



Scholars Research Library

J. Comput. Method. Mol. Design, 2011, 1 (4):35-45
(<http://scholarsresearchlibrary.com/archive.html>)



ISSN : 2231- 3176
CODEN (USA): JCMMDA

Docking Studies of few C-3 Substituted Azapteridines as Hepatitis C Virus RNA-Dependent RNA Polymerase inhibitors

Anil M. Manikrao^{1*}, Niranjan S. Mahajan¹, Rahul D. Jawarkar^{1*}, Devidas T. Mahajan²,
Vijay H. Masand², Taibi. ben. Hadda³

¹Department of Pharmaceutical Chemistry, Sahyadri College of College of Pharmacy, Methawade, Sangola, Solapur, Maharashtra, India

²Department of Chemistry, VidyaBharati Mahavidyalaya, Camp, Amravati, Maharashtra, India

³Laboratoire Chimie des Matériaux, Faculté des Sciences, Université Mohammed Premier, Oujda, Morocco

ABSTRACT

Docking studies of few C-3 Substituted Azapteridines which act as Hepatitis C Virus RNA-Dependent RNA Polymerase inhibitor was performed by using MOE 2009. The docking studies reveal that majority of the Azapteridine derivatives interacted with Hepatitis C Virus RNA-Dependent RNA Polymerase through hydrogen bonding as well as hydrophobic interactions. The present analysis is useful in future drug design.

Keywords: - Azapteridines, drug design, hydrogen bonding, Hydrophobic interaction, MOE 2009.

INTRODUCTION

Hepatitis C virus (HCV) infection is a major cause of liver failure, and is responsible for the majority of liver transplants. An estimated 170 million people have been infected by HCV [1], which in most cases establishes a chronic infection that puts them at risk for cirrhosis and hepatocellular carcinoma. The current standard of treatment is pegylated interferon-alpha (IFN- α) in combination with ribavirin. This treatment provides a sustained response in about 80% of patients infected with genotype 2 and 3 viruses, but only about 40% of those infected by a genotype 1 HCV [2]. These drugs also result in potentially severe side effects, causing a significant number of patients to withdraw from treatment [3].

HCV is a positive strand RNA virus that encodes at least four enzymatic activities. These include the NS2 protease, a protease and a helicase encoded by NS3 and, a polymerase encoded by NS5B. HCV is strictly dependent on its RNA-dependent RNA polymerase (RdRp) for genome replication, and this polymerase is an attractive target for development of inhibitors [4]. The RdRp shares a core structure common to other polymerases [5-9], however, sequence homology with other polymerases is limited to the catalytic site. There is a high degree of sequence diversity within the HCV genome across isolates, due to the low fidelity of the RdRp. This presents a challenge for identifying efficacious anti-HCV drugs, both because of the structural variability in the initial drug target and the potential for the rapid development of resistance. The development of a new generation of inhibitors has attracted the attention of many researchers working in the design, synthesis, and molecular modeling studies of reversible and selective inhibitors.[10] The determination of the 3D structure of Hepatitis C Virus RNA-Dependent RNA Polymerase by X-ray crystallography has opened the way for molecular modeling studies. To get better insight into the Inhibitory mechanism of Hepatitis C Virus RNA-Dependent RNA Polymerase, a series of Azapteridine derivatives were collected from literature [11] and studied against Hepatitis C Virus RNA-Dependent RNA Polymerase enzyme by molecular docking .

MATERIALS AND METHODS

Experimental

Structures of all Azapteridine derivatives were collected from literature [11]. The compounds were sketched and converted into three dimensional structures using the program Chem Draw [12] . Since the main goal of this study was to perform docking to understand binding between ligand (Azapteridine analogues) and receptor (Hepatitis C Virus RNA-Dependent RNA Polymerase PDB ID-2gir) was chosen to get fruitful results [13]. During docking most of the default settings were applied except that the number of retain were 30 instead of 10 during docking in MOE. Protein structures were first repaired, Ramchandran plot was plotted to ascertain the health of protein (Figure 1) and then appropriately protonated in the presence of ligand using the Protonate3D [14] process in MOE. Proteins prepared in this manner were applied directly for docking. It is well documented in literature [15] that if a crystallographic structure of the protein complexed with a relatively close analog of the ligand is available, "ligand-based docking" may be performed. In this procedure, one or more conformations of the candidate ligand are fitted to the crystallographic structure of the known ligand by optimizing the similarity in electrostatic and steric potentials. The experimental structure of the "template" ligand is then deleted, leaving the candidate ligand docked to the protein. In addition, the conformation of the fitted ligand may be simultaneously optimized during the fitting. The same strategy was used to get best docking results. The default procedure using Triangle Matcher placement method with London dG scoring was used for the docking runs.

