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Research Article

ANTICANCER, ANTIMICROBIAL, DNA CLEAVAGE AND DOCKING STUDIES OF BINARY COMPLEXES OF CU(II) , CO(II) AND ZN(II) WITH 4-CHLORO-2-(2-HYDROXY) NAPHTHYLIDENE AMINO BENZOTHIAZOLE SCHIFF BASE

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ABSTRACT

A series of binary complexes of Cu(II), Co(II) and Zn(II) containing 4-chloro-(2-hydroxy)Naphthylidene aminobenzothiazole Schiff base were synthesised. These compounds were characterised by elemental analysis, FT-IR, UV-Vis, mass, TGA, molarconductance and SEM. From the electronic spectra and magnetic moment values the geometry of the complexes was determined. TGA data confirmed that there is no coordinated water molecules in the complexes. The binding mode of the Schiff base ligand to metal ions through azomethine nitrogen, oxygen of aldehyde was confirmed through the absorption bands appeared in the IR spectrum of the binary complexes. Anticancer activity of the compounds revealed that Schiff base ligand and its Cu(II), Zn(II) complexes have shown greater activity against the HeLa and MCF-7 cell lines. The complete cleavage of CT-DNA occured with the Cu(II), Co(II) complexes, where as no cleavage was observed in the Schiff base ligand and Zn(II) complex wells. The In vitro antimicrobial assessment of the Schiff base and its complexes have displayed that complexes have shown more activity than its free ligand. Docking studies were carried out on ligand to illustrate binding mode of ligand in to different active sites which are Pencillin binding protein 4 of Staphylococcus aureus , Penicillin Binding Protein 4 (dacB) of E.coli and Homo sapiens cyclin dependent kinase.

Keywords: Binary metal complexes, IR, SEM, Antibacterial, DNA cleavage anticancer activity.

INTRODUCTION

Cervical cancer is the most widely recognised reason for Malignancy demise in women.About 70% of cervical cancers occur in developing and low income countries. Chemotherapy is used to shrink cervical cancer and decrease the tumour growth. In recent years, the development of metal complexes as anticancer drugs has attracted much attention[1-6]. Inspite of, some platinum- based drugs cisplatin, carboplatin have already achieved success in chemotherapy, there has been given considerable importance to the non platinum-based transistion metal compexes with satisfactory anticancer activities, less toxicities and specific antitumour pathway which is different to platinum-based drugs[7]. Synthesis of metal complexes by using Schiff base ligands make them as effective and stereospecific catalysts, they show biological activity, other transformation of organic and inorganic chemistry. Schiff bases have been reported to show a variety of biological applications like antifungal, antibacterial, clinical and herbicidal activities by virtue of the azomethine linkage, which is responsible for these actions[8-10]. Among Schiff bases, heterocyclic compounds containing N, O and S have played vital role in medicinal chemistry. Molecules like Vit.B_1, Coenzyme Cocarboxylase and Pencillin containing thiazole as key component and which is accountable for various biological activities of these compounds. Benzothiazole is one of the most important heterocyclic compound, which posses immense activities like antitumour[11], antimicrobial[12], anticonvulsant[13], antitubercular[14], anthelminthic[15],

antioxidant[16], analgesic[17], antifungal[18], anti-inflammatory[19], antileishmanial[20], antiulcer[21], schistosomicidal[22] and diuretic[23] activities. The importance of 2-amino benzothiazoles in Medicinal and Bioorganic chemistry with applications in drug discovery and development for the treatment of diabetes[24], epilepsy[25-27], inflammation[28], amytropic Lateral sclerosis[29], analgesia[30], tuberculosis[31] and viral infections[32] make them previlaged scaffold in drug discovery. For example, in the treatement of amyotrophic Lateral sclerosis[33] marketed Riluzole drug is used which is a 2-aminobenzothiazole compound. N-aryl substituted 2-aminobenzothiazole is serving as a potential inhibitor of retinoic acid metabolism for cancer treatment[34,35].

In view of the importance of 2-aminobenzothiazole, we report the synthesis, characterisation and biological studies on binary metal complexes of Cu(II),Co(II) and Zn(II) containing 4-chloro-2-(2-hydroxy)Naphthylidene aminobenzothiazole Schiff base ligand. Anticancer activity of the Schiff base and its metal complexes were screened against the HeLa and MCF carcinoma. CT-DNA is used for cleavage experiments and gram positive and negative strains were used to know the bacterial activity of the ligand and its complexes.Metal complexes have shown greater activity than its free ligand. The docking studies were concluded that the binding mode of the ligand with protein active sites were predicted using docking technique. The dock score values of ligand molecule was showed a correlation with their inhibitory activity against Staphylococcus aureus Pencillin binding protein 4 ,E.Coli Penicillin Binding Protein 4 (dacB) and Homo sapiens cyclin dependent kinase.

MATERIALS AND METHODS

CHEMICALS

All the chemicals including metal(II) chlorides were purchased from sigma aldrich. The reagents and solvents used were of analytical grade.

