ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 09, Issue, 09, pp.8585-8601, September, 2018

RESEARCH ARTICLE

STRUCTURE AND SELECTIVITY OF BINDING SITES IN HIV PROTEIN BY 6-AMINOQUINOLINES MOLECULES: DOCKING AND MOLECULAR MECHANICS STUDIES

Tapash Kumar Barman and *Chitrani Medhi

Department of Chemistry, Gauhati University, Guwahati-781014, India

ARTICLE INFO

Article History:
Received 17th June, 2018
Received in revised form
26th July, 2018
Accepted 19th August, 2018
Published online 30th September, 2018

Key words:
Docking, Amino acid, active site, Hydrophobic, Hydrophilic, HIV.

ABSTRACT

Due to drug binding, deformation of HIV-RT enzyme in several regions at the active sites may take place. The drug resistance is the major problem in anti-AIDS drug therapy and development of new potent drugs has been one of the major challenges for researchers to tackle this problem. So, it is important to understand the features of inhibitor binding within HIV protein which is very important for designing more effective drugs. So the binding features of several 6-aminoquinolines are carried to understand the nature of hydrogen bonds and amino acid selectivity. There is significant variation of binding features of these molecules.

INTRODUCTION

Prediction of ligand-protein interaction is one of the crucial tasks. It relies on the accurate docking algorithm used in selecting the active site for binding by a particular ligand. Again molecular flexibility of ligand in binding is also accountable factor in assessing the ligand-protein interaction (Cecchetti *et al.,* 2000; Cecchetti *et al.,* 1995; Cecchetti *et al.,* 1996; Cruciani *et al.,* 1996; Mahmood *et al.,* 1993; Palu *et al.,* 1984; García-Sosa *et al.,* 2011). However, it is possible to attempt certain molecules with respect to substituents for comparing structural and conformational changes in ligand-protein interactions. The anti-AIDS drugs usually bind at specific active sites of RT virus. The biological properties of these drugs are quite similar (Cecchetti *et al.,* 2000; Cecchetti *et al.,* 1995; Cecchetti *et al.,* 1996; Cruciani *et al.,* 1996). So, it is essential to identify the active sites in protein for accessing ligands completely at the binding sites. Entry of these drugs inside the cavity of a particular active site for interaction with several groups and atoms inside the cavity are rather crucial. It is important to understand the accessibility of 6-aminoquinolines having various substituents inside the cavity. However, it is also important to examine the selectivity of active sites by these molecules for distinguishing binding modes. The docking studies can be used to identify several sites and binding modes, which depend especially the on the shapes of cavities and amino acid sequences of the active sites. There are several methods for analysing the binding of ligands inside biomolecules, but the molecular docking protocols have been successfully applied in several studies where the applications of other quantum mechanical computational methods are not feasible (Mahmood *et al.,* 1993; Palu *et al.,* 1984; García-Sosa *et al.,* 2011; Matsuyama *et al.,* 2010; Garg *et al.,* 1999). So, before proceeding to high level computational methods, it is essential to locate the binding positions of molecules within RT virus. Selection of active sites and estimating binding affinity of molecules within these sites might be related to the emergence of drug resistant, which is very important in the development of new drugs (Matsuyama *et al.,* 2010; Garg *et al.,* 1999; Tzoupis *et al.,* 2014; Cappelli *et al.,* 2011; Zhou *et al.,* 2014; Musmuca *et al.,* 2009; Ghosh and Brindisi, 2015). So, understanding of the ligand interaction in polymerase active site of HIV-RT is essential particularly for these molecules (Cecchetti *et al.,* 2000). The structures of HIV protein are obtained from Protein Data Bank (1EBZ.pdb).

There are several amino acid sequences in this structure. The study on several set of protein structures can provide the benefit of exploring a larger region of conformational space, as well as exploring chemical space to identify compounds that would bind well into the protein. The lowest energy ligand binding pose obtained from several docking protocols may be used. By doing so the target hits and scoring functions were subsequently predicted from several docking programs. The internal energies of the docked structures can be used to detect best possible interaction sites. When the ligands are docked inside the protein one can select the positions where ligands get deep interactions.

Corresponding author: **Chitrani Medhi,**
Department of Chemistry, Gauhati University, Guwahati-781014, India.

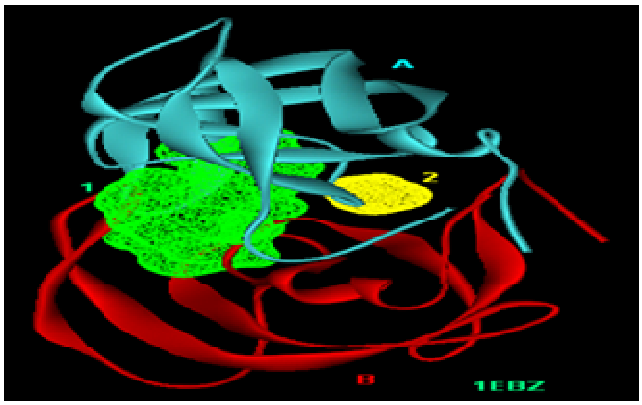
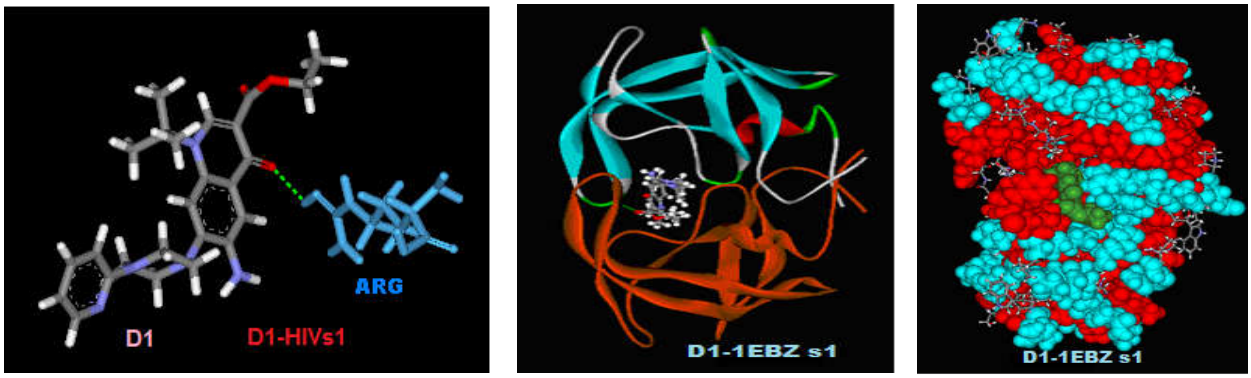


Figure 1. Active Sites in HIV-1EBZ Molecule (*Blue : Sequence A. Red: Sequence B. Green: Active Site1. Yellow: Active Site2*)



Figures 2(a). Interaction of D1 with portion of HIV-1EBZ (ARG) at site1 Charmm Minimised Complex(b) Interaction of D1 with portion of HIV-1EBZ (ARG) at site1 Charmm Minimised Complex Ribbon Structure. *(Blue-Sequence A. Red-Sequence B. White/Grey- Drug D1)*(c) Interaction of D1 with portion of HIV-1EBZ (ARG) at site1 Charmm Minimised Complex with Hydrophobic and Hydrophilic regions. (*Blue-Hydrophilic. Red- Hydrophobic. Green-Drug*)

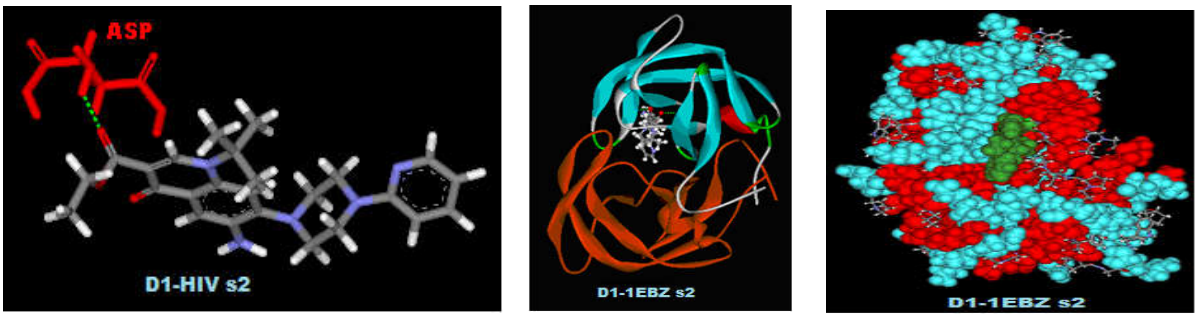
MATERIALS AND METHODS

The procedure as implemented in CHARMM, semi-flexible docking has been carried out (Discovery Studio, Accelyrs). In the sense that the structure of target molecule, the protein was kept rigid and the ligand molecule was made flexible within certain degrees of freedom. The water molecules and already existed ligand molecule were removed before performing docking studies. Similarly, the ligand, aminoquinolones are prepared and docking studies are carried to understand the active site selectivity of these molecules and also the conformation of ligand within the hydrophobic or hydrophilic region within the active sites. This will enable to understand the preference of amino acid sequences by these molecules within the active sites. Then the binding ability of these molecules may be compared. The presently used structure-based approaches to ligand design for HIV inhibitors are based on the crystal structure report of the receptor-ligand complexes (García-Sosa *et al.,* 2011; Matsuyama *et al.,* 2010). The LIGANFIT protocol of CHARMM package is used in this study (Discovery Studio, Accelyrs). This method is used in several docking studies in drug design (Matsuyama *et al.,* 2010; Garg *et al.,* 1999; Tzoupis *et al.,* 2014; Cappelli *et al.,* 2011; Zhou *et al.,* 2014).

