INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

BIOLOGICAL EVALUATION AND VEGFR2 KINASE STUDIES OF METAL (II) COMPLEXES DERIVED FROM *N*-BENZOYLATEDTETRAZOLO[1,5-*A*]PYRIMIDINE LIGANDS WITH CIPROFLOXACIN DRUG

SH. Mohamed Yusuf[1], Haleel Azees Khan[1,2]* and TK. Shabeer[1]

[1]Post-Graduate and Research Department of Chemistry, The New College (Autonomous), Chennai-600 014, India (University of Madras).
[2]Instrumentation & Chemical Division, Bahrain Training Institute (Ministry of Education) Isa Town, Bahrain.

ABSTRACT

Ametal(II) mononuclear complexes of Cu(II) (1) & Zn(II) (2) holding tetrazole core ligand have been synthesized and described by spectral methods. The five coordinated square pyramidal geometry was determined by means of electronic absorption and EPR spectral methods. All the complexes exhibit significant antimicrobial activity against Gram (–ve) and Gram (+ve) bacterial as well as fungal strains, which were analyzedbyresazurin reduction assay.The complexes interact strongly with the receptor kinase VEGFR2 through the interaction of π-π, σ-π and hydrogen bonds, and form a stable complex through covalent interactions such as hydrophobic and van der Waals interactions and their binding energy values -24.04 (**1**) and -9.41 (**2**) kcal/mol for the complexes, respectively.

Keywords: Copper(II) & Zinc(II) complexes and Molecular Docking.

1. INTRODUCTION

Molecular binding can be defined as an optimization task used to evaluate the "optimal" orientation of a specific protein of interest and a ligand that binds to DNA, and to evaluate an intermolecular complex structure formed between two or more molecules. In modern drug design, molecular binding is widely used to understand drug-receptor interaction[1,2]. Binding is a method in which two molecules combine in three-dimensional space. In addition, regression or informational prediction functions may be useful to calculate the free enthalpy of ligand binding. There are various tools, software and servers to combine calculations. They can be rigid, flexible and semi-flexible. Thus, the center of computational biology develops gracefully from day to day. It is a promising field that reduces the time and cost of biological research related to drug discovery and molecular interaction.

Researchers draw attention to transition metal complexes due to their wide range of application due to their flexible coordination behaviors, different structural and electronic features[3, 4]. The development of new coordination compounds for biochemical applications is a complex task that requires significant synthetic efforts. Studies on the interaction of transition metal complexes with DNA have been the subject of exciting research and have become one of the most important issues due to their possible use in bio-organic chemistry. However, these platinum-based chemotherapy drugs are covalently linked to DNA and include autotoxicity, nephrotoxicity, peripheral neuropathy and neurotoxicity, nausea, myelotoxicity, resistance to tumors, low selectivity, and so on. These side effects are badly affected.

In this context, the focus is on the development and synthesis of new products based on metals

which are more efficient, target-specific, less toxic and non-covalent DNA binding[6-8]. In this sense, the five-membered N-heterocyclic azole and the tetrazole and its derivatives have attracted great interest and have been carefully studied for their interaction with DNA, the specific properties of site binding, and many applications. They were suitable candidates for cancer treatment and these coordination compounds, DNA secondary structure probes, photo-dividers and antitumor drugs[9]. These motifs forced us to learn about the synthesis of tetrazole Cu (II) and Zn (II) based complexes. Therefore, our study will provide new useful information for the development of metal-pharmaceutical preparations for antitumor therapy.

2. EXPERIMENTAL PART

2.1. MATERIALS

Metal(II) perchlorate hexahydrate (M = Cu(II) & Zn(II)) and other commercially available reagents (5-aminotetrazole, ethylacetoacetate, 4-(diethylamino)benzaldehyde, benzoyl chloride, sodium thiosulphate, sodium bicarbonate, sodium sulphate, iodine and pyridine). It was purchased from Aldrich and used without further purification. The solvent for the synthesis was reactive or better and was purified according to standard procedure[10].

2.2. Physical measurement

Melting points were determined using an electro-thermal capillary apparatus and reported as uncorrected values. Elemental analyzes were performed using Carlo-Erba element analyzer model 1106. FT IR spectra were recorded on a Thermo-Nicolet 6700 FT-IR spectrophotometer using a KBr pellet in the range 4000-400 cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were recorded in 400 MHz Advance Bruker spectrometer using CDC13 as solvent. Mass data of the ligand were obtained in the JEOL DX-303 mass spectrometer and the mass data of the complexes were obtained from the Thermo-Finnigan LCQ Advantage MAX 6000 ESI spectrometer. UV-Vis spectra were recorded on a Shimadzu UV-2450 UV-Vis spectrophotometer using acetonitrile as solvent. The molar conductivity of the complexes was measured using the Elico CM-88 digital conductivity bridge model using a freshly prepared solution of complexes in dimethylformamide. X-band EPR spectra were recorded at room temperature on a Varian EPR-E-112 spectrometer using DPPH (2,2'-diphenyl-1-picrylhydrazyl) as a chamber marker.

2.3. Molecular docking studies

Molecular binding studies were performed using the tools of Auto Dock Tools (ADT) version 1.5.6 and Auto Dock version 4.2.5.1[8]. The structures of the synthesized tetrazole-based complexes were converted from the mol format of OPENBABEL to PDB format. The crystal structure of the PDB ID: VEGFR2 (4AGC) was downloaded from the protein database (http://www.rcsb.org./pdb).

