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Synthesis, Characterisation, biological activity and Docking studies of ternary metal complexes of Cu(II) and Co(II with 4-chloro-2-(2-Hydroxy) Naphthylidene Amino Benzothiazole Schiff base and Glycine ligands

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Abstract

The ternary complexes of Cu(II), Co(II) containing 4-chloro-(2-hydroxy)Naphthylidene aminobenzothiazole Schiff base and Glycine ligandwere 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 are no coordinated water molecules in the complexes. The binding mode of the Schiff base ligand to metal ions through azomethine nitrogen, oxygen of aldehyde and N, O donors of the glycine ligand was confirmed through the absorption bands appeared in the IR spectrum. Anticancer activity of the compounds revealed that Schiff base ligand and its Cu(II), Co(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) ternary complexes. 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: Ternary metal complexes, IR, SEM, Antibacterial, DNA cleavage anticancer activity.

1. 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 (Messori et al. 2000); (Wong et al. 1999); (Galanski et al. 20005); (Dyson et al. 2006); (Clark, 2003); (Wagenknecht et al. 2000). 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 (Rosenberg et al. 1969). 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 (Abd El-halim et al. 2011); (Priya et al. 2009); (Ravoof et al. 2010). 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 (Racane et al. 2012), antimicrobial (Patel & Shaikh, 2010) anticonvulsant (Nadeem et al. 2009), antitubercular (Navin et al. 2011), anthelminthic (Himaja et al. 2011), antioxidant (Kuntal Hazra et al. 2011), analgesic (Verma et al. 2014), antifungal (Filomena Corbo et al. 2013), anti-inflammatory (Muttu et al. 2010), antileishmanial (Carole Di Giorgio et al. 2002), antiulcer (Pareek et al. 2010), schistosomicidal (Mona & Mahran et al. 2007) and diuretic (Shahar Yar et al. 2009) activities. The importance of 2-amino benzothiazoles in Medicinal and Bioorganic chemistry with applications in drug discovery and development for the treatment of diabetes (Suter & Zutter, 1967), epilepsy (Hays et al. 1994); (Jimonet et al. 1999); (He et al. 2002), inflammation (Sawhney at al. 1978), amytropic Lateral sclerosis (Bensimon et al. 1994), analgesia (Foscolos et al. 1977) , tuberculosis (Shirke et al. 1990) and viral infections (Paget et al. 1969) make them previlaged scaffold in drug discovery. For example, in the treatement of amyotrophic Lateral sclerosis (McDonnell et al. 2010) 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 (Massari et al. 2010); (Van Heusden et al. 2002); (Aelterman et al. 2001).

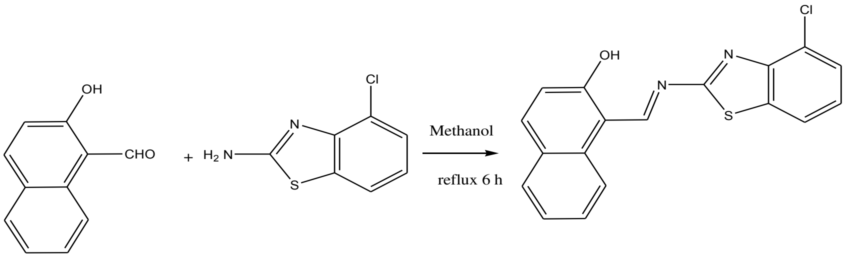
In view of the importance of 2-aminobenzothiazole, we report the synthesis, characterisation and biological studies of ternary metal complexes of Cu(II) and Co(II) containing 4-chloro-2-(2-hydroxy) Naphthylidene aminobenzothiazole Schiff base and glycine ligands. Anticancer activity of the Schiff base 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.

2. 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)

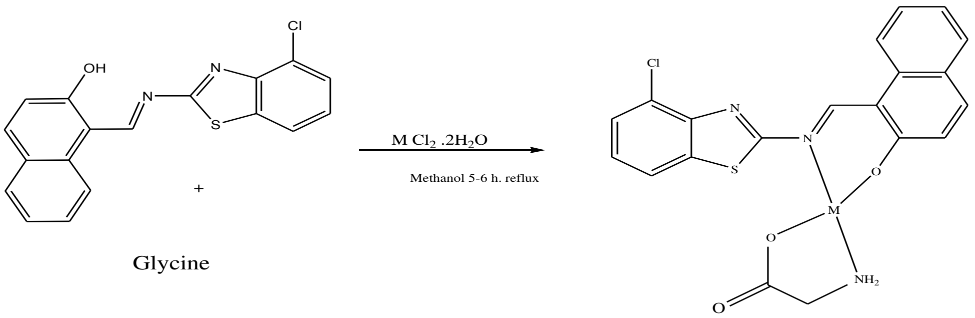
The Schiff base ligand 4-chloro-2-(2-Hydroxy) Naphthylidene Amino Benzothiazole (L) was obtained from the condensation reaction of 2-hydroxy-1-naphthaldehyde (1.72g. 10mmol) and 2-amino benzothiazole (1.5g. 10mmol) in methanol for reflux for 6 h.



