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Molecular docking studies of indole derivatives containing cyanide group as hepatitis C Ns5b polymerase inhibitor

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Abstract
Hepatitis C is a liver disease caused by the Hepatitis C virus (HCV) and the infection has affected approximately 180 million people around the world. Nonstructural protein 5B (NS5B) polymerase is a viral protein found in HCV and it plays a major role in the replication of the virus. Over the decade, it has been found that inhibition of the enzyme prevents the replication of the virus and thus treats the disease. In this study, molecular docking was performed on a series of indole derivatives by Autodock 4.2 into active sites of NS5B polymerase enzyme.

Keywords: Hepatitis C, NS5B polymerase, Indole derivatives, Molecular docking.

1. Introduction
Previous studies over the decade have demonstrated that compounds with indole nucleus possess many therapeutic properties. This includes antimicrobial, anti-viral, antitubercular, anti-inflammatory, anticancer, antidiabetic, anticonvulsant, antimicrobial, antioxidant, antidepressant activities [1, 2]. Nonstructural protein 5B (NS5B) polymerase is a viral protein found in HCV. NS5B polymerase plays a critical role in the replication of the virus.NS5B polymerase contains subunits that have additional roles during the infection process that are independent of RNA synthesis [3]. Therefore, this protein has become an attractive target in drug designing as inhibition of the protein prevents the virus from affecting normal cellular processes as well as inhibiting HCV RNA synthesis. Great variability is possible with the inhibitors of HCV as multiple allosteric binding sites are present on NS5B polymerase [4]. The enzyme has four binding sites which are non-nucleoside inhibitor (NNI) site I, II, III and IV. NNI site I and II are located in the thumb domain, while III and IV are closer to the active site in the palm domain. The upper section of the thumb domain, approximately 30Å from the active site at the juncture of the thumb and finger loop is the target of indole derivatives. This will interfere with conformational changes required for the formation of productive RNA/enzyme complex thus inhibiting the elongation process [5].

2. Methods and Materials
2.1 Preparation of protein molecule
Crystal structure of target protein was retrieved from Protein Data Bank (PDB ID: 3UPI). The protein was bounded with inhibitor, 4, 5-dihydrofurano indole. The ligand 4, 5-dihydrofurano indole was removed from the target protein using protein data bank data base. The energy of the target protein was minimized using USCF Chimera. The protocol of USCF Chimera prepares the proteins by inserting missing atoms in incomplete residues, modeling missing loop regions, deleting alternate conformations, removing water molecules, standardizing atom Names, protonating titratable residues using predicted pKs.

2.2 Preparation of ligands
The structures of 2-phenylindole derivatives were selected from the literature S.Y. Liao *et al*. 2009. In this literature, 3D quantitative structure-activity relationship (QSAR) and docking studies have been carried out for 43 2-phenylindole derivatives with anticancer activity against human breast cancer cell line. These compounds were found possess anticancer activity by preventing the polymerization of the α/β tubulin dimers to functional microtubules. Chem Sketch was downloaded and the 2D structures of the selected ligands were drawn. The 2D structure of the ligands was converted into 3D structure using Marvin Sketch. The 3D structure of the compounds will be saved in protein databank (pdb) format. The pdb format of the ligands will be viewed in USCF Chimera and the energy of the ligands will be minimized using USCF Chimera.

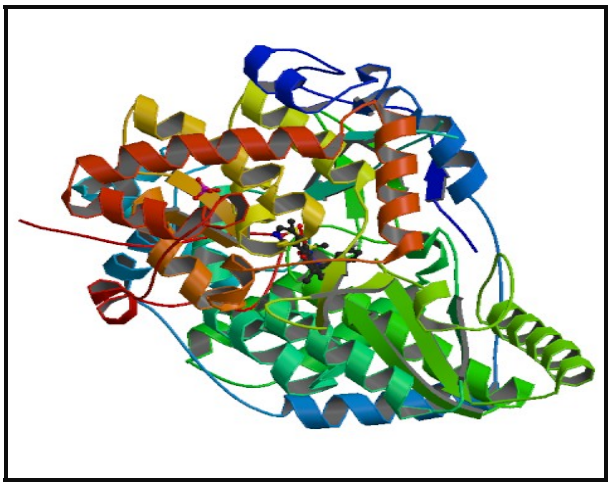


Fig 1: Ribbon structure of target protein (PDB ID: 3UPI)n

2.3 Validation of software

Software method validation was performed in Autodock 4.2 using pdb protein 3UPI. The x-ray crystal structure of 3UPI was recovered from PDB. The co-crystallized ligand 4, 5-dihydrofurano indole was redocked and the docked position was compared to the crystal structure position by calculating RMSD value.

