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RESEARCH ARTICLE

Synthesis and Identification of New Macrocyclic Ligand and Study its Pharmaceutical Properties as Anti-bacterial Reagent by Using Molecular Docking Study

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Abstract

The macrocyclic ligand (5, 7, 12, 14-tetraisobutyl-5, 12-dimethyl -1, 4, 8, 11-tetraazacyclotetradeca-7, 14-diene) was prepared by the reaction between ethylene diamine and 4-methyl pentan-2-one with the help of the perchloric acid as a precipitant reagent. It characterized by FTIR, ^{1}HNMR and UV –Visible techniques. The prepared macrocyclic ligand combine with protein by the application of molecular docking study and it compared with a well-known and standard drug which is gentamycin. The results indicated that the recorded compound is very effective against the toxic bacteria on its cell area and may be in the future become an effective treatment regent by conduct further studies.

Keywords: *Macrocyclic ligand, Molecular Docking, Gentamycin, Staphylococcus aureus.*

Introduction

Hetero cyclic compounds from typical tetrahedral, square planar and octahedral complexes with transition metals via coordination through N atoms and a strongly electron donating atom such as O and S atoms [1]many such complexes used as drugs such as cadmium complex with 4-(2-pyridyl azo) resorcinol reagent which is used as an anti-tumor drug [2] many of these reagents act as inhibitors of heavy metal ions in human body by preventing its absorption by the blood [3]The most interest inorganic chemistry of researchers, is the chemistry of the large ring compounds because these compounds are touch with the human and his biological processes which are within the body directly, for example be a site to link the metal protein inside the body (metal-protein) [4,5].

Minerals are one of the components of structure of living organisms which are in the form of complexes and we can be realized the importance of these compounds through the importance of iron in hemoglobin magnesium in chlorophyll and cobalt in vitamin B12 [6, 11], The preparation of a new complex of a large ring compound simulates biologically within the structure of the organism and must be highly stable and geometrical structures must be can selective during the selection of the metal through their collagen ability[12, 15]. These processes depend on the number, location and the type of species of the donor atoms, as well as the radius of the central atom [16, 18].The importance of macro cyclic ligands and their complexes used as antigens, anti-cancer, antibacterial and anti-inflammatory, reagent as well as it uses as herbicides and uses in various chemical industries [19, 21].One important and modern applications of macrocyclic ligand is the molecular docking study. This study utilizes a desktop calculator that can be used to detect it as drug or not this based on the structure selection and the binding of small molecules to desired target for example protein [22, 25].

To accomplish molecular docking study we must follow the following steps: The first step is to set the goal of the drug so that we can make one of the large parts is the target of the main goals enzymes or proteins or organizational elements The second step is to predict the shape of three-dimensional or can be determined by using high-precision methods such as electron microscopy or NMR

the three dimension shape of the target. The third step identification of the structure with the selected part (target) and assigns the resulting bonds through the fusion example of hydrogen bonds or Vander Falls forces [26]. The fourth step is to work on comparing the used or known drugs and the knowledge of the most successful and candidate and working on these compounds in the laboratory or in the body of the organism the end of the resultant date my it can be used clinically [27]. Staphylococcus aureus is a bacterial bacterium that was proposed in 1882 by the scientist Alexander Johnson and is in the organism's body static but appears when the skin is exposed to scratches [28, 29].

These bacteria are the main cause of infection in hospitals and are dangerous when complications occur and cause inflammations at the site where surgery is performed [30, 31]. And S. *ureus* is one of the most danger bacteria which is the responsible of inflammation of the bone marrow and bone tissue and it's painful and difficult to treat [32, 33].

Experimental Materials

All the used reagents were of analar R- grade water was doubly distilled and stored in glass stoppered flasks.

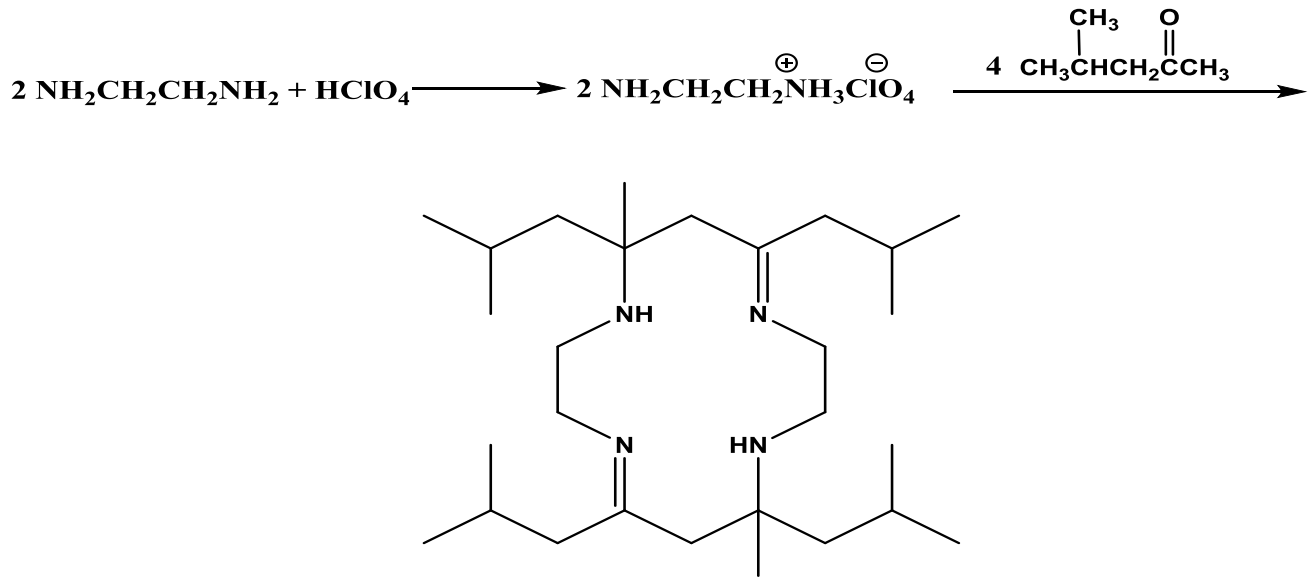
Spectral Measurement

The structure of prepared macrocyclic ligand was confirmed by means of many techniques FTIR, ^{1}H-NMR and U.V-Visible spectroscopy. FTIR spectrum was recorded for the ligand by using FTIR–84005 Shimadzu spectroscopy with KBr discs in the range (400-4000) cm^{-1}.UV-Visb.Spectrum was recorded for the aqueous solution of the ligand by using UV-1650 PPC Shimadzu spectroscopy with quartz cell. All these analysis were done at chemistry department labs of college of science at University of Babylon / Iraq except the HNMR which done at Tehran University.

Preparation of Ligand [34]

The reagent(5,7,12,14-tetraisobutyl-5,12-dimethyl-1,8, 11-tetraaza cyclo tetradeca-7,14-diene) was prepared by the reaction between (4-methyl 2-benthanone) and ethylene diamine in 60 % perchloric acid this solution stirred and during stirring the perchloric acid was added slowly for 30 min. them the solution was put in the ice bath until a brown color solution was obtained the solution was left 2-3 days at this situation and a yellow crystals was obtained filtered and washed with cold methanol and then we get a light yellow crystals, m.p.= 290c°.

The reagent was identified by FTIR, HNMR and UV. Visb. Spectroscopy its color is light yellow at a maximum wave length (350 nm) scheme 1 shows the steps of these reaction.



Scheme 1: Show the reaction to prepare the ligand under this study

Molecular Docking Study

Molecular docking was performed through several programs. The first one is to select the target which is a bacterial protein which is obtained from the Protein Data Bank (PDB) at the Research Collaborative for Structural Bioinformatics (http:// www. RCSB.org) [35].Then from of the target protein improved by a program (Molegro

Virtual Docker and Chimera 1.8.1) [36]. (Http: //www.rbvi.ucsf.edu/chimera) [37]. (Chem Draw) program used to draw the structures (http://chemistry.com.pk/software/ chemdraw -tra-12) which is made up of crystalline structures [38].The process of docking between the protein and the ligand was done by using PyRx software (ver. 0.8). Another program VINA WIZARD was used to give the thence, their graphical interface which had been analyzed with LigPlot + software [39, 40].

Results and Discussion

FTIR Data

The important vibrational bonds of the ligand as showed in Table (1) were as the following stretching frequency of N-H group was observed theat 3184 cm^{-1} and the stretching frequency of C=N group was observed at 1510 cm^{-1}. The carbonyl group of the ketone at 1650-1700 cm^{-1} was disappeared Figure (1) show the FTIR spectrum of the prepared ligand.

Table 1: **Important FTIR absorption bonds of the ligand**

Assignment (cm^{-1})	The ligand
υ (C-N)	1340
υ (C=N)	1510
υ (N-H)	3184
υ (C-C)	2991
υ (C-H)	3004

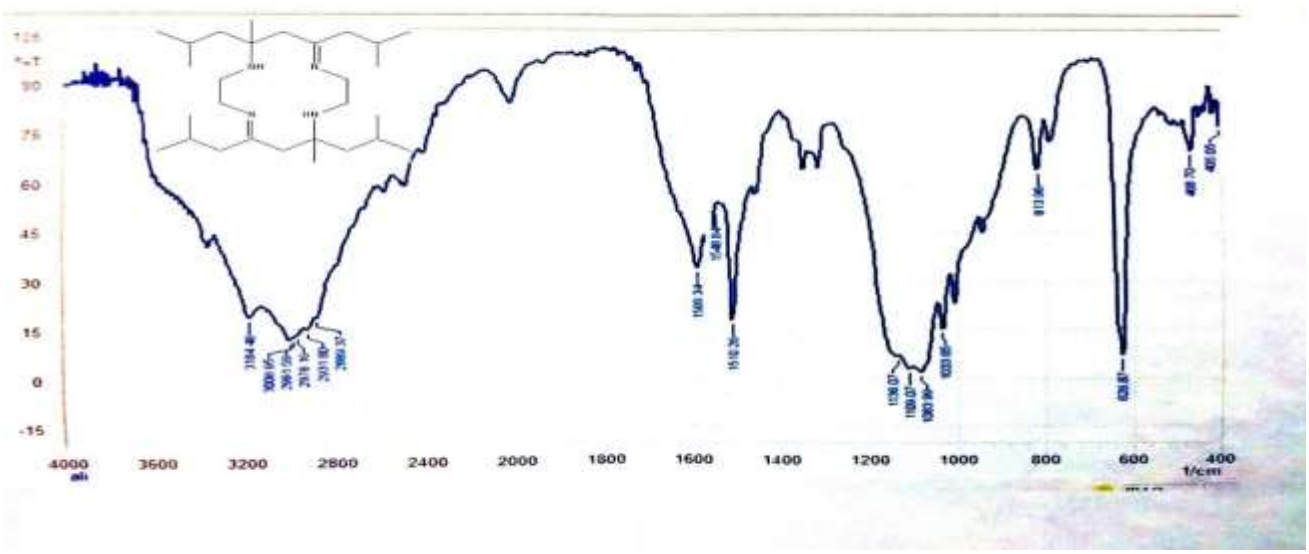


Figure 1: FT-IR spectrum of Ligand

UV-Visb. Data

The electronic spectrum of the reagent shows many bands as shown in Figure 2: λ max is (282, 350 nm).

