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Original Article

Molecular docking study of catechol-*O*-methyltrasferase inhibitor on enoyl acyl carrier protein reductase of *mycobacterium tuberculosis*

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ABSTRACT

Tuberculosis, a disease largely observed to be caused by *Mycobacterium tuberculosis* bacteria, affects mainly to the lung. Most of anti-tubercular drug therapy leads to development of multi-drug resistant tuberculosis (MDR-TB) or extensive-drug resistant tuberculosis (XDR-TB) caused by extensive usage of anti-TB drugs. COMT inhibitor which are used in treatment of Parkinson's disease have some similarity to the enoyl acyl carrier protein reductase (InhA) of *Mycobacterium tuberculosis*, which is responsible for synthesis of mycolic acid, essential component of bacterial cell wall. Molecular docking study of COMT inhibitor was performed on enoyl acyl carrier protein reductase (InhA) of *Mycobacterium tuberculosis* having PDB ID 1ENY by using Biomed CAche software. Docking study performed on 150 molecules of COMT inhibitor out of them twelve best poses [C1 (-93.395), C2 (-92.433), C48 (-91.890), C54 (-94.873), C56 (-92.810), C94 (-90.849), C102 (-97.113), C114 (-92.958), C125 (-95.436), C142 (-90.994), C145 (-97.534)] are selected and evaluated for Biological activity by using PASS Online and Toxicity study performed by using OSIRIS property explorer. C48 having a good binding affinity to InhA and shows better anti-tubercular activity (Pa-0.419, Pi-0.0026) and have no major toxicity found by OSIRIS property explorer.

KEY WORDS: Molecular docking, COMT inhibitor, InhA, Tuberculosis, Parkinson Disease.

INTRODUCTION

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* bacteria, the causative microbe that most oftenly affects the lungs. About 1/3rd of the world's population has latent Tuberculosis, which means that people have been affected by TB bacteria but not yet ill with disease and cannot transmit the disease. Tuberculosis mostly affects young adults. About half million children fell ill with TB. Frequent usages of tobacco, greatly increases the risk of TB and morbidity. More than 20% of worldwide Tuberculosis cases are attributable to smoking. In 2012, around 8.6 million people fell ill with TB and 1.3 million died. In 2012, 5, 30,000 children became ill with TB and more than 74,000 HIV negative children died from TB [1]. Every year, around 8 million people affected by the TB, this subsequently claims the lives of nearly 2 million. This means over 4900 deaths per day and more than 95% of these are found in developing countries. In 2002, WHO estimated that if this disease was left unchecked, then it may leads more than 36 million more deaths by the year 2020 [2]. Most of anti-tubercular drugs remain largely unchanged over the last four decades. The wide spread use of these anti tubercular drug and time needed to remove infections may leads resistance to *Mycobacterium tuberculosis* strains. Multi-drug resistance tuberculosis (MDR-TB) is a resistance developed by the causative organism to the first-line pharmacotherapy drugs like Isoniazid (INH) and rifampicin. The effective treatment of MDR-TB requires long term combination therapy of second-line drugs in combination with first-line drugs. However, it may lead to extensive drug resistance tuberculosis (XDR-TB) to *Mycobacterium tuberculosis* strains which are resistant to INH + Rifampicin combination therapy as well as second-line drug like ciprofloxacin and moxifloxacin. XDR-TB is extremely difficult to treat because only remaining drug exhibit very low potency and have high toxicity, so its needs to identify new anti-tubercular agent as an urgent priority [3, 4].

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COMT Inhibitor: Catechol-*O*-methyltrasferase (COMT) is the enzyme involved in deactivation of catecholamines and drug with a catechol structure [5]. COMT is Mg^{2+} dependent enzyme found in both peripheral and central nervous system that catalyse the transfer of methyl group from S-adenosyl L-methionine (SAM) to one hydroxyl group of substrate which have catechol moiety via S_N2 type reaction [6]. In Parkinson's disease level of dopamine is decreased, Levodopa is widely used for the treatment of Parkinson's disease [7]. Levodopa is converted by dopa decarboxylase (DDC) enzyme to dopamine in both peripheral tissue and brain. Levodopa itself does not cross the blood brain barrier (BBB), so to allow higher concentration of levodopa to reach the brain and to reduce the peripheral as well as brain toxicity of the drug, it is co administered in combination of peripheral dopa decarboxylase inhibitor like carbidoapa. Levodopa is also converted into 3-O-methyl dopa (3-OMD) by COMT in both peripheral tissues as well as in brain [8]. 3- OMD is not useful in treatment of Parkinson's disease and it may be harmful in circulation and the brain of Parkinsonian patient [9]. Thus selective COMT inhibitor improves the bio-availability of levodopamine [10].

InhA Inhibitor: InhA (Enoyl acyl carrier protein reductase) is the primary molecular target of the first line anti-tubercular drug isoniazid [11]. InhA catalyse the reduction of long chain tarns-2-enoyl-acyl-carrier protein in the type II fatty acid biosynthesis pathway of *Mycobacterium tuberculosis*. Inhibition of InhA, decrease the biosynthesis of the mycolic acid which are essential component of the mycobacterium cell wall [12]. Isoniazid is a pro-drug, so it must be first activated by mycobacterial catalase peroxidase KatG enzyme into its acyl radical active form. The adduct resulting from covalent binding of the activated isoniazid to the co-substrate NADH or its oxidization product NAD^+ which act as potential InhA inhibitor [13]. Activation of InhA by KatG enzyme shows drug resistant to *Mycobacterium tuberculosis* strains there for direct inhibition of InhA without a requirement of activation would be a promising agent for the development of new drug molecule against the drug resistant of *Mycobacterium tuberculosis* [14].