2.4 Molecular docking

The ligand-flexible docking studies were performed using Lamarckian Genetic Algorithm of the Autodock 4.2 program [7]. This program is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. This is achieved through rapid grid based energy evaluation and efficient search of torsional freedom

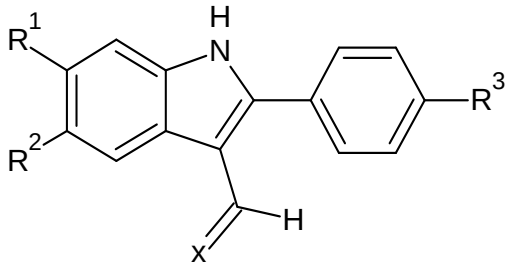
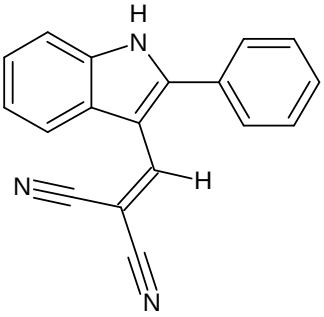
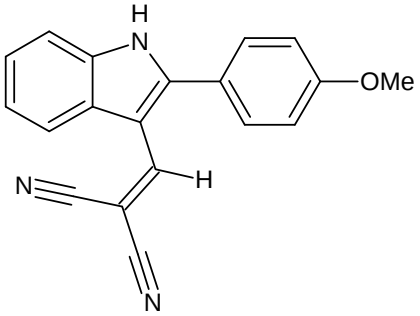
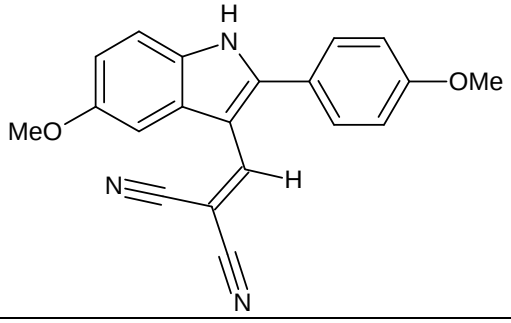
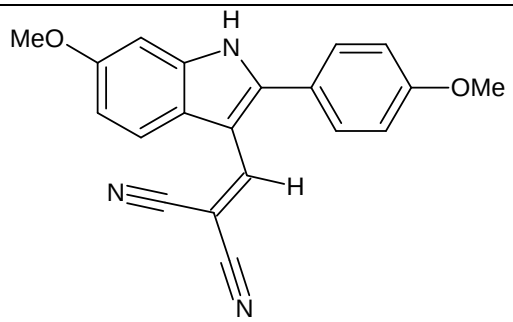
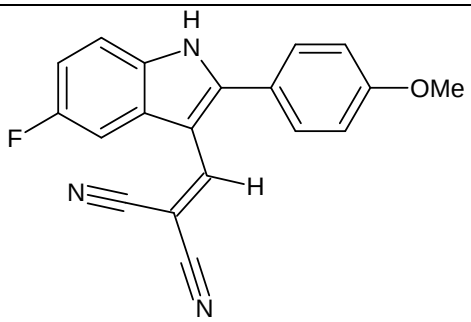
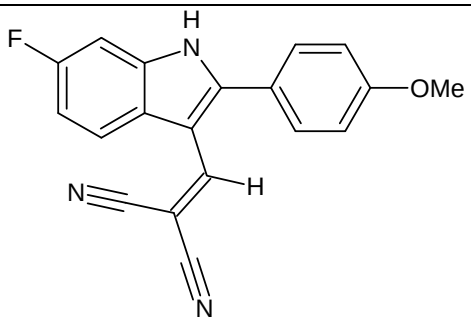