RESULTS AND DISCUSSION

There are several binding sites in this HIV protein (Figure 1). Molecular docking simulation was carried out and also the scoring functions at the active site are also recorded (Tables 2.1- 2.6). The mode of binding of molecule at site is shown in

The values of the scoring functions of different methods are not equal but it is possible to find the selectivity of active site from the internal energy values. Maximum negative values of internal energies are taken to analyse the ligand-protein structures. Likewise the several molecules are taken for docking studies at the active site. The molecules D1-D11 are also capable of entering inside the cavity of site 1. The exact mode of binding of this molecule will be analysed from the docked structures (Figures 2-23). The values of scoring function of different methods vary significantly. The variation of internal energies for the most favoured conformations of molecules ranges from -1.451 kcal/mol to -8.451 kcal/mol. It is important to analyse the type of substituent and its orientation in the cavity of protein. The different interactions of substituents and groups within protein may provide some useful information on the effectiveness in binding inside the active site, which is very important in the development of new anti HIV agents. The values of top scoring solution are always referred in receptor-ligand docking. The internal energy values are shown in Tables 2.1-2.6 , and the maximum negative values for the docked structure is considered to be the most favoured position of ligand-protein binding. The variation of scoring functions for different docking protocol is large. Again the docked structures are minimised and geometries of ligands inside the pocket are further analysed. Figures 2-23 show the ligand-protein interaction at the minimized conformation of ligands within the active sites. The electrostatic stabilization energies are large for some molecules, D4, D5, D7 & D8 and the maximum negative value -113.055 kcal/mol (Table 3.1-3.2). The vander Waals stabilization energies are shown in Table 4.

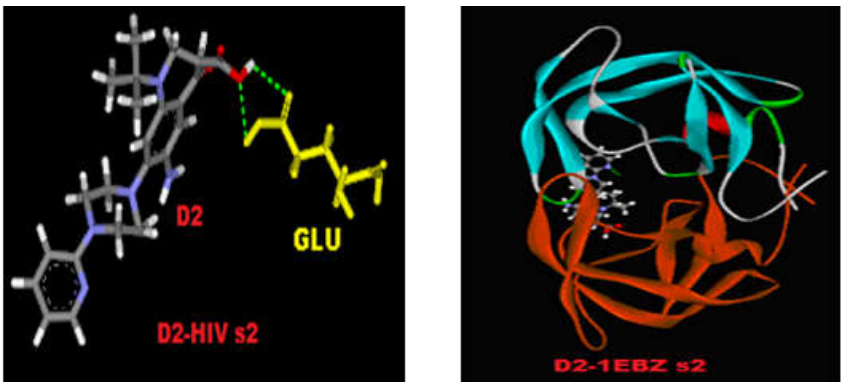


(a) (b) (c)

Figures 3(a) Interaction of D1 with portion of HIV-1EBZ (ASP) at site2 Charmm Minimised Complex Figure 3b Interaction of D1 with portion of HIV-1EBZ (ASP) at site2 Charmm Minimised Complex Ribbon Structure. (*Blue-Sequence A. Red- Sequence B. White/Grey- Drug D1*) © Interaction of D1 with portion of HIV-1EBZ (ASP) at site2 Charmm Minimised Complex with Hydrophobic and Hydrophilic regions. (*Blue-Hydrophilic. Red- Hydrophobic. Green-Drug*)

(a) (b) (c)

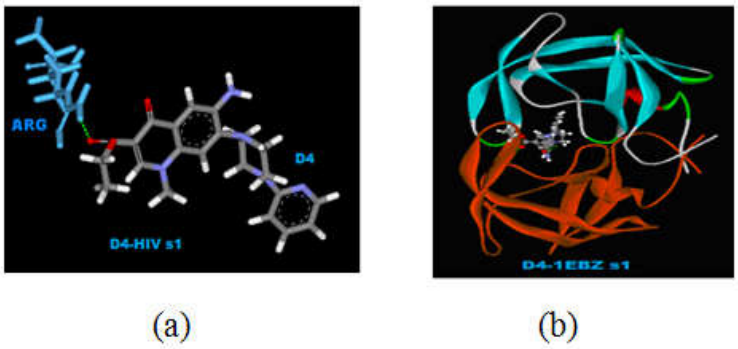
Figures 4(a) Interaction of D2 with portion of HIV-1EBZ (ASP pair & GLY) at site1 Charmm Minimised Complex (b) Interaction of D2 with portion of HIV-1EBZ (ASP pair & GLY) at site1 Charmm Minimised Complex Ribbon Structure. (*Blue-Sequence A. Red-Sequence B. White/Grey- Drug D2*) (c) Interaction of D1 with portion of HIV-1EBZ (ARG) at site1 Charmm Minimised Complex with Hydrophobic and Hydrophilic regions. (*Blue-Hydrophilic. Red- Hydrophobic. Green-Drug*)



Figures 5(a) Interaction of D2 with portion of HIV-1EBZ (GLU) at site2 Charmm Minimised Complex(b)Interaction of D2 with portion of HIV-1EBZ (GLU) at site2 Charmm Minimised Complex Ribbon tructure. (*Blue-Sequence A. Red- Sequence B. White/Grey- Drug D2*)

(a) (b)

Figures 7(a) Interaction of D3 with portion of HIV-1EBZ (ASP) at site2 Charmm Minimised Complex.(b) Interaction of D3 with portion of HIV-1EBZ (ASP) at site2 Charmm Minimised Complex Ribbon Structure. (*Blue-Sequence A. Red- Sequence B. White/Grey- Drug D3*)



Figures 8(a) Interaction of D4 with portion of HIV-1EBZ (ARG) at site1 Charmm Minimised Complex(b) Interaction of D4 with portion of HIV-1EBZ (ARG) at site1 Charmm Minimised Complex Ribbon Structure. (*Blue-Sequence A. Red- Sequence B. White/Grey- Drug D4*)

Figures 9(a) Interaction of D4 with portion of HIV-1EBZ (ASP) at site2 Charmm Minimised Complex.(b) Interaction of D4 with portion of HIV-1EBZ (ASP) at site 2 Charmm Minimised Complex Ribbon Structure. (*Blue-Sequence A. Red- Sequence B. White/Grey- Drug D4*)

Figures 10(a) Interaction of D5 with portion of HIV-1EBZ (ARG & GLY) at site1 Charmm Minimised Complex.(b) Interaction of D5 with portion of HIV-1EBZ (ARG & GLY) at site1Charmm Minimised Complex Ribbon Structure. (*Blue-Sequence A. Red- Sequence B. White/Grey- Drug D5*)