MATERIALS AND METHODS

Ligand binding site similarity between COMT and InhA: Xie and Bourne developed a Sequence Order Independent Profile-Profile Alignment (SOIPPA) algorithm; their studies implied an evolutionary relationship between the NAD-binding rossman fold and the SAM dependent methyltrasferase, through similarity between their co-factor binding sites. NAD and SAM include adenine as a common fragment. COMT inhibitors are drugs that block the ligand binding sites of COMT in presence of SAM co-factor. It is possible to that COMT may possess a ligand binding pocket similar to those found in protein domains belonging to the NAD binding rossman fold as their co-factor binding sites are similar [15]. Figure 1 shows ligand binding site similarity between COMT and InhA. In figure 1 Green colour indicates COMT, purple colour indicates SAM- cofactor, and red colour indicates ligand; blue colour indicates InhA, orange colour indicates NAD cofactor and yellow colour indicates ligand.

Preparation of ligand for docking: Ligand was prepared by using Biomed CAche software and correct atom type (including valence, hybridization states, geometry, H-bond, and bond category) was defined. After beautifying, the final structure was analysed for energy minimization to get stable ligand.

Preparation of protein structure for docking: Three dimensional crystal structure of InhA [Enoyl-acyl carrier protein (ACP) – reductase] having resolution 2.20 °A was retrieved from the RCSB (Research Collaboratory for Structural Bio-informatics) protein data bank under the PDB id is 1ENY. 1ENY having enzyme classification (EC#) is 1.3.1.9, 1 means: oxidoreductase, 1.3 means: acting on CH-CH group of donors, 1.3.1 means: with NAD^+ or $NADP^+$ as acceptor, 1.3.1.9 means: Enoyl-acyl carrier protein (ACP) – reductase. 1ENY having a structure weight is 29057.13, length 268 and ligand located in 1ENY is NAD (Nicotinamide Adenine Dinucleotide).

Ramachandran plot shows the Phi-Psi torsion charges for all residues in the structure. G-factor analysis indicates how unusual, or out-of-the-ordinary, a property is. If the value is below -0.5 it will considered as unusual and if below -1.0 it will considered as highly unusual

Cleaning of protein structure include removal of water molecules and add some required information such as hydrogen atoms, atom hybridization, and correct bond type for HET group and standard residues. If residues are missing or incomplete, it may be necessary to correct their structures. After cleaning, protein structure analysed for energy minimization. Minimum energy for final protein structure was 4726.4703 Kcal/mol. **Figure 2** shows the ribbon structure of Protein (1ENY). Active site of protein was characterized by selecting residue, water and HET's group at the 5°A radius. Sequence analysis

of amino acid of protein structure was done by using Bio-med Cache software. Initially it display sequence in 1-letter code, converted it into 3-letter code.

Docking of ligand into active site: CAche automates the docking of ligand into active site by using genetic algorithm with a fast simplified potential of mean force (PMF) [16]. Docking of ligand into an active site is sensible when both contain any PMF atom types. Dock ligand into active site by selecting "dock into active site" by using Bio-med Cache software. Dialog box appear, select the ligand and active site and select the docking option: run in target window; scoring function: PMF; calculation type: dock; ligand: flexible and active site: rigid. Run the docking process which displays the status of docking calculation. After completion of docking process final dock score was analysed from chemical spread sheet. Ligand which shows dock score in between -90.00 to -100.00 were selected as a best poses. **Figure 3 to 14** shows structure of best poses [17-22]. Best poses were analysed for different properties like conformation minimum energy, connectivity index, dipole moment, electron affinity, di-electric energy, steric energy, ionization potential, logP, shape index, solvent accessible surface area etc. by using project leader. Construct correlation coefficient matrix of properties having Pearson correlation is more than 0.5.

Toxicity prediction of best poses by using OSIRIS property explorer: OSIRIS property explorer determines toxicity risk like mutagenic, tumorigenic, irritant, reproductive effect; clogP, solubility, molecular weight, drug likeness and drug score. Prediction results are valued and colour coded, properties with high risk of undesired effect like mutagenicity is shown in red, whereas a green colour indicates drug conform behaviour.

Biological activity prediction by using PASS: PASS (Prediction of Activity Spectra for Substances) is a software product which is designed as a tool for evaluating the general biological activity of an organic drug like molecule. PASS predicts biological activity based on the structure of compound. It can be used to estimate biological activity profile for virtual molecules prior to their chemical synthesis and biological testing.

RESULTS AND DISCUSION

Ramachandran plot shows that for the 1ENY.pdb out of total 268 residues 200 (88.9%) were in the most favoured region, 23 (10.2%) in the additional allowed region, 1 (0.4%) in the generally allowed region and only 1 (0.4%) residue found in the disallowed region residue. The colouring or shading on the plot represent the different region, the darkest area (sown in red) correspond to the core region which represent the most favoured combination of Phi-Psi value. **Figure 15** shows the Ramachandran plot of 1ENY.

G-factor analysis indicates how unusual, or out-of-the-ordinary, a property is. If the value is below -0.5 it will considered as unusual and if below -1.0 it will considered as highly unusual. **Table 1** shows G-factor analysis.

Sequence analysis of amino acid of protein structure was done by using Biomed CAche software Active site of amino acids of the enzyme determined by docking NAD and ligan.Amino acid conserved by NAD & Ligand are : SER13, GLY14, ILE15, ILE16, THR17, SER19, SER20, ILE21, ALA22, PHE41, ASP42, ILE47, LEU63, ASP64, VAL65, GLN66, HIS93, SER94, ILE95, GLY96, PHE97, MET98, MET103 ,GLY104, ILE122,MET147,ASP148,PHE149,MET155,PRO156, ALA157, ILE158, MET161, LYS165, VAL189, ALA190, ALA191, GLY192, PRO193, ILE194, THR196, ALA198, MET199, ILE 202,LEU 207, ALA 211, ILE 215, LEU 218 within 5°A radius. Hydrogen bond plays an important role for structure and functions of biological molecules. **Figure 16** shows the H-bond distances between ligand and protein residues. In binding mode, six H-bond of length 2.42°A, 2.87°A, 2.84°A, 3.00°A, 2.85°A & 2.80°A and 2.75°A were found in between the ligand and protein residues at SER20, ILE21, ASP64, VAL65, LYS165, ILE194. In order to find out a new and effective drug for tuberculosis treatment molecular docking study of COMT inhibitor on InhA of *Mycobacterium tuberculosis* was performed using Biomed CAche software. Around one hundred fifty ligands were docked into active site of InhA (PDB id: 1ENY) and dock score of individual was calculated. Best poses were selected on the basis of molecule having dock score between -90.00 to -100.00. Out of one hundred fifty ligands total twelve best poses were found. **Table 2** show best poses with dock score. **Figure 17 to 28** shows the docking of ligand into active site. Selected best poses were analysed for different properties like dipole moment, electron affinity, di-electric energy, steric energy, ionization potential, logP, shape index, solvent accessible surface area etc. by using project leader. **Table 3** shows properties of best poses. Pearson coefficient of different properties was calculated. More than 0.5 value of Pearson coefficient selected for the construction of correlation matrix. Correlation matrix shows the how properties are correlated to each other. **Table 4** shows the