Fig 2: Molecular structure of 2-phenylindole

Table 1: List of Docked Compounds

No.	Compound ID	Structure of Compound	IUPAC Name of Compound
1.	1A		[(2-phenyl-1H-indol-3-yl)methylidene]propanedinitrile
2.	2A		{[2-(4-methoxyphenyl)-1H-indol-3-yl]methylidene}propanedinitrile
3.	3A		{[5-methoxy-2-(4-methoxyphenyl)-1H-indol-3-yl]methylidene}propanedinitrile

4.	4A		{[6-methoxy-2-(4-methoxyphenyl)-1H-indol-3-yl]methylidene}propanedinitrile
5.	5A		{[5-fluoro-2-(4-methoxyphenyl)-1H-indol-3-yl]methylidene}propanedinitrile
6.	6A		{[6-fluoro-2-(4-methoxyphenyl)-1H-indol-3-yl]methylidene}propanedinitrile

3. Results

Table 2: Docking Results of Ligands

No.	Ligands	Ligand efficiency	Inhibition constant (µM)	Intermolecular energy	Van der Waals dissolution energy	Electrostatic energy	Total internal	Unbound energy
1.	1A	-0.37	1.72	-8.16	-8.19	-0.03	-0.47	-0.47
2.	2A	-0.38	765260	-8.64	-8.68	-0.04	-0.51	-0.51
3.	3A	-0.4	216040	-9.39	-9.42	-0.03	-0.54	-0.54
4.	4A	-0.35	1.2	-8.38	-8.35	-0.03	-0.47	-0.47
5.	5A	-0.36	852320	-8.53	-8.61	-0.03	-0.53	-0.53
6.	6A	-0.34	2.14	-8.03	-8.0	-0.03	-0.5	-0.5

Table 3: Binding energy, Interaction Residues, Hydrogen Bonds and Hydrogen Bond Distance of Ligands

No.	Ligands	Binding energy (kJ/mol)	Interacting residues	Hydrogen Bond	Hydrogen bond distance (Å)
1.	1A	-7.86	ARG 200, LEU 384, MET 414, PRO 197, SER 196, SER 368, TYR 415, TYR 448	25A:UNK0:N1	2.584
2.	2A	-8.34	ARG 200, HIS 467, LEU 384, MET 414, PRO 197, SER 196, SER 368, TYR 415, TYR 448	26A:UNK0:N1	2.628
3.	3A	-9.09	ARG 200, HIS 467, LEU 384, MET 414, PRO 197, SER 196, SER 368, TYR 415, TYR 448, VAL 201	27A:UNK0:N1	2.651
4.	4A	-8.08	ASN 411, GLY 410, ILE 447, LEU 384, MET 414, PRO 197, TYR 415, TYR 448	NO HYDROGEN BOND	
5.	5A	-8.28	ARG 200, HIS 467, LEU 384, MET 414, PRO 197, SER 196, SER 368, TYR 415, TYR 448	29A:UNK0:NI	2.626
6.	6A	-7.73	ARG 200, ASN 411, GLY 410, ILE 447, LEU 384, MET 414, TYR 415, TYR 448	NO HYDROGEN BOND	--