2.4. Synthesis of precursor (PC)

2.4.1. Ethyl 7-(4-(diethylamino)phenyl)-4,7-dihydro-5-methyltetrazolo[1,5-a] pyrimidine-6-carboxylate (PC)

A mixture of 5-aminotetrazole (5 mmol), 4-(Diethylamino) benzaldehyde (5 mmol) and ethylacetoacetate (0.64 g, 5 mmol) in isopropyl alcohol (5 ml) were added to a round bottom flask and iodine (0.5 mmol) was added to the stirred mixture. at reflux temperature (82-85 ° C)[7]. After heating to reflux for 4 hours, the whole mixture was cooled to room temperature and a sodium thiosulfate solution (10 M, 2 ml) was added, the precipitated solid was filtered and washed with cold methanol (2 ml), then the crude product was dried and recrystallized from hot ethanol and dried in *vacuo*.

Yield: 1.4 g (80.12%). M.p.: 214 ^{o}C.Anal. $C_{18}H_{24}N_6O_2$ (FW: 356.42 g/mol): Calculated (%): C, 60.66; H, 6.79; N, 23.58. Obtained (%): C, 60.62; H, 6.72; N, 23.55.IR (υ/cm^{-1}): 3240 (–NH), 1701 (C=O), 1442 (N=N, tetrazole). NMR: ^{1}H (400 MHz: $CDCl_3$: Me_4Si) δ (ppm): 1.17–1.20 (t, 3H), 1.24–1.60 (t, 6H), 2.43 (s, 3H), 3.41–3.50 (m, 2H), 4.16–4.22 (m, 4H), 6.73 (s, 1H), 7.20–7.36 (m, 5H), 11.04 (s, 1H). ^{13}C ($CDCl_3$: Me_4Si) δ (ppm): 164.89, 148.65, 146.09, 139.71, 128.97, 128.86 and 127.33 (aromatic carbons), 99.30, 60.53, 59.64, 44.2, 19.43, 14.01, 13.20.EI:357.5 ($M^{\cdot+}$+H, 85.0%).

2.5. Synthesis of ligand(L)

2.5.1.Ethyl4-benzoyl-5-methyl-7-(4-(diethyl amino)phenyl)-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carboxylate (L)

The benzoyl chloride (0.86 g, 6.2 mmol) was added dropwise to a solution of the precursor (PC) (1.76 g, 6.2 mmol) and pyridine (1.48 g, 18.8 mmol) in dry toluene (30 ml) at 0 °C. The reaction mixture was stirred for 1 hour and heated to reflux for 2.5 hours. The mixture was then cooled to room temperature and the organic phase was washed with a saturated solution of $NaHCO_3$ (2.5 ml) and dried over anhydrous Na_2SO_4. The solid product was recrystallized from ethanol and dried in *vacuo*.

Yield: 3.1 g (92.13%). Colour: Colorless. M.p.: 220 °C. Anal.$C_{25}H_{28}N_6O_3$ (FW: 460.22 g/mol): Calculated (%): C, 65.20; H, 6.13; N, 18.25.

Obtained (%): C, 65.17; H, 6.09; N, 18.23.IR (υ/cm^{-1}): 1698 (C=O, benzoyl), 1444 (N=N, tetrazole). NMR: ^{1}H ($CDCl_3$: Me_4Si) δ (ppm): 1.18–1.20 (t, 3H), 1.26–1.61 (t, 6H), 2.45 (s, 3H), 3.43–3.52 (m, 2H), 4.17–4.23 (m, 4H), 6.74 (s, 1H), 7.21–7.37 (m, 5H), 8.02–8.15 (m, 5H). ^{13}C ($CDCl_3$: Me_4Si) δ (ppm): 168.21 ($Ar_{benzoyl}$–C=O), 164.87, 148.68, 147.07 ($Ar_{benzoyl}$), 146.02, 139.73, 128.92 ($Ar_{benzoyl}$), 128.95, 128.82 and 127.31 (aromatic carbons), 99.30, 60.53, 59.67, 44.2, 19.43, 14.01, 13.20. EI: 461.5 ($M^{\cdot+}$+H, 12.0%).

2.6. Synthesis of mononuclear metal(II) complexes

To A methanolic solution (25 ml) of ligand (L, 1.0 mmol) was added to a methanolic solution (25 ml) of metal perchlorate hexahydrate (II) (1.0 mmol) and sodium ciprofloxacin with continuous stirring and refluxed for 2 hours. The resulting solution was filtered and kept separate for slow evaporation. The product obtained was washed with cold methanol and dried under vacuum. Efforts to obtain crystals in different conditions and solvents were not successful.