Scheme 1. Formation Schiff base ligand (L)

Synthesis of 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 and 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. In this Schiff base, metal salts and glycine were dissolved in 1:1:1 ratio.



M= Cu, Co

(proposed structure of ternary metal complexes)

Scheme 2. Formation of ternary 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 were 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 was determined. The SEM/EDX images were obtained from a Hitachi SEM analyser.

3. 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 is 1:1:1 for ternary complexes (Schiff base ligand: metal: glycine).

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) ternary complex [$C_{20}H_{14}N_3O_3SCl$ Cu]	475	Green	49.46 (50.52)	2.51 (2.94)	7.96 (8.84)
Co(II) ternary complex [$C_{20}H_{14}N_3O_3SCl$ Co]	470.5	Thick brown	49.32 (51.01)	2.49 (2.97)	8.16 (8.93)

^{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).

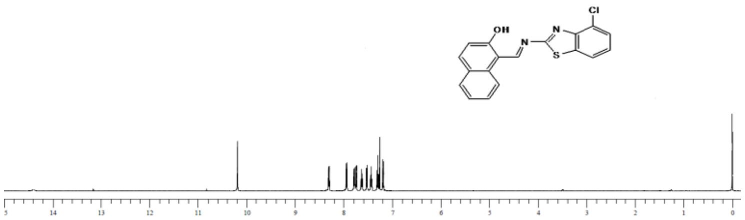


Fig. 1 ^{1}H NMR spectrum of Schiff base ligand

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 (Sharma et al. 1980). The absorption band at 694nm region for the Co(II) ternary complex suggesting tetrahedral environment. The magnetic moment value of the ternary Co(II) complex was found to be 4.41BM. Which also pin pointed the tetrahedral geometry for the complex (Lever et al. 1967). The absorption bands at 592nm appeared in the UV-Vis spetra of the Cu(II) ternary complex which is due to the transistion [$^2B_{1g} \longrightarrow {}^2A_{1g}$] (Joseph et al. 2012). This is characteristic of square planar environment. The square planar geometry was proposed for ternary Cu(II) complex, based on their magnetic moment value which was found to be 1.78BM.

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} $\upsilon_{(O\text{-}H)}$, 1597cm^{-1} $\upsilon_{(C=N\ \text{azomethine})}$,1620cm^{-1} $\upsilon_{(C=N\ \text{thiazole ring})}$ and 677-680cm^{-1}$\upsilon_{(C\text{-}S\text{-}C)}$.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 (Ramesh et al. 2003). 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. Additionally, in the Cu(II),Co(II) ternary complexes the antisymmetric and symmetric stretching COO$^-$ frequencies of free aminoacid observed at 1660cm^{-1} and 1430cm^{-1} were shifted to a lower frequency 1537-1550cm^{-1} and 1360-1415cm^{-1} respectively indicating that the terminal coordination mode of carboxylate group of the aminoacid to metal ion is via deprotonation (Yin et al. 2004).The bands at 1620cm^{-1} $\upsilon_{(C=N\ \text{thiazole ring})}$ 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) (Maurya et al. 2004).

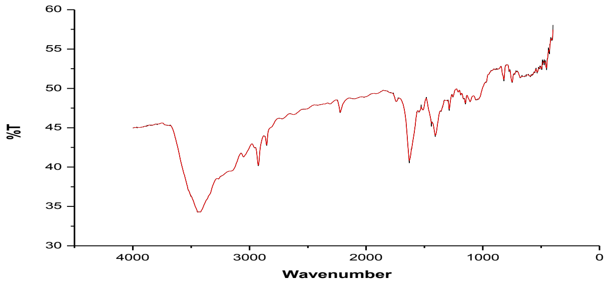


Fig.2 IR spectrum of Cu(II) ternary complex

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. The ternary Cu(II) and Co(II) complexes do not any weight loss up to 300^{0}C but between 300-400^{0}C there is a significant weight loss occurred, which may be due to the removal of chlorine atoms as HCl gas, carbon, sulphur and nitrogen in the organic moiety 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. The TGA of Cu(II) ternary complex has shown in Fig.3