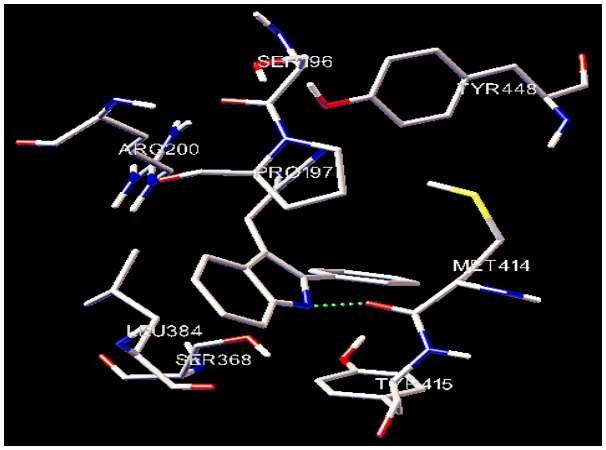


Fig 3: Interacting residues of ligand 1A with NS5B

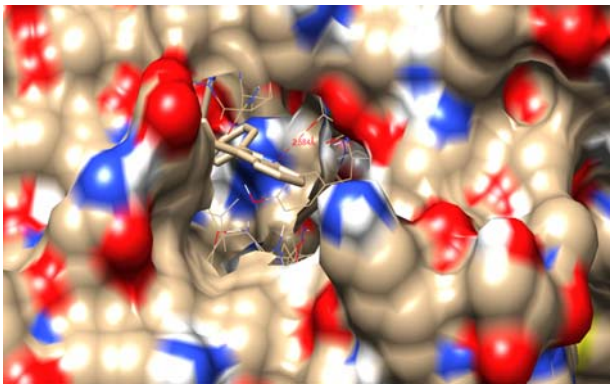


Fig 4: Surface view of Ligand 1A with NS5B

The binding energy of ligand 1A with NS5B polymerase enzyme is -7.86kJ/mol. The ligand efficiency of 1A was -0.37 and the Van der Waals dissolution energy were found to be -8.19. This ligand has an intermol energy of -8.16 with a total internal of -0.47. The electrostatic energy of this ligand is -0.03 and the inhibition constant is 1.72uM. The docking of the ligand with NS5B has 8 Van der Waals interacting residues at binding site which are ARG 200, LEU 384, MET 414, PRO 197, SER 196, SER 368, TYR 415 and TYR 448. Ligand 1A exhibited 1 hydrogen bond interactions with amino acid, MET 414 in the active site. The hydrogen bond is formed between amino group of indole ring in the ligand and oxygen in the carboxyl group of amino acid with a hydrogen bond distance of 2.584Å.

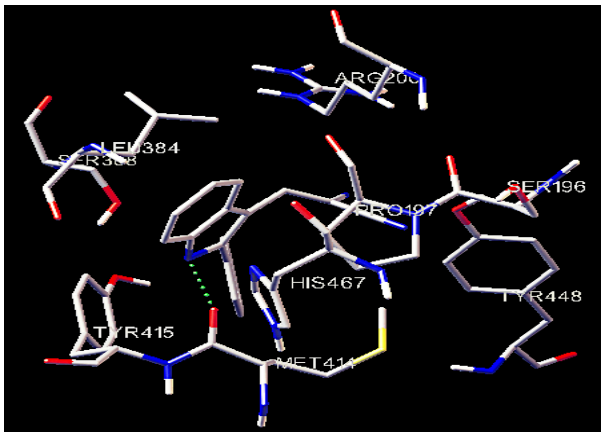


Fig 5: Interacting residues of ligand 2A with NS5B

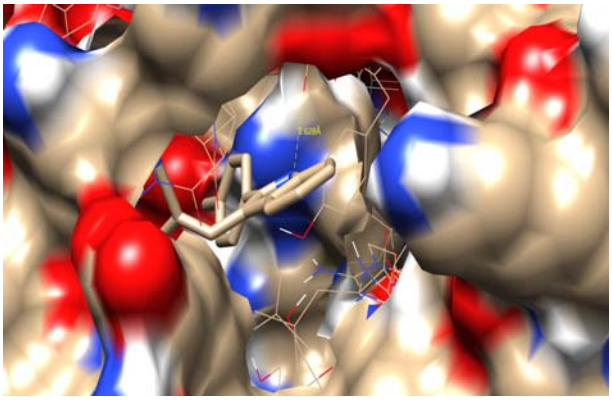


Fig 6: Surface view of ligand 2A complexed with NS5B

The binding energy of ligand 2A with NS5B polymerase enzyme is -8.34kJ/mol. The ligand efficiency of 2A was -0.38 and the Van der Waals dissolution energy were found to be -8.68. This ligand has an intermol energy of -8.64 with a total internal of -0.51. The electrostatic energy of this ligand is -0.04 and the inhibition constant is 765.26 nM. The docking of the ligand with NS5B has 9 Van der Waals interacting residues at binding site which are ARG 200, HIS 467, LEU 384, MET 414, PRO 197, SER 196, SER 368, TYR 415 and TYR 448. Ligand 2A exhibited 1 hydrogen bond interactions with amino acid, MET 414 in the active site. The hydrogen bond is formed between amino group of indole ring in the ligand and oxygen in the carboxyl group of amino acid with a hydrogen bond distance