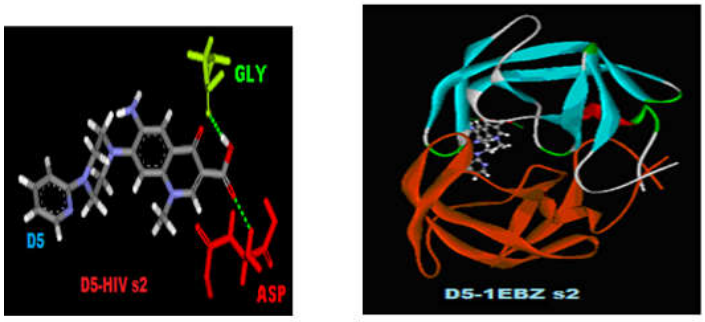
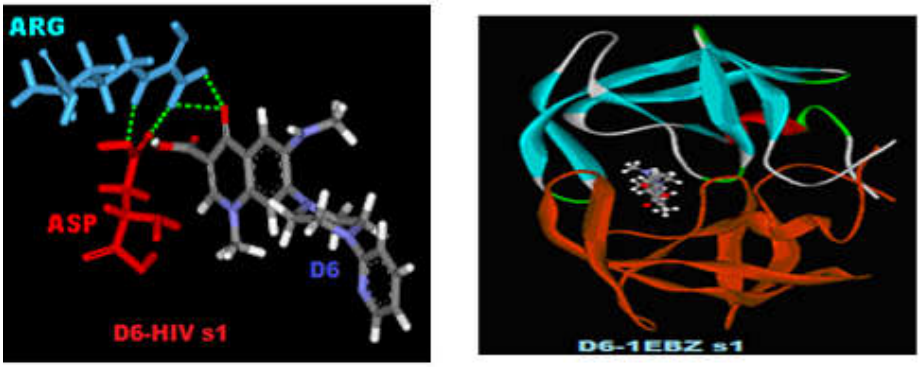
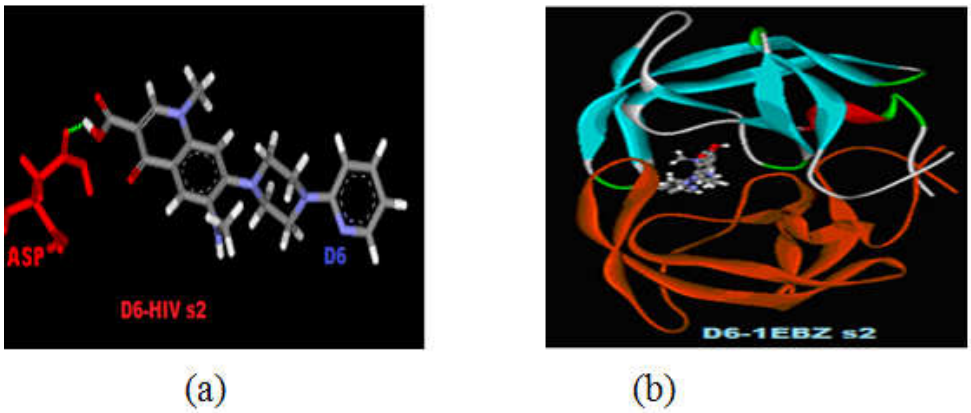


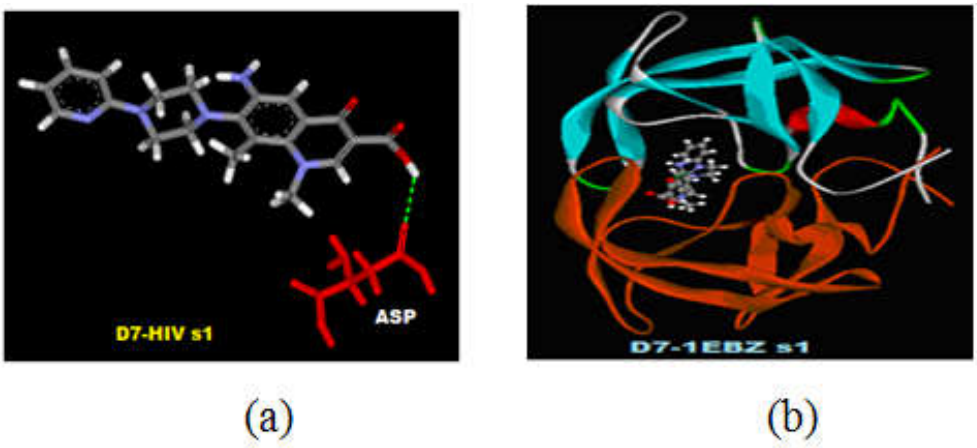
Figure s11(a) Interaction of D5 with portion of HIV-1EBZ (ASP & GLY) at site2 Charmm Minimised Complex.(b) Interaction of D5 with portion of HIV-1EBZ (ASP & GLY) at site2Charmm Minimised Complex Ribbon Structure.
(*Blue-Sequence A. Red- Sequence B. White/Grey- Drug D5*)



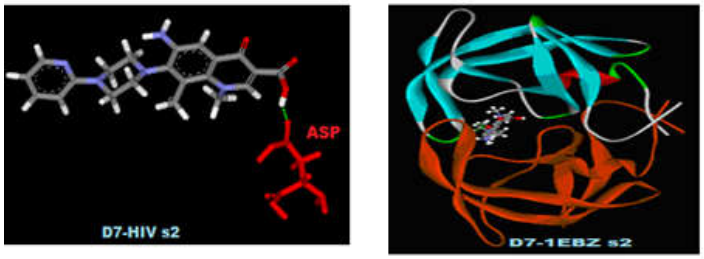
Figures 12(a) Interaction of D6 with portion of HIV-1EBZ (ASP & ARG) at site1 Charmm Minimised Complex.(b) Interaction of D6 with portion of HIV-1EBZ (ASP & ARG) at site1Charmm Minimised Complex Ribbon Structure. (*Blue-Sequence A. Red- Sequence B. White/Grey- Drug D6*)



Figures 13(a) Interaction of D6 with portion of HIV-1EBZ (ASP) at site2 Charmm Minimised Complex. (b) Interaction of D6 with portion of HIV-1EBZ (ASP) at site2.Charmm Minimised Complex Ribbon Structure. (*Blue-Sequence A. Red- Sequence B. White/Grey- Drug D6*)

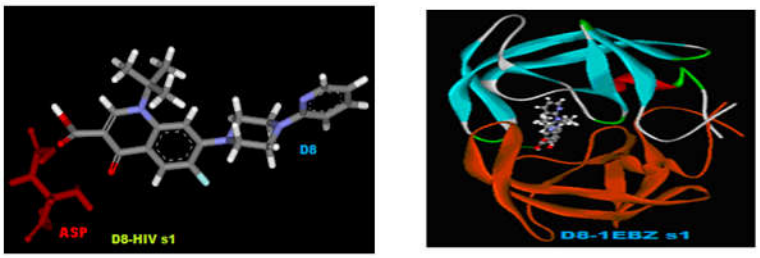


Figures 14(a) Interaction of D7 with portion of HIV-1EBZ (ASP) at site1 Charmm Minimised Complex.(b) Interaction of D7 with portion of HIV-1EBZ (ASP) at site1Charmm Minimised Complex Ribbon Structure. (*Blue-Sequence A. Red- Sequence B. White/Grey- Drug D7*)



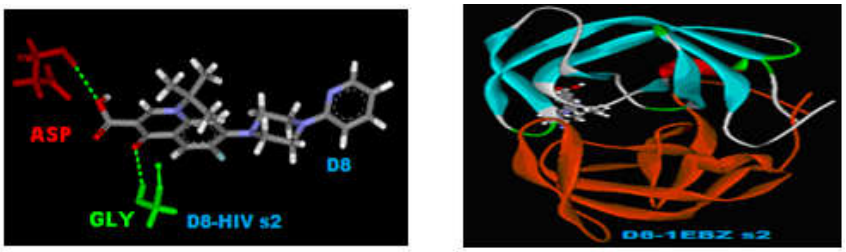
(a) (b)

Figures 15(a) Interaction of D7 with portion of HIV-1EBZ (ASP) at site2 Charmm Minimised Complex.(b) Interaction of D7 with portion of HIV-1EBZ (ASP) at site 2 Charmm Minimised Complex Ribbon Structure. (*Blue-Sequence A. Red- Sequence B. White/Grey- Drug D7*)



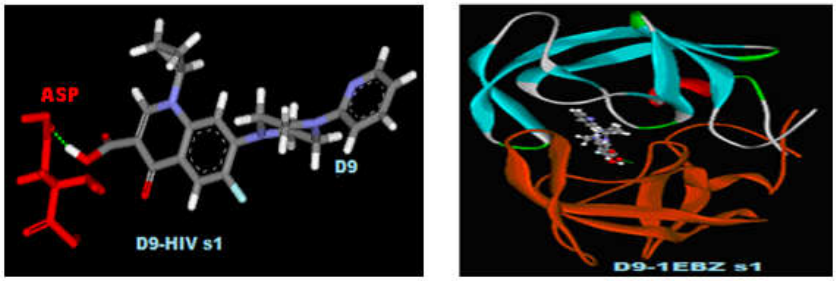
(a) (b)

Figures 16(a) Interaction of D8 with portion of HIV-1EBZ (ASP) at site1 Charmm Minimised Complex. (b) Interaction of D8 with portion of HIV-1EBZ (ASP) at site1.Charmm Minimised Complex Ribbon Structure. (Blue-Sequence A. Red- Sequence B. White/Grey- Drug D8)