Synthesis of Schiff base ligand (L)

The Schiff base ligand 4-chloro-2-(2-Hydroxy) Naphthylidene Amino Benzothiazole (L) was obtained from the condensation reaction of 2-hydroxy-1-naphthaldehyde (1.72g. 10mmol) and 2-amino benzothiazole (1.5g. 10mmol) in methanol for reflux for 6 h. (Scheme. 1: Formation Schiff base ligand (L)).

Synthesis of binary&ternary Metal complexes

The ligand(2mmol) was dissolved in 20-30mL of chloroform and solution of the metal salts(1mmol) in 10mL of methanol was added dropwise to the ligand solution with continuous stirring and the mixture refluxed overnight. The pH of the solution was maintained between 7-8 by adding ammonical buffer to the solution. Then the volume of the solution was reduced to about 10mL and complexes were precipitated with dry diethyl ether. The precipitate was filtered, washed with water and cold ethanol then dried at room temperature. The resulting metal complexes were1:2 ratio (Metal:Ligand) binary complexes. (Scheme. 2: Formation of binary metal complexes).

Instruments

The percentage of the elements (C, H, N) present in the ligand and complexes were determined by using Perkin Elmer Elemental analyser. The IR spectra of the compounds were recorded on Prestige-21 Insrument, by using KBr pellet in the range 4000-400 cm^{-1}. Elico Electronic Digital conductivity meter was used to know the molar conductance of the metal complexes. The electronic spectra of the compounds was carried out in DMSO using a SHIMADZU UV-2600 spectrophotometer. The proton nmr of the ligand was recorded at 200 MHz and 300 MHz on Varian Gemini Spectrometer and TMS is used as an internal standard. To analyse molecular weight of the compounds VG AUTOSPEC mass spectrometer was used and which is performed through ESI technique. Thermogravimetric analysis of the metal complexes was carried on a Mettler Toledo Star system in the temperature range 50-1000^{o}C and heating rates were controlled by 15^{o}C min^{-1}. A Gouy balance model 7550 using $Hg[Co(NCS)_4]$ as standard is operated to examine the magnetic moment values of the metal complexes. By using Polmon instrument (model No. MP-96) the melting point of the ligand and decomposition temperature of the complexes were determined. The SEM/EDX images were obtained from a Hitachi SEM analyser.

Antibacterial activity

By using disc diffusion method[36] the invitro antibacterial activities of the Schiff base ligand and its metal complexes were analysed. All the metal complexes were screened against gram positive(eg. (i) Bacillus subtilis (ii) Staphylococcus aureus) and gram negative (eg.(iii) Pseudomonas putida (iv) Escherichia coli) bacterium. One day prior to the experiment, the bacterial cultures were

inoculated in broth(inoculation medium) and incubated overnight at 37^0C. Inoculation medium containing 24h grown culture was added aseptically to the nutrient medium and mixed thoroughly to get the uniform distribution. This solution was poured (25mL in each dish) into petri dishes and then allowed to attain room temperature. Wells (6mm in diameter) were cut in the agar plates using proper sterile tubes. Then, wells were filled upto the surface of agar with 0.1mL of the test compounds dissolved in DMSO (200µM/mL). The plates were allowed to stand for an hour, in order to facilitate the diffusion of the drug solution. Then the plates were incubated at 37^0C for 24h and the diameter of the inhibition zones were read.

DNA cleavage studies

The compounds were dissolved in DMSO and added separately to the CT-DNA (Calf Thymus DNA) sample and add Hydrogen Peroxide. The sample mixtures were incubated at 37ºC for 1 hour. The electrophoresis of the samples was done according to the following procedure. Weigh 0.25grams of agarose and dissolve it in 25 ml of 1x TAE buffer (121.1g Tris base, pH 8.0, 0.5 M EDTA, 57.1ml of Glacial acetic acid for 1 ltr) by boiling. When the gel attains approximately 55ºC, pour it into the gel cassette fitted with comb. Let the gel to solidify. Carefully remove the comb, place the gel in the electrophoresis chamber flooded with TAE buffer. Load DNA sample with bromophenol blue carefully into the wells, along with standard DNA marker and pass the constant 100 V of electricity till the dye front reaches the end of gel. Remove the gel and carefully stain with ETBR solution (10 µg/ml) for 10-15 min and destain the gel and observe the bands under UV transilluminator.

In vitro anticancer activity

The HeLa cervical cancer cell lines and MCF-7 breast adino carcinoma cancer cell lines were purchased from NCCS, Pune. The cells were maintained in DMEM supplemented with 10 % FBS and the antibiotics penicillin/streptomycin (0.5 mL^{-1}), in atmosphere of 5% CO_2 /95% air at 37^0 C. For the MTT assay, HeLa cells were plated in 96 well plate at 5.0 X 10^3 cells were per well in culture medium and incubated overnight at 37^0 C.

Cell viability

Cell viability of the HeLa and MCF-7 carcinoma was evaluated by the MTT Assay with three independent triplicate experiments of six concentrations of compounds (5, 10, 25, 50 75 and 100 µM). After 24 hrs of incubation, each treatment was withdrawn and MTT solution (0.5 mg / mL^{-1}) was added to each well and plates were incubated at 37^0 C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 560 nm on a microplate reader[37].