Docking Algorithms

Docking programs are of two classes, "direct" and "unbiased." Despite of the disadvantage of making assumptions about the potential energy landscape to save computational time direct docking softwares such as DOCK have the benefit of speed. Unbiased methods such as AutoDock, FTDOCK and MOE-Dock perform with few assumptions about the potential energy landscape. Thus at the expense of computation time, they find final docked solutions that the

direct method might have missed. Here we report the use of MOE-Dock by Chemical Computing Group Inc.⁵, which has the advantage flexible docking as well as integration with a graphical interface as well as with other modules, such as analysis, molecular mechanics, and molecular dynamics.

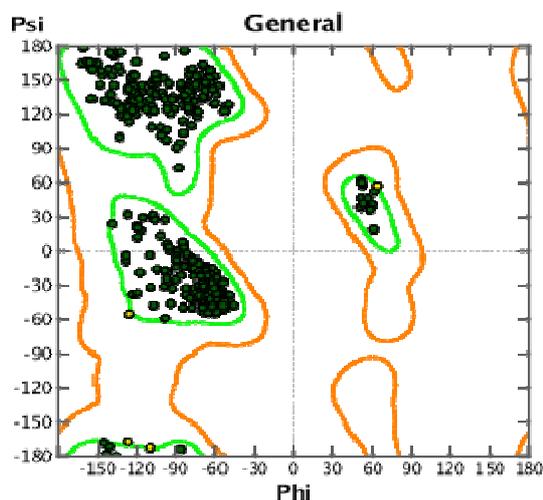


Figure:-1 Ramchandran Plot (pdb- 2gir) after energy and residue optimization

Docking Simulations

In MOE London dG scoring is used as default setting to calculate the exact confirmation and configuration of the ligand to find the best molecule with minimum binding energy and it can be used to develop potential drug molecules against the disease. The London dG scoring function estimates the free energy ΔG of binding of the ligand from a given pose. The functional form is a sum of terms:

$$\Delta G = c + E_{flex} + \sum_{h-bonds} c_{HB} f_{HB} + \sum_{m-lig} c_M f_M + \sum_{atoms\ i} \Delta D_i$$

where C represents the average gain/loss of rotational and translational entropy; E_{flex} is the energy due to the loss of flexibility of the ligand (calculated from ligand topology only); f_{HB} measures geometric imperfections of hydrogen bonds and takes a value in $[0,1]$; C_{HB} is the energy of an ideal hydrogen bond; f_M measures geometric imperfections of metal ligations and takes a value in $[0,1]$; C_M is the energy of an ideal metal ligation; and D_i is the desolvation energy of atom i . The difference in desolvation energies is calculated according to the formula

$$\Delta D_i = c_i R_i^3 \left\{ \iiint_{u \notin A \cup B} |u|^{-6} du - \iiint_{u \notin B} |u|^{-6} du \right\}$$

Where A and B are the protein and/or ligand volumes with atom i belonging to volume B ; R_i is the solvation radius of atom i (taken as the OPLS-AA van der Waals sigma parameter plus 0.5 Angstrom); and C_i is the desolvation coefficient of atom i . Atoms are categorized into ~12 atom

types for the assignment of the C_i coefficients. MOE 2009.10 was run on a Windows XP based Pentium Dual Core 3.00 GHz PC (with 2GB RAM).