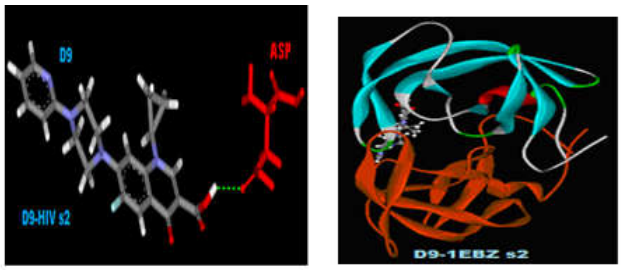
(a) (b)

Figures 17(a) Interaction of D8 with portion of HIV-1EBZ (ASP & GLY) at site2 Charmm Minimised Complex. (b) Interaction of D8 with portion of HIV-1EBZ (ASP & GLY) at site2 Charmm Minimised Complex Ribbon Structure. (*Blue-Sequence A. Red- Sequence B. White/Grey- Drug D8*)



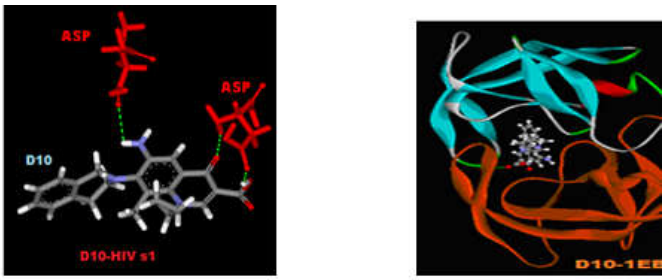
(a) (b)

Figures 18(a) Interaction of D9 with portion of HIV-1EBZ (ASP) at site1 Charmm Minimised Complex.(b) Interaction of D9 with portion of HIV-1EBZ (ASP) at site1.Charmm Minimised Complex Ribbon Structure. (*Blue-Sequence A. Red- Sequence B. White/Grey- Drug D9*)



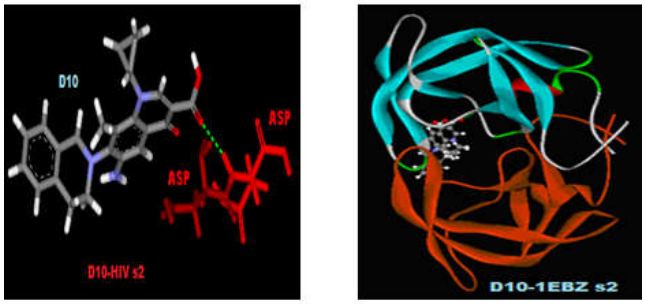
(a) (b)

Figures 19(a) Interaction of D9 with portion of HIV-1EBZ (ASP) at site2 Charmm Minimised Complex.(b) Interaction of D9 with portion of HIV-1EBZ (ASP) at site2.Charmm Minimised Complex Ribbon Structure. (*Blue-Sequence A. Red- Sequence B. White/Grey- Drug D9*)



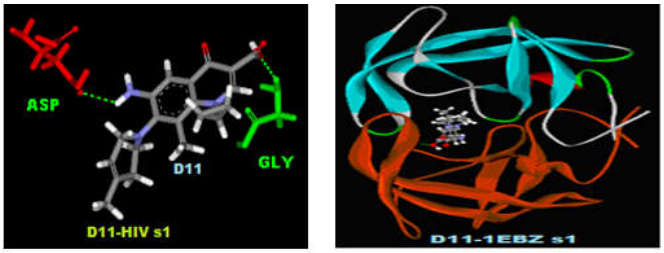
(a) (b)

Figures 20(a) Interaction of D10 with portion of HIV-1EBZ (ASP & ASP) at site1 Charmm Minimised Complex.(b) Interaction of D10 with portion of HIV-1EBZ (ASP & ASP) at site1.Charmm Minimised Complex Ribbon Structure. (*Blue-Sequence A. Red- Sequence B. White/Grey- Drug D10*)



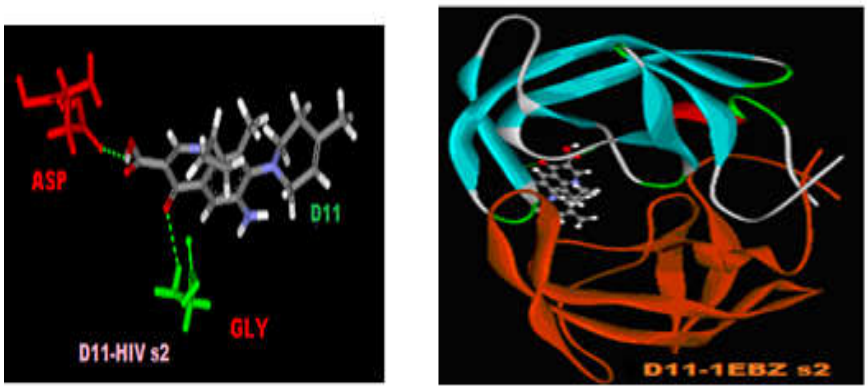
(a) (b)

Figures 21(a) Interaction of D10 with portion of HIV-1EBZ (ASP pair) at site2 Charmm Minimised Complex. (b) Interaction of D10 with portion of HIV-1EBZ (ASP pair) at site2. Charmm Minimised Complex Ribbon Structure. *(Blue-Sequence A. Red-Sequence B. White/Grey- Drug 10)*



(a) (b)

Figures 22(a) Interaction of D11 with portion of HIV-1EBZ (ASP & GLY) at site1 Charmm Minimised Complex.(b) Interaction of D11 with portion of HIV-1EBZ (ASP & GLY) at site1.Charmm Minimised ComplexRibbon Structure. (*Blue-Sequence A. Red- Sequence B. White/Grey- Drug D11*)



Figures 23(a) Interaction of D11 with portion of HIV-1EBZ (ASP & GLY) at site2 Charmm Minimised Complex.(b) Interaction of D11 with portion of HIV-1EBZ (ASP & GLY) at site2.Charmm Minimised Complex Ribbon Structure. ***(Blue-Sequence A. Red-Sequence B. White/Grey- Drug D11)***

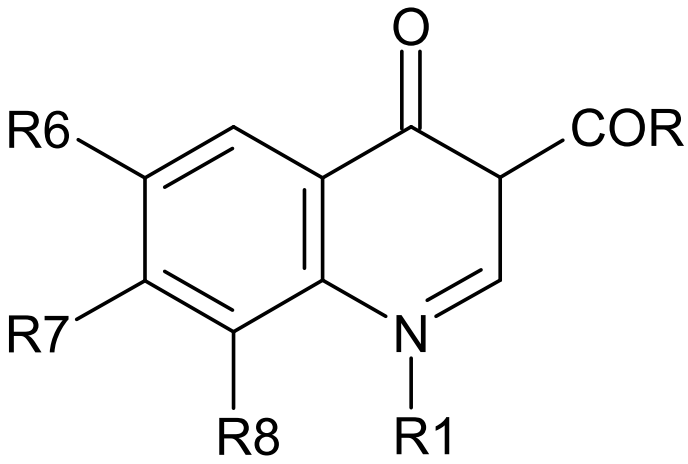


Table 1. Structures of Drugs used in the study

Table 2.1. Docking scores and internal energies of docked HIV (1EBZ) -DRUG complexes: Cryst Drug(Crystal structure)and Drug D1

Drugs	Docking Site In HIV	LigScore1 Dreiding	LigScore2 Dreiding	-PLP1	-PLP2	-PMF	Dock score	Internal energies (kcal/mol)
Cryst Drug	HIVs1	7.24	7.31	175.58	157.05	239.82	131.149	-18.726
		7.05	7.04	175.91	158.76	241.18	128.846	-18.726
		6.91	7.22	167.73	148.42	241.25	124.525	-18.726
		6.89	6.75	163.82	148.9	234.54	121.087	-18.726
D1	HIVs1	4.1	5.52	88.65	72.97	126.24	62.983	-0.162
		4.09	5.51	88.46	72.76	126.25	62.981	-0.162
		2.55	4.38	79.31	63.18	128.32	59.408	-3.831
		2.82	5.23	89.01	69.75	135.66	59.038	-0.385
		2.84	5.23	88.94	69.74	135.74	59.037	-0.385
		3.42	4.34	79.02	68.98	133.7	52.267	-3.224
		2.28	4.46	85.09	67.97	124.96	51.089	-1.915
		2.34	4.47	85.87	68.04	122.43	50.95	-1.915
		3.5	4.45	77.24	67.34	126.89	50.565	-3.224
		3.34	4.56	75.94	60.64	116.02	47.898	-0.189
	HIVs2	2.84	4.04	67.2	51.9	90.33	44.945	-1.27
		2.2	4.36	58.08	44.52	81.16	41.721	-0.201
		2.55	4.22	50.44	40.37	86.07	39.616	-0.232
		2.28	3.59	33.08	29.45	64.62	36.649	-1.258
		2.02	3.07	59.6	46.28	91.5	36.423	-2.59
		1.84	3.77	47.66	35.88	81.29	36.004	-1.161
		1.8	3.39	47.2	40.77	69.75	34.555	-1.218
		2.15	3.12	51.15	40.09	79.17	31.949	-1.097
		1.92	4.28	49.97	37.53	84.55	38.568	0.759
		1.92	3.07	65.15	50.65	86.8	38.19	0.026