Docking studies

The synthesized ligand was selected for performing molecular docking studies. Molecules were built using Maestro build panel and prepared by LigPrep 2.0 application. Crystal structures of *Staphylococcus aureus* Pencillin binding protein 4, *E.coli* Penicillin Binding Protein 4 (dacB) and Homo sapiens cyclin dependent kinase (pdb id: 3HUN[38] 2EXB[39] 2XNB[40]) were downloaded from protein data bank (www.rcsb.org). GLIDE 5.6[41] was used for molecular docking. The proteins were prepared using protein preparation module applying the default parameters. Grid was generated around the active site of the protein by selecting the cocrystalized ligand. Receptor van der Waals scaling for the nonpolar atoms was kept 0.9[42] Low energy conformation of the ligands were selected and docked into the grid using standard precision (SP) docking mode. Dock pose of each ligand was analysed for interactions with the receptor.

RESULTS AND DISCUSSION

Physical characteristics of the complexes

The metal complexes were colored, stable at room temperature and non-hygroscopic in nature. On heating, they melt at high temperatures. The complexes were insoluble in water and are soluble in DMSO.

Elemental analysis

The analytical data of the Schiff base ligand and its complexes were represented in **Table-**1. The data reveals that the experimental values have shown for each of the compounds were similar to that of its theoretical values. These values confirmed the metal to ligand ratio is1:2

^{1}H NMR

The proton nmr of the Schiff base ligand was monitored by the peak ratios in the spectra recorded in DMSO(d^6). The aromatic proton for the ligand is a multiplet at 7.24-8.02ppm. The hydroxy proton of the aldehyde appeared as broad singlet at 10.12ppm (ph-OH), and a singlet at 8.5ppm due to azomethine proton

(CH=N) (Fig. 1: ^{1}H NMR spectrum of Schiff base ligand).

UV-Vis spectra and magnetic measurements

The UV-Vis spectra of the Schiff base ligand and its complexes were taken in DMSO. In the ligand, two very strong bands were observed at 269nm and 366nm which is assigned to π-π* and n-π* transitions of the aromatic ring and C=N chromophore[43]. The electronic spectrum of Co(II) binary complex shows absorption band at 690nm region which corresponds to transition [$^4A_2$(F)- $^4T_1$(P)] implying tetrahedral environment. The magnetic moment value of the Co(II) complex was found to be 4.35BM.which is also pinpointed the tetrahedral geometry for the complex[44]. The absorption band at 590nm appeared in the UV-Vis spetra of the Cu(II) complex, which is due to the transistion [$^2B_{1g} \longrightarrow {}^2A_{1g}$][45] This is characteristic of square planar environment. The square planar geometry was proposed for the Cu(II) complex, based on its magnetic moment value which was found to be 1.81BM . However, the diamagnetic Zn(II) complex exhibits only the intraligand and charge-transfer bands at 270nm, 392nm respectively and were expected to have a tetrahedral geometry[46]. (Fig. 2: UV-Vis spectrum of Zn(II) binary complex).

Infrared spectra

The IR spectral data has shown significant shifts in the stretching frequencies of the ligand to the metal complexes. The most characteristic bands of the Schiff base ligand appeared at 3431cm^{-1} $\upsilon_{(O-H)}$, 1597cm^{-1} $\upsilon_{(C=N\ azomethine)}$,1620cm^{-1} $\upsilon_{(C=N\ thiazole\ ring)}$ and 677-680cm^{-1}$\upsilon_{(C-S-C)}$.The azomethine band at 1597cm^{-1} of the ligand underwent a shift in all the complexes, indicating the bonding of azomethine nitrogen to the metal ion[47]. The phenolic c-o stretching band appeared at 1352cm^{-1} in the Schiff base ligand which is shifted to lower frequency, suggest a weakening of the υ(C-O) vibration hence a bond should form between O-M atoms. This also confirms the deprotonation of the OH group on complexation. The bands at 1620cm^{-1} $\upsilon_{(C=N\ thiazole\ ring)}$ and677-680cm^{-1}$\upsilon_{(C-S-C)}$ were unchanged after complexation which affirms that the thiazole group itself does not coordinate to metal atoms by neither nitrogen nor sulphur atoms. In the low frequency region 440-468cm^{-1} is ascribed to (M-N) and the region 555-580cm^{-1} could be assigned υ (M-O)[48] (Fig. 3:IR spectrum of Co(II) binary complex).

Thermogravimetric analysis

TGA gave the thermal stabilities of the metal complexes under nitrogen atmosphere with a heating rate of 15^{0}C per minute from 50-1000^{0}C. In the present work all the metal complexes do not show any significant weight loss upto 220^{0}C indicating the absence of coordinated water molecules in these metal complexes. The binary Co(II) and Cu(II) complexes started to decompose at 290-300^{0}C and gradual decrease in the weight loss occurs upto 600^{0}C. After that a straight line is obtained specifying the formation of cobalt as residue and copper as its oxide. From the data it is concluded that all the metal complexes were stable at ordinary temperature. Based on the above studies the structures were proposed for Schiff base ligand and its metal complexes (Fig. 4: TGA of Cu(II) binary complex).