RESULT AND DISCUSSION

The purpose of the Dock application is to search for favorable binding configurations between small to medium-sized ligands and a not-too-flexible macromolecular target, which is usually a protein. For each ligand, a number of configurations called *poses* are generated and scored in an effort to determine favorable binding modes. The search for binding modes is usually constrained to a specific, small region of the *receptor* called the *site*. As one would expect the predictive power of Docking correlates with the degrees of freedom of the system. Docking results are expected to be reliable when the ligands are molecules with limited flexibility, and the site is not significantly larger than the ligand. Ligands having up to 10 rotatable bonds can be handled reasonably. However, if there is extra pharmacophore information, docking more flexible ligands may still produce reasonable results. The Docking workflow is divided into stages. For each stage, multiple methods are available, and new methods can easily be integrated. The stages are: **Conformational Analysis**. If ligand conformations are not supplied via a conformation database, Docking can be used to generate conformations from a single 3D conformer by applying a collection of preferred torsion angles to the rotatable bonds. Bond lengths and bond angles will not be altered. Rings will not be flexed. **Placement**. A collection of poses is generated from the pool of ligand conformations using one of the placement methods like Alpha Triangle in which Poses are generated by superposition of ligand atom triplets and triplets of receptor site points **Rescoring (1)**. Poses are generated by the placement methodology can be rescored using one of the available methods. Typically, scoring functions emphasize favorable hydrophobic, ionic and hydrogen bond contacts like **London dG Scoring (default in MOE)** function estimates the free energy of binding of the ligand from a given pose. **Refinement** Poses resulting from the placement stage can be refined using either the explicit molecular mechanics forcefield method or the grid-based energetics method and **Rescoring (2)**. Poses resulting from the refinement stage can be rescored using one of the scoring schemes.

In the present docking analysis, all twelve c-3 substituted Azapteridine derivatives were docked into active site of Hepatitis C Virus RNA-Dependent RNA Polymerase enzyme. Docking results for twelve Azapteridine derivatives were given in table no-1. For our studies, X-ray crystal structure of Hepatitis C Virus RNA-Dependent RNA Polymerase enzyme was taken from PDB entry 2gir, having resolution of 2.0 Å.

Docking analysis reveal that the most active compound (compound no-12) interacted with receptor through nonpolar and hydrophobic interactions, there is no hydrogen bond formation reported during docking analysis (figure no-2). The number of conformations generated by compound 12 were 18 which indicated that flexibility is an important parameter for the ligand to docked deeply within the binding pocket of Hepatitis C Virus RNA-Dependent RNA Polymerase enzyme. The energy of conformation for compound 12 was -9.9366 which indicate compound is active at lowest energy of conformation. There exist a good correlation between IC_{50} and energy of conformation which suggest that the most active compound 12 have lowest energy of conformation (as shown in table no-1). Further a careful inspection of the binding pocket

indicated that compound 12 adopted a position in a hydrophobic cage surrounded by Trp 528, Arg 422, Met 423, Leu 497, Leu 474, His 475 and Ser 476. These amino acid residue approach closely to the

ligand but do not have any qualifying strong interactions (i.e. hydrogen bonds) may be classified as non-bonded residues that have a significant effect on the orientation and binding of the ligand, but which may be spread out over a number of pairwise contacts, each of which is relatively weak. Quinoline ring in compound 12 approaching hydrophobic region of receptor which involves amino acid residues like Arg 422, Met 423 and Trp 528 (as shown in figure no- 2 (a))

Table :-1 Docking results for C-3 Substituted Azpteridine derivatives.

compound no	S	rmsd_refine	E_conf	E_place	E_score1	E_refine	No. of conf
1	-11.7888	3.07243	-38.965	-45.533	-8.2433	-11.789	15
2	-11.6148	3.00503	-36.867	-53.487	-8.7094	-11.615	15
3	-12.4556	2.73222	-46.43	-50.405	-8.8986	-12.456	16
4	-11.9546	1.71762	-43.639	-40.008	-8.5578	-11.955	17
5	-12.2287	1.5915	-32.442	-43.271	-8.2025	-12.229	13
6	-13.1063	1.94384	0.5887	-48.582	-8.397	-13.106	16
7	-12.5119	1.91239	-17.986	-45.367	-8.5353	-12.512	11
8	-14.7077	1.01005	-28.204	-79.129	-8.528	-14.708	14
9	-14.1815	1.97697	3.09402	-67.367	-8.1086	-14.182	11
10	-16.2314	2.4748	-1.9265	-58.854	-8.9242	-16.231	22
11	-15.9153	2.65673	7.50051	-58.066	-8.7908	-15.915	10
12	-14.6086	2.01911	-9.9366	-49.641	-8.2756	-14.609	18

