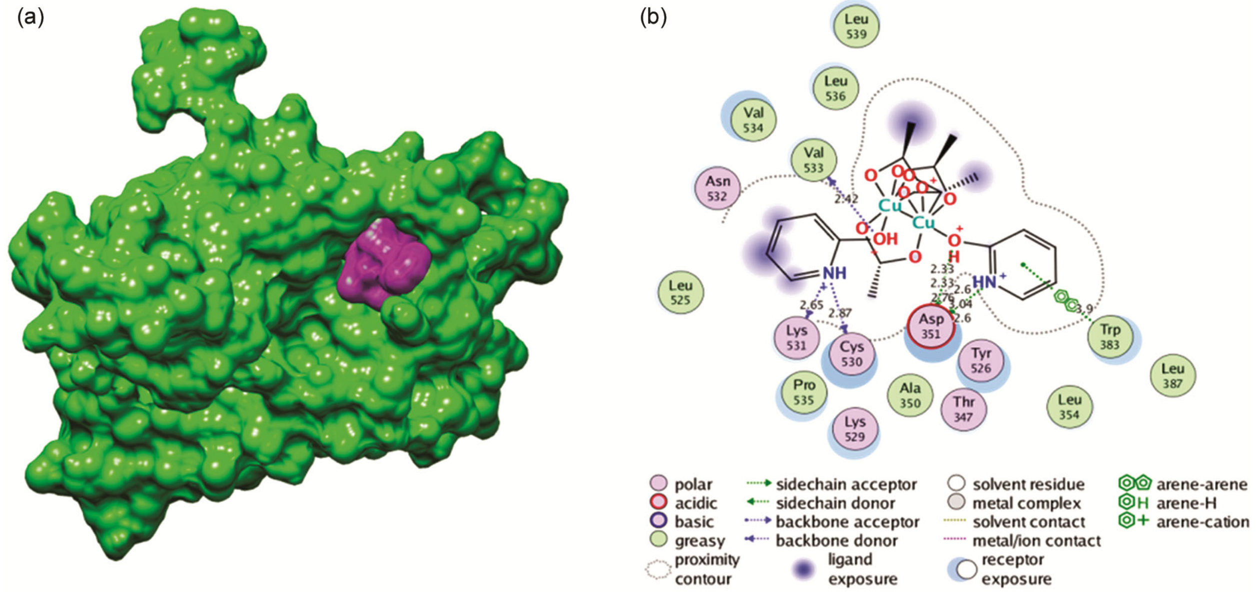


Fig. 12 — (a) 3D view and (b) 2D view docked complex of Cu_2TAP and target protein Human estrogen receptor alpha (PDB ID: 3ERT)