[Cu(L)(drug)ClO_4] (1)

Yield: 0.47 g (90.03 %). Colour: Green. Anal.$C_{42}H_{45}N_9O_{10}ClFCu$ (FW: 953.86 g/mol): Calculated (%): C, 52.89; H, 4.76; N, 13.23. Obtained (%): C, 52.84; H, 4.73; N, 13.20. IR (υ/cm^{-1}): 1685 υ(C=O, benzoyl), 1555 $\upsilon(COO)_{asy}$, 1363 $\upsilon(COO)_{sym}$. 1430 υ(N=N, tetrazole ring), 531 υ(Cu–O), 463 υ(Cu–N). UV-Vis, λ_{max}, nm: 284 (3,748), 371 (561), 620 (410). EPR: $g_{||}$ = 2.18, $g_{\perp}$ = 2.03 and $A_{||}$ = 163. μ_{eff} = 1.75 BM.ESI: 953.85[Cu(L)(drug)ClO_4(*m/z)*]; 854.48 [Cu(L)(drug)(*m/z)*].

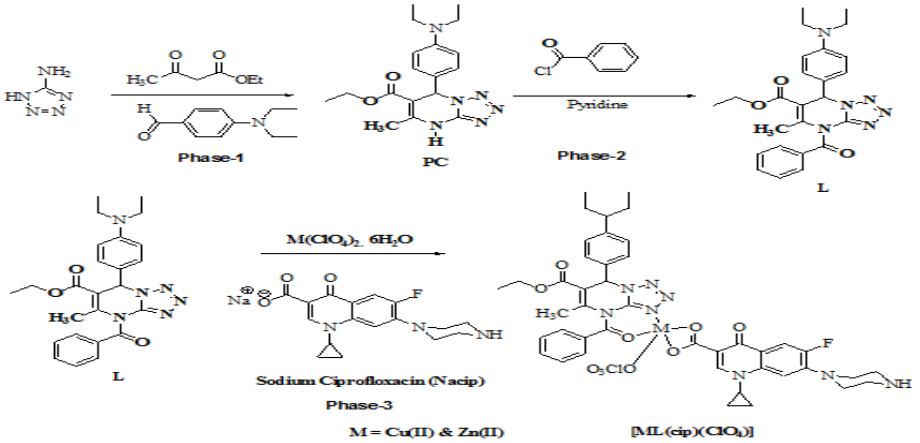
[Zn(L)(drug)ClO_4] (2)

Yield: 0.41 g (78.39 %). Colour: Yellow. Anal.$C_{42}H_{45}N_9O_{10}ClFZn$(FW: 955.71 g/mol): Calculated (%): C, 52.78; H, 4.75; N, 13.19. Obtained (%): C, 52.75; H, 4.71; N, 13.17. IR (υ/cm^{-1}): 1683 υ(C=O, benzoyl), 1554 $\upsilon(COO)_{asy}$, 1365 $\upsilon(COO)_{sym}$. 1432 υ(N=N, tetrazole ring), 530 υ(Cu–O), 464 υ(Cu–N). UV-Vis, λ_{max}, nm:271 (4,326), 370 (1,428), 455 (890), 490 (430). ESI: 955.75 [Zn(L)(drug)ClO_4 (*m/z)*]; 856.38 [Zn(L)(drug) (*m/z)*].

3. RESULT AND DISCUSSION

3.1. Synthesis of ligands and complexes

Complexes of metals (Cu (II) **1** and Zn (II) **2**) were synthesized for the development of novel potent chemotherapeutic anticancer drugs. The synthesis of the ligand (L) was followed by the procedure described in the literature[11] and the metal complexes of type [**M(L)(drug)ClO_4**] were obtained from the ligand in the presence of MCIO4. $6H_2O$ (M = Cu (II) and Zn (II)). The proposed molecular structure of the complexes is based on spectroscopic data, elemental analysis and magnetic susceptibility values. The above synthesized complexes 1 and 2 were stable in air and dissolved in DMSO and DMF. Analytical data suggest a ratio of 1:1:1 (M:L:D) for Cu(II) and Zn(II) complexes. The presence of lattice water or uncoordination MeOH was confirmed by TGA analysis. The molar conductivity corresponding to the Cu(II) and Co(II) complexes presents low values and in this way a structural formula of non-electrolyte for these complexes can be assigned. The magnetic susceptibilities of the complexes at room temperature were consistent with trigonalbipyramid geometry around the central metal ion. The overall complexes preparation performed in three phases is shown under **Scheme 1**.



Scheme. 1: Synthetic route for the synthesis of mononuclear metal(II) complexes of *N*-benzoylatedtetrazolo[1,5-*a*]pyrimidine ligands and sodium ciprofloxacin (Nacip) drug

3.2. Spectral characterization

3.2.1. Infrared spectroscopy

IR spectra of metal (II) complexes (1 and 2) of ligand (L) gave information about their coordination regimes and analyzed by comparing with free ligand and drug. In the experimental section, an important wave number of precursors, ligands, drugs and mononuclear complexes is given. The IR spectrum of the precursor (PC) shows a sharp band at 1701 cm^{-1} depending on the presence of the acetyloxy group; The band appearing at about 3240 cm^{-1} comes from the second amino group (NH)[12, 13]. The precursor and ligand show an average sharp band in the 1430 and 1432 cm^{-1} regions, respectively for (N=N) tetrazole ring. The removal of the precursor sec-amino band (PC) by the emergence of a new band of about 1698 cm^{-1} after isolation indicates the formation of a ligand (L).