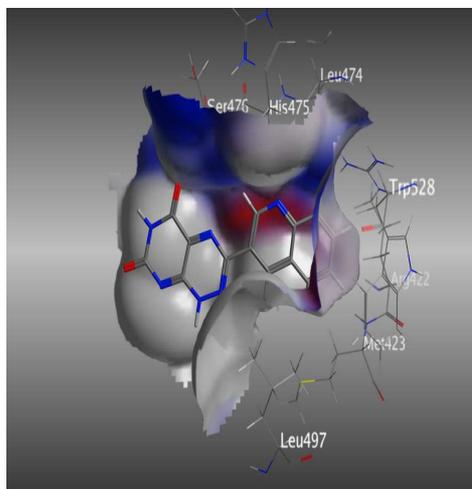
S - The final score, which is the score of the last stage that was not set to none, *rmsd_refine*- The root mean square deviation between the pose before refinement and the pose after refinement, *E_conf*- The energy of the conformer. If there is a refinement stage, this is the energy calculated at the end of the refinement. Note that for Forcefield refinement, by default, this energy is calculated with the solvation option set to Born, *E_place* - Score from the placement stage, *E_score1*- Score from the rescoring stage(s), *E_refine*- Score from the refinement stage and *No. of conf*- number of conformations generated by ligand.

Final docked position of flat azapterinine ring showed shape complementarity with flat hydrophobic region of the receptor which revealed that azapteridine ring significantly involved in hydrophobic interaction with receptor.(as shown in figure 2 (a) and (b)).

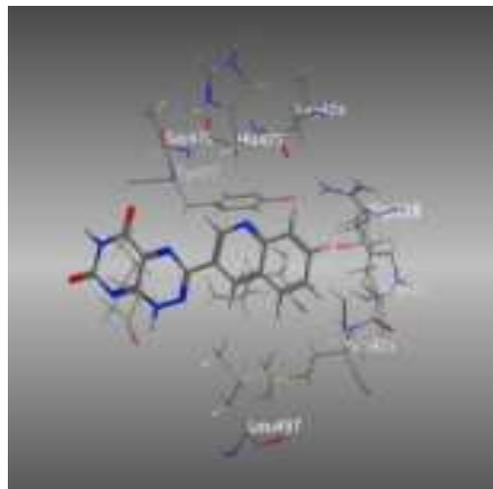
Second most active compound 11 interact with receptor through hydrogen bonding wherein carbonyl group of azapteridnine ring form hydrogen bond with Arg 422 residue .

The hydrogen bonding score was found to be 20% and interatomic distance was recoded to be 1.98 Å⁰. The C-3 substituted phenoxy phenyl group impart flexibility to the azapteridine ring and interacted with residues Ile 482, Leu 419 and Leu 474 through nonbonding interactions.(as shown in figure 2 (c)). Apparently, the residue Arg 422 may contribute to the binding and stabilization of compound 11 in the cavity space of RNA polymerase. The energy of conformation for compound 11 was observed to be 7.50051 which suggest that flexibility of phenoxy phenyl group may not significantly affecting the biological activity.(as shown in table no-1).The number

of conformation generated by compound 11 is merely due to the phenoxy phenyl substituent at c-3 position.