correlation matrix. Toxicity of best poses was predicted in OSIRIS property explorer. OSIRIS predicts toxicity risk like mutagenic, tumorigenic, irritant, reproductive effect; clog P, solubility, drug likeness and drug score. **Table 5** shows the toxicity prediction of best poses. PASS online predict the biological activity and adverse effect of best poses. Biological activity like anti-tuberculosis and anti-Parkinson were evaluated by using PASS online software. **Table 6** shows the biological activity and adverse effect of best poses. Pa is probability "to be active" and Pi is probability "to be inactive".

CONCLUSION

In order to find out a new and effective drug for tuberculosis treatment molecular docking study of COMT inhibitor on InhA of *Mycobacterium tuberculosis* was performed using Biomed CAche software. Best poses were selected on the basis of ligand having a good dock score (Between -90.00 to -100.00). Twelve molecules with best dock scores have id C1 (-93.395), C2 (-92.433), C48 (-91.890), C54 (-94.873), C56 (-92.810), C94 (-90.849), C102 (-97.113), C114 (-92.958), C125 (-95.436), C142 (-90.994), C145 (-97.534) selected and further evaluated for toxicity prediction by OSIRIS property explorer and biological activity by PASS online software. Compound id C48 having a good predicted binding affinity to InhA and shows better anti-tubercular activity (Pa-0.419, Pi-0.0026) and have no major toxicity predicted by OSIRIS property explorer. This molecule can be further optimized for anti-tubercular activity.

Table 1: G-Factor analysis

Parameter	Score	Average Score
Dihedral angels Phi-Psi distribution Chi 1- Chi 2 distribution Chi 1 only Chi 3 – Chi 4 Omega-	-0.26 -0.93 -0.27 0.34 -0.15	-0.29
Main chain co-valet forces Main chain bond length Main chain bond angles	-0.20 -0.46	-0.35
Overall average		-0.30

Table 2: Best poses with dock score

Id	Dock Score	Id	Dock Score
C1	-93.395	C94	-90.849
C2	-92.433	C102	-97.113
C48	-91.890	C114	-92.958
C54	-94.873	C125	-93.436
C56	-92.810	C142	-90.994
C74-A	-90.552	C145	-97.534

Table 3: Properties of best poses

ID	Properties						
	Dipole moment	**Electron affinity**	**logP**	**Molar refractivity**	**Molecular weight**	**Shape index**	**Solvent accessible surface**
C1	8.615	1.234	3.626	107.824	439.390	25.62	415.6
C2	3.555	0.792	0.557	104.141	432.383	24.14	410.9
C48	0.519	0.303	2.234	102.589	388.419	24.27	427.1
C54	4.148	1.361	0.053	125.220	501.455	18.99	475.6
C56	5.351	1.372	1.369	117.893	455.429	26.07	445.4
C74-A	2.811	1.477	3.246	89.586	339.304	19.75	335.5
C94	6.929	1.230	1.883	102.067	389.383	22.68	385.9
C102	7.881	1.528	1.759	113.121	444.400	26.60	427.1
C114	7.721	1.057	1.400	90.624	337.375	20.34	357.8
C125	7.484	1.360	0.069	115.553	473.401	27.05	445.9
C142	4.859	1.735	2.393	95.755	413.173	21.70	345.6
C145	3.179	1.737	2.451	94.333	382.289	21.24	362.8

Table 4: Correlation matrix

	Dock score	**Connectivity index**	**Molar refractivity**	**Mol.wt**	**Shape index**	**Solvent Accessibility Surface Area**	**Valence Connectivity Index**
Dock score	1	0.6255	0.5836	0.5648	0.5797	0.5711	0.5568
Connectivity index	0.6255	1	0.9659	0.9789	0.9708	0.9605	0.9552
Molar refractivity	0.5836	0.9659	1	0.9489	0.9575	0.9724	0.9833
Molecular weight	0.5648	0.9789	0.9489	1	0.9588	0.9484	0.9658
Shape index	0.5797	0.9708	0.9575	0.9588	1	0.9641	0.9555
Solvent Accessibility Surface Area	0.5711	0.9605	0.9724	0.9484	0.9641	1	0.9769
Valence Connectivity Index	0.5568	0.9552	0.9833	0.9658	0.9555	0.9769	1

TABLE 5: Toxicity predictions of best poses

ID	Toxicity risk				clog P	Solubility	Drug likeness	Drug score
	Mutagenic	Tumorigenic	Irritant	Reproductive effect				
C1	YES	NO	NO	NO	2.95	-3.75	-4.03	0.29
C2	YES	YES	YES	NO	0.77	-6.02	0.67	0.10
C48	NO	NO	NO	NO	3.12	-2.75	-3.80	0.41
C54	NO	NO	NO	NO	-0.88	-3.29	-3.76	0.35
C56	NO	NO	NO	NO	0.76	-4.62	3.04	0.31
C74-A	YES	NO	NO	NO	3.29	-4.72	-4.04	0.28
C94	YES	NO	NO	NO	2.24	-3.29	1.25	0.57
C102	YES	NO	NO	NO	2.31	-3.04	-2.61	0.24
C114	NO	NO	NO	NO	1.83	-1.70	2.77	0.88
C125	YES	NO	NO	NO	1.36	-2.00	-5.58	0.38
C142	NO	NO	NO	NO	3.34	-8.78	-6.58	0.21
C145	YES	NO	NO	NO	2.45	-6.93	-6.13	0.20