The IR spectra of the complexes showed a significant difference in terms of ligand and drug ciprofloxacin. The formation of the complexes is confirmed by significant changes in the frequencies of the benzoyl group of (C = O) and the tetrazole ring (N = N) of the ligand (L). After the complexation, these bands moved at lower frequencies indicating the coordination of oxygen and nitrogen atoms of benzoyl and tetrazole groups with metal ions, respectively. Free ciprofloxacin shows stretching vibration induced by υ(O - H), υ(C=O) and υ(C-O), at 3364, 1729 and 1265 cm^{-1}, respectively. After the complexation of -OH, the stretching disappears and significant changes in the vibration of the ciprofloxacin group (C=O) stretch show the coordination of ciprofloxacin and metal ion between metal ion(II) through υ(O-H) and (C=O) groups (**Fig. 1**). Due to the emergence of two new non-ligand peaks in the 531 and 463 cm^{-1}region ν(M-O) and ν(M-N) modes, respectively. These spectral observations also confirm the coordination of the metal ion with the ligand (the nitrogen atom of the benzoyl group and the nitrogen atom of the tetrazole) and the ciprofloxacin (the oxygen atom of the hydroxyl and carbonyl groups).

3.2.2. Nuclear magnetic resonance & Mass spectroscopy

The ^{1}H and ^{13}C NMR spectra of the precursor (PC) and ligand (L) at ambient temperature confirm their formation ($CDCl_3$-d_1 solvents).The ^{1}H NMR spectrum of the precursor and ligand shows a triplet signal for the acetyloxy methyl group at δ 1.17-1.20 ppm (**Fig. 2**). A triplet (1.24-1.60 ppm) and a multiple (4.16-4.22 ppm) are assigned for methyl and methylene protons respectively of the $N(C_2H_5)_2$ substituent attached to the aromatic ring. The precursor showed a singlet for the proton 5-CH_3 and the quartet -CH_2CH_3 signals at δ 2.43 and δ 3.41-3.50 ppm, while the ligand signaled at 2.45 and δ 3.43-3.52 ppm respectively. The precursor and ligand show a singlet signal at δ 6.73 and 6.74 ppm, respectively, assigned to the 7-CH-group. Aromatic protons of precursor and ligand appeared in the range of 8.02 to 8.15 ppm. The precursor shows a peak at 11.04 ppm, which can be attributed to the N-H proton of the secondary amine, and this peak disappeared in the NMR spectrum of the ligand after benzoylation. The ^{13}C NMR spectrum of the precursors and ligand exhibit methylene and carbon resonance at 59.64-59.67 ppm, respectively. The entire ligand confirmed the presence of the benzoyl ring by signal in the 168.21 ppm region.

Mass spectral studies confirmed the formulae proposed for the precursor and ligand, which show the corresponding molecular ion peak $[PC + H]^+$ and $[L + H]^+$. The electrospray ionization mass of the mononuclear complexes was recorded in positive mode, indicating some significant peaks corresponding to the various parts of the complexes. The information collected from mass spectral data is in agreement with the data obtained from the elemental analysis.

3.2.3. Electronic absorption & molar conductivity measurements

Electronic spectra of ligand (**L**) and their mononuclear metal (II) complexes (**1** and **2**) were recorded at room temperature in acetonitrile.

The electronic spectrum of the ligand shows a dense band in the region of 266-276 nm corresponding to the various ligand charge transfer transitions (π-π* and n-π*). Compared to the ligand, the charge transfer transitions band appeared with a red shift for the entire mononuclear copper (II) complex and suggested the coordination of the ligand with the metal ion (II). In the UV region, the mononuclear copper(II) complex (1) shows three bands at 284, 371 and 620 nm; this may be due to the permissible transitions like intra-ligand CT (π-π*), ligand-metal CT (n-π*) and d-d transitions, respectively. These electronic spectral data reveal square pyramidal geometry for copper (II) complexes[14, 15].

The zinc (II) complex isolated in this study is colorless. Electronic spectra show bands in the 271, 370, 455 and 490 nm regions due to charge-transfer and intra-ligand transitions. Metal ligand charge transfer bands can be assigned at 450 nm. The electronic spectrum recommends conjugation between the ligand and drug units. In the absence of this conjugation, the spectra will be similar to those

reported for the zinc(II) complexes of the Schiff bases of acetone and the benzaldehyde of *S*-methyldithiocarbazate[16, 17]. In the latter, two separate bidentate N-S ligands are coordinated to metal ions tetrahedrally with d^{10} manner. Zinc (II) ions generally form tetrahedral complexes, but in this case four plane chelate rings are expected to produce a square pyramidal geometry along with perchlorate ion. The zinc(II) complex being discussed has less solubility in non-polar organic solvents than the copper(II) complex in which spectroscopic evidence points to square pyramidal structures.

The aforesaid analytical and spectral data are completely consistent with the proposed square pyramidal geometry for the mononuclear copper(II) (**1**; **a**) and zinc(II) (**2**; **b**) complexes.

The molar conductivity measurements for the mononuclear metal (II) complex were carried out at room temperature in DMF (10-3 M). The low molar conductivity values (23 and 17; Ω–1 cm2 mol–1) obtained for mononuclear copper(II) and zinc(II) complexes (1 and 2) indicate that not all complexes are electrolytic in nature.