(a) Compound 12



(b) Compound 12

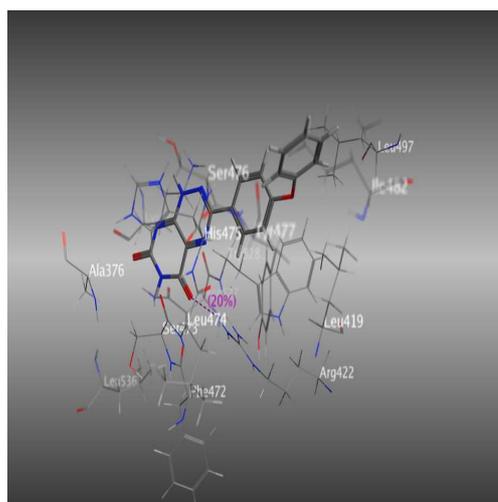


Figure no - 2 (c) Compound 11

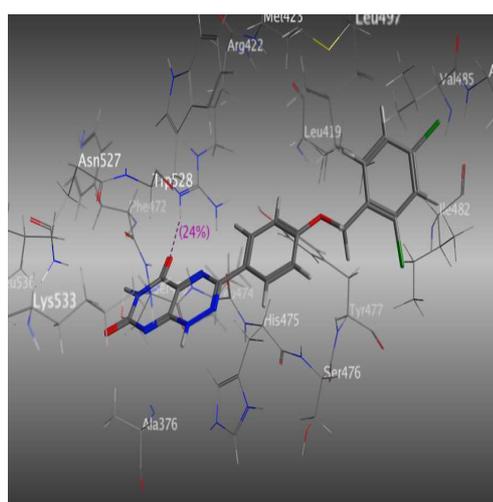


Figure no 2 (d) Compound-10

Furthermore compound 10 and 9 interacted with RNA polymerase through hydrogen bonding with residue Arg 422 in which hydrogen bond score for compound 10 was observed to be 24% and for compound 9, hydrogen bonding score was 23 % . The docking results for compound 9 and 10 revealed that biphenyl group in compound 9 was oriented horizontally in the hydrophobic cage of receptor wherein ,it is interacted with residues Leu 497, Leu 419 and Ile 482. These residue may be involved in flexible alignment of compound 9 in the hydrophobic cage of receptor. (As shown in figure 2 (e))

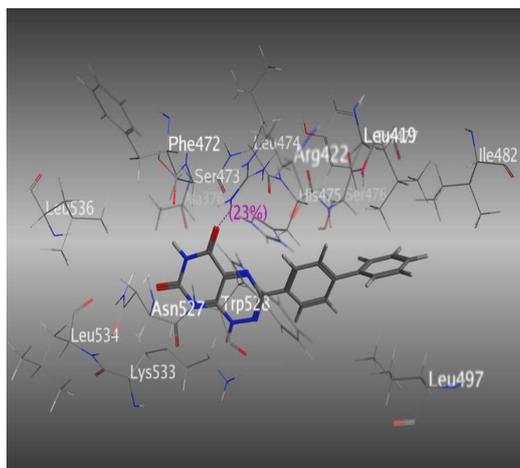


Figure no 2(e) Compound- 9

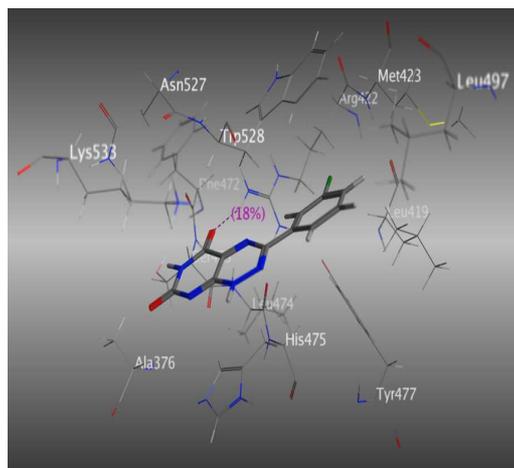


Figure no 2(f) Compound-8

In compound 10, substituent at c-3 position facing closely to the amino acid residue Ile 482, Leu 419 and Val 485 which suggest that compound 10 had close contact with the receptor surface which may offer proper fit between drug and receptor. (as shown in figure 2(d)). The energy of conformation for compound 9 and 10 was observed to be 3.09402 and -1.9265 which suggest that compound 10 elicited superior biological response as compare to compound 9 due to low energy of conformation. (as shown in table no-1). Moreover the number of conformation generated by compound 10 was 22 which evidently intend that flexibility is a crucial parameter for proper alignment as well as stability of ligand into the receptor cavity (as shown in table no-1).

Compound 7 reported no hydrogen bonding interaction with receptor whereas compound 8 forms hydrogen bond with Arg 422 residue. In compound 7, phenyl group is placed between residues Leu 419, Trp 528, His 475, Leu 497 and Arg 422 revealed that compound entered into the receptor pocket by posing the phenyl group at front which was orienting horizontally into the receptor pocket. (as shown in figure 2 (g)).