SEM and EDX analysis

The surface morphology of the Schiff base ligand and its complexes was determined by using scanning electron microscope. Schiff base ligand has shown different morphology compared to its complexes. Ligand shows bar with layered structure and Cu(II) complex has shown agglomerate and flakes like structures respectively. The composition of the elements present in the compounds were obtained from the EDX analysis (Fig. 5: SEM and EDX images of Cu(II) binary complex).

Mass and Molar conductance

The formula weight(338.5) of the ligand coincides with the molecular ion peak in the mass spectrum. The molecular ion peak of the ligand at(m/z= 339), the mass spectrum of all the metal complexes were shown in Table-2 The low molar conductance values of the metal complexes disclosed that all the metal complexes were non-electrolytic in nature[49].

In vitro antibacterial activity

The Schiff base ligand and its metal complexes were tested against gram positive and gram negative bacterial strains. Ampicillin and Ceftriaxone are used as standard drug for gram positive and gram negative strains respectively. The zone of inhibition values of the compounds are tabulated in Table. From these values it is concluded that metal complexes have shown greater activity than its free ligand. This is explained on the basis of chelation theory and overtones concept[50]. In the present work, all the binary metal complexes have shown more activity on gram negative strain **E.coli** and binary Cu(II) and Zn(II) complexes shows moderate to less activity on S.aures. Finally all the metal

complexes have shown good activity on other two bacterial strains **B.subtillis** and **P.putida** .

Standard Drug for Gram Positive: Ampicillin
Standard Drug for Gram Negative: Ceftriaxone
Table 3: Zone of inhibition in (mm) [concentration(100 µg)]

DNA cleavage experiment

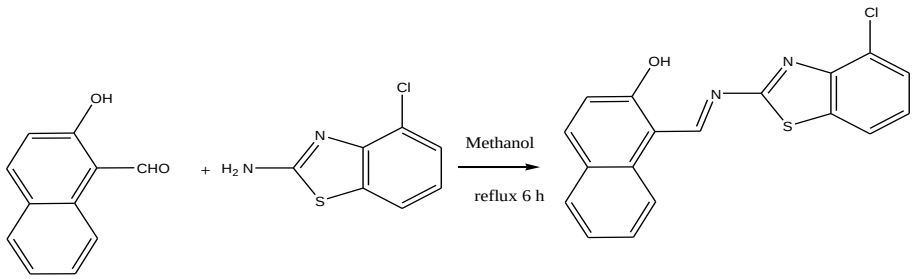
In the present work CT-DNA is used for the cleavage experiment.The results illustrated that all metal complexes can interact with CT-DNA in the presence of H_2O_2. From Fig. it is implied that the complete DNA cleavage occurs with Cu(II) and Co(II) complexes where as Zn(II) binary complex and free ligand exhibit no cleavage activity. The metal complexes can catalyse the production of highly reactive hydroxyl radicals from H_2O_2. These hydroxyl radicals participate in the oxidation of the deoxyribose moiety, followed by the hydrolytic cleavage of the sugar-phosphate backbone. The general oxidative mechanisms proposed account for DNA cleavage by hydroxyl radicals via abstraction of a hydrogen from sugar units. It also anticipate the release of specific residues arising from transformed sugars, depending on the position from which hydrogen atom removed[51] (Fig. 6: *DNA cleavage activity of free ligand and its metal complexes* [M] Marker [1] Control(CT-DNA)+H_2O_2 [2] Ligand+DNA+ H_2O_2 [3] Cu(II) binary complex+DNA+ H_2O_2[4] Co(II) binary complex+DNA+ H_2O_2).

Anticancer activity

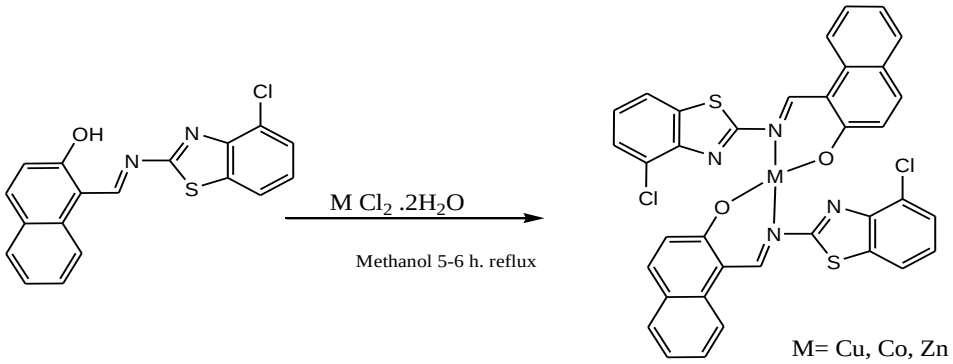
To know the antiproliferative activity of the Schiff base ligand and its metal complexes on cancer cells, we used these compounds to treat with HeLa(Cervicalcarcinoma) and MCF-7(breast adino carcinoma) at the concentration of 5,10,25, 50, 75,100µM. For 48h. The untreated cells were used as control. MTT assay was used to check the cell growth inhibition of the compounds. The Inhibitory concentration 50(IC_{50}), defined as the concentration required to reduce the size of the cell population by 50%. The IC_{50} values obtained for the ligand and itsmetal complexes against HeLa and MCF-7 cell lines are given in Table-4 In the present work Ligand and its complexes have shown significant cytotoxicity against HeLa and MCF-7 cell lines. Low IC_{50} values obtained for the Cu(II) and Zn(II) binary complexes indicated that these compounds have pronounced antitumour activity on HeLa cancer cell line. The Cu(II) complex has shown greater antitumour activity than free ligand against MCF-7 cell line, but Co(II) and Zn(II) binary complexes low activity than free ligand (**Table 4)**.