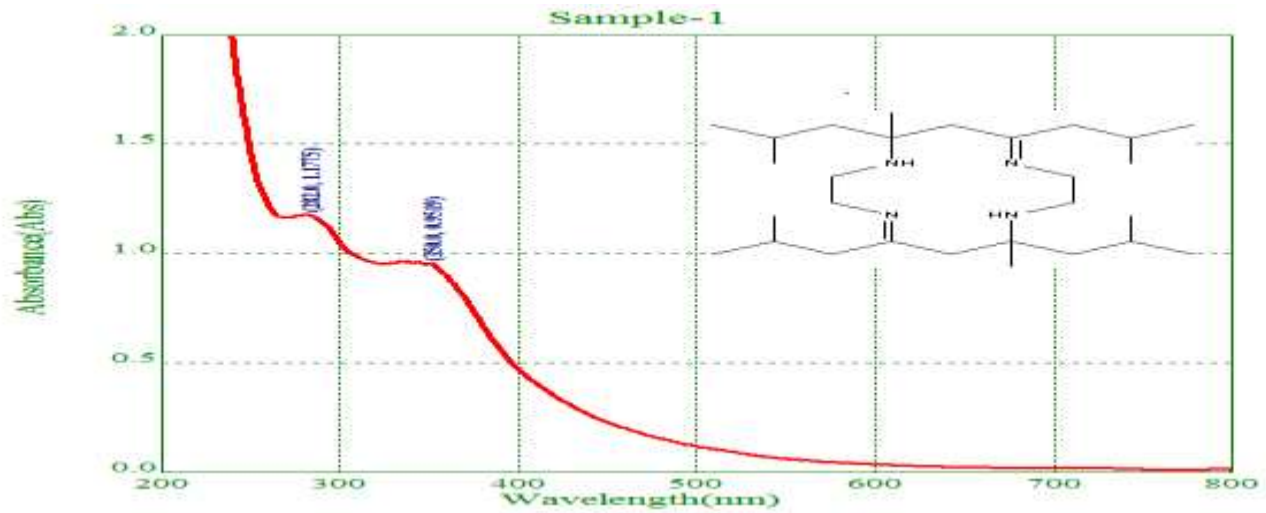


Figure 2: UV-Visb. Spectrum of ligand

^{1}H-NMR Data

^{1}HNMR spectrum shows chemical shift (δ ppm) as below the signals of eight CH_3 groups or two CH_3 groups of attached at sp3-hybridized carbon atom was equivalent and appeared as one singlet in the range of (0.9-1.3) ppm. Protons of the two CH_3 groups which bound with azomethine carbon resonated in weak field (2.0-2.4) ppm (Figure 2). The methylene protons of NH-CH_2 and =N-CH_2 groups in the ligand resonated as multiples in the regions of (2.5-3) and (3-4) ppm, respectively. The broadened signals corresponded to the protons of the two amino groups. Figure 3 show the HNMR spectrum of the ligand.

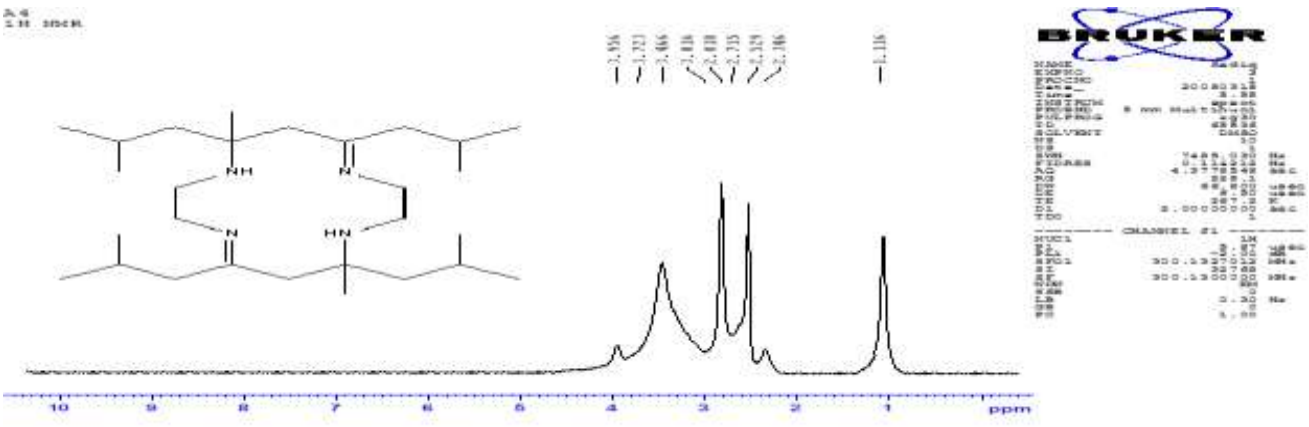


Figure 3: ^{1}HNMR spectrum of Ligand

Molecular Docking Study for the Ligand

This computational study was applied to the prepared ligand against the bacteria under this study for agents : fibrinogen–binding protein (Efb), hemolysin ß (HIß), enterotoxin E, iron-regulated surface determinant C, exofoliative toxin D, protein A, clumping factor A, the target PDB codes were: 3D5S, 3K55, 3WGL, 4LFD, 5C2Z, 5CBO and 5JQ6 of S. aureus. The results showed in table 2 which are expressed as binding affinity (Kcal/ mol.), root mean square deviation-upper bound (RMSD/UB**)** and root mean square deviation-lower bound (RMSD/LB) which are the average distance between the atoms Figures (4,17) show the interactions between the ligand and the proteins.

Molecular Docking Study for the Gentamicin Drug

This computational study was applied to gentamicin drug against the bacteria under this study for the same agents who were used for ligand. The target PDB codes were the same also which were used for ligand .The result showed in table 3 as had done with the ligand under studying Figures (18, 31) show the interactions between the gentamicin and the proteins under studying.

Table 2: the results of Molecular docking study for ligand

Target (PDB code)	Binding affinity (Kcal/mol)	RMSD/upper bound	RMSD/lower bound
3D5S	-6.5	7.64	3.282
3K55	-8.0	7.524	0.946
3WGL	-8.2	4.83	1.005
4LFD	-7.6	35.336	33.166
5C2Z	-6.4	15.608	21.031
5CBO	-7.9	7.366	1.319
5JQ6	-7.3	23.002	19.842