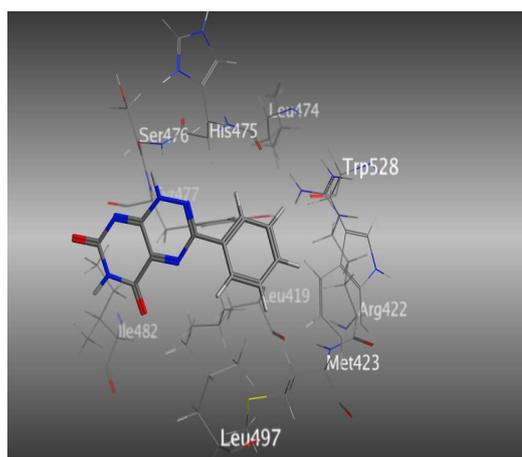


Figure no 2(g) Compound -7

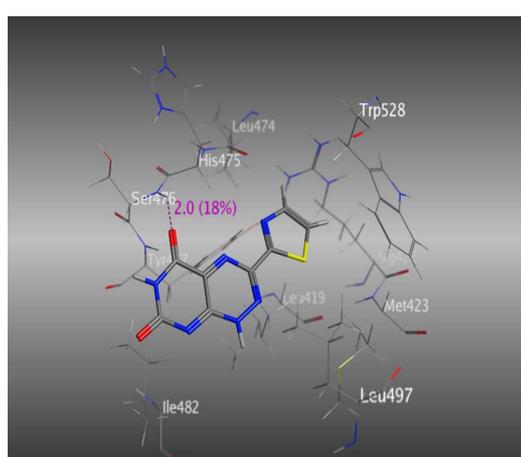


Figure no (2h) Comp-6

The energy of conformation for compound 7 (IC₅₀ - 1.31) was observed to be -17.986 which suggest the stable interaction between drug and receptor. (Table no-1). In compound 8, *c*-3 substituted phenyl ring which have further chloro substitution at meta position is in close proximity of Leu 419 residue and orienting horizontally in the receptor pocket surrounded by residues Leu 497, Trp 528, Met 423. (as shown in figure 2 (f)). These amino acids are non bonded residues which are involvd in flexible alignment as well as orientation of ligand into the receptor cavity. The energy of conformation for compound 8 (IC₅₀- 0.28) was observed to be -28.204 which imply that activity of compound correspond to the lowest energy of conformation.(as shown in table no-1).

Carbonyl group of azapteridine ring in compound 6 form hydrogen bond with Ser 476 residue of receptor and hydrogen bonding score was found to be 20%. Azapteridine ring orient closely near the residues Tyr 477, Ile 482 and His 475 also contributed to some of the interactions to stabilize the complex. The energy of conformation for compound 6 (IC₅₀ - 0.50) was observed to be 0.5887 which propose that compound is moderately active which match up the IC₅₀. (as shown in figure no- 2(h)). *C*-3 substituted thiazole ring positioned horizontally in the hydrophobic cage of receptor in which thiazole ring is surrounded by His 475, Trp 528 and Met 423 stabilized the drug receptor complex.

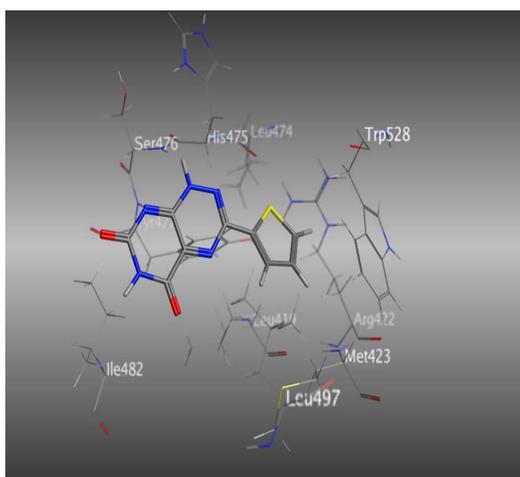


Figure no 2(i) Compound 5

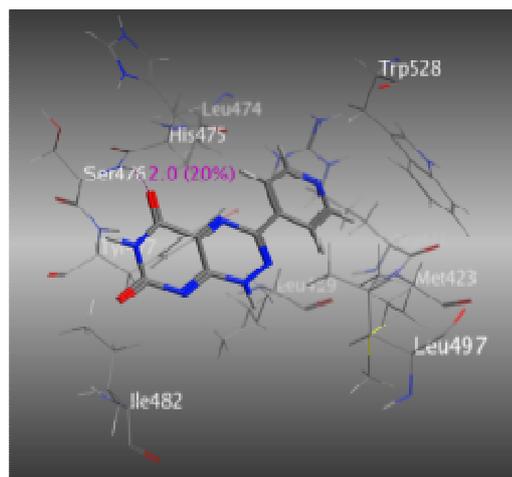


Figure no 2(j) Comp-4

Compound 5 interacted with receptor through nonbonded interaction. Azapteridine ring positioned closely toward Ile 482, Ser 476 and Tyr 477. The Azapteridine ring was sandwiched between the Ser 476 and Tyr 477 residues. (as shown in figure no- 2(i)). The thiophene ring at *c*-3 position is in close proximity to the residue Trp 528 and orient closely near residues Leu 419, Arg 422, Met 423 and Trp 528. The energy of conformation for compound 5 (IC₅₀ - 1.16) was observed to be -32.442 which suggest that substitution of thiophen ring at *c*-3 position have moderate impact on biological profile of compound 5 (Table no-1).