TABLE 6: Biological activities of best poses

Id	Biological activity			Adverse effect		
	Pa	Pi	Biological activity	Pa	Pi	Adverse effect
C1	0.414	0.026	Anti-parkinsonian	0.906	0.004	Postural hypotension
	0.274	0.087	Anti-tubercular	0.705	0.025	Urine retention
C48	0.250	0.087	Anti-parkinsonian	0.930	0.003	Galactorrhea
	0.419	0.026	Anti-tubercular	0.871	0.006	Ulcer
C74-A	0.248	0.099	Anti-parkinsonian	0.932	0.003	Urine discoloration
	0.359	0.045	Anti-tubercular	0.646	0.046	Hepatotoxicity
C94	0.234	0.101	Anti-parkinsonian	0.897	0.003	chorea
	0.234	0.099	Anti-tubercular	0.594	0.028	Cataract
C102	0.399	0.029	Anti-parkinsonian	0.898	0.005	Postural hypotension
	0.252	0.108	Anti-tubercular	0.712	0.010	Hypotonia
C114	0.650	0.005	Anti-parkinsonian	0.760	0.023	Fibrillation
	0.305	0.068	Anti-tubercular	0.742	0.075	Twitching
C142	0.991	0.002	Anti-parkinsonian	0.311	0.140	Fasciculation
C145	0.556	0.004	Anti-parkinsonian	0.310	0.030	Hypnotic

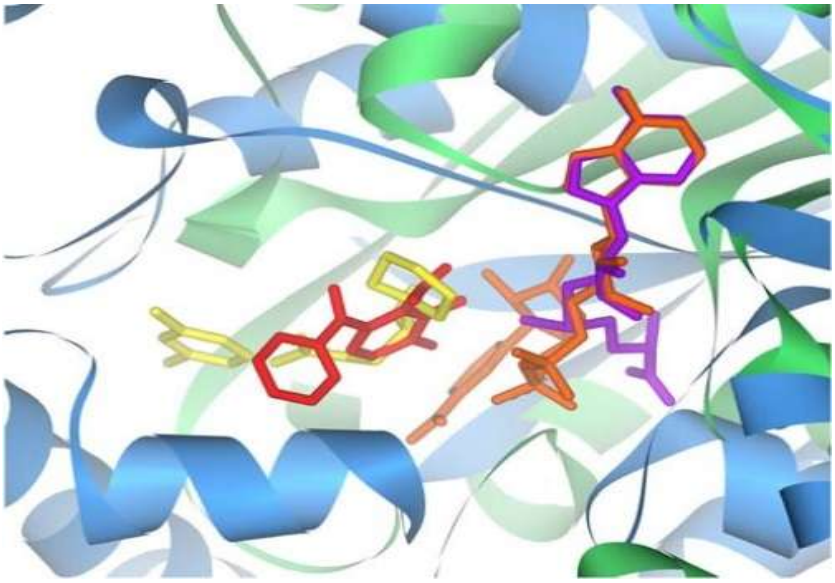

Fig. 1: Ligand binding site similarity between COMT and InhA [15]

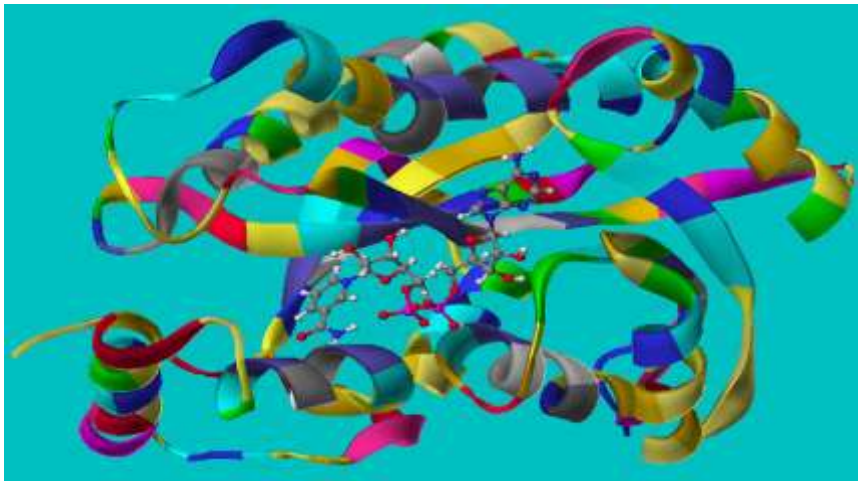

Fig. 2: Ribbon structure of 1ENY protein

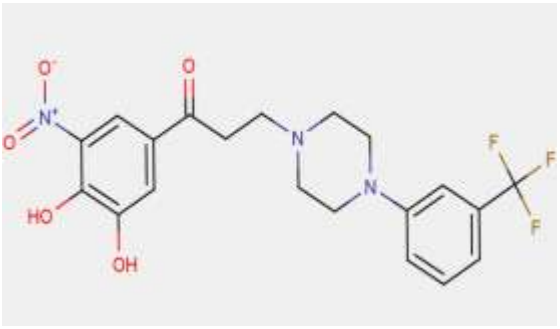

Fig. 3: Structure of best poses C1 [17]