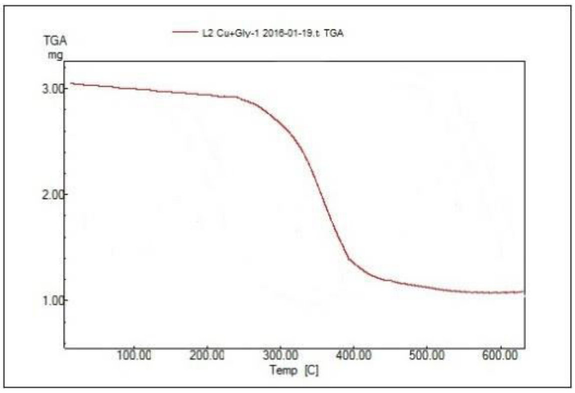


Fig.3 TGA of Cu(II) ternary complex

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) ternary complex has shown agglomerate and flakes like structures. The composition of the elements present in the compounds were obtained from the EDX analysis

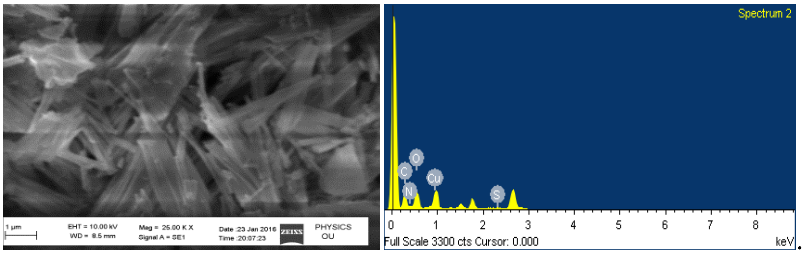


Fig. 4 SEM and EDX images of Cu(II) ternary complex

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 metal complexes were shown in Table. The low molar conductance values of the metal complexes disclosed that the metal complexes were non-electrolytic in nature (Geary et al. 1971).

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)(gly)]	475	498 [M+3]	12.2
[Co(L)(gly)]	470.5	471 [M^+]	10.8

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 (Emaraet al. 2010). In the present work, ternary metal complexes shows more activity on gram negative strain **E. coli** and gram positive strain **S. aures**. Finally 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

Compound	**Psuedomonas putida(Pp)**	**Escherichia coli (E.coli.)**	**Staphylococcus Aures(Sa)**	**Bacillus subtilis (Bs)**
Cu(II)ternary complex	8	11	12	9
Co(II)ternary complex	7	10	11	7
Standard	8	8	8	8

Fig.5 Cu(II) ternary complex

Fig.6 Co(II) ternary complex

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), Co(II) binary and ternary 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 anticipates the release of specific residues arising from transformed sugars, depending on the position from which hydrogen atom removed (Prativel et al. 1991).

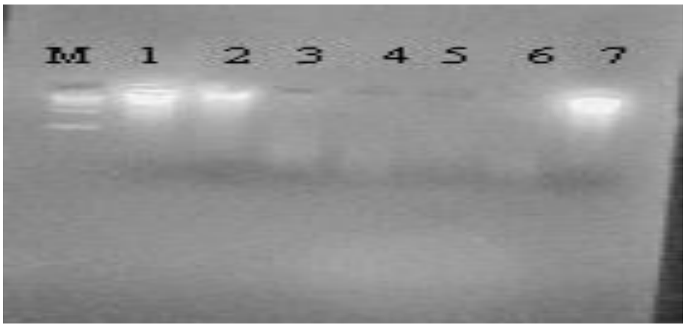


Fig:7 DNA cleavage activity of free ligand and its ternary metal complexes

[M] Marker [1] Control(CT-DNA)+H_2O_2 [2] Ligand+DNA+ H_2O_2 [5] Cu(II) ternary complex+DNA+ H_2O_2 [6] Co(II) ternary 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 its metal 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. The higher the IC_{50} values of the Cu(II) ternary, Co(II) ternary complexes than free ligand suggested that these complexes have lesser antiproliferative activity than the ligand. The Cu(II), Co(II) ternary complexes have shown greater antitumour activity than free ligand against MCF-7 cell line.

Table:4

S.No.	COMPOUND	IC$_{50}$ values (μM)	
		HeLa	MCF-7
1	Schiff base ligand	16.87	76.18
2	Cu(II) ternary complex	51.0	18.68
3	Co(II) ternary complex	70.4	38.11

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.