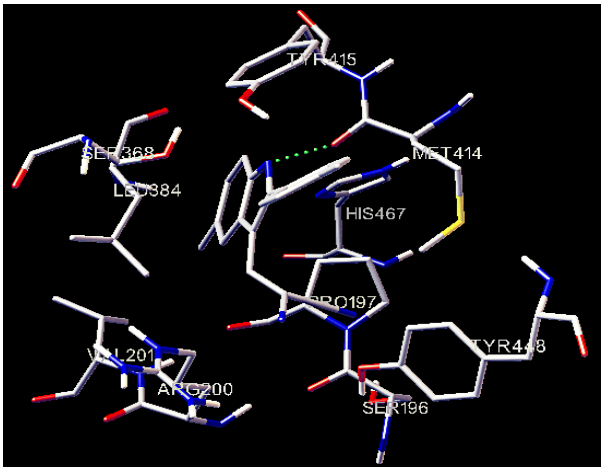


Fig 7: Interacting residues of ligand 3A with NS5B

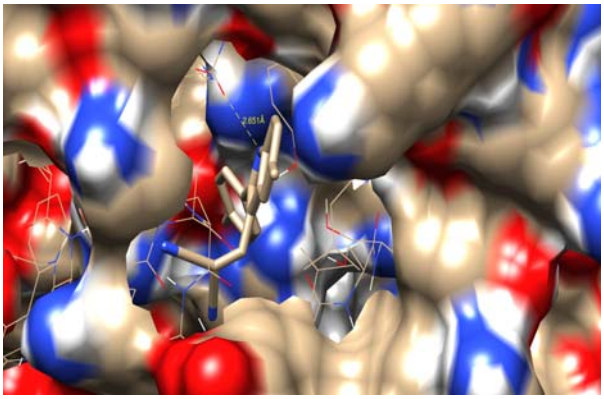


Fig 8: Surface view of ligand 3A complexed with NS5B

The binding energy of ligand 3A with NS5B polymerase enzyme is -9.09kJ/mol. The ligand efficiency of 3A was -0.4 and the Van der Waals dissolution energy were found to be -9.42. This ligand has an intermol energy of -9.38 with a total internal of -0.54. The electrostatic energy of this ligand is -0.03 and the inhibition constant is 216.04 nM. The docking of the ligand with NS5B has 10 Van der Waals interacting residues at binding site which are ARG 200, HIS 467, LEU 384, MET 414, PRO 197, SER 196, SER 368, TYR 415, TYR 448, and VAL 201. Ligand 2A exhibited 1 hydrogen bond interactions with amino acid, MET 414 in the active site. The hydrogen bond is formed between amino group of indole ring in the ligand and oxygen in the carboxyl group of amino acid with a hydrogen bond distance of 2.615Å.