3.2.4. Electron paramagnetic resonance (EPR) spectroscopy

EPR spectra of the paramagnetic copper(II) complex (**1**) were recorded at RT (25 °C) in the X-band region. The trend $g_{||}$ (2.18) >$g_{\perp}$(2.03) >g_e (2.0023) observed for the Cu(II) complex indicating that the unpaired electron is present in the $d_{x^2-y^2}$orbital of the copper ion [18, 19]. A Cu(II) complex of five coordinates retain the square pyramidal geometry and provides a well-resolved axial ESR spectrum; Therefore, the EPR study of the copper(II) complex provided supportive evidence for the absorption spectroscopic results.

3.2.5. In vitro antimicrobial activity

In vitro antimicrobial activities of N-benzoylatedtetrazolo[1,5-*a*]pyrimidine -based metal(II) complexes (**1** & **2**)were tested against two Gram (–ve)(*Shigelladysenteriae*and *Vibrio cholerae*), two Gram (+ve) (*Bacillus cereus* and *Streptococcus faecalis*) human bacterial,and one human fungal (*Candida krusei*)strain by resazurin dye reduction assay. Resazurin is a non-toxic redox indicator dye used in various cytotoxicity assays to assess cell growth. It is a blue non-fluorescent dye that turns pink under the influence of oxidoreductase enzymes in living cells. A color change from blue to pink shows living cells. When the color of the dye remains blue, this indicates that there is no activity of living cells[20].In the present assay, the antimicrobial activity is dose dependent and the compounds are added at various concentrations. The minimum inhibitory concentration (MIC) is the lowest concentration of antimicrobial agent that inhibits the apparent growth of the microorganism after the necessary incubation period. Test compounds kill bacteria and fungal cells during the incubation period. This is derived from a discoloration in the corresponding cavities. The antibacterial and antifungal activity evaluated using test organisms is shown in Figure **5**&**6**. The results obtained in Figures **5** and **6** are shown in the **Table. 1**.

Copper (II) complex **1** shows moderate activity against test organisms, but **1**, Gram (+) bacteria shows higher activity against Bacillus cereus and Candida krusei fungus. Although complex **2** zinc (II) is not effective against all bacterial and fungal strains tested, complex **2** shows poor activity against Gram (-and) Shigelladisenteriae. As a rule, the test compounds were less active than the reference positive control erythromycin (antibacterial) and amphotericin B (antifungal). A possible explanation of the antimicrobial activity of the complexes may be related to the lipophilicity of the central metal, which then promotes the formation of a hydrogen bond between the donor group and the active center of the cell components, as well as penetration through the lipid layer of the cell group. This may result in destruction of the cell permeability barrier to interfere with normal cell operations. Increased inhibitory activity of metal complexes may also be related to the nature of metal ions and ligands, the presence of co-ligands, the geometry of complexes, steric and pharmacokinetic factors[21–23].

3.2.6. Vascular Endothelial Growth Factor Inhibitor (VEGFR2) studies

Vascular endothelial growth factor (VEGF) plays an important role in tumor angiogenesis, and it is believed that inhibition of the signaling pathway is an effective therapeutic option for cancer treatment[8, 24]. An increase in VEGF kinase receptor in various human tumors. And expression levels correlate with poor prognosis and clinical stage in patients with a solid tumor[25, 26]. Therefore, the VEGFR2 signal is an attractive therapeutic target in the treatment of cancer. Monoclonal anti-humanized anti-VEGF antibody (bevacizumab)[27] and many small molecules of the VEGFR2 kinase inhibitor were considered anti-angiogenic drugs and demonstrated clinical benefit in the treatment of some tumors with controlled side effects[28-30]. For these reasons, we grew up by examining the results of the interaction between the VEGFR2 kinase receptor and the synthesized metal complexes.

To examine the selection of the binding site, the synthesized complexes (**1** and **2**) were d0cked to the 3D structure of BSA ID (PDB): 3V03 using Auto Dock, and the binding results were sequenced in **Table 2**. The predicted active sites consisted of amino acids for complex **1** such as ASN 923, LYS 920, LEU 840, CYS 919, PHE 916, GLU 917, LEU 1035, VAL 899, ALA 866, ICE 915, VAL 916, VAL 914, VAL 867, LYS 868, LEU 889, GLU 885, CYS 1045, ASP 1046, VAL 848, LYS 868, ASP 1046, LEU 889, GLY 841, CYS 1045, PHE 1047, ALA 1050, GLY 841, LEU 840, GLY 922, VAL 848, LYS 920, CYS 919, PHE 921, GLU 850, GLU 917, PHE 918, ALA 866, LEU 1035, ALA 866, VAL 916, VAL 899, GLY 922 and GLU 885[8]. **Fig. 7** provides information on energetically encouraging images, binding sites and complex binding positions. It can be seen that the complexes are possibly associated with the hydrophobic pocket and that all complexes have five hydrogen-binding interactions, one of which is the ASN 923 formed between the hydrogen atom of the ligand and the CO atom of the ligand. CYS 919 hydrogen atom and N atom of tetrazole ring. The other three, HIS 1088, GLY 1224 and PHE 1223, are formed between hydrogen atoms and O and N atoms of the drug.

The binding energy of the complexes is reduced in the order 1 > 2. According to the results that complex **1** exhibits a binding energy value greater than complex **2**, the relative binding energy is the binding affinity between the stronger VEGFR2 and the target molecules. The negative relative binding mode of complex **1** means stronger binding affinity to VEGFR2 by playing a key role in the metabolism of VEGFR2 and in the transport chamber. Results from molecular docking studies were the interaction of VEGFR2 with the complexes, electrostatic interactions, van der Waals, for example hydrogen bonds interaction (π–π & σ–π).