Table 2.2. Docking scores and internal energies of docked HIV(1EBZ) -DRUG complexes: Drug D2 and Drug D3

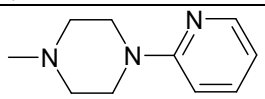
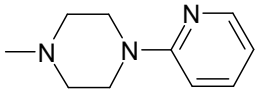
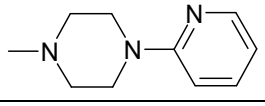
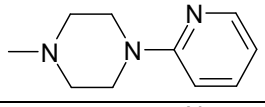
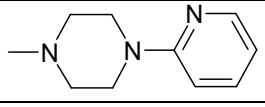
Drugs	Docking Site	LigScore1 Dreiding	LigScore2 Dreiding	-PLP1	-PLP2	-PMF	Dock score	Internal energies (kcal/mol)
D2	HIVs1	4.26	5.73	93.89	81.07	121.2	67.808	-2.045
		4.51	5.74	95.24	84.49	128.29	65.071	-2.139
		4.19	5.22	88.01	77.79	128.91	63.866	-2.463
		4.24	5.6	91.29	81.27	124.95	61.187	-2.112
		4.38	4.54	87.52	75.77	119.09	59.715	-4.287
		2.88	4.17	86.41	72.23	110.7	58.972	-2.186
		3.66	4.91	86.7	78.67	128.47	57.056	-2.567
		3.68	4.93	87.44	78.52	132.06	56.902	-3.543
		3.73	4.93	87.93	79.96	130.95	55.404	-2.995
		4.25	4.34	86.77	74.73	115.49	55.215	-2.69
D2	HIVs2	2.09	2.76	27.55	26.79	61.59	42.041	6.303
		1.8	1.23	34.66	33.66	65.43	19.428	1.793
		0.91	1.1	30.95	34.11	57.75	14.003	0.85
		1.47	2.58	29.04	31.38	57.62	13.559	1.31
		1.6	1.55	24.91	27.74	64.26	11.954	3.812
		1.5	1	42.06	36.65	67.01	11.858	1.233
		1.12	1.47	33.95	29.54	65.33	6.321	4.704
		0.87	-0.19	27.46	26.47	60.05	6.052	8.407
		0.92	0.27	45.45	41.11	63.54	3.038	8.047
		0.77	2	27.13	24.29	54.78	1.56	0.436
D3	HIVs1	3.62	4.93	76.02	62.41	117.47	75.33	-7.516
		3.68	5.63	88.79	70.75	125.68	75.248	-7.946
		3.31	5.16	84.41	65.97	125.91	75.218	-7.349
		3.03	4.84	89.91	70.3	124.89	74.441	-7.349
		3.57	5.51	93.39	72.59	126.66	74.421	-7.284
		3.7	5.43	95.6	76.26	123.83	73.694	-7.697
		3.55	5.43	92.57	73.98	131.54	73.214	-7.556
		3.55	5.15	86.32	72.04	119.11	72.414	-7.516
		3.21	5.38	89.57	72.26	119.12	72.363	-7.036
		4.09	5.02	80.64	65.42	123.29	71.893	-7.025
D3	HIVs2	3.14	4.45	62.73	51.23	86.34	51.707	-6.917
		3.47	4.61	62.28	50.02	89.24	50.282	-6.918
		3.6	4.65	59.27	48.59	87.84	49.326	-2.473
		2.74	3.71	38.89	30.26	66.11	48.194	-6.396
		2.38	3.75	45.49	38.8	67.14	43.726	-7.415
		2.93	4.13	58.1	47.31	82.27	43.66	-6.317
		2.72	4.05	56.58	48.1	82.16	42.019	-5.275
		2.7	3.62	38.75	33.83	80.6	39.649	-5.938
		2.43	3.66	38.92	27.96	61.5	39.309	-6.36
		2.54	3.95	51.14	40.96	75.39	38.334	-5.964

Mode of binding and relevance of active site selectivity:

The entry of ligand at the active site and selectivity of amino acid sequences is considered to be important in ligand-protein interaction. It is important to examine the size of the ligand that can easily enter into the pocket of active site. Selectivity of protein residue by these molecules is different and it is likely that the conformation of the substituents could be responsible for the selectivity of amino acid residues. The binding ability of these molecules depend on various factors, however it is essential to analyse the variation of amino acid selectivity with respect to substituents. Table 1 displays the 2D structure of substituents and the entry of these molecules at the active sites could be related to the size of these substituents. The conformational flexibility of molecule inside the cavity and variation of amino acid selectivity may also depend on type the substituents. The root mean square deviations (RMSD) of the ligand-protein binding are calculated from the docking trajectory of the most stable ligand amino acid bonded structures (Tables 2.1-2.6). The stable structures are obtained when the ligand was docked at several active sites of HIV protein, and the active sites are shown in Figure 1 and variation of the stabilization energies are given in Tables 3.1 and 3.2. After performing docking studies of ligands at the various active sites, the binding energies of ligands inside the region of the active sites were further analysed. The methods used in docking study are useful for finding the preferred location of ligand at a particular site of protein and conformation of the ligand within the binding region can be distinctly visualised. The most favourable structure was obtained from the scoring functions and internal energies. Figures 2-23 show that the position of molecules and the pattern of interaction at the active sites, and D1 and D2 are favourably stabilized within site 1 and located at amino acid residue ARG(Argenine) , GLY(Glycine), ASP(Aspartic acid). There is a hydrogen bond between –NH group of molecule and O=C- group of amino acid. The internal energies of the most stable conformation are given in Tables 2.1-2.6. The substituent is found towards the hydrophobic region, whereas only one hydrogen bond is formed between amino acid and ligand. Similarly, for the 6-Aminoquinolones, the size of the substituent is important for entering inside the active sites. Also, most of the molecules preferably bonded within the amino acid residue ASP (Aspartic acid) and GLY (Glycine). From these structures, it is possible that there must be some relevance of amino acid selectivity and the strength of .

Table 2.3. Docking scores and internal energies of docked HIV(1EBZ) -DRUG complexes: Drug D4 and Drug D5

Drugs	Docking Site	LigScore1 Dreiding	LigScore2 Dreiding	-PLP1	-PLP2	-PMF	Dock score	Internal energies (kcal/mol)
D4	HIVs1	4.33	5.95	84.31	70.45	102.67	77.198	-7.679
		3.99	5.64	80	65.2	103.98	77.006	-7.774
		3.99	5.63	79.88	64.99	103.93	76.994	-7.774
		3.73	5.38	76.39	62.12	107.72	75.618	-8.07
		3.95	5.37	84.97	68.76	93.86	73.785	-7.729
		3.86	5.48	77.69	63.68	105.01	73.274	-7.565
		4.19	5.81	86.87	73.74	98.34	72.472	-8.451
		4.11	5.7	69.44	54.93	128.93	72.343	-7.655
		3.87	5.55	81.81	68.77	103.36	72.178	-7.732
		4.08	5.63	88.65	73.91	95.55	71.341	-8.114
D4	HIVs2	3	4.54	77.68	61.19	98.31	55.396	-7.564
		2.82	4.1	54.56	39.48	78.62	55.164	-8.019
		2.96	4.52	51.08	37.87	83.37	52.645	-7.724
		2.42	3.89	48.12	35.62	77.49	51.945	-7.615
		2.66	3.87	54.63	38.07	78.72	51.848	-7.304
		2.98	4.54	54.43	39.83	82.17	51.83	-7.115
		2.85	4.29	48.75	36.91	77.68	51.55	-7.928
		2.74	4.03	49.6	37.99	78.83	50.858	-8.193
		2.81	4.36	49.26	35.95	81.09	50.781	-7.147
		2.55	3.81	53.23	39.51	76.4	48.677	-7.859
D5	HIVs1	4.7	5.94	73.07	62.6	115.25	71.153	-4.843
		4.8	5.85	74.56	63.52	115.21	69.392	-4.365
		4.32	5.63	82.97	67.63	114.67	66.491	-4.455
		4.12	5.49	84.62	68.69	113.25	65.802	-3.69
		3.83	5.33	81.39	67.88	112.04	64.918	-3.179
		3.6	5.44	79.27	63.18	113.28	52.746	-4.121
		3.11	4.41	64.63	51.4	102.87	52.558	-4.121
		2.61	4.21	65.84	60.28	100.94	42.495	-3.086
		1.29	3.42	54.12	43.03	99.51	33.847	-3.69
		0.56	2.81	57.36	47.86	99.63	31.679	-3.179
D5	HIVs2	2.53	2.85	62.94	55.84	68.17	62.626	-6.705
		3.56	4.26	38.21	30.13	59.52	52.604	-6.474
		2.67	2.69	60.59	60.33	66.68	52.326	-6.661
		2.85	3.92	69.55	66.55	63.75	51.99	-6.539
		2.87	3.71	47.16	35.06	61.54	51.664	-6.085
		2.98	4.18	45.78	36.44	64.85	51.114	-6.698
		2.9	4.17	46.55	36.45	65.49	50.682	-6.689
		2.98	4.36	49.29	38.43	72.31	50.307	-6.746
		3.63	4.32	39.98	30.95	63.43	49.838	-6.772
		2.53	2.85	62.94	55.84	68.17	62.626	-6.705