In compound 4, *C*-5 carbonyl group of azapteridine form hydrogen bond with Ser 476 residue of receptor wherein percent of hydrogen bonding was observed to be 20 %. Further *C*-7 carbonyl

group is in close proximity to the Tyr 477 and Ile 482 but no interaction was observed. Moreover, substitution of pyridine ring at C-3 position reported nonbonding interaction with residues Leu 419, Trp 528, Met 423, Leu 497 and His 475. (as shown in figure 2(j)). These residues may have certain hydrophobic kind of interaction with receptor. The energy of conformation for compound 4 (IC_{50} - 0.92) was observed to be -43.639 which intend that substitution of pyridine ring at C-3 position have less to moderate impact on biological activity. (as shown in table no-1).

No bonded interaction was reported in compound 3 wherein Azapteridine ring was positioned slight vertically in the hydrophobic cage of receptor pocket and is in close proximity to the residue Tyr 477 and Ile 482.(as shown in figure 2(k)). The cyclohexane ring at C-3 position orient vertically (due to its flexible nature) in the receptor pocket and may have hydrophobic interaction with the residues Met 423, Arg422, Trp 528, His 475, Leu 474 and Leu 497. The residues may be engaged in flexible orientation of cyclohexane ring into the receptor pocket. The energy of conformation for compound 3 was reported to be -46.43 which intend that substitution of cyclohexane ring at C-3 position have moderate impact on biological activity (IC_{50} - 0.58) . (as shown in table no-1).

Compound 1 and 2 have no bonding interaction with receptor. In compound 1, tertiary butyl group is in close proximity to the residues Leu 419, Arg422, Met 423, Tyr 474 and Arg 501.(as shown in figure no 2 (m)). Azapteridine ring in compound 1 orient horizontally in the hydrophobic pocket of receptor surrounded by residues Trp528, His 475, Ser 476, Lys 533, and Lys 531. These residues may engaged in hydrophobic interaction as well as flexible alignment of azapteridine ring into the receptor cavity. The energy of conformation for compound 1 was found to be -38.965 (as shown in table no-1)which revealed that substitution of tertiary butyl group have less impact on Biological activity of compound 1 (IC_{50} - 2.37) .

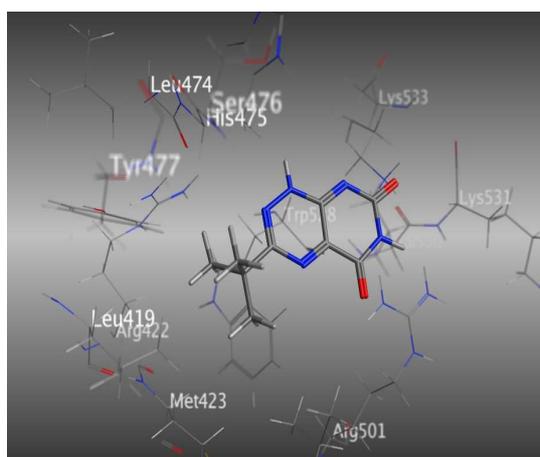


Figure no 2 (m) Compound 1.

In compound 2, C-3 substituted pentyl group in close contact with Leu 419 residue of receptor and may engaged in hydrophobic interaction with hydrophobic surface of receptor.(as shown in figure 2 (l)). Tertiary pentyl group positioned vertically within the