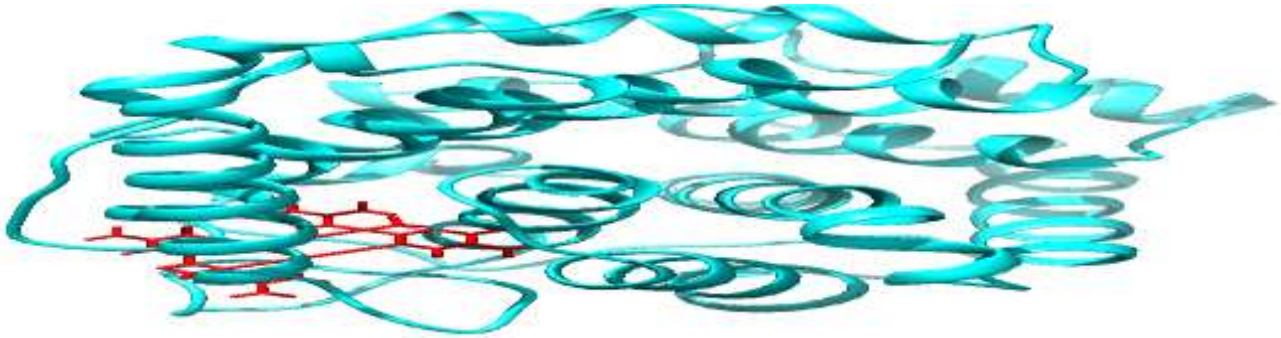


Figure 4: 3D5S-Ligand

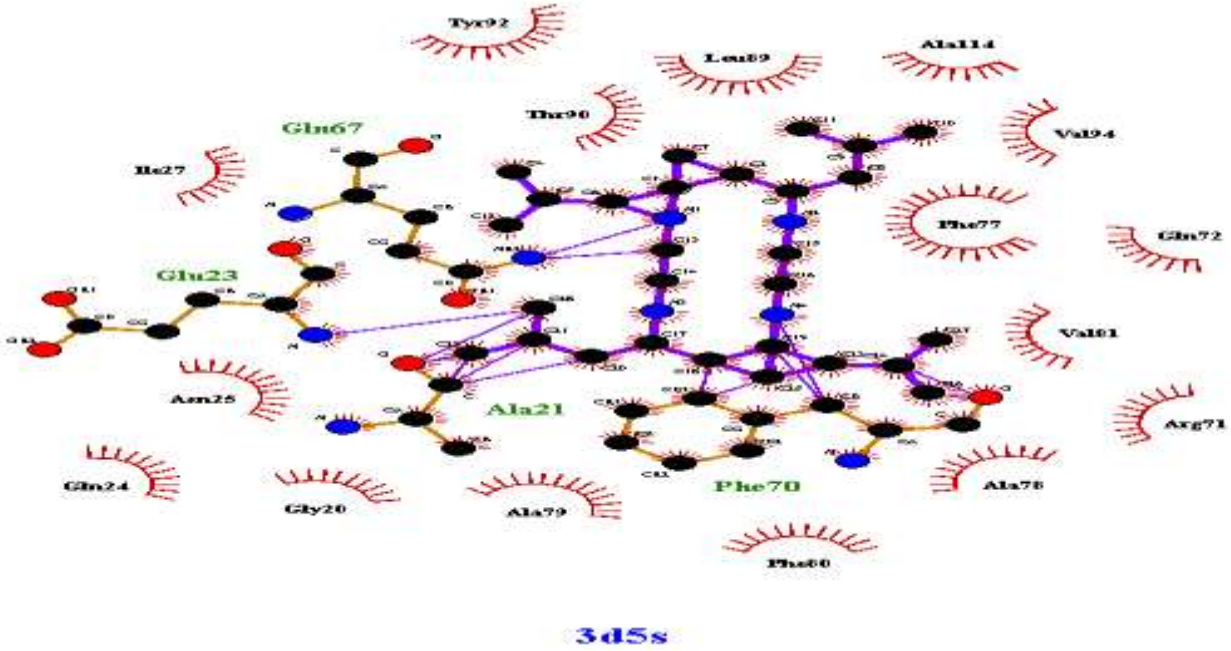


Figure 5: 3D5S-Ligand shows: non-ligand bond. Corresponding atoms and non-ligand residues Ala78, Arg71, Val81, Gln72, Phe77, Val94, Ala114, Leu89, Tyr92, Thr90, Ile27, Asn25, Gln24, Gly20, Ala79 and Phe80 involved in hydrophobic Interactions

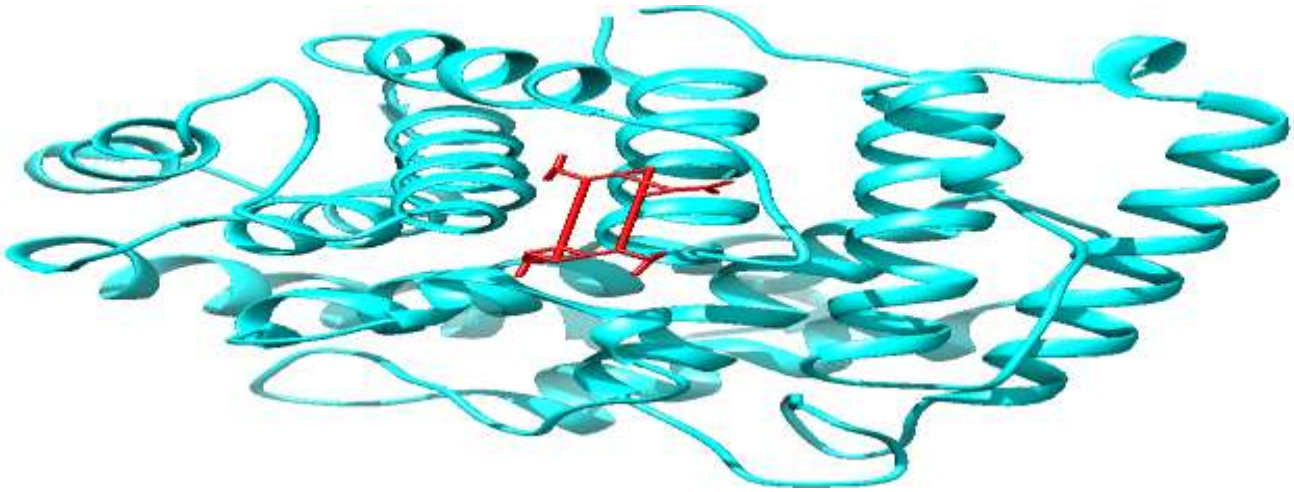


Figure 6: 3K 55-Ligands

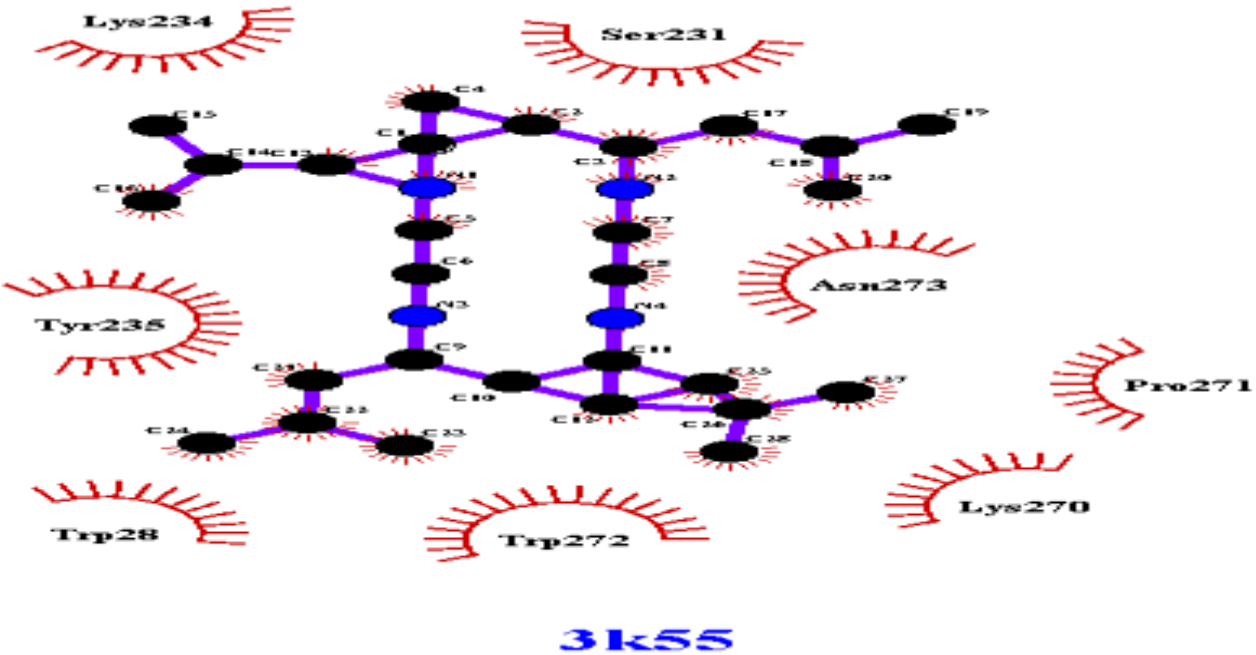


Figure 7: 3K55-Ligand shows: Corresponding atoms and non-ligand residues Ala78, Arg71, Val81, Gln72, Phe77, Val94, Ala114, Leu89, Tyr92, Thr90, Ile27, Asn25, Gln24, Gly20, Ala79 and Phe80 involved in hydrophobic interactions