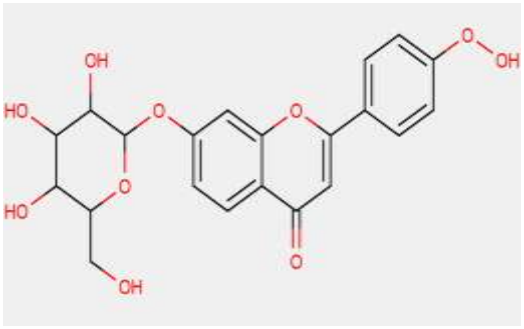

Fig. 4: Structure of best poses C2 [17]

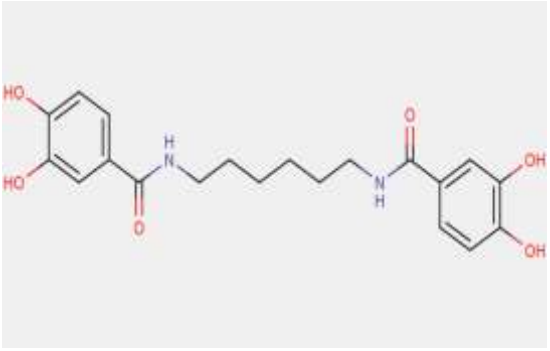

Fig. 5: Structure of best poses C48 [18]

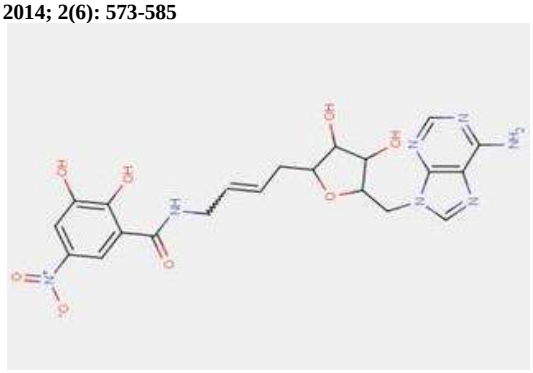

Fig. 6: Structure of best poses C54 [18]

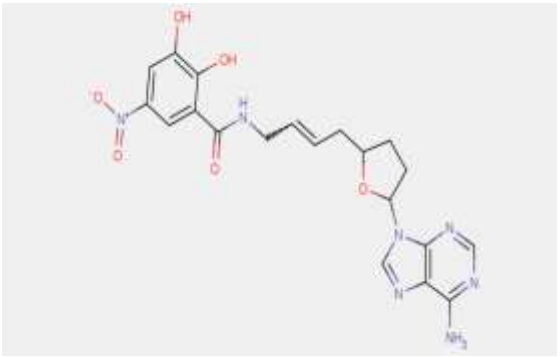

Fig. 7: Structure of best poses C56 [18]

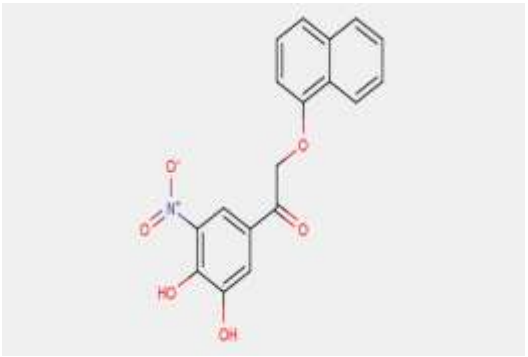

Fig. 8: Structure of best poses 74-A [19]

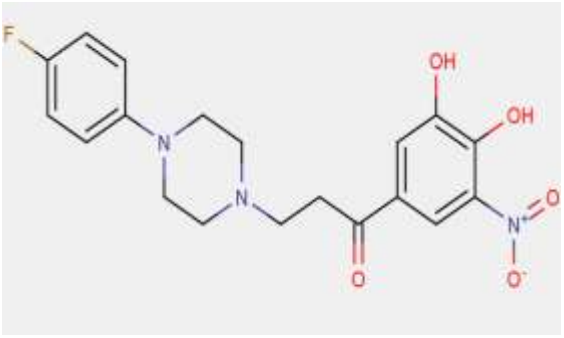

Fig. 9: Structure of best poses C94 [19]

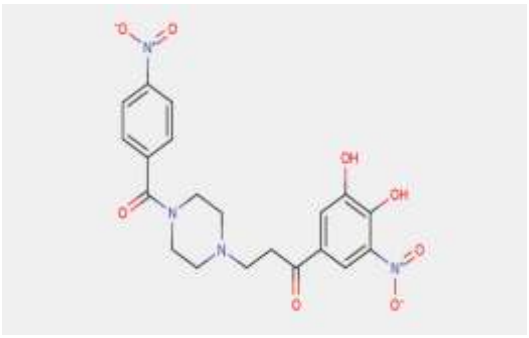

Fig. 10: Structure of best poses C102 [19]

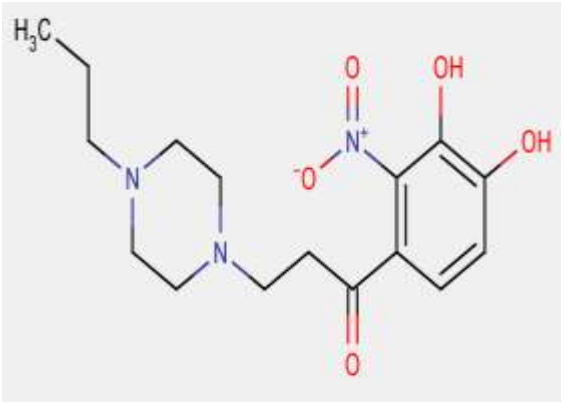

Fig. 11: Structure of best poses C114 [20]

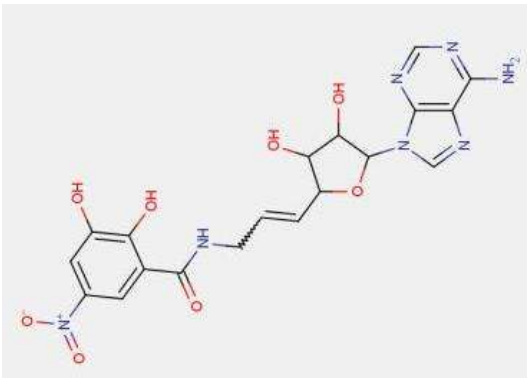

Fig. 12: Structure of best poses C125 [21]