Sl.No.	Drug	R1	R6	R7	R8	R
1	D1	*t*-Bu	NH_2		H	OEt
2	D2	*t*-Bu	NH_2		H	OH
3	D3	c-Pr	NH_2		H	OH
4	D4	Me	NH_2		H	OEt
5	D5	Me	NH_2		H	OH

Continue……

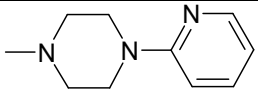
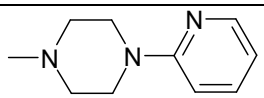
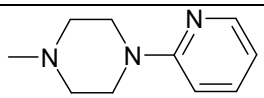
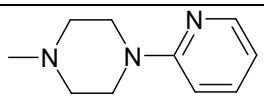
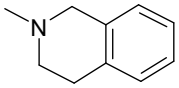
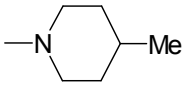
6	D6	Me	NHMe		H	OH
7	D7	Me	NH_2		Me	OH
8	D8	*t*-Bu	F		H	OH
9	D9	c-Pr	F		H	OH
10	D10	c-Pr	NH_2		Me	OH
11	D11	c-Pr	NH_2		Me	OH

Table 2.3. Docking scores and internal energies of docked HIV(1EBZ) – DRUG complexes: Drug D4 and Drug D5

Drugs	Docking Site	LigScore1 Dreiding	LigScore2 Dreiding	-PLP1	-PLP2	-PMF	Dock score	Internal energies (kcal/mol)
D4	HIVs1	4.33	5.95	84.31	70.45	102.67	77.198	-7.679
		3.99	5.64	80	65.2	103.98	77.006	-7.774
		3.99	5.63	79.88	64.99	103.93	76.994	-7.774
		3.73	5.38	76.39	62.12	107.72	75.618	-8.07
		3.95	5.37	84.97	68.76	93.86	73.785	-7.729
		3.86	5.48	77.69	63.68	105.01	73.274	-7.565
		4.19	5.81	86.87	73.74	98.34	72.472	-8.451
		4.11	5.7	69.44	54.93	128.93	72.343	-7.655
		3.87	5.55	81.81	68.77	103.36	72.178	-7.732
		4.08	5.63	88.65	73.91	95.55	71.341	-8.114
D4	HIVs2	3	4.54	77.68	61.19	98.31	55.396	-7.564
		2.82	4.1	54.56	39.48	78.62	55.164	-8.019
		2.96	4.52	51.08	37.87	83.37	52.645	-7.724
		2.42	3.89	48.12	35.62	77.49	51.945	-7.615
		2.66	3.87	54.63	38.07	78.72	51.848	-7.304
		2.98	4.54	54.43	39.83	82.17	51.83	-7.115
		2.85	4.29	48.75	36.91	77.68	51.55	-7.928
		2.74	4.03	49.6	37.99	78.83	50.858	-8.193
		2.81	4.36	49.26	35.95	81.09	50.781	-7.147
		2.55	3.81	53.23	39.51	76.4	48.677	-7.859
D5	HIVs1	4.7	5.94	73.07	62.6	115.25	71.153	-4.843
		4.8	5.85	74.56	63.52	115.21	69.392	-4.365
		4.32	5.63	82.97	67.63	114.67	66.491	-4.455
		4.12	5.49	84.62	68.69	113.25	65.802	-3.69
		3.83	5.33	81.39	67.88	112.04	64.918	-3.179
		3.6	5.44	79.27	63.18	113.28	52.746	-4.121
		3.11	4.41	64.63	51.4	102.87	52.558	-4.121
		2.61	4.21	65.84	60.28	100.94	42.495	-3.086
		1.29	3.42	54.12	43.03	99.51	33.847	-3.69
		0.56	2.81	57.36	47.86	99.63	31.679	-3.179
D5	HIVs2	2.53	2.85	62.94	55.84	68.17	62.626	-6.705
		3.56	4.26	38.21	30.13	59.52	52.604	-6.474
		2.67	2.69	60.59	60.33	66.68	52.326	-6.661
		2.85	3.92	69.55	66.55	63.75	51.99	-6.539
		2.87	3.71	47.16	35.06	61.54	51.664	-6.085
		2.98	4.18	45.78	36.44	64.85	51.114	-6.698
		2.9	4.17	46.55	36.45	65.49	50.682	-6.689
		2.98	4.36	49.29	38.43	72.31	50.307	-6.746
		3.63	4.32	39.98	30.95	63.43	49.838	-6.772
		2.53	2.85	62.94	55.84	68.17	62.626	-6.705

Table 2.4. Docking scores and internal energies of docked HIV(1EBZ) -DRUG complexes:Drug D6 and Drug D7

Drugs	Docking Site	LigScore1 Dreiding	LigScore2 Dreiding	-PLP1	-PLP2	-PMF	Dock score	Internal energies (kcal/mol)
D6	HIVs1	4.16	5.52	76.92	58.09	119.05	86.299	-7.458
		4.33	5.56	77.53	60.49	116.53	83.294	-7.528
		4.37	6.16	95.11	75.4	122.38	83.152	-7.218
		4.73	6.31	85.83	65.44	118.74	83.121	-7.791
		4.23	5.66	77.76	58.26	118.85	82.304	-7.142
		4.21	5.67	89.19	69.91	124.87	82.153	-7.798
		4.23	6.03	89.32	71.41	127.87	81.928	-7.332
		4.01	5.52	82.14	62.64	118.17	80.766	-8.022
		3.76	4.79	70.62	57	114.44	80.325	-7.399
		3.03	5.17	77.04	59.9	126.32	78.784	-7.314
D6	HIVs2	3.17	3.86	65.8	61.87	83.14	61.429	-6.857
		2.26	3.6	50.12	41.22	67.48	45.319	-7.701
		2.02	3.32	54.28	46.31	77.12	44.857	-6.839
		2.7	4.22	62.55	56.34	97.23	44.687	-6.936
		1.93	3.21	55.01	46.81	74.53	43.891	-7.421
		2.77	3.98	58.27	46.39	76.43	43.783	-7.582
		2.44	3.78	58.94	47.6	79.23	43.36	-7.363
		2.49	3.08	56.23	48.47	86.43	42.589	-6.939
		2.45	3.9	44.11	31.71	70.92	41.105	-7.218
		2.88	4.19	53.28	45.18	74.82	40.239	-6.685
D7	HIVs1	4.37	6.11	98.94	76.88	120.03	76.642	-5.078
		4.62	5.92	88.81	70.02	113.03	73.695	-4.853
		4.68	5.9	91.25	71.88	119.61	73.069	-4.802
		4.71	5.68	89.03	72.23	117.81	72.565	-4.63
		4.57	6.17	100.14	78.01	131.16	71.571	-5.124
		4.77	5.73	83.08	66.71	116.24	70.886	-4.816
		4.2	5.69	93.68	74.95	119.94	70.84	-4.654
		4.96	6.14	81.4	66.54	120.29	68.561	-4.861
		3.92	5.95	97.27	75.66	127.64	68.004	-4.942
		4.09	6.01	93.91	73.57	125.25	67.809	-5.087
D7	HIVs2	3.74	4.32	70.61	60.62	95.46	76.638	-3.443
		2.82	3.14	64.05	56.27	94.78	73.363	-5.052
		2.38	3.74	69.32	54.45	91.07	62.712	-2.645
		3.31	3.87	73.89	69.98	76.26	60.558	-3.994
		2.34	2.9	66.46	58.7	79.92	58.181	-5.169
		1.91	3	59.33	53.81	92.1	52.809	-5.033
		2.08	3.64	57.68	50.3	91.36	51.926	-5.078
		2.89	3.77	67.89	53.96	94.12	50.891	-3.298
		2.34	3.4	63.4	48.19	94.75	45.803	-3.895
		2.07	3.91	68.64	56.69	92.21	45.383	-4.26