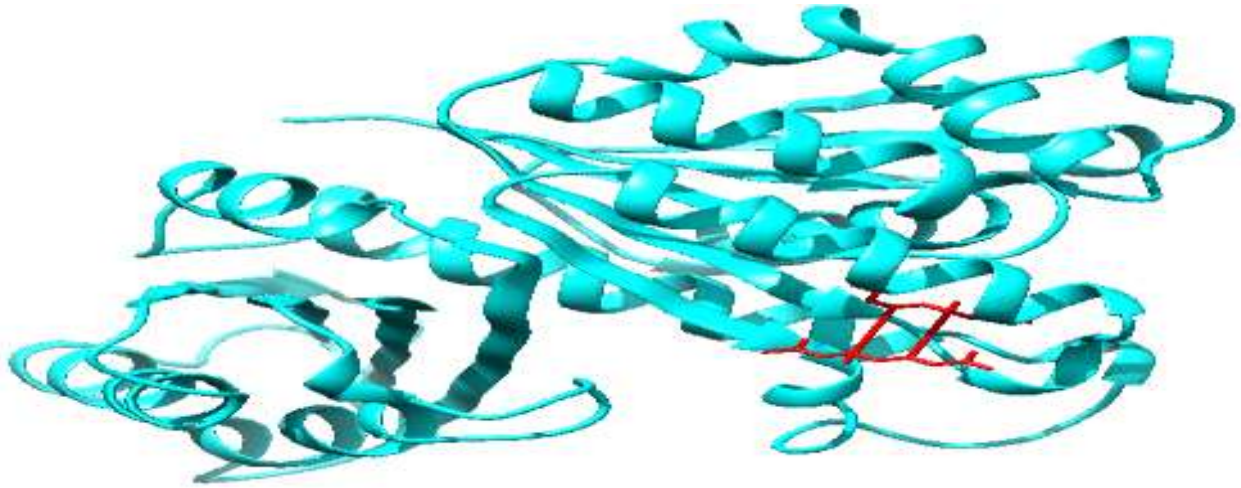


Figure 8: 3WGL- Ligand

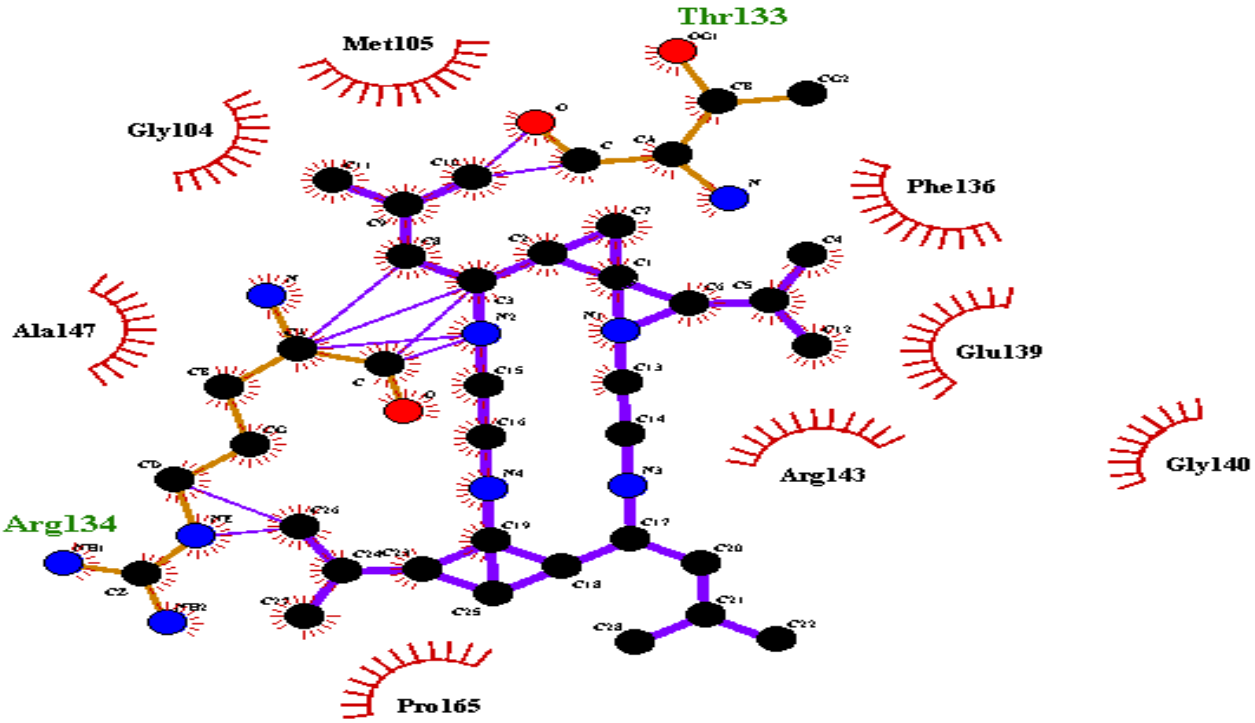


Figure 9: 3WGL-Ligand shows: non-ligand bond. Corresponding atoms and non- ligand residues Arg143, Gly140, Glu139, Phe136, Met105, Gly104, Ala147 and Pro165 interactions

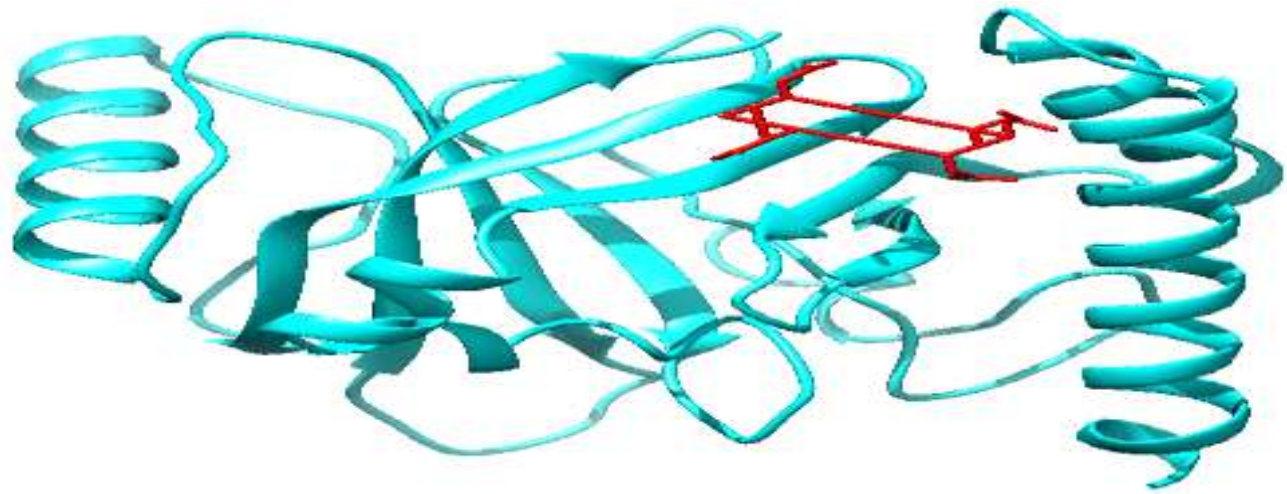


Figure 10: 4LFD- Ligand

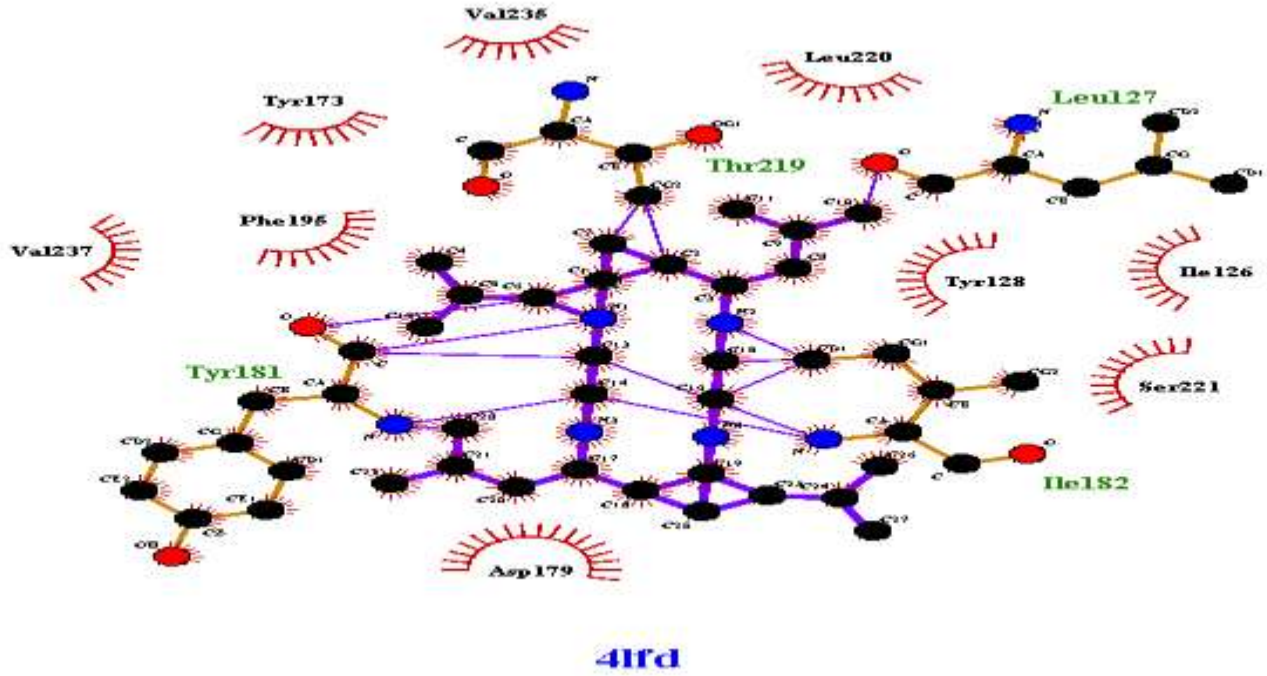


Figure 11: 4LFD- Ligand shows: non-ligand bond. Corresponding atoms and non-ligand residues Ser221, Ile126, Tyr128, Leu220, Val235, Tyr173, Phe195, Val237 and Asp179 interactions

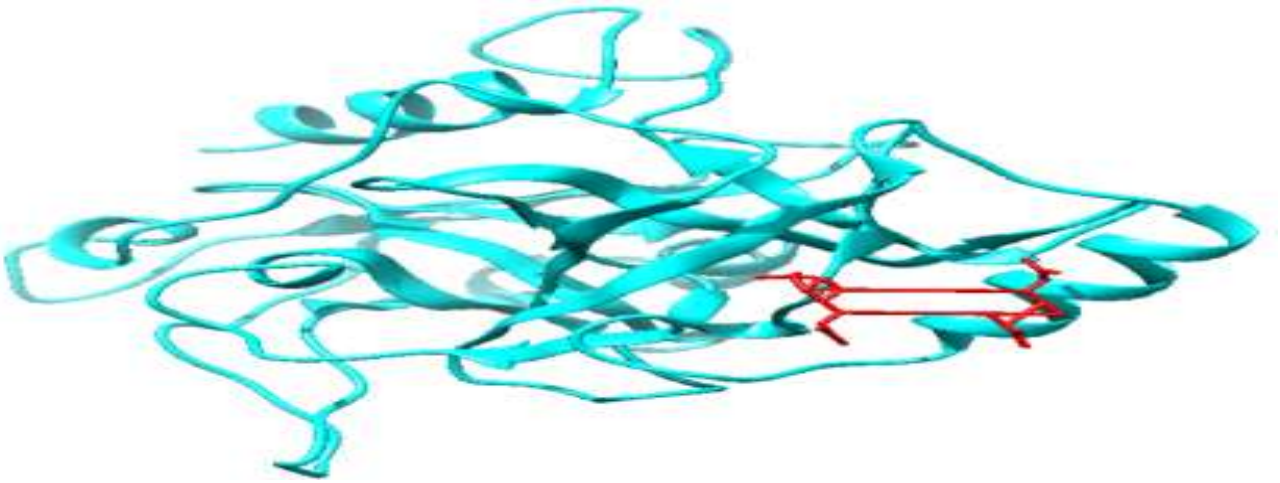


Figure 12: 5C2Z- Ligand

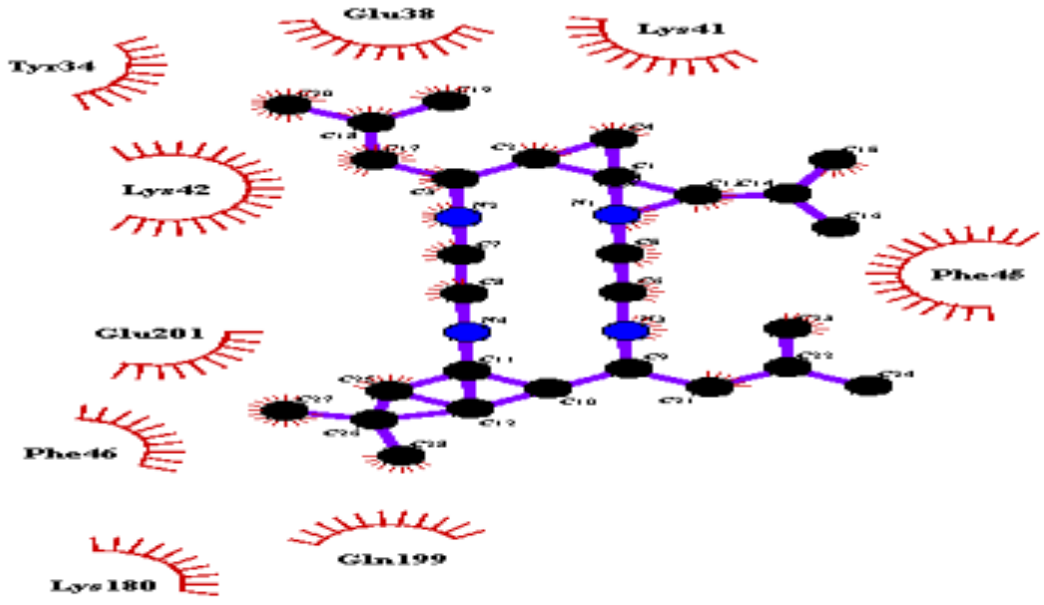


Figure 13: 5C2Z- Ligand shows: Corresponding atoms and non-ligand residues Phe45, Lys41, Glu38, Tyr34, Lys42, Glu201, Phe46, Lys180 and Gln199 interactions