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

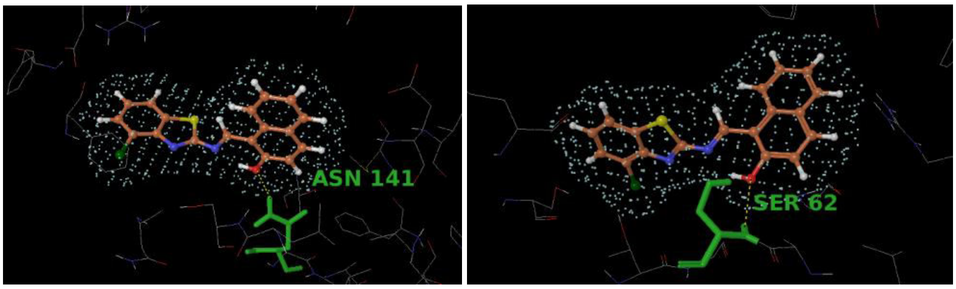


Fig.8 Docked pose of ligand in the protein active site showing the hydrogen bond interaction (yellow lines) with ASN 141 in Staphylococcus aureus (pdb.id-3HUN) and SER 62 *E.Coli* (pdb.id-2EXB)

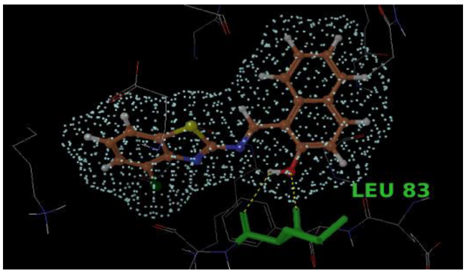


Fig.9 Docked pose of ligand in the protein active site showing the hydrogen bond interaction (yellow lines) with LEU 83 in Homo sapiens cyclin dependent kinase **(pdb.id-2XNB)**

References

1. Abd El-halim, H. F., Omar, M. M., & Mohamed G. G. (2011) Spectrochim. Acta A, 78, 36.
2. Arun Pareek et al., (2010) Scholars Research Library, Der Pharma Chemica, 2(5), 281-293.
3. Bensimon, G., Lacomblez, L., & Meininger, V. (1994). New Engl. J. Med. 330, 585.
4. Carole Di Giorgio et al., (2002). Antimicrobial Agents and Chemotherapy. 46, 2588–2594.
5. Clark, M. J. (2003) Coord. Chem. Rev, 236, 209.
6. Dyson, J., & Sava G. (2006). Dalton Trans, 1929.
7. Emara, A. A. A. et al., (2010). Spectrochim. Acta A. 77, 117–125.
8. Filomena Corbo et al., (2013). European Journal of Medicinal Chemistry. 64, 357 – 364.
9. Foscolos, G., Tsatsas, G., Champagnac, A., & Pommier, M. (1977). Ann. Pharm. Fr. 35, 295.
10. Geary, W. J. Et al., (1971). Coord. Chem. Rev. *7*, 81–122.
11. Galanski, M., Jakupec, M. A., & Keppler B. K. (2005). Curr. Med.Chem. 12, 2075.
12. Hays, S. J., Rice, M. J., Ortwine. D. F., Johnson, G., Schwartz, R. D., Boyd, D. K., Copeland, L. F., Vartanian, M. G., & Boxer, P. A., (1994). J. Pharm. Sci. 83, 1425.
13. He, Y., Benz, A., Fu, T., Wang, M., Covey, D. F., Zorumski, C. F., & Mennick, S.(2002) Neuropharmacology 42, 199.
14. Himaja, M. et al., (2011). International research journal of pharmacy. 2(1), 114-117.
15. Jimonet, P., Audiau, F., Barreau, M., Blanchard, J. C., Boireau, A., Y. Bour, Y., Coleno, M. A., Doble, A., Doerflinger, G., Huu, C. D., Donat, M. H., Duchesne, J. M., Ganil, P., Gueremy, C., Honore, E., Just, B., Kerphirique, R., Gontier, S., Hubert, P., Laduron, P. M., Le Blevec, J., Meunier, M., Miquet, J. M., Nemecek, C., Pasquet, M., Piot, O., Pratt, J., Rataud, J., Reibaud, M., Stutzmann, J. M., & Mignani, S.(1999). J. Med. Chem. 42, 2828.
16. Joseph, J., Nagashri, K., & Janaki, G. B. (2012). Eur. J. Med. Chem. 49,151–163.
17. Kuntal Hazra et al., (2011). Der Chemica Sinica. 2(2), 149-157.
18. Lever A. B. P. Et al., (1968). *Inorganic* Electronic Spectroscopy, second ed., Elsevier, New York.
19. Massari, S., Daelemans, D., Barreca, M. L., Knezevich, A., Sabatini, S., Cecchetti, V., Marcello, A., Pannecouque, C., & Tabarrini, O. (2010). J. Med. Chem. 53, 641.
20. Maurya, R. C., 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."(2004). Journal of Molecular Structure. vol. 687, pp. 35.
21. (a)McDonnell, M. E., Vera, M. D., Blass, B. E., Pelletier, J. C., King, R. C., Fernandez-Metzler, C. Smith, G. R., Wrobel, J., Chen, S., Wall, B. A., & Reitz, A. B. Bioorg. Med. Chem. 20, 5642. (b) Cheah, B. C., Vucic, S., Krishnan, A. V., & Kiernan, M. C.(2010). Curr. Med. Chem.17, 1942.
22. Messori, L., Casini, A., Vullo, D., Haroutiunian, S.G., Dalian, E.B., & Orioli P. (2000). Inorg. Chim. Acta. 303,283.
23. Mohammad Shahar Yar et al., (2009) Acta Poloniae Pharmaceutical n Drug Research.66 387-392.
24. Mona A., & Mahran et al., (2007). Molecules. 12, 622-633.