Table 2.5. Docking scores and internal energies of docked HIV(1EBZ) -DRUG complexes:Drug D8 and Drug D9

Drugs	Docking Site	LigScore1 Dreiding	LigScore2 Dreiding	-PLP1	-PLP2	-PMF	Dock score	Internal energies (kcal/mol)
D8	HIVs1	3.22	5.15	96.47	83.7	116.17	64.852	-0.84
		2.98	4.78	105.58	86.44	118.34	63.749	-0.711
		2.96	4.67	94.29	78.36	124.2	63.428	0.575
		2.95	5.3	98.76	83.9	123.27	62.987	-1.088
		2.84	5.19	101.09	88.07	129.04	62.972	0.264
		3.23	5.35	89.79	74.3	122.24	61.323	0.753
		3.43	5.07	88.62	74.51	130.06	61.193	0.028
		3.43	5.64	107.55	89.16	118.29	60.466	10.284
		3.08	5.12	88.15	75.23	129.73	60.224	-0.746
		2.82	4.97	87.12	73.16	114.92	58.2	0.534
D8	HIVs2	2.49	4.19	53.69	42.77	84.53	43.824	0.647
		2.45	3.76	50.89	43.02	70.25	38.821	-0.311
		2.95	4.55	51.84	41.46	71.59	37.308	0.125
		2.21	3.97	41.77	37.4	72.52	37.176	-0.728
		1.74	3.39	49.64	40.85	75.69	34.14	-1.545
		1.89	3.39	41.41	36.18	74	33.099	0.403
		3.14	4.14	55.15	44.27	80.3	32.832	0.789
		1.91	3.57	41.9	36.45	77.92	31.917	1.388
		2.83	4.33	41.58	34.69	69.88	31.574	0.977

Continue …..

		1.41	3.61	41.18	32.18	78.04	30.578	-1.568
D9	HIVs1	4.14	5.91	102.06	88.71	140.76	82.468	-6.651
		4.38	5.7	79.48	62.06	117.38	81.657	-6.858
		4.06	5.97	101.39	86.71	142.24	80.947	-6.718
		3.75	4.81	67.3	54.36	114.79	80.267	-6.876
		3.71	5.64	106.64	90.53	141.88	79.313	-6.676
		3.7	5.63	91.48	73.02	125.46	79.24	-6.848
		3.67	5.45	96.98	79.35	119.09	75.825	-6.619
		4	5.39	88.67	71.1	120.36	73.398	-6.933
		3.39	5.35	97.08	78.33	126.37	73.081	-6.727
		3.82	5.2	86.92	69.06	118	72.737	-6.814
D9	HIVs2	2.25	3.44	75.58	63.3	96.83	66.627	-5.592
		2.63	3.77	78.61	68.35	82.71	63.751	-5.479
		2.43	3.51	54.22	47.38	80.34	49.904	-6.58
		2.4	3.81	48.88	37.24	80.38	47.744	-6.147
		1.86	3.72	44.71	35.28	76.05	46.55	-6.14
		3.15	4.35	54	44.1	77.3	45.621	-5.869
		1.41	3.45	79.22	63.83	95.07	44.974	-6.549
		2.07	3.48	38.31	28.92	77.95	44.845	-6.561
		2.98	3.96	63.26	52.9	81.14	43.762	-6.456
		1.91	3.33	41.67	35.09	64.93	42.531	-5.801

Table 2.6. Docking scores and internal energies of docked HIV (1EBZ) -DRUG complexes: Drug D10 and Drug D11

Drugs	Docking Site	LigScore1 Dreiding	LigScore2 Dreiding	-PLP1	-PLP2	-PMF	Dock score	Internal energies (kcal/mol)
D10	HIVs1	3.97	5.26	89.88	81.16	124.68	62.863	-2.884
		4.18	4.65	82.52	78.64	116.35	59.194	-4.057
		3.94	4.52	83.61	77.41	120.44	58.221	-4.299
		3.56	4.41	85.91	77.78	120.48	57.787	-5.062
		4.3	4.54	79.04	75.05	110.11	55.917	-2.953
		3.64	4.72	87.61	78.81	122.31	55.443	-2.365
		3.79	5.22	88.29	82.2	122.76	54.577	-3.185
		3.12	3.67	84.31	77.35	116	51.699	-5.396
		3.5	4.52	83.93	80.4	117.1	50.834	-5.067
		4.03	4.41	74.97	70.41	112.09	50.283	-3.962
D10	HIVs2	3.58	4.72	50.85	44.93	82.19	52.712	-5.396
		3.82	4.8	49.81	44.52	84.47	52.581	-5.062
		3.93	5	55.01	48.86	81.98	52.383	-5.044
		4.02	4.88	49.51	42.77	77.92	52.144	-4.299
		3.66	4.61	41.79	40.96	75.73	51.37	-4.749
		3.53	4.65	53.5	46	78.49	51.169	-4.728
		3.59	4.67	54.95	47.01	81.46	50.765	-4.269
		3.42	4.57	43.67	43.13	71.57	49.124	-4.758
		3.39	4.18	42.94	40.16	74.5	48.006	-5.067
		3.23	4.37	42.01	41.14	77.29	47.87	-4.741
D11	HIVs1	4.77	5.76	80.63	73.81	116.61	65.163	-3.826
		3.95	5.36	77.48	70.9	118.17	62.209	-3.494
		4.38	5.59	80.35	72.96	113.66	60.171	-4.597
		4.47	5.43	80.27	72.77	112.98	59.769	-3.535
		4.33	5.57	79.35	71.93	112.92	59.104	-3.692
		4.1	5.44	77.94	69.25	113.85	58.769	-4.007
		3.42	5.02	78.36	72.24	114.59	56.373	-4.167
		3.51	5.19	78.42	71.36	117.19	56.313	-4.053
		3.65	5.3	79.68	71.99	114.82	55.971	-4.313
		3.46	5.24	74.91	66.03	104.01	55.471	-3.559
D11	HIVs2	3.15	3.93	55.85	49.11	84.18	56.491	-3.666
		3.46	4.75	57.33	49.82	82.17	52.153	-4.377
		3.73	4.74	55.36	49.73	82.4	51.925	-4.597
		3.6	4.62	52.28	45.48	78.74	50.31	-3.692
		3.47	4.76	57.9	50.25	82.36	50.185	-4.167
		3.26	4.53	50.17	44.33	80.57	49.052	-4.053
		3.36	4.58	52.59	46.46	81.41	49.038	-4.377
		3.23	4.55	47.01	41.81	78.87	44.981	-3.535
		3.31	4.35	45.29	41.44	75.18	44.578	-4.313
		3.34	4.43	45.9	41.53	72.71	44.497	-3.559

Table 3.1 The van der Waals and electrostatic energies along with RMS gradient of the complexes obtained from CHARMm minimization of HIV(1EBZ)-DRUG complex

Drug	Drug-HIV Complex/Site	Vander Waals Energies kcal/mol	Electrostatic Energies kcal/mol	RMS Gradiant kcal/molxA^0
D1	HIVs1	-1535.891	-12492.432	0.070
	HIVs2	-1474.243	-12521.900	0.073
D2	HIVs1	-1487.287	-12531.276	0.086
	HIVs2	-1482.228	-12569.329	0.074
D3	HIVs1	-1504.737	-12702.670	0.078
	HIVs2	-1453.075	-12670.415	0.074
D4	HIVs1	-1518.623	-12678.603	0.061
	HIVs2	-1462.930	-12473.312	0.077
D5	HIVs1	-1509.674	-12512.200	0.069
	HIVs2	-1455.682	-12482.657	0.066
D6	HIVs1	-1491.678	-12499.309	0.074
	HIVs2	-1483.931	-12638.273	0.075
D7	HIVs1	-1491.949	-12463.385	0.099
	HIVs2	-1468.116	-12562.753	0.076
D8	HIVs1	-1483.094	-12502.273	0.073
	HIVs2	-1467.726	-12489.179	0.073
D9	HIVs1	-1513.106	-12618.173	0.075
	HIVs2	-1449.653	-12499.566	0.064
D10	HIVs1	-1494.324	-12531.122	0.080
	HIVs2	-1491.509	-12492.124	0.074
D11	HIVs1	-1498.488	-12637.428	0.080
	HIVs2	-1471.522	-12577.298	0.095
Cryst-Drug	HIVs1	-1518.265	-12506.835	0.076

Table 3.2. Electrostatic stabilisation energies calculation of CHARMm minimised drug-HIV(1EBZ) complex (kcal/mol)