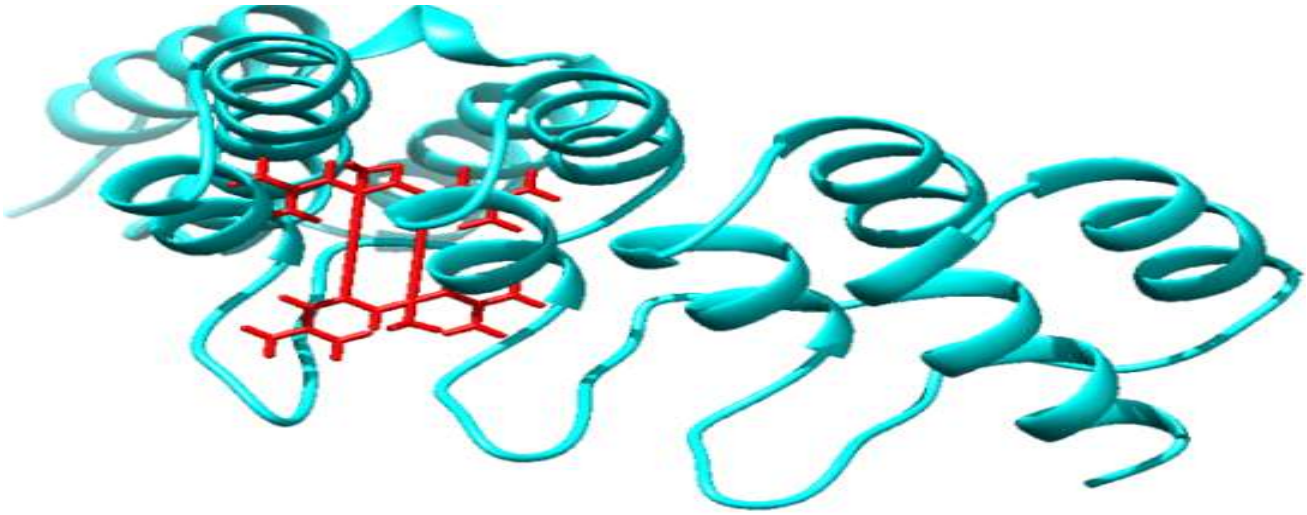


Figure 14: 5CBO- Ligand

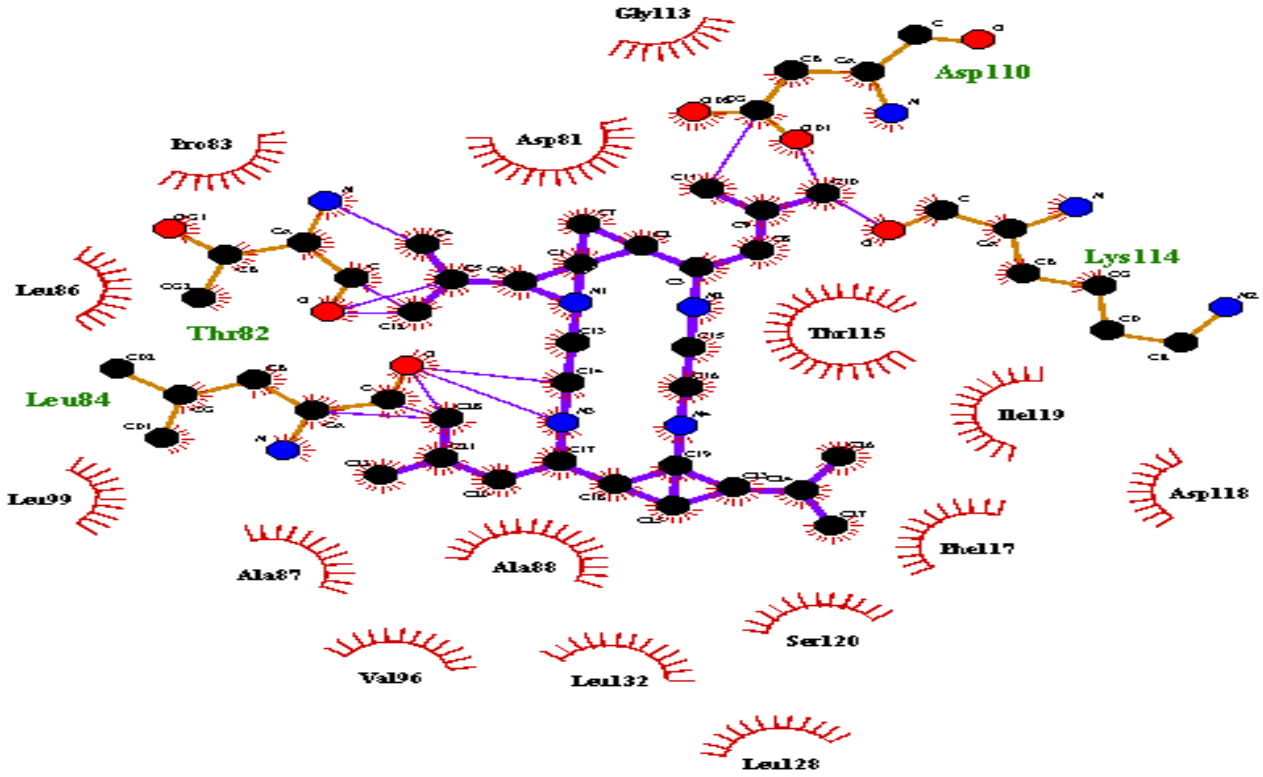


Figure 15: 5CBO- Ligand shows non-ligand bond. Corresponding atoms and non-ligand residues Leu128, Ser120, Phe117, Asp118, Ile119, Thr115, Gly113, Asp81, Pro83, Leu86, Leu99, Ala87, Ala88, Val96 and Leu132 interactions