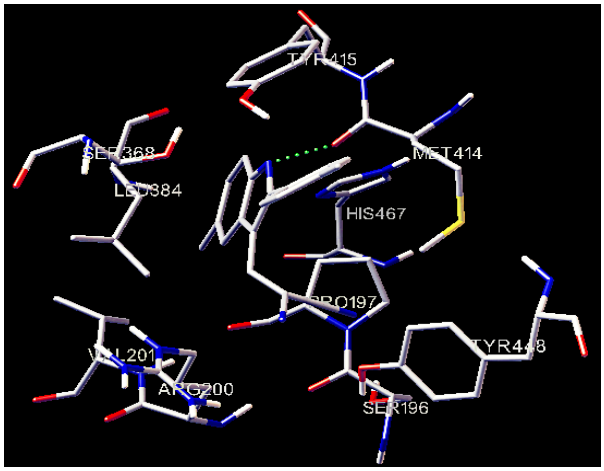


Fig 9: Interacting residues of ligand 3A with NS5B

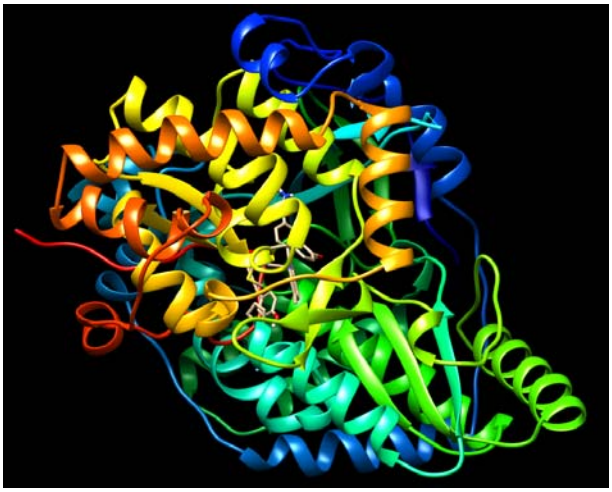


Fig 10: Ribbon view of ligand 3A complexed with CDK2

The binding energy of ligand 4A with NS5B polymerase enzyme is -8.08kJ/mol. The ligand efficiency of 4A was -0.35 and the Van der Waals dissolution energy were found to be -8.35. This ligand has an intermol energy of -8.38 with a total internal of -0.47. The electrostatic energy of this ligand is -0.03 and the inhibition constant is 1.2 uM. The docking of the ligand with NS5B has 8 Van der Waals interacting residues at binding site which are ARG 200, HIS 467, LEU 384, MET 414, PRO 197, SER 196, SER 368, TYR 415, TYR 448 and VAL 201. Ligand 4A did not form a hydrogen bond with the amino acid residues in the active side of NS5B polymerase enzyme.

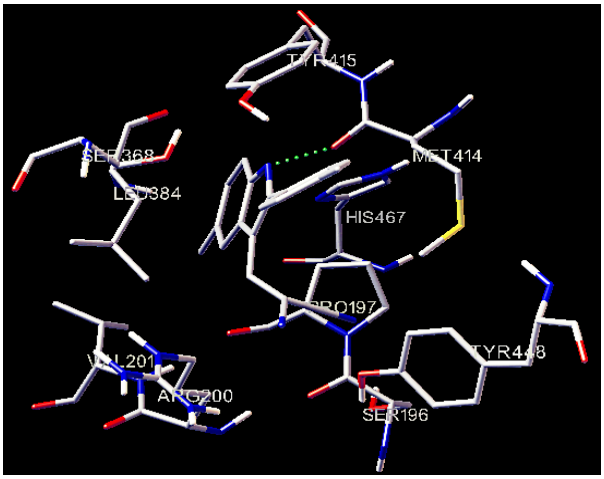


Fig 11: Interacting residues of ligand 5A with NS5B

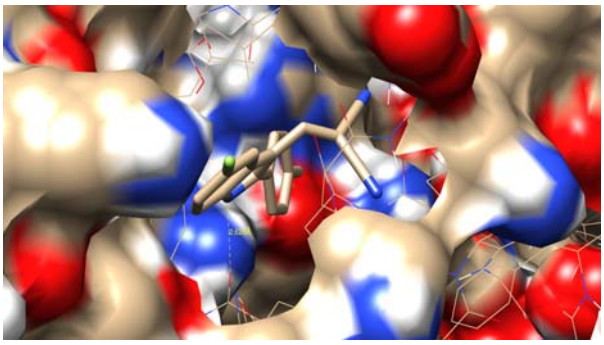


Fig 12: Surface view of ligand 5A with NS5B

The binding energy of ligand 5A with NS5B polymerase enzyme is -8.28kJ/mol. The ligand efficiency of 5A was -0.36 and the Van der Waals dissolution energy were found to be -8.61. This ligand has an intermol energy of -8.53 with a total internal of -0.53. The electrostatic energy of this ligand is -0.03 and the inhibition constant is 852.32nM. The docking of the ligand with NS5B has 9 Van der Waals interacting residues at binding site which are ARG 200, HIS 467, LEU 384, MET 414, PRO 197, SER 196, SER 368, TYR 415 and TYR 448. Ligand 5A exhibited 1 hydrogen bond interactions with amino acid, MET 414 in the active site. The hydrogen bond is formed between amino group of indole ring in the ligand and oxygen in the carboxyl group of amino acid with a hydrogen bond distance of 2.626Å