25. Muttu, C. T. et al., (2010). International journal of research in ayurveda & pharmacy. 1(2), 522 – 528.
26. Nadeem Siddiqui et al., (2009). Acta Chim. Slov. 56, 462–469.
27. Navin Patel, B. et al., (2011). Journal of Enzyme Inhibition and Medicinal Chemistry. 26(4), 527–534.
28. Paget, C. J., Kisner, K., Stone, R. L., & Delong, D. C. (1969) J. Med. Chem.12, 1016.
29. Patel & Shaikh (2010). Journal of Sciences, Islamic Republic of Iran. 21(2)121-129.
30. Prativel, G., Pitie, M., Bernadou, J., & Meunier, B. (1991). Angew. Chem. Int. Ed. Eng. 30, 702–704.
31. Priya, N. P., Arunachalam, S., Manimaran, A., Muthupriya, D., & Jayabalakrishnan C. (2009). Spectrochim. Acta A. 72, 6706.
32. Rosenberg, B., VanCamp, L., Trosko J. E.,& Mansour V. H. (1969). *Nature*. 222, 385.
33. Ravoof, T. B. S. A., Crouse, K. A., Tahir, M. I. M., How, F. N. F., Rosli R., & Watkins D. J. (2010). Trans. Met. Chem, 35, 871.
34. Racane, L. et al., (2012). European Journal of Medicinal Chemistry. 55,108e116.
35. Ramesh, R., & Sivagamasundari, M. (2003). Synth. React. Inorg. Met-Org. Chem. 33, 899.
36. Sawhney, S. N., Arora, S. K., Singh, J. V., Bansal, O. P., & Singh, S. P. (1978). Indian J. Chem. 16B, 605.
37. Sharma, R. K., Singh R. V. et al. (1980). Tandon, Inorg. Nucl. Chem. 42, 1382.
38. Shirke Bobade, V. G., Bhamaria, R. P., Khadse, B. G., & Sengupta, S. R. (1990). Indian Drugs. 27(6), 350.
39. Suter, H., & Zutter H. (1967) Helv. Chim. Acta.50, 1084.
40. (a) Van Heusden, J., Van Ginckel, R., Bruwiere, H., Moelans, P., Janssen, B., Floren, W., van der Leede, B. J., van Dun, J., Sanz, G., Venet, M., Dillen, L., Van Hove, C., Willemsens, G., Janicot, M., & Wouters, W. (2002). Br. J. Cancer. 86, 605. (b) Aelterman, W., Lang, Y., Willemsens, B., Vervest, I., Leurs, S., & De Knaep, F., (2001). Org. Process Res. Dev. 5, 467.
41. Verma, A. K. et al., (2014). Indian J.Pharm.Biol.Res. 2(3), 84-89.
42. Wong, E., & Giadomenico C. M. (1999). Chem.Rev, 99, 2451.
43. Wagenknecht, H. A., Stemp, E. D. A., & Barton J. K. (2000). J. Am. Chem. Soc, 122, 1.
44. Yin, H. D., Wang, Q. B., & Xue, S. C. (2004). J. Organmet. Chem. 689, 2480–2485.