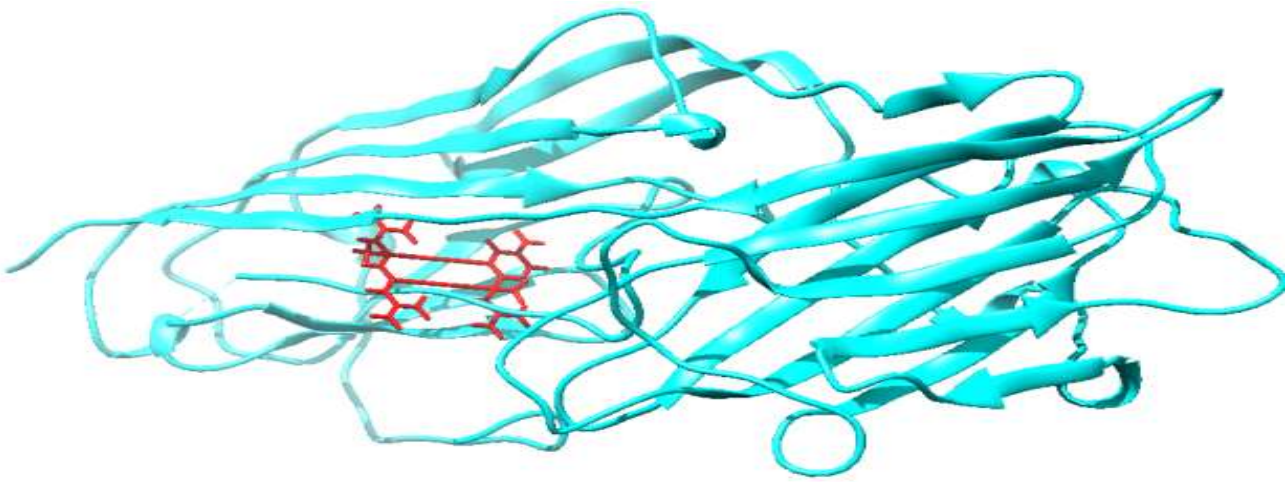


Figure 16: 5JQ6- Ligand

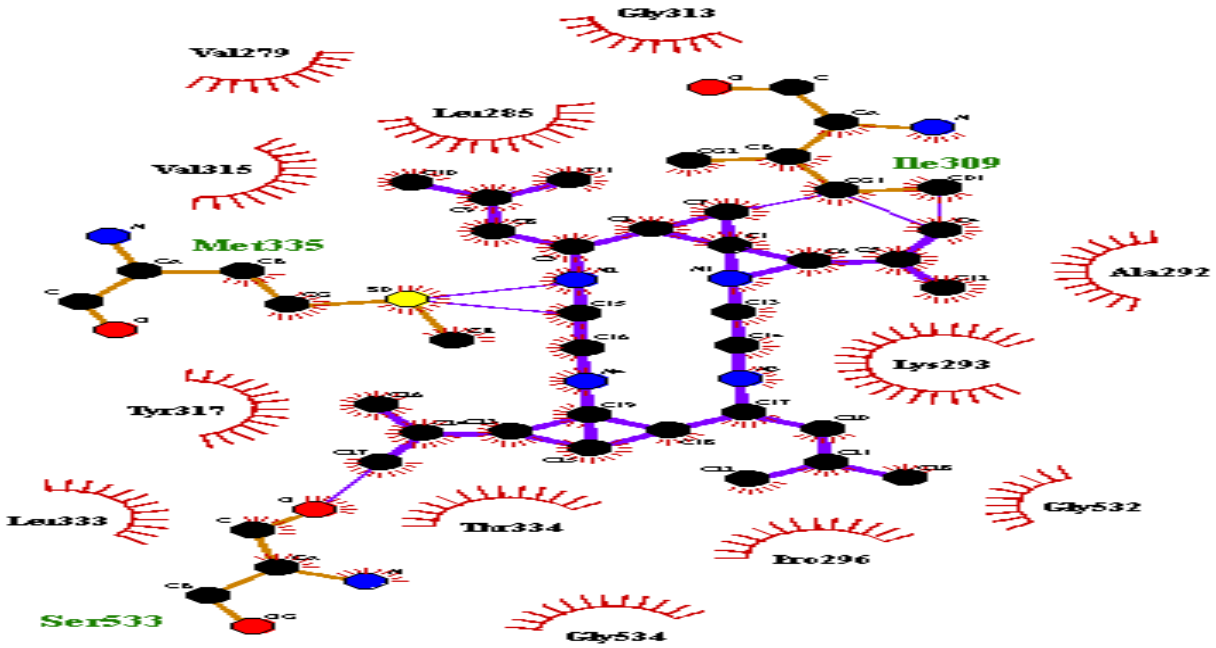


Figure 17: 5JQ6- Ligand shows: non-ligand bond. Corresponding atoms and non-ligand residues Thr334, Gly534, Pro296, Gly532, Lys293 Ala292, Gly313, Leu285, Val279, Val315, Tyr317, Leu333 interactions

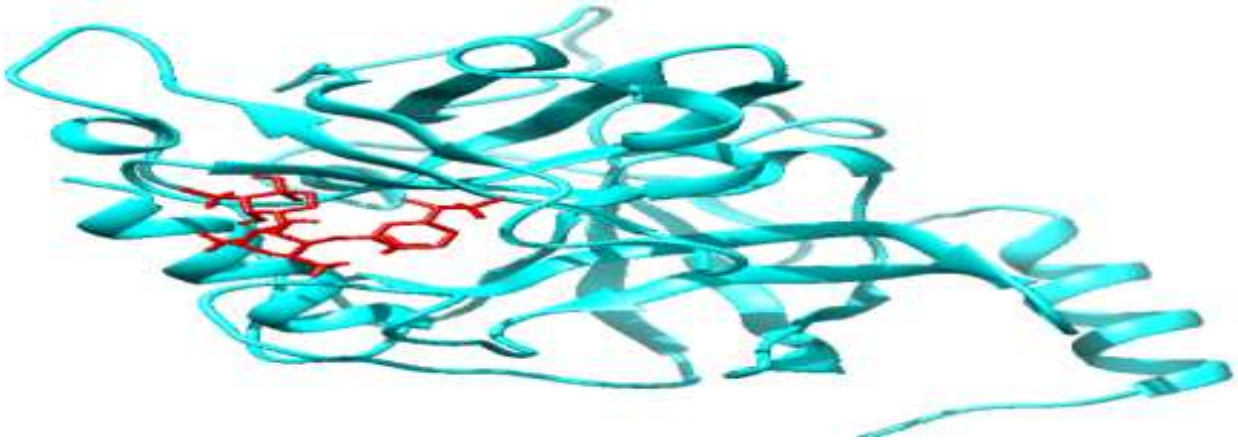


Figure 18: 5C2Z- gentamicin

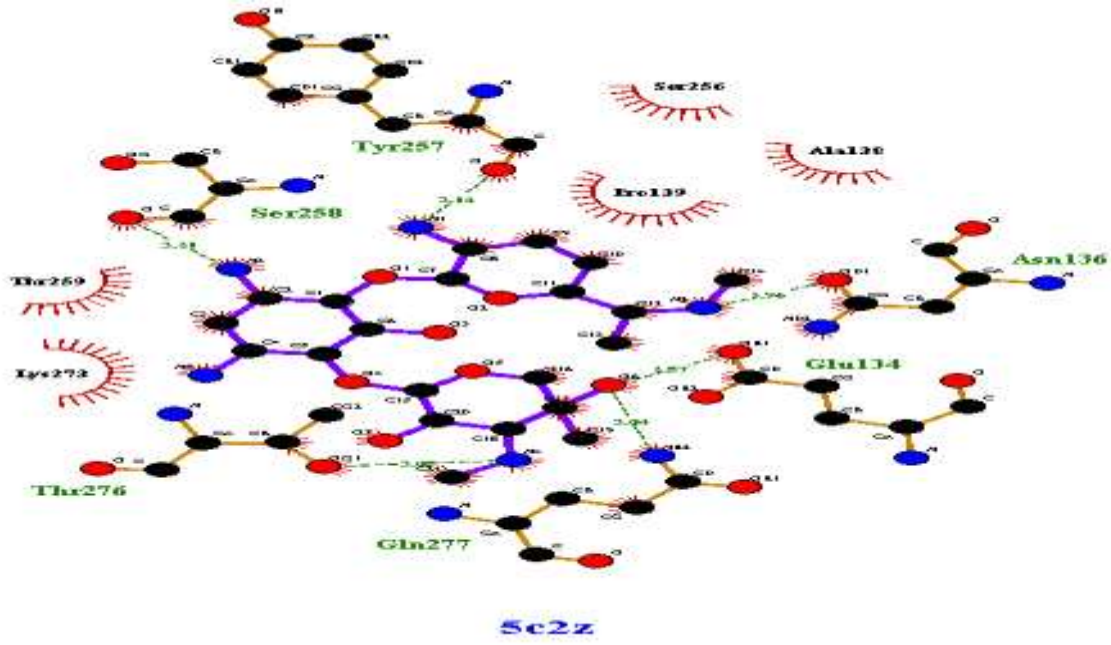


Figure 19: 5C2Z-gentamicin shows: non-ligand bond. Corresponding atoms and non-ligand residues Pro139, Ala138, Ser256, Thr259 and Lys273 interactions .Hydrogen bond between A and Glu134 and Asn136 and Tyr257 and Ser258 and Thr276 and Gln277 shown by green lines

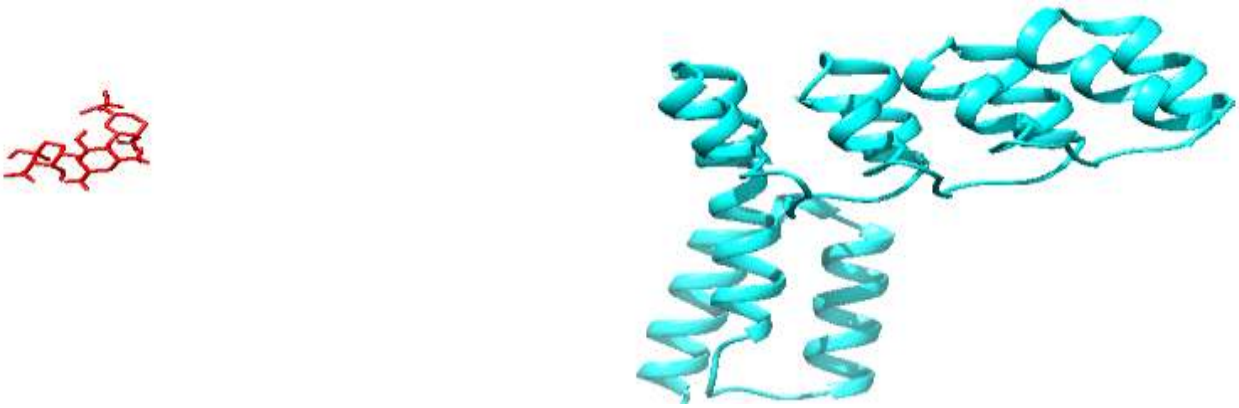
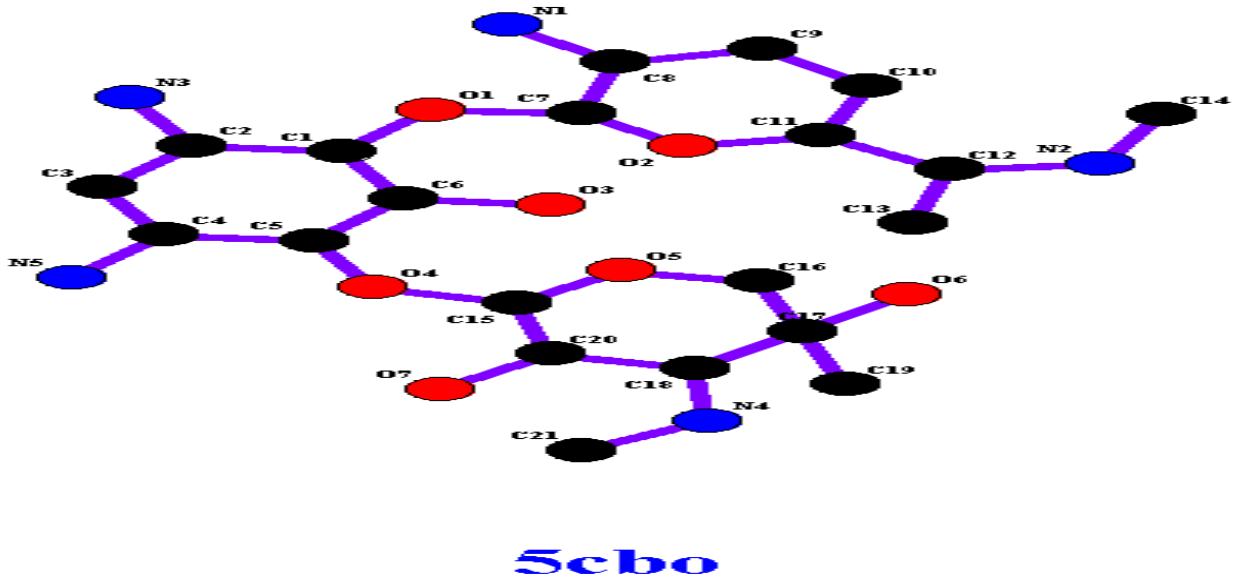