Docking results

To gain insight of the binding mode, ligand was docked into the ligand active site that showed hydrogen bond interaction with ASN 141 for *Staphylococcus aureus* Pencillin binding protein 4, SER 62 for E .Coli Penicillin Binding Protein 4 (dacB) and LEU 83 for Homo sapiens cyclin dependent kinase. The binding mode of the ligand with protein active sites were predicted using docking technique. The dock score values of ligand showed a correlation with their inhibitory activity against Staphylococcus aureus Pencillin binding protein 4 ,E.Coli Penicillin Binding Protein 4 (dacB) and Homo sapiens cyclin dependent kinase. The dock score values were given in Table 5: Dock score of Ligand.



Scheme. 1: Formation Schiff base ligand (L)



(Proposed structure of binary metal complexes)
Scheme. 2: Formation of binary metal complexes

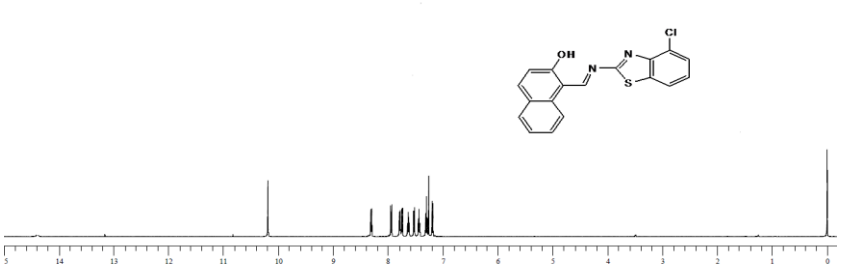


Fig. 1: ^{1}H NMR spectrum of Schiff base ligand

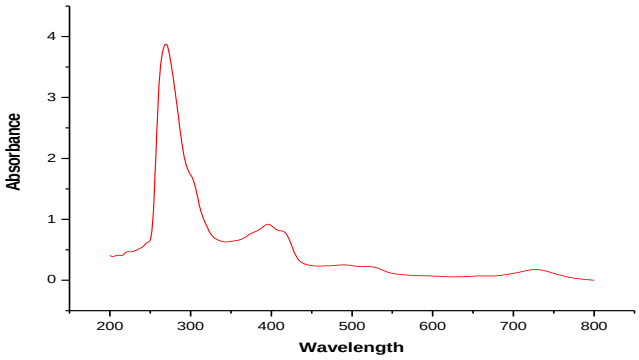