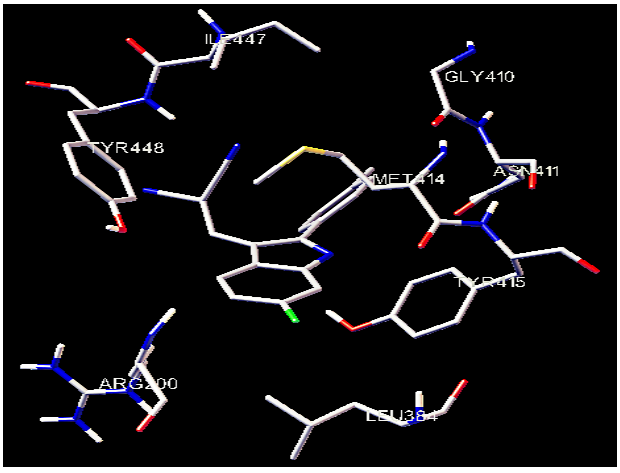


Fig 13: Interacting residues of ligand 6A with NS5B

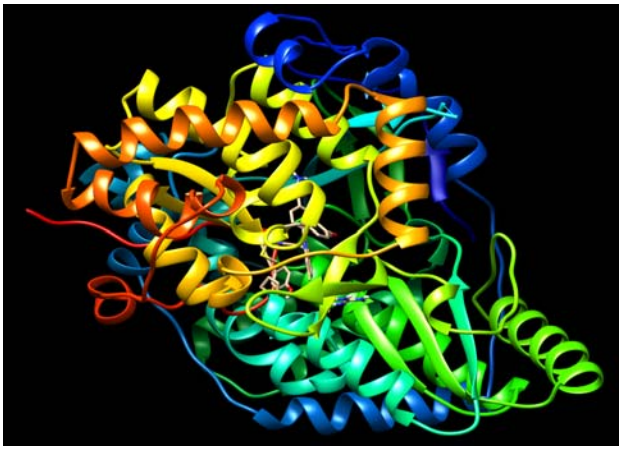


Fig 14: Ribbon view of ligand 6A complexed with NS5B

The binding energy of ligand 6A with NS5B polymerase enzyme is -7.73kJ/mol. The ligand efficiency of 4A was -0.34 and the Van der Waals dissolution energy were found to be -8.0. This ligand has an intermolecular energy of -8.03 with a total internal of -0.5. The electrostatic energy of this ligand is -0.03 and the inhibition constant is 2.14 μM. The docking of the ligand with NS5B has 8 Van der Waals interacting residues at binding site which are ARG 200, ASN 411, GLY 410, ILE 447, LEU 384, MET 414, TYR 415 and TYR 448. Ligand 6A did not form a hydrogen bond with the amino acid residues in the active side of NS5B polymerase enzyme.

4. Discussion

Computational techniques are routinely used in modern drug design to help in understanding the drug-receptor interaction. It has been shown in many literatures that molecular docking can strongly support and help the design of novel, more potent inhibitors by revealing the mechanism of drug-receptor interaction. The present docking study is carried out for six compounds against target protein NS5B polymerase enzyme (3UPI) within the active site which is MET 414. Table 2 and 3 show the binding energy and the inhibition constant of the selected ligands respectively. This docking study revealed that maximum number of the selected compounds has good binding energy with the target protein which range from -7.73 to -9.09 kJ/mol. The ligands 3A and 2A showed the highest binding energy of -9.09 kJ/mol and -8.34 kJ/mol, with ligand efficiency of 0.4 and 0.38 respectively. These molecules are completely surrounded by the amino acid residues in the active site pocket as shown in Figure 3 and Figure 4. However compound 4A and 6A did not form hydrogen bond with the target protein although it has a high binding energy of 8.08 kJ/mol and 7.73 kJ/mol respectively. Compounds 4A and 6A have a methoxide and a fluoride molecule at the R1 position respectively. The presence of these molecules at the R1 position has prevented the formation of hydrogen bond between the compounds and the target protein. Besides that, the residue in the active site which is involved in the hydrogen bonding of most of the ligands is MET 414.

5. Conclusion

Molecular docking study was performed in a series of indole derivatives with cyanide group as inhibitors of NS5B polymerase enzyme (3UPI). Almost all of the selected ligands showed good binding energy together with good inhibition constant. Of all the ligands docked, compound 3A and 2A exhibit the highest binding energy which is -9.09 kJ/mol and 8.34 kJ/mol respectively. This study has provided a theoretical framework to drug design of 2-phenylindole derivatives as novel inhibitors of NS5B polymerase enzyme of Hepatitis C virus.

6. References

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