Figure 20: 5CBO-gentamicin



5cbo

Figure 21: 5CBO-gentamicin

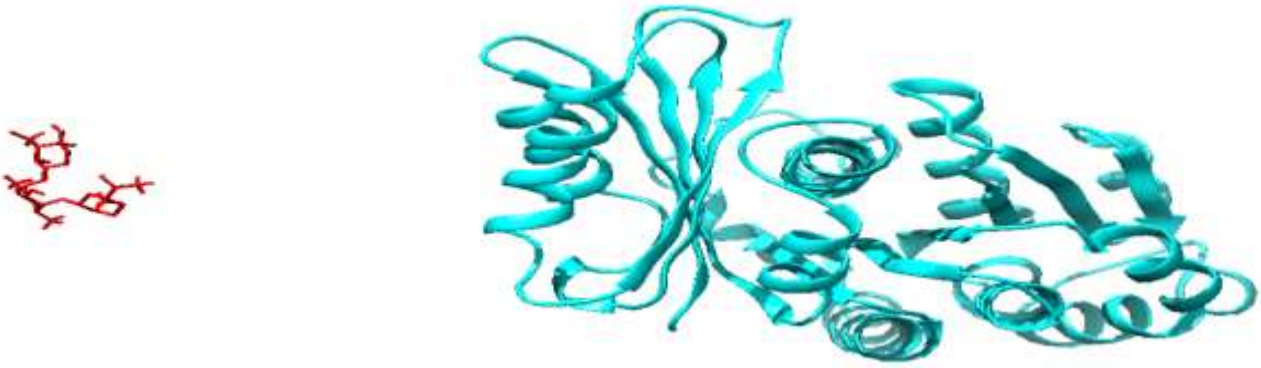
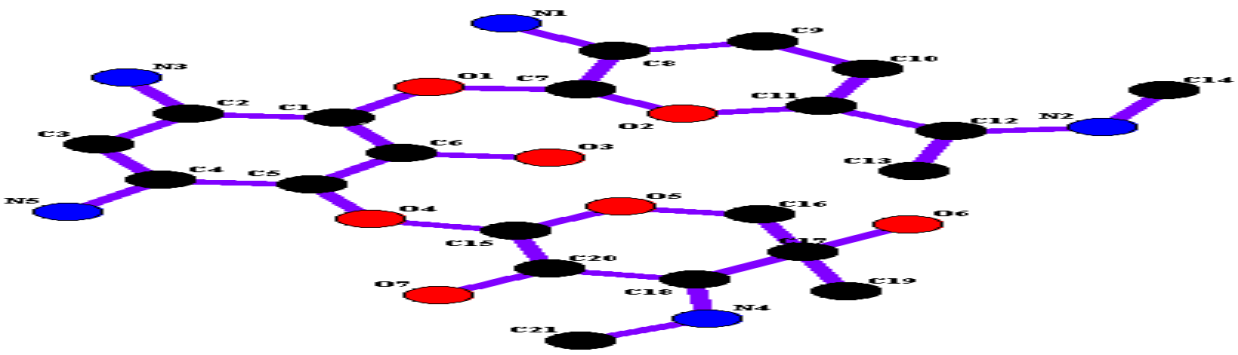


Figure 22: 3WGL-gentamicin



3wgl

Figure 23: 3WGLGgentamicin

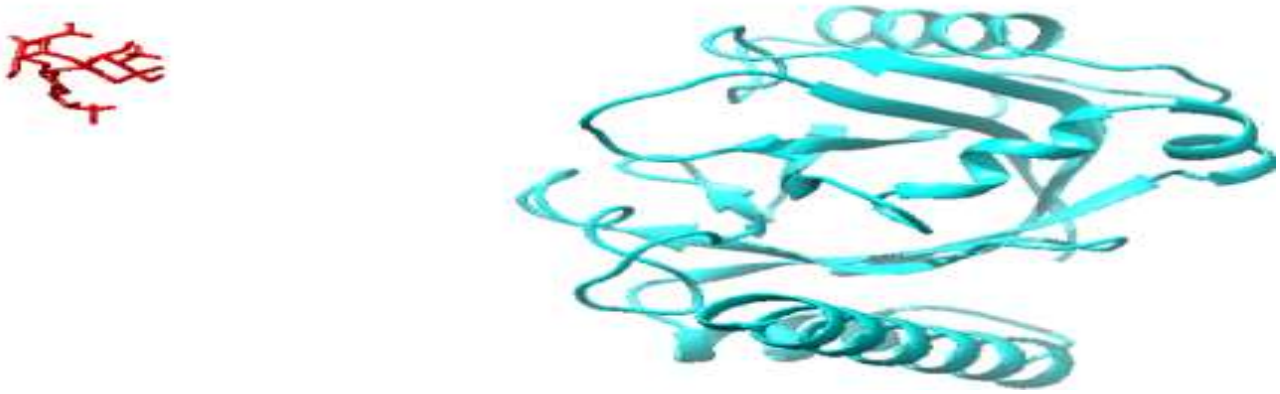
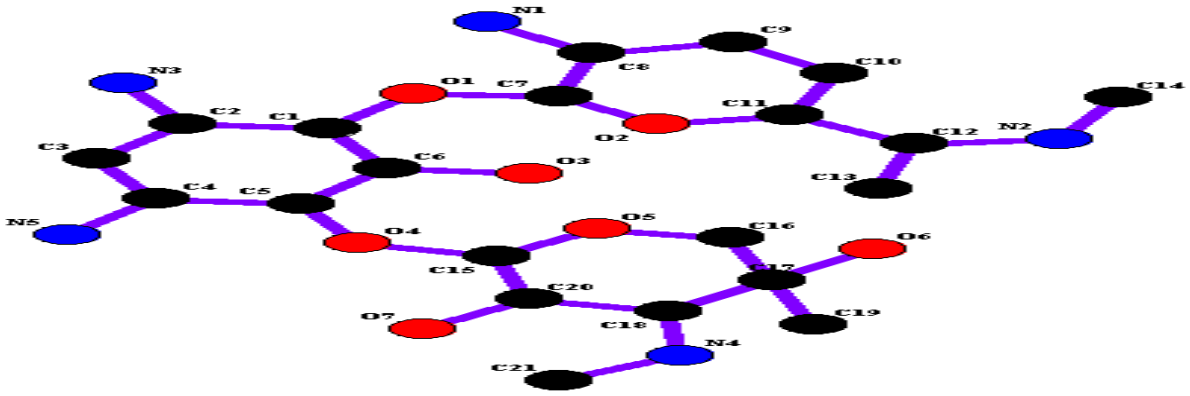


Figure 24: 4LFD- gentamicin



4lfd

Figure 25: 4LFD-gentamicin shows

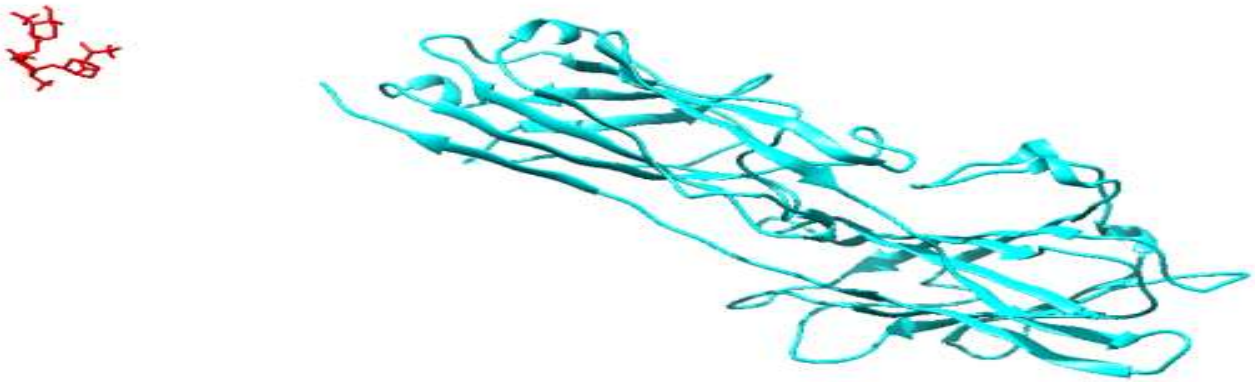
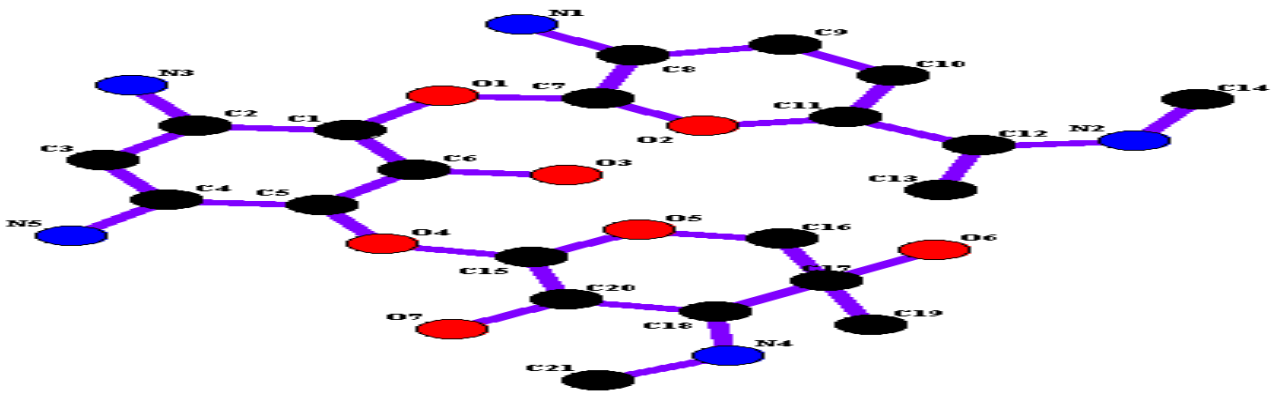