Fig. 2: UV-Vis spectrum of Zn(II) binary complex

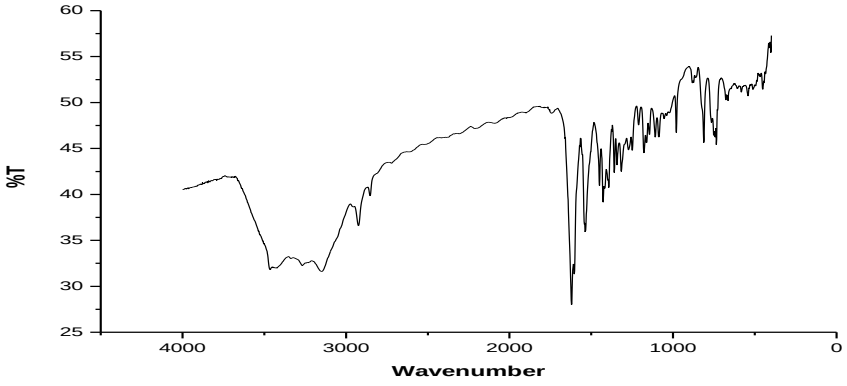


Fig. 3: IR spectrum of Co(II) binary complex

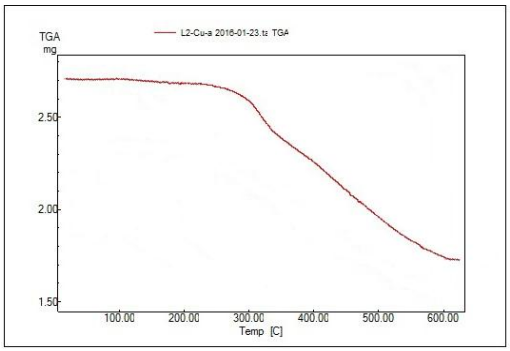


Fig. 4: TGA of Cu(II) binary complex

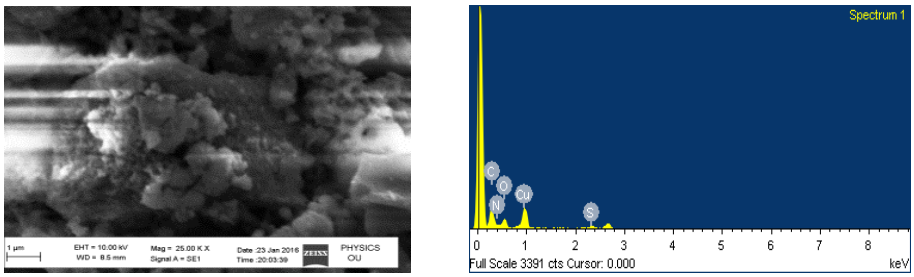


Fig. 5: SEM and EDX images of Cu(II) binary complex

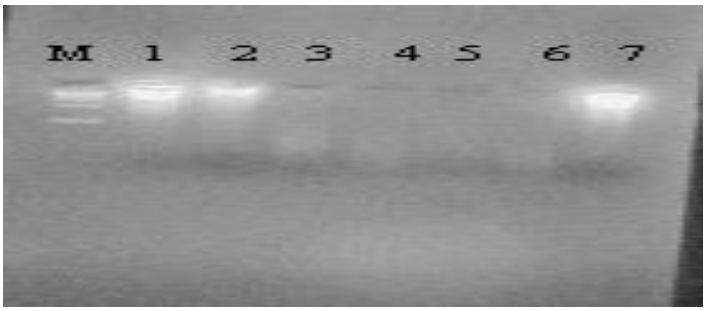


Fig. 6: *DNA cleavage activity of free ligand and its metal complexes*
[M] Marker [1] Control(CT-DNA)+H_2O_2 [2] Ligand+DNA+ H_2O_2 [3] Cu(II) binary complex+DNA+ H_2O_2[4] Co(II) binary complex+DNA+ H_2O_2

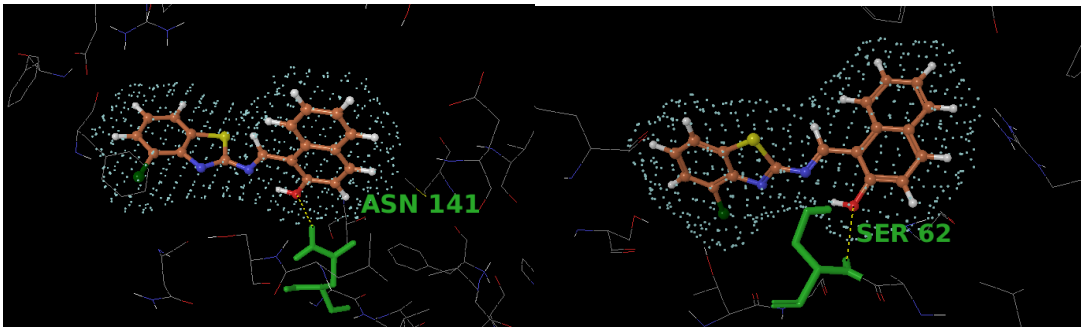


Fig. 7: Docked pose of ligand in the protein active site showing the hydrogen bond interaction (yellow lines) with ASN 141 in Staphylococcus aureus (pdb.id-3HUN) and SER 62 *E.Coli* (pdb.id-2EXB)

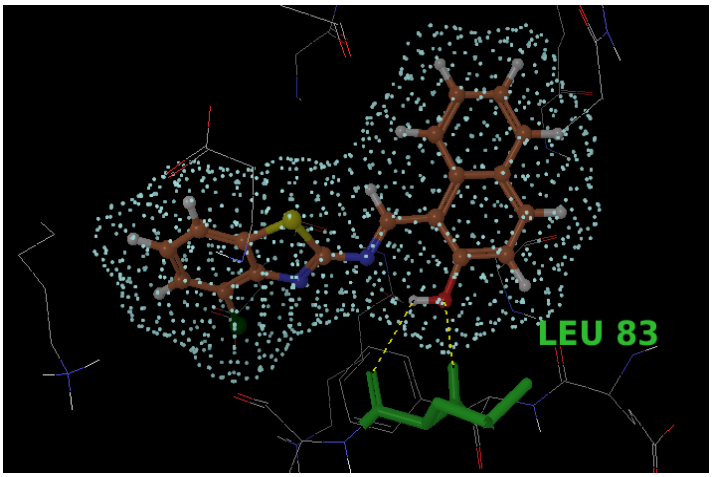


Fig. 8: Docked pose of ligand in the protein active site showing the hydrogen bond interaction (yellow lines) with LEU 83 in Homo sapiens cyclin dependent kinase (pdb.id-2XNB)