4.CONCLUSION

Thus, two metal(II) mononuclear complexes of type [M(L)(drug)Cl_4] containing a ligand with a tetrazole core were synthesized and identified by analytical and spectral methods. Based on spectral studies, square pyramidal geometry was given for both complexes. All the synthesized metal complexes show profound antimicrobial activities against the tested microorganisms. Cu(II) complex show higher antibacterial and antifungal activities compared to the Zn(II) complex. These results also indicate that a combination of metal and ligand environment with drug plays a vital role in increasing the degree of inhibition. Analysis of the molecular binding of complex 1 and 2 with the VEGFR2 kinase receptor indicates the interaction of π-π, σ-π and hydrogen bonds. The strong activity of the complex 1 can be related to the potent effect of non-conjugated electrons around the copper (II) metal ion. All encouraging chemical and molecular comparison results show that complex 1 is a suitable candidate for use as an anticancer drug. Further research is needed to evaluate the pharmacological properties of biological activity *in-vitro* and *in-vivo* to explain the true mechanism.

5. ACKNOWLEDGMENT

The authors gratefully acknowledge. Prof. N. Sakthivel, Department of Biotechnology, Pondicherry University, Pondicherry-605 014, India, for making fruitful discussions about molecular coupling studies.

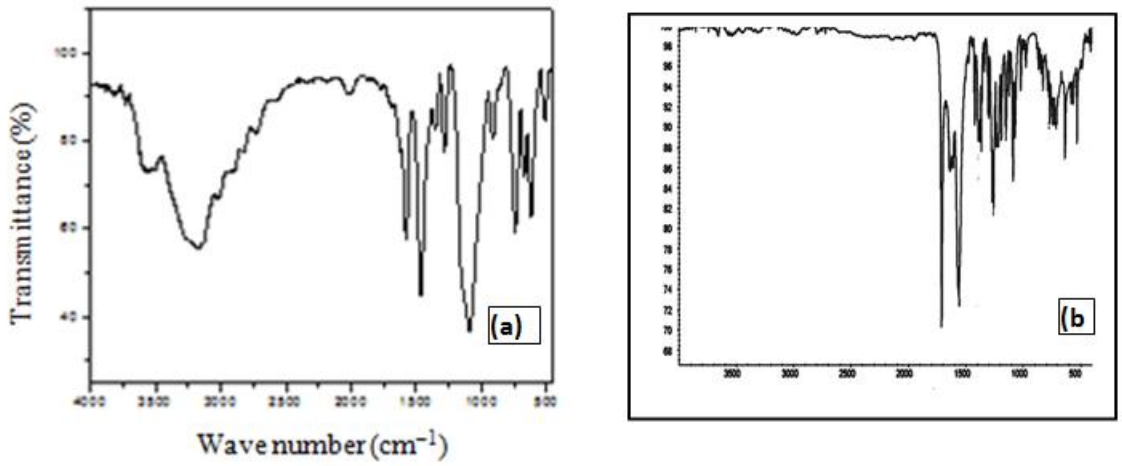


Fig. 1: FT IR spectra of the precursor(PC) (a) and *N*-benzoylatedtetrazolo [1,5-a]pyrimidine-based copper(II) (1)

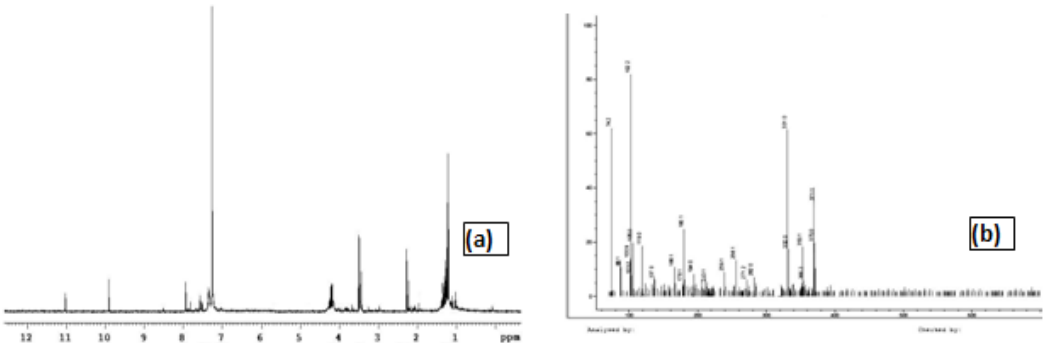


Fig. 2: ^{1}H NMR (a) and EI mass (b) spectra of the *N*-benzoylatedtetrazolo[1,5-a]pyrimidine precursor HL