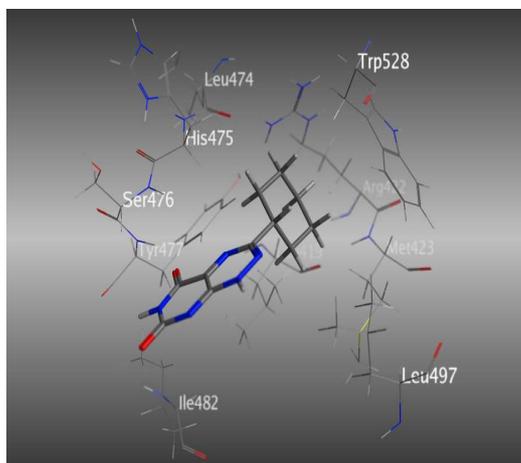


Figure no 2 (k) Comp-3

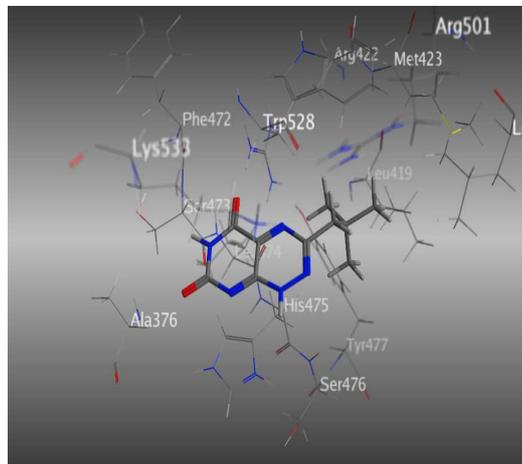


Figure no 2 (l) Compound -2

Binding packet of receptor surrounded by residues His 475, Tyr477, Ser 476, Leu 419 and Ser 473. This residue comprises hydrophobic core of receptor cavity and may engaged in hydrophobic interaction with compound 2. Moreover azapteridine ring in compound 2 is in close proximity to the His 475, Ala 376 and Ser 473. These residues contributed some of the interactions to stabilize the complex. The energy of conformation for compound 2 was observed to be -36.867 (as shown in table no-1) which suggest that substitution of tertiary pentyl group at C-3 position have significant impact on biological activity of compound 2 (IC₅₀- 0.66). Noticeably, Increase in number of carbon atom enhances the activity of compound 2 as compared to compound 1.

Acknowledgement

We are thankful to Sumit O. Bajaj, C. Eugene Bennett Department of Chemistry, West Virginia University, and Morgantown, West Virginia 26506-6045, USA for providing computational data and helpful discussions.

REFERENCES

- [1] Lavanchy D and McMahon B. Academic Press:, San Diego. **2000**, 185-202.
- [2] Kenny-Walsh E. *Clin. Liver Dis.* **2001**, 5, 969-77.
- [3] Fried MW *Hepatology*, **2002**, 36, S237-44.
- [4] De Francesco R, Tomei L, Altamura S, Summa and V Migliaccio G *Antiviral Res.* **2003**, 58, 1-16.
- [5] Bressanelli S, Tomei L, Rey FA, De Francesco R *J. Virol.* **2002**, 76, 3482-3492.
- [6] Lesburg C A, Cable M B, Ferrari E, Hong Z, Mannarino A F and Weber P C *Nat. Struct. Biol.* **1999**, 6, 937-43.

- [7] Labonte P, Axelrod V, Agarwal A, Aulabaugh A, Amin A and Mak P *J. Biol. Chem.*, **2002**, 277, 38838-46.
- [8] Adachi T, Ago H, Habuka N, Okuda K, Komatsu M, Ikeda S and Yatsunami K *Biochim. Biophys. Acta*, **2002**, 1601, 38-48.
- [9] Wang M N, K K Cherney, M M, Chan L, Yannopoulos CG, Bedard J, Morin N, Nguyen-Ba N, Alaoui-Ismaili M.H, Bethell R C and James M N *J. Biol. Chem.*, **2003**, 278, 9489-95.
- [10] Sloley B D, Urichuk L J, Morley P, Durkin J, Shan J J, Pang P K, Coutts R T *J. Pharm. Pharmacol.* **2000**, 52, 451.
- [11] T Middleton, H B Lim, D Montgomery, T Rockway, D Liu, L Klein, W Qin, J E Harlan, W M Kati and A Molla, *Letters in Drug Design & Discovery*, **2007**, 4, 1-8.
- [12] <http://www.cambridgesoft.com>
- [13] <http://www.rcsb.org/pdb/>:Hepatitis C Virus RNA-Dependent RNA Polymerase (PDBId.2gir).
- [14] H Singh, S S Marla, M Agarwal *Int. J. Comp. Sci.* **2007**, 33 (1), 1-7.
- [15] N. C. Cohen, *Guidebook on Molecular modeling in Drug Design*, Academic Press, **1996**, 253-257.