Table 1: Elemental analysis of the synthesised compounds

Compound	M.wt.	Colour	% Analysis found (Cal.)		
			C	H	N
Ligand ($C_{18}H_{11}N_2OSCl$)	338.5	Yellow	61.16 (63.8)	2.98 (3.25)	7.94 (8.27)
Cu(II) binary complex $[C_{36}H_{20}N_4O_2S_2Cl_2$ Cu]	739	Brown	56.42 (58.45)	2.45 (2.70)	7.32 (7.57)
Co(II) binary complex $[C_{36}H_{20}N_4O_2S_2Cl_2$ Cu]	734	Brown	56.35 (58.85)	2.34 (2.72)	7.41 (7.63)
Zn(II) binary complex $[C_{36}H_{20}N_4O_2S_2Cl_2$ Cu]	740	White	55.36 (58.38)	2.26 (2.76)	7.16 (7.57)

Table 2: Mass and Molar conductance values of the compounds

Compound	Calculated mass(m/z)	Obtained mass(m/z)	Conductance
Ligand[$C_{18}H_{11}N_2OSCl$]	338.5	339 [M^+]	-
[$Cu(L)_2$]	739	740 [M+1]	10.3
[$Co(L)_2$]	734	734 [M^+]	8.9
[$Zn(L)_2$]	740	742 [M+2]	9.5

Table 3: Zone of inhibition in (mm) [concentration (100 µg)]

Compound	Psuedomonas Putida (Pp)	Escherichia coli (E.coli.)	Staphylococcus Aures(Sa)	Bacillus subtilis (Bs)
Cu(II)binary complex	8	7	4.5	6.5
Co(II)binary complex	6	7.5	7	6
Zn(II)binary complex	5	4.5	4	3

Table 4:

S.No.	COMPOUND	IC_{50} values (µM)	
		HeLa	MCF-7
1	Schiff base ligand	16.87	76.18
2	Cu(II) binary complex	7.59	73.30
3	Co(II) binary complex	21.13	156.3
4	Zn(II) binary complex	15.23	80.17

Table 5: Dock score of Ligand

	Ligand
	Dock score (K cal/mol)
Staphylococcus aureus **(pdb id: 3HUN)**	-6.007
E.coli **(pdb.id-2EXB)**	-4.445
Homo sapiens **(pdb.id-2XNB)**	-8.186

REFERENCES

1. Messori L, Casini A, Vullo D, Haroutiunian SG, Dalian EB and Orioli P. Inorg Chim Acta. 2000;303:283.
2. Wong E and Giadomenico CM. Chem Rev. 1999;99:2451.
3. Galanski M, Jakupec MA and Keppler BK. Curr Med Chem. 2005;12:2075.
4. Dyson J and Sava G. Dalton Trans. 2006;1929.
5. Clark MJ. Coord Chem Rev. 2003;236:209.
6. Wagenknecht HA, Stemp EDA and Barton JK. J Am Chem Soc. 2000;122:1.
7. Rosenberg B, VanCamp L, Trosko JE and Mansour VH. Nature. 1969;222:385.
8. Abd El-halim HF, Omar MM and Mohamed GG. Spectrochim Acta A. 2011;78:36.
9. Ravoof TBSA, Crouse KA, Tahir MIM, How FNF, Rosli R and Watkins DJ. Trans Met Chem. 2010;35:871.
10. Priya NP, Arunachalam S, Manimaran A, Muthupriya D and Jayabalakrishnan C. Spectrochim Acta A. 2009;72:6706.
11. Racane L. European Journal of Medicinal Chemistry. 2012;55:108e116.
12. Patel and Shaikh. Journal of Sciences, Islamic Republic of Iran. 2010;21(2):121-129.
13. Nadeem Siddiqui. Acta Chim Slov. 2009;56:462–469.
14. Navin Patel. Journal of Enzyme Inhibition and Medicinal Chemistry. 2011;26(4):527–534.
15. Himaja M. International research journal of pharmacy. 2011;2(1):114-117.
16. Kuntal Hazra. Der Chemica Sinica. 2011;2(2):149-157.
17. Abhay Kumar Verma. Indian J Pharm Biol Res. 2014;2(3):84-89.
18. Filomena Corbo. European Journal of Medicinal Chemistry. 2013;64:357–364.
19. Muttu CT. International journal of research in ayurveda and pharmacy. 2010;1(2):522–528.
20. Carole Di Giorgio. Antimicrobial Agents and Chemotherapy. 2002;46:2588–2594.
21. Arun Pareek. Scholars Research Library, Der Pharma Chemica. 2010;2(5):281-293.
22. Mona A and Mahran. Molecules. 2007;12:622-633.
23. Mohammad Shahar Yar. Acta Poloniae Pharmaceutica n Drug Research. 2009;66:387-392.
24. Suter H, Zutter H. Helv Chim Acta. 1967;50:1084.
25. Hays SJ, Rice MJ, Ortwine DF, Johnson G, Schwartz RD, Boyd DK, Copeland LF, Vartanian MG and Boxer PA. J Pharm Sci. 1994;83:1425.
26. Jimonet P, Audiau F, Barreau M, Blanchard JC, Boireau A, Bour Y, Coleno MA, Doble A, Doerflinger G, Huu CD, Donat MH, Duchesne JM, Ganil P, Gueremy C, Honore E, Just B, Kerphirique,R.; Gontier,S.; Hubert,P.; Laduron,P.M.; Le Blevec,J.; Meunier M, Miquet JM, Nemecek C, Pasquet M, Piot O, Pratt J, Rataud J, Reibaud M, Stutzmann JM and Mignani S. J Med Chem.1999;42:2828.
27. He Y, Benz A, Fu T, Wang M, Covey DF, Zorumski CF and Mennick S. Neuropharmacology. 2002;42:199.
28. Sawhney SN, Arora SK, Singh JV, Bansal OP and Singh SP. Indian J Chem. 1978;16B:605.
29. Bensimon G, Lacomblez L and Meininger V. New Engl J Med. 1994;330:585.
30. Foscolos G, Tsatsas G, Champagnac A and Pommier M. Ann Pharm Fr. 1977;35:295.
31. Shirke Bobade VG, Bhamaria RP, Khadse BG and Sengupta SR. Indian Drugs.1990;27(6): 350.
32. Paget CJ, Kisner K, Stone RL and Delong DC. J Med Chem.1969;12:1016.
33. (a)McDonnell ME, Vera MD, Blass BE, Pelletier JC, King RC, Fernandez-Metzler C, Smith GR, Wrobel J, Chen S, Wall BA and Reitz AB. Bioorg Med Chem. 2012;20:5642. (b) Cheah BC,