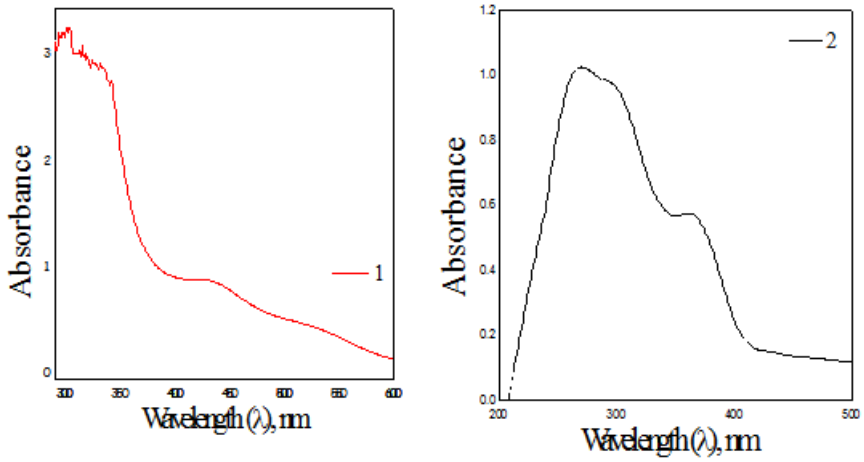


Fig. 3: Electronic spectra of the *N*-benzoylated tetrazolo[1,5-*a*] pyrimidine mononuclear copper(II) (a) and zinc(II) (b) complexes

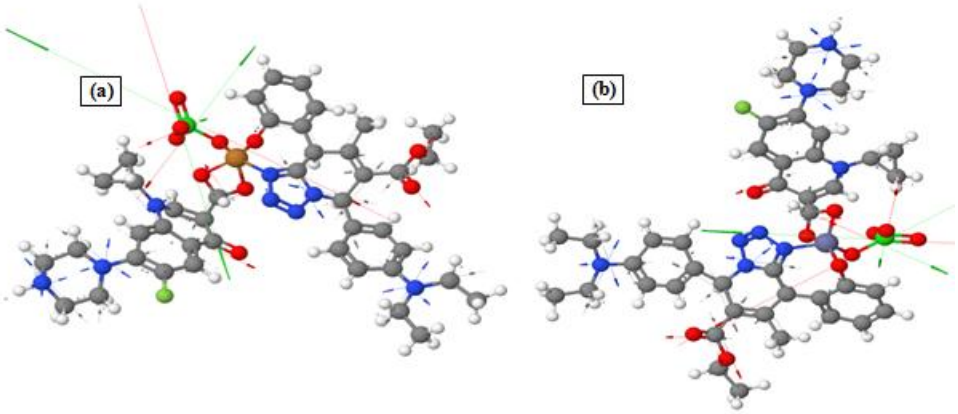


Fig. 4: Optimized geometries of complexes 1 (a) & 2 (b)

Fig. 5.Antibacterial activity of the *N*-benzoylatedtetrazolo[1,5-*a*]pyrimidine-based copper(II) complex (1) against bacterial strain *Bacillus cereus* by resazurin reduction assay

C – Control – Dye + *Bacillus cereus* + without complex
C1 (**1**) + Dye + *Bacillus cereus*

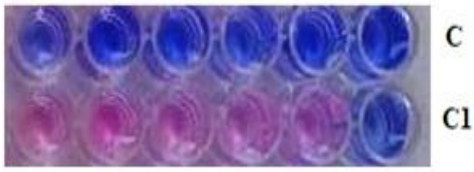


Fig. 6: Antifungal activity of the *N*-benzoylatedtetrazolo[1,5-*a*]pyrimidine-based copper(II) complex (1) against fungal strain *Candida krusei* by resazurin reduction assay

C – Control – Dye + Candida krusei + without complex
C1 (**1**) + Dye + Candida krusei

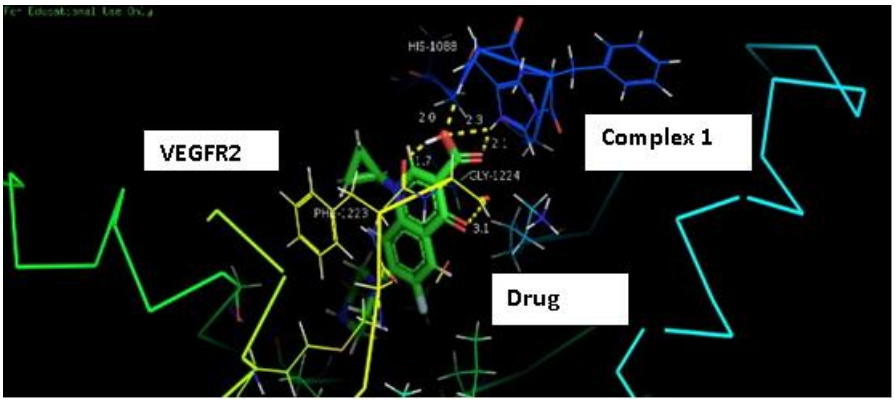


Fig. 7: Molecular fused models of the drug and copper (II) complex based on *N*-benzolated tetrazolo[1,5-*a*]pyrimidine were present in 8 Å

Table 1: Antimicrobial activity of the *N*-benzoylatedtetrazolo[1,5-*a*]pyrimidine-based copper(II) complexes by resazurin reduction assay as MIC values (µg/mL)

Compounds	Gram (-ve)		Gram (+ve)		Fungal Strains
	S. d	V. c	B. c	S. f	C. k
1	10.50	21	5.20	9.25	8.25
2	7.25	25	14.12	12.50	-
Erythromycin	2.56	5.25	4.12	0.88	-
Amphotericin-B	-	-	-	-	0.98

S. d =Shigelladysenteriae; V. c = Vibrio cholerae; B. c = Bacillus cereus;
S. f = Streptococcus faecalis;C. k = Candida krusei.