Figure 26: 5JQ6- gentamicin



5jq6

Figure 27: 5JQ6-gentamicin

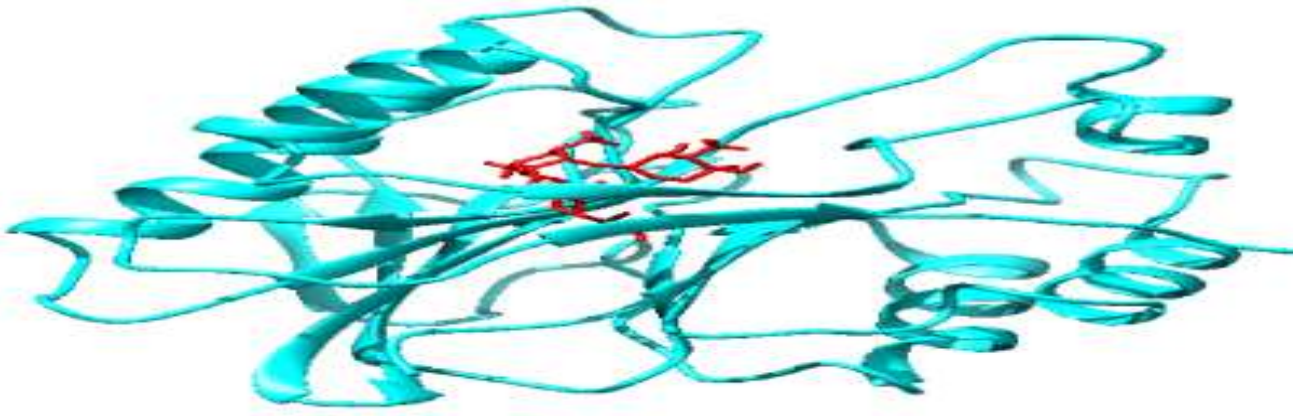


Figure 28: 3k55-gentamicins

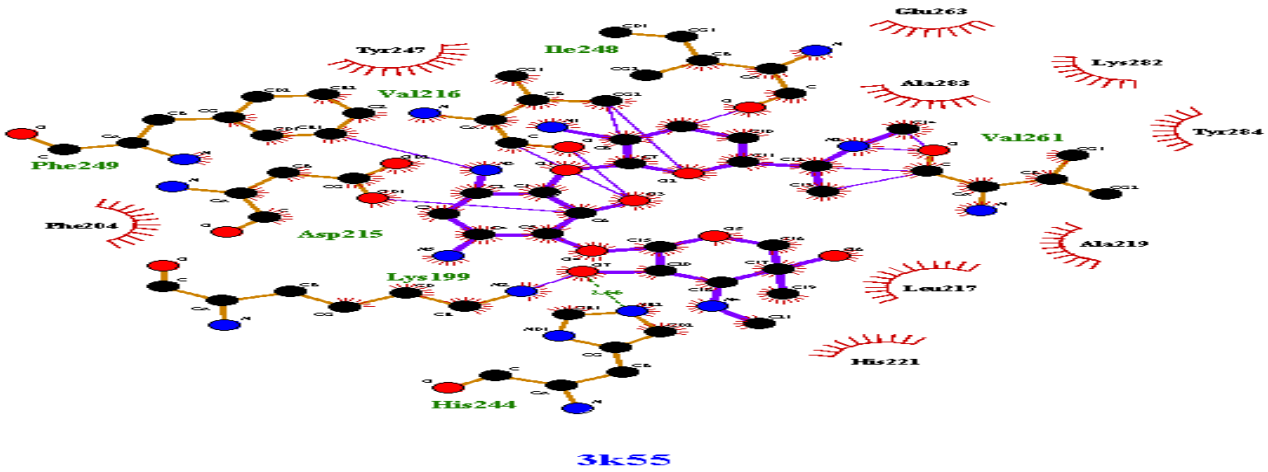


Figure 29: 3k55-gentamicin shows: non-ligand bond. Corresponding atoms and non- ligand residues His221, Leu217, Ala219, Tyr284

Table 3: The results of Molecular docking study for gentamicin

Target (PDB code)	Binding affinity (Kcal/mol.)	RMSD/upper bound	RMSD/lower bound
3D5S	-5.3	24.612	21.19
3K55	-7.1	8.586	3.236
3WGL	-6.8	2.008	1.251
4LFD	-6.9	6.909	2.222
5C2Z	-6.3	34.354	31.669
5CBO	-5.4	5.083	2.278
5JQ6	-5.8	6.478	2.398

References

1. Latlmer GW JR (1968) Journal Talanta, 15: 1.
2. Harvey Dihel, John Ellin Boe (1960) Anal. Chem., 32: 1121.
3. Adely KG, Al FH Hussein (2001) National J.B. of Chemistry 1: 87.
4. Archibald SJ (2009) Coordination chemistry of macrocyclic ligands, Annu. Rep. Prog. Chem., Sect. A: In org. Chem., 105: 297-322.
5. Chu Z, Huang W, Wang L, Gou S (2008) Chiral 27-membered [3+3] Schiff-base macrocycles and their reactivity with firs-row transition metal ions, Polyhedron. 27: 1079-1092.
6. Kilpin KJ, Henderson W, Nicholson □BK (2007) Synthesis, characterisation and biological activity of cycloaurated organogold (III) complexes with imidate ligands, Polyhedron, 26: 204-213.
7. Dudev T, Lim C (2001) Modeling Zn2+ Cysteinate Complexes in Proteins, J. Phys. Chem., 105: 10709-10714.
8. David SS, Meggers E (2008) Inorganic chemical biology: from small metal complexes in biological systems to metalloproteins, Curr. Opin. Chem. Biol., 12: 194-197.
9. Flora SJS, Pachauri V (2010) Chelation in Metal Intoxication, Int. J. Environ. Res. Public Health, 7: 2745-2788.
10. M Salavati-Niasari, F Davar (2006) Inorg. Chem. Commun. 9: 304-309.
11. PS Pan, FA Curtius, CLI Medina, LA Liotta, et al (2006) Bio org. Med. Chem. 14: 4731-4739.

12. A Harriman, R Ziessel (1998) Co ord. Chem. Rev., 171: 331.
13. F M Raymo (2002) Adv. Mater., 14: 401.
14. R Ballardini, V Balzani, A Credi, M T Gandolfi, M Venturi (2001) Acc. Chem. Res., 34: 445.
15. V Balzani, A Credi, M Venturi (2003) Molecular Devices and Machines. A Journey into the Nanoworld (Weinheim: Wiley-VCH.
16. B P Rosen (2006) Top. Curr. Genet., 14: 485.
17. A J Thomson, H B Gray (1988) Curr. Opin. Chem. Biol., 2: 155.
18. V E Mironov, I D Isaev, Vvedenie V Khimiyu, Vneshnesfernykh Kompleksnykh Soedinenii (1986) Metallov v Rastvorakh (Introduction in the Chemistry of Outer-Sphere Complex Compounds of Metalsin Solutions) (Krasnoyarsk: Krasnoyarsk University.
19. El FF Dacunha, TC Ramalho, CA Taft, RB Dealencastro (2006) Lett. Drug Des. Disc., 3: 17.
20. N Raman, C Thangaraja (2005) Pol. J. Chem., 79: 123-134.
21. R Kumar, R Singh (2006) Russ. J. Co ord. Chem. 32: 192-198.
22. Pagadala NS, Syed K, Tuszynski J (2017) Software for molecular docking: A review. Biophysical Reviews, 9(2):91-102.
23. Wong CF (2015) Flexible receptor docking for drug discovery. Expert Opinion on Drug Discovery, 10(11):1189-1200.
24. Ferreira LG, Dos Santos RN, Oliva G, Andricopulo AD (2015) Molecular docking and structure based drug design strategies. Molecules, 20(7):13384-13421.
25. Yuriev E, Ramsland PA (2013) Latest developments in molecular docking: 2010-2011 in review. Journal of Molecular Recognition, 26(5):215-239.
26. Phillips MA, Stewart MA, Woodling DL, Xie ZR (2018) Has Molecular Docking Ever Brought us a Medicine. In Molecular Docking. In tech Open.
27. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN (2000) Bourne PE. The protein data bank. Nucleic Acids Research, 28(1):235-242
28. Orenstein A (2013) The discovery and naming of Staphylococcus aureus, Gevonden op.
29. Rosenbach AJF (1884) Mikro-organismen bei den Wund-infections-krankheiten des Menschen, JF Bergmann.
30. Gordon RJ, Lowy FD (2008) Pathogenesis of methicillin-resistant Staphylococcus aureus infection, Clinical infectious diseases 46: S350-S359.
31. Klein E, Smith DL, Laxminarayan R (2007) Hospitalizations and deaths caused by methicillin-resistant Staphylococcus aureus, United States, 1999-2005. Emerging infectious diseases 13: 1840.
32. Richards MJ, Edwards JR, Culver DH, Gaynes RP (1999) Nosocomial infections in medical intensive care units in the United States, Critical care medicine, 27: 887-892.
33. Naber CK (2009) Staphylococcus aureus bacteremia: epidemiology, pathophysiology, and management strategies, Clinical infectious diseases, 48: S231-S237.
34. Bodie E D (1978) Inorganic Syntheses, XVIII, John Wily and Sons.
35. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE (2000) The protein data bank, Nucleic acids research, 28: 235-242.
36. Thomsen R, Christensen MH (2006) MolDock: a new technique for high-accuracy molecular docking, Journal of medicinal chemistry 49: 3315-3321.
37. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE (2004) UCSF Chimera-a visualization system for exploratory research and analysis, Journal of computational chemistry, 25: 1605-1612.
38. Mills N (2006) ChemDraw Ultra 10.0 Cambridge Soft, 100 Cambridge Park Drive, Cambridge, MA 02140. Www. Cambridge soft.
39. Dallakyan S, Olson AJ (2015) Small-molecule library screening by docking with PyRx. Chemical Biology, Methods and Protocols, 12: 243-250.
40. Laskowski R A, Swindells MB (2011) LigPlot+: Multiple ligand-protein interaction diagrams for drug discovery, Journal of Chemical Information and Modeling, 51: 2778-2786.