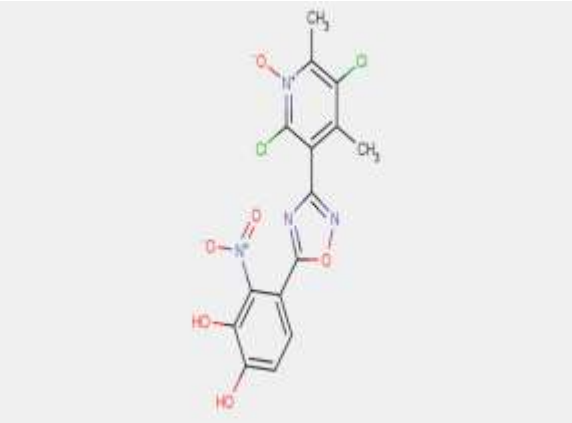

Fig. 13: Structure of best poses C142 [22]

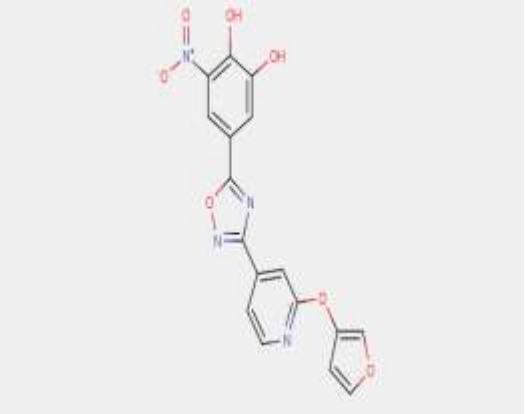

Fig. 14: Structure of best poses C145 [22]