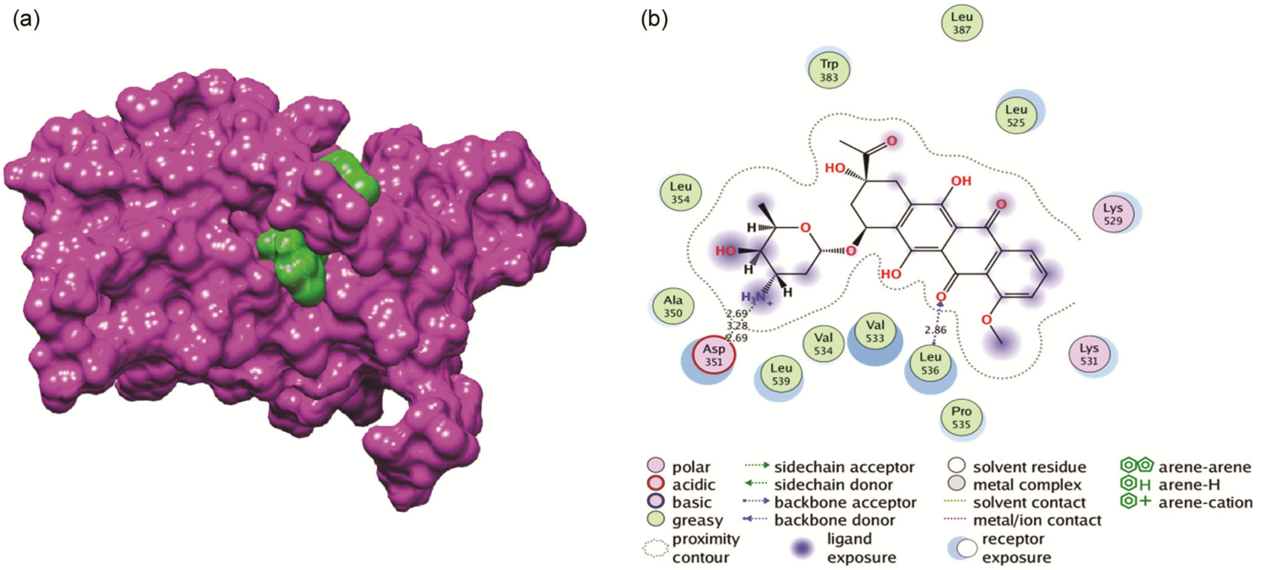
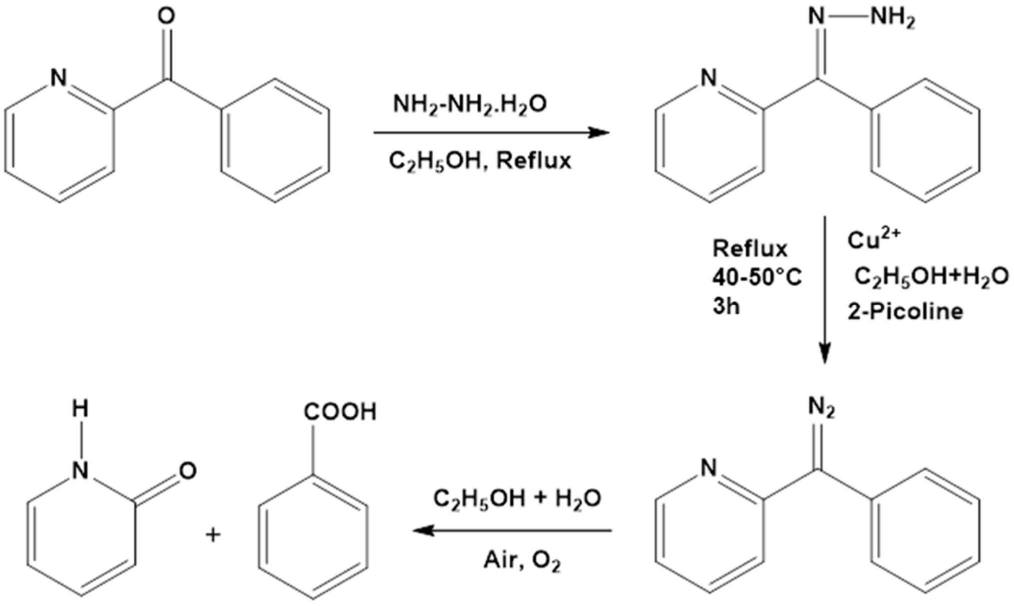


Fig. 13 — (a) 3D view (b) 2D view of docked complex of Doxorubicin and target protein Human estrogen receptor alpha (PDB ID: 3ERT)

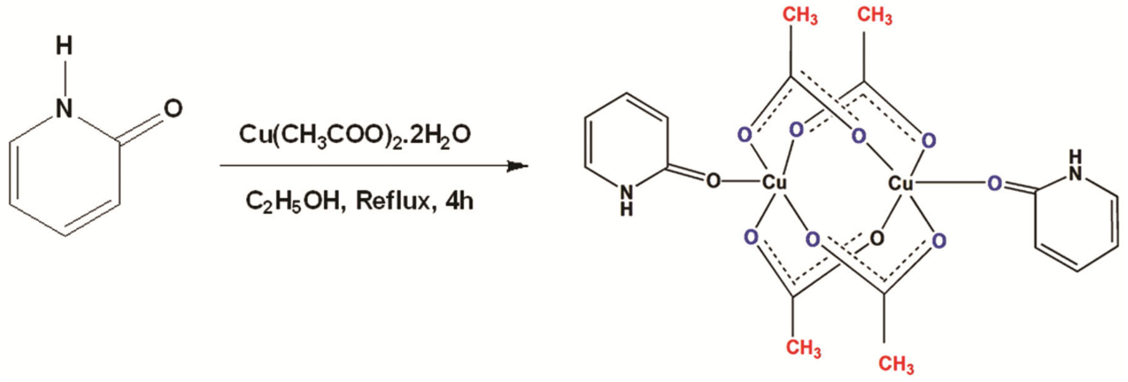
software and thus prepared protein was saved as PDB formed.