Vucic S, Krishnan AV and Kiernan MC. Curr Med Chem. 2010;17:1942.
34. Massari S, Daelemans D, Barreca ML, Knezevich A, Sabatini S, Cecchetti V, Marcello A, Pannecouque C and Tabarrini O. J Med Chem. 2010;53:641.
35. (a) Van Heusden J, Van Ginckel R, Bruwiere H, Moelans P, Janssen B, Floren W, van der Leede BJ, van Dun J, Sanz G, Venet M, Dillen L, Van Hove C, Willemsens G, Janicot M and Wouters W. Br J Cancer. 2002;86:605. (b) Aelterman W, Lang Y, Willemsens B, Vervest I, Leurs S and De Knaep F. Org Process Res Dev. 2001;5:467.
36. Devi GS, Muthu AK, Kumar DS, Rekha S, Indhumathi R and Nandhini R. International Journal of Drug Development and Research. 2013;1:105–109.
37. Monks A. J Natl Cancer Inst. 1991;83:757–766.
38. Vikas N, Savitha N, Varun Sood, Prasad K, Gayathri A and Gopal B. Molecular Basis for the Role of Staphylococcus aureus Penicillin Binding Protein 4 in Antimicrobial Resistance. Journal of Bacteriology. 2010;192:134–144.
39. Crystal Structure of Penicillin Binding Protein 4 (dacB) from Escherichia coli, both in the Native Form and Covalently Linked to Various Antibiotics. Hiroyuki Kishida, Satoru Unzai, David I Roper, Adrian Lloyd, Sam-Yong Park and Jeremy RH. Tame, Biochemistry. 2006; 45:783-792.
40. Discovery and Charecterization of 2-Anilino-4-(Thiazol-5-yl) Pyrimidine Transcriptional CDK Inhibitors as Anticancer Agents. David Blake G and Peter Fischer M. Chemistry & Biology. 2010;17:1111–1121.
41. Schrödinger LLC Glide. Version 4.0. New York, NY2005.
42. Friesner RA. Glide, a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy, J Med Chem. 2004;47:1739–1749.
43. Sharma RK, Singh RV and Tandon JPJ. Inorg Nucl Chem.1980;42:1382.
44. Lever ABP. Inorganic Electronic Spectroscopy, second ed., Elsevier, New York,1968.
45. Joseph J, Nagashri K and Janaki GB. Eur. J Med Chem. 2012;49:151–163.
46. (a) Tarafdera MTH, Kasbollah A, Crouse KA, Ali AM, Yamin BM and Fun HK. Polyhedron. 2001;20:2363–2370. (b) Arjmand F and Muddassir M. Chirality. 2011;23:250–259.
47. Ramesh R and Sivagamasundari M. Synth React Inorg Met-Org Chem. 2003;33:899.
48. Maurya RC and Rajput S. Oxovanadium(IV) complexes of bioinorganic and medicinal relevance: synthesis, characterization and 3D molecular modeling and analysis of some oxovanadium(IV) complexes involving the O, N-donor environment of pyrazolone-based sulfa drug Schiff bases. Journal of Molecular Structure. 2004;687:35.
49. Geary WJ. Coord Chem Rev. 1971;7:81–122.
50. Emara AAA. Spectrochim Acta A. 2010;77:117–125.
51. Prativel G, Pitie M, Bernadou J and Meunier B. Angew Chem Int Ed Eng.1991;30:702–704.