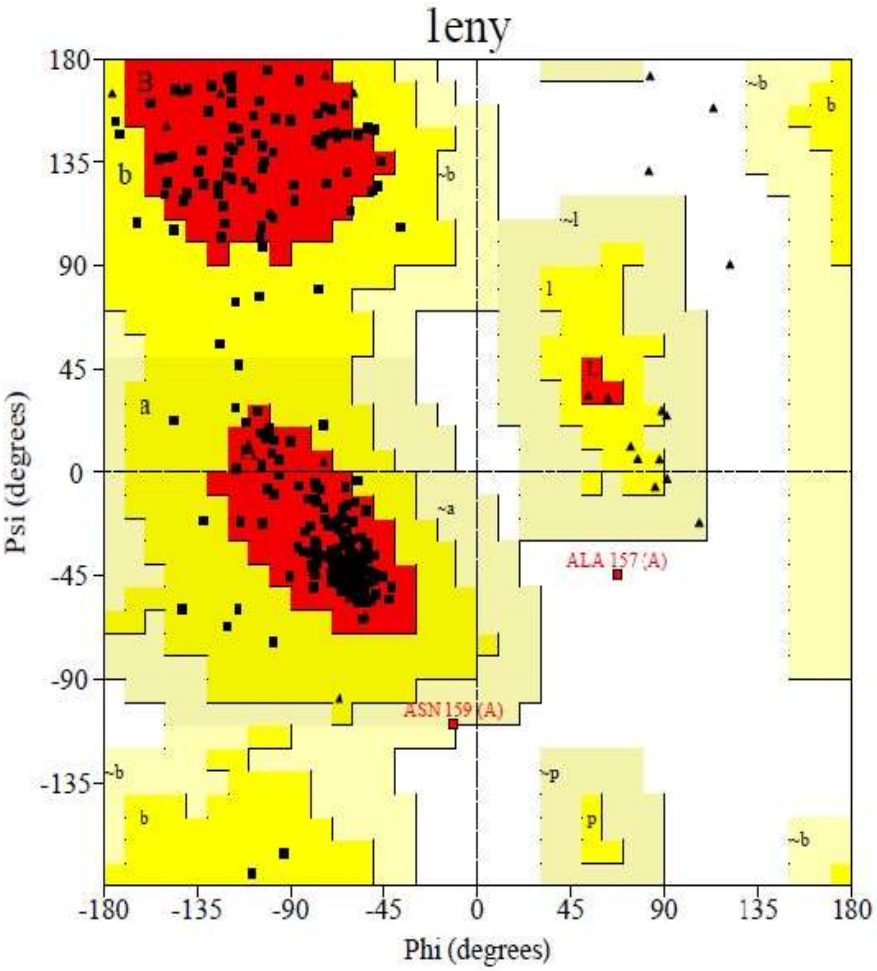

Fig. 15: Ramachandran plot

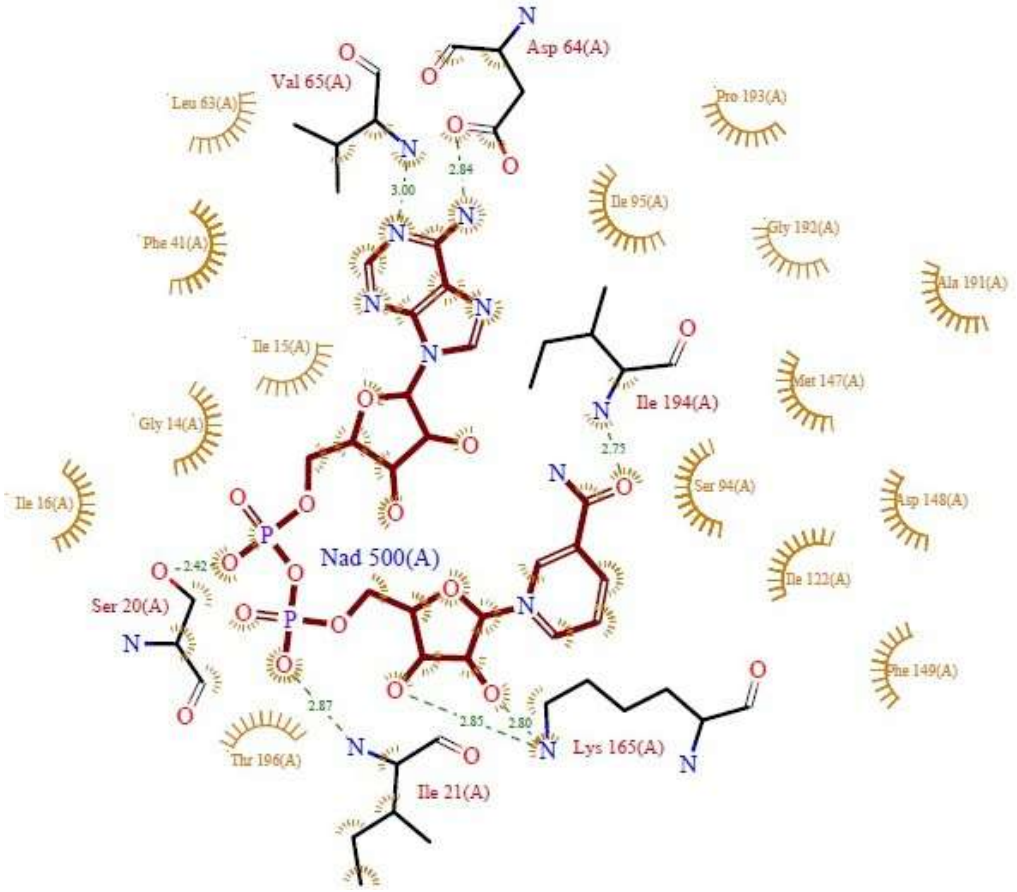

Fig. 16: H-bond distances between ligand and protein residues

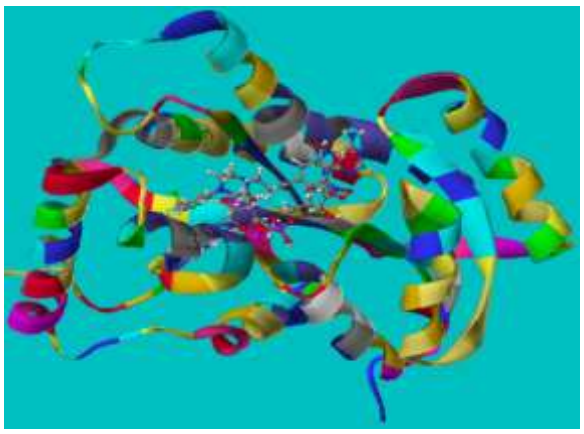

Fig. 17: Docking of ligand (C1) into active site

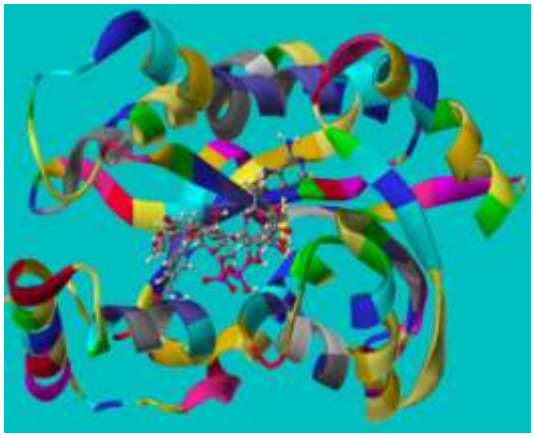

Fig. 18: Docking of ligand (C2) into active site

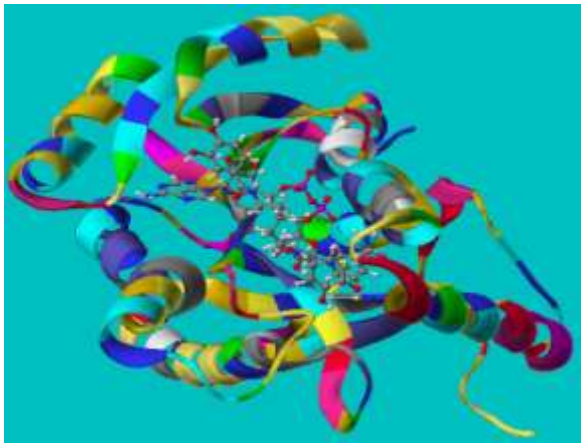

Fig. 19: Docking of ligand (C48) into active site

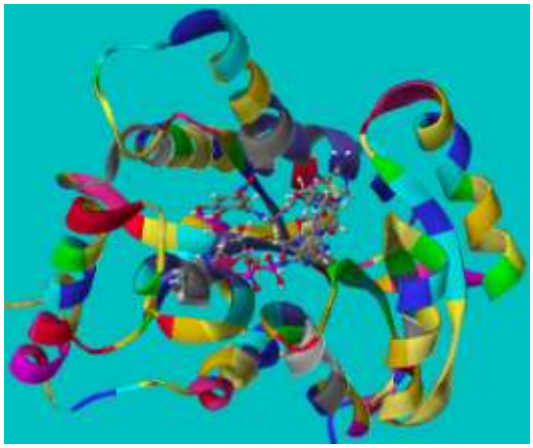

Fig. 20: Docking of ligand (C54) into active site

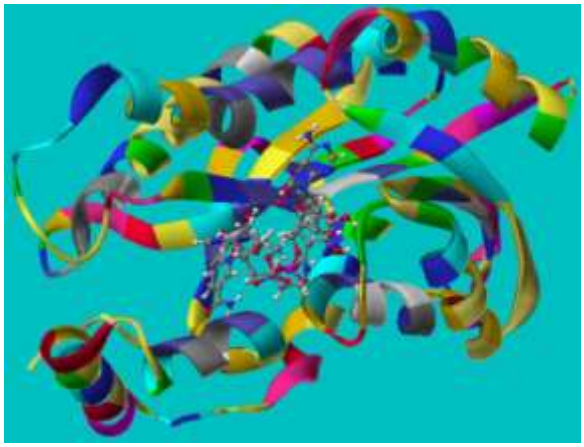

Fig. 21: Docking of ligand (C56) into active site