Molecular docking

Computational drug discovery techniques[15, 22-27] in the recent day of Pharmaceutical research have been successful in molecular modelling with different algorithm-based programming software. The ligand and protein binding scores according to the algorithm-based program thereby may use any software for protein and ligand interactions for the best results[22]. Automated docking along with a graphical user interface, Auto Dock tool was utilized to generate grids, calculate dock score and evaluate the conformers of the activators bound in the active site of protein as targets. Energy minimization was done in ACD/Chem Sketch . The minimized structures were then subjected to docking studies. A Lamarckian genetic algorithm method, implemented in the program PyRx 0.8 (Auto Dock vina) was employed. This software is used for the estimation of energy during the interaction and to identify the best flexible



Scheme 1 — Synthesis of ligand [hydrazinylidene(phenyl)methyl]pyridine (HPMP)



Scheme 2 — Synthesis of tetrakis(μ-acetato)bis(2-pyridone)dicopper(II)(Cu_2TAP) complex

Table 3— Physicochemical properties

Mol. Formula	$C_{18}H_{26}Cu_2N_2O_{10}$
Mol. Wt.	557.50 g/mol
Number of heavy atoms	32
Number of aromatic heavy atoms	12
Fraction C*sp*^{3}	0.44
Number of rotatable bonds	2
Number of H-bond acceptors	10
Number of H-bond donors	2
Mo. Refractivity	101.26
TPSA	139.56 Å²

ligand pose with minimum energy. The scoring function is based on the intermolecular interaction of ligand and protein during docking. As per the genetic algorithm, all the torsions were allowed to rotate during docking.

The grid map was centred at particular residues of the protein and was generated with grid dimension prepared (center_x = 22.45, center_y = 5.49 and center_z = 21.61). The Lamarckian genetic algorithm and the pseudo-Solis and Wets methods were applied for minimization, using default parameters[22-26]. The complex structures were modelled using modelling software Pymol (1.1 version, Delano Scientific LLC, San Carlos, CA, USA), Chimera (1.10.1 version UCSF Resources for biocomputing visualization and informatics, NIH, CA, USA), and 2D pose view using Discovery Studio Visualizer[27].

Conclusions

The paddle wheel complex, Tetrakis(μ-acetato) bis(2-pyridone)dicopper(II), has been prepared unexpectedly during the attempts to prepare a mixed ligand complex using $Cu(CH_3COO)_2$, HPMP (Hydrazone) and 2-picoline in ethanol-water mixture.

The hydrazone, HPMP, has been oxidized by Cu^{2+} giving a diazo compound which on subsequent reaction yielded 2-pyridone. The compound has four bridged acetato ligands with the neutral 2-pyridone ligands coordinated to each Cu^{2+}. This gives rise to a paddle-wheel complex with an unusual five-coordinate Cu^{2+} complex. The two Cu^{2+} centres come closer because of the bridging acetato ligands. The bonding by acetato and 2-pyridone has been confirmed by the IR spectrum. EPR revealed anti-ferromagnetic interaction between the two copper(II) centers. The complex showed good anticancer activity against MCF-7 breast cell line cultures with an IC_{50} value of 62.5µg/mL (13.62 µM). When the concentration of the complex increased the cell viability decreased linearly. The fluorescence microscope examination also inferred the cell mortality as the concentration of Cu_2TAP increased. Molecular docking studies for the possible interaction of Cu_2 TAP with human estrogen receptor alpha protein (PDBID3ERT) have been computed and compared with Doxorubicin. The binding affinity of Cu_2TAP with 3ERT protein is –8.40 kcal mol, greater than that of Doxorubicin (–7.90 kcal/mol). The paddle wheel dinuclear copper(II) complex can be extended for its use as an anticancer drug and for any possible optoelectronic applications.

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