Table 2: Docking results of the *N*-benzoylated tetrazolo[1,5-*a*]pyrimidine -based metal(II) complexes 1&2 with VEGFR2.

Complexes	Binding Energy		
	Final total internal energy	Torsional free energy	Estimated Binding energy
1	–25.73	1.69	**–24.04**
2	–10.83	1.42	**–9.41**

6. REFERENCES

1. Sharma NK, Jha KK and Priyanka J. Adv Sci Res. 2010;1:67–72.
2. Lounnas V, Ritschel T, Kelder J, Mcguire R, Bywater RP and Foloppe N. Comput Struct Biotechnol J. 2013;5:1–14.
3. Alvarez N, Veiga N, Iglesias S, Torre MH and Facchin G. Polyhedron. 2014;68:295–302.
4. Solanki A, Kumar SB, Doshi AA and Prabha CR. Polyhedron. 2013;63:147–155.
5. Wang Y, He QY, Sun RWY, Che CM and Chiu JF. Eur J Pharmacol. 2007;554:113-122.
6. Rajendiran V, Murali M, Suresh E, Sinha S, Somasundaram K and Palaniandavar M. Dalton Trans. 2008;148-163.
7. Haleel A, Mahendiran D, Veena V, Sakthivel N and Kalilur Rahiman A. Mater Sci Eng C. 2016;68: 366–382.
8. Jayakumar S, Mahendiran D, Srinivasan T, Mohanraj G and Kalilur Rahima A. J Photochem Photobiol B. 2016;155:66-77.
9. Terenzi A, Barone G, Palumbo Piccionello A, Giorgi G, Guarcello A, Portanova P, Calvaruso G, Buscemi S, Vivona N and Pace A. Dalton Trans. 2010;39:9140–9145.
10. Armarego WLF and Perrin DD. Purification of Laboratory Chemicals. Pergamon press. Fourth ed., 1996;80.
11. Zeng LY and Cai C. J Comb Chem. 2010;12:35–40.
12. Mosalkova AP, Voitekhovich SV, Lyakhov AS, Ivashkevich LS, Gaponik PN and Ivashkevich OA. Z Anorg Allg Chem. 2012;638:103–110.
13. Degtyarik MM, Gaponik PN, Naumenko VN, Lesnikovich AI and Nikanovich MV. Spectrochim Acta Part AMol Biomol Spectrosc. 1987;43:349–353.
14. Hathaway BJ, Dudley RJ and Nichols PJ. J Chem Soc A. 1969;1845–1848.
15. Hathaway BJ, Proctor IN, Slade RS and Tomlinson AA. J Chem Soc A. 1969;2219–2224
16. Iskander F, Mishrikey MM, E1-Sayed L and El-Toukhy. J Inorg Nucl Chem. 1979;41:815–823.
17. Melson GA, in Melson (Ed.) GA. Coordination Chemistry of the Macrocyclic Compounds, Plenum, New York. 1979.
18. Prabhakar B, Lingaiah P and Laxma Reddy K. Polyhedron 9. 1990;805–811.
19. Hathaway BJ. Copper in Comprehensive Coordination Chemistry; Wilkinson G, Ed.; Pergamon Press: Oxford. 1987;5:533.
20. Thirunarayanan A, Raja S, Mohanraj G and Rajakumar P. RSC Adv. 2014;4:41778–41783.
21. Tweedy BG. Phytopathology 55. 1964:910–914.
22. Malhota L, Kumar S and Dhindsa KS. Indian J Chem. 1993;32A:457–459.
23. Thangadurai TD and Natarajan K. Trans Met Chem. 2001;26:500–504.
24. Shibuya M and Claesson-Welsh L. Exp Cell Res. 2006;312:549–560.
25. Samoto K, Ikezaki K, Ono M, Shono T, Kohno K, Kuwano M and Fukui M. Cancer Res. 1995;55: 1189-1193.
26. Guetz GD, Uzzan B, Nicolas P, Cucherat M, Morere JF, Benamouzig R, Breau JL and Perret GY. Br J Cancer. 2006;94:1823–1832.
27. Ferrara N, Hillan KJ, Gerber HP and Novotny W. Nat Rev Drug Disc. 2004;3:391–401.
28. Strumberg D. Drugs Today. 2005;41:773–784.
29. Harris PA, Boloor A, Cheung M, Kumar R, Crosby RM, Davis-Ward RG, Epperly AH, Hinkle KW, Hunter III RN, Johnson JH, Knick VB, Laudeman CP, Luttrell DK, Mook RA, Nolte RT, Rudolph SK, Szewczyk JR, Truesdale AT, Veal JM, Wang L, Stafford JA. J Med Chem. 2008;51:4632–4640.
30. Rini BI, Escudier B, Tomczak P, Kaprin A, Szczylik C, Hutson TE, Michaelson MD, Gorbunova VA, Gore ME, Rusakov IG, Negrier S, Ou YC, Castellano D, Lim HY, Uemura H, Tarazi J, Cella D, Chen C, Rosbrook B, Kim S and Motzer RJ. The Lancet. 2011;378:1931–1939.