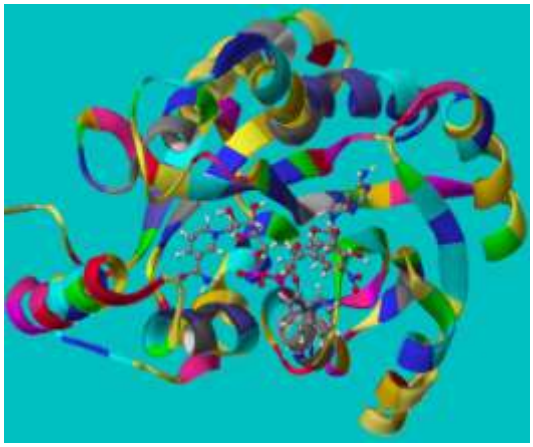

Fig. 22: Docking of ligand (C74-A) into active site

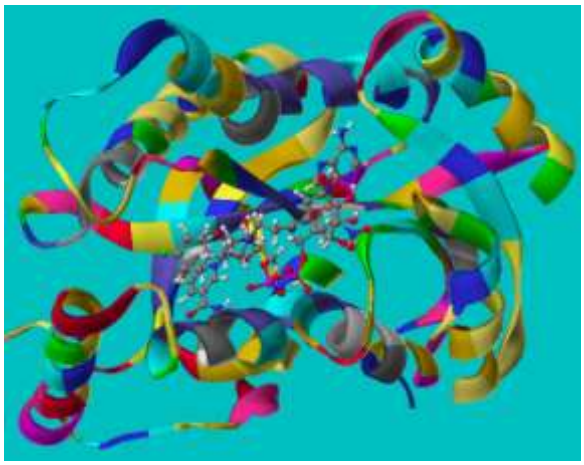

Fig. 23: Docking of ligand (C94) into active site

Fig. 24: Docking of ligand (C102) into active site

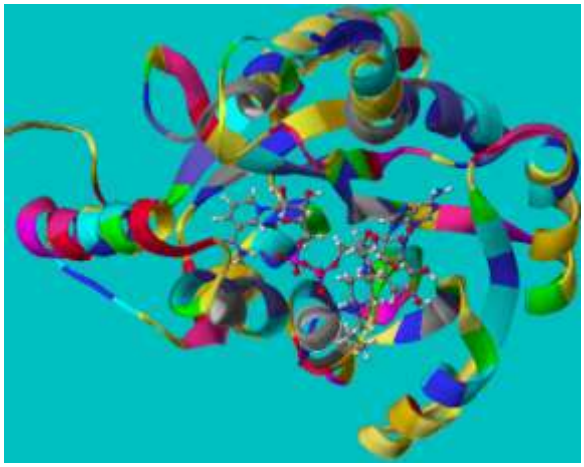

Fig. 25: Docking of ligand (C114) into active site

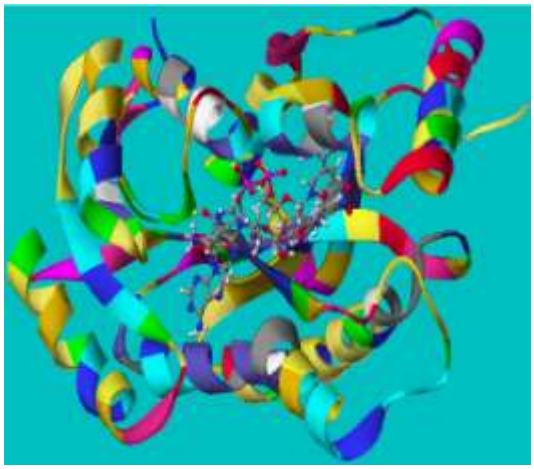

Fig. 26: Docking of ligand (C125) into active site

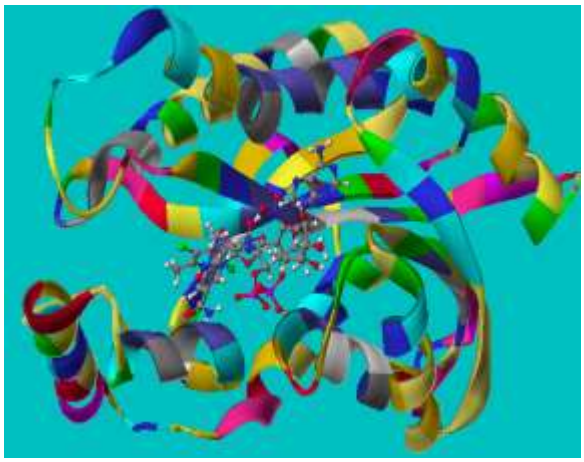

Fig. 27: Docking of ligand (C142) into active site

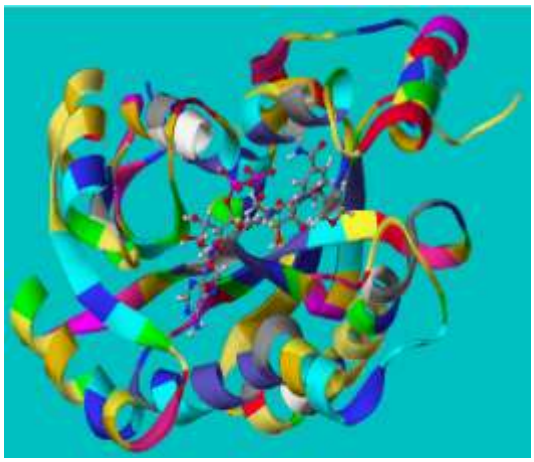

Fig. 28: Docking of ligand (C145) into active site

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