Drug	Drug-HIV Complex/Site	Electrostatic Energy of mini-Complex	Electrostatic Energy of HIV	Electrostatic Energy of Drug	Electrostatic Stabilisation Energy of Mini-Complex
D1	HIVs1	-12492.432	-12322.959	-41.177	-128.296
	HIVs2	-12521.900	-12322.959	-41.177	-157.763
D2	HIVs1	-12531.276	-12322.959	-46.445	-161.872
	HIVs2	-12569.329	-12322.959	-46.445	-199.925
D3	HIVs1	-12702.670	-12322.959	-30.757	-348.953
	HIVs2	-12670.415	-12322.959	-30.757	-316.698
D4	HIVs1	-12678.603	-12322.959	-34.467	-321.177
	HIVs2	-12473.312	-12322.959	-34.467	-115.885
D5	HIVs1	-12512.200	-12322.959	-28.183	-161.058
	HIVs2	-12482.657	-12322.959	-28.183	-131.515
D6	HIVs1	-12499.309	-12322.959	-25.665	-150.685
	HIVs2	-12638.273	-12322.959	-25.665	-289.649
D7	HIVs1	-12463.385	-12322.959	-27.370	-113.055
	HIVs2	-12562.753	-12322.959	-27.370	-212.424
D8	HIVs1	-12502.273	-12322.959	-32.511	-146.802
	HIVs2	-12489.179	-12322.959	-32.511	-133.708
D9	HIVs1	-12618.173	-12322.959	-23.537	-271.677
	HIVs2	-12499.566	-12322.959	-23.537	-153.070
D10	HIVs1	-12531.122	-12322.959	-12.006	-196.157
	HIVs2	-12492.124	-12322.959	-12.006	-157.159
D11	HIVs1	-12637.428	-12322.959	-11.009	-303.460
	HIVs2	-12577.298	-12322.959	-11.009	-243.330
Cryst-Drug	HIVs1	-12506.835	-12322.959	-42.086	-141.790

hydrogen bonds formed in the most favourable conformation of ligand inside the cavity. Initially the active sites in the protein have been selected to find the binding ability of these molecules. Figure 1 represents the active sites present in HIV-1.

Molecular docking protocol has been used to find the selectivity of active sites by these drugs, which is examined from the variation of the binding energies as well as internal energies for these sites. The most important active region has been detected from these energy values (Tables 2.1-2.6).

Table 4. van der Waals stabilisation energies obtained from CHARMm minimised drug-HIV(1EBZ) complex (kcal/mol)

Drug	Drug-HIV Complex/Site	VDW Energy of mini-Complex	VDW Energy of HIV	VDW Energy of Drug	VDW Stabilisation Energy of Mini-Complex
D1	HIVs1	-1535.891	-1482.245	-2.829	-50.818
	HIVs2	-1474.243	-1482.245	-2.829	10.830
D2	HIVs1	-1487.287	-1482.245	-5.438	0.395
	HIVs2	-1482.228	-1482.245	-5.438	5.454
D3	HIVs1	-1504.737	-1482.245	-3.115	-19.378
	HIVs2	-1453.075	-1482.245	-3.115	32.285
D4	HIVs1	-1518.623	-1482.245	-1.836	-34.543
	HIVs2	-1462.930	-1482.245	-1.836	21.150
D5	HIVs1	-1509.674	-1482.245	-0.669	-26.761
	HIVs2	-1455.682	-1482.245	-0.669	27.231
D6	HIVs1	-1491.678	-1482.245	-1.530	-7.904
	HIVs2	-1483.931	-1482.245	-1.530	-0.156
D7	HIVs1	-1491.949	-1482.245	-2.838	-6.867
	HIVs2	-1468.116	-1482.245	-2.838	16.967
D8	HIVs1	-1483.094	-1482.245	-1.939	1.090
	HIVs2	-1467.726	-1482.245	-1.939	16.458
D9	HIVs1	-1513.106	-1482.245	-0.108	-30.754
	HIVs2	-1449.653	-1482.245	-0.108	32.699
D10	HIVs1	-1494.324	-1482.245	-2.654	-9.425
	HIVs2	-1491.509	-1482.245	-2.654	-6.610
D11	HIVs1	-1498.488	-1482.245	-3.660	-12.583
	HIVs2	-1471.522	-1482.245	-3.660	14.383
Cryst-Drug	HIVs1	-1518.265	-1482.245	-12.931	-23.089

Table 5.1 Interaction distances (Å) of Drugs: D1, D2, D3, D4 and D5 with the nearest amino acid residues of HIV(1EBZ) in the CHARMm Minimised Structures

Drug	Complex/site	Interacting Atoms	Type of Hydrogen Bonds	Distance(Å)
D1	HIVs1	B:GLY148:HN - :D1:O33	-NH------O=C-	2.131
	HIVs2	A:ASP30:HN - :D1:O33	-NH------O=C-	2.180
		A:GLY48:HN - :D1:O32	-NH------O=C-	1.985
D2	HIVs1	B:GLY148:HN - :D2:O26	-NH------OH-C-	2.349
		B:GLY148:HN - :D2:O31	-NH------O=C-	2.380
		B:GLY148:O-:D2:H43	--C=O -----H-O-C-	2.040
	HIVs2	B:ARG108:HH22 - :D2:N23	-NH ------N=C-	2.170
		B:GLU134:OE1-:D2:H43	-C=O------H-O-C-	2.006
		B:GLU134:OE2-:D2:H43	-COH------H-O-C-	2.481
D3	HIVs1	B:ASP130:HN - :D3:O29	-NH------O=C-	2.085
		B:GLY148:HN - :D3:O30	-NH------O=C-	2.178
		B:ASP129:OD2-:D3:H48	-C=O------ H-O-C-	2.392
	HIVs2	A:GLY48:HN - :D3:O29	-NH------O=C-	2.109
		A:GLY48:O-D3:H48	-C=O------H-O-C-	2.171
D4	HIVs1	A:ARG8:HH22 - :D4:O17	-NH------O=C-	2.321
		B:GLY148:HN - :D4:O30	-NH-----O=C-	1.920
	HIVs2	A:ASP29:HN - :D4:O30	-NH------O=C-	2.141
		A:ASP30:HN - :D4:O30	-NH------O=C-	2.032
D5	HIVs1	A:ARG8:HH22 – D5:O27	-NH-----O=C-	1.675
	HIVs2	A:ASP29:HN - :D5:O28	-NH------O=C-	2.193
		A:ASP30:HN - :D5:O28	-NH------O=C-	2.219
		A:GLY48:HN - :D5:O27	-NH------O=C-	2.051
		A:GLY48:O-:D5:H37	-C=O------H-O-C-	1.873

Most of these molecules are selective for active site 1 than the other sites. The search for active site has been performed within the volume of 273.63 Å. Tables 2.1-2.6 show the comparison of docking scores obtained from various docking methods. The variation of docking scores obtained from these methods is similar for these active sites.

It is noted that the internal energies computed with various docking protocols are not very different. The structure of molecules at the interaction sites and the type of hydrogen bonding are shown in Tables 5.1 to 5.3. Although these molecules can bind within the same active site or region, interactions of groups within the cavity and changes of

Table 5.2 Interaction distances (Å) of Drugs: D6, D7, D8, D9, D10, D11 and Crystal drug with the nearest amino acid residues of HIV(1EBZ) in the CHARMm Minimised Structures.

Drug	Complex/site	Interacting Atoms	Type of Hydrogen Bonds	Distance (Å)
D6	HIVs1	B:ASP129:OD2-D6:H45	-C=O------H-O-C-	2.194
	HIVs2	B:ARG108:HH12 - :D6:N20	-NH------N-C-	2.469
		A:ASP29:OD1-D6:H49	-C=O------H-N-	2.303
D7	HIVs1	A:ARG8:HH22 - :D7:O28	-NH------O=C-	1.904
	HIVs2	A:ILE50:HN - :D7:O28	-NH------O=C-	2.139
		B:ILE150:HN - :D7:O28	-NH------O-C-	2.209
		A:GLY49:O-D7:H49	-C=O------H-N-	2.280
D8	HIVs1	B:GLY148:HN - :D8:O26	-NH------OH-C-	2.028
	HIVs2	A:GLY48:HN - :D8:O31	-NH------O=C-	1.848
		A:ASP30:OD1-D8:H43	-C=O------H-O-C-	2.323
D9	HIVs1	B:ASP130:HN - :D9:O29	-NH------O=C-	2.085
	HIVs2	NIL		NIL
D10	HIVs1	B:GLY148:HN - :D10:O29	--NH-----O=C-	1.881
		B:ASP129:OD2-D10:H44	-C=O------H-O-C-	2.354
	HIVs2	A:ASP30:HN - :D10:O29	-NH------O=C-	2.253
		A:GLY48:HN - :D10:O28	-NH------O=C-	2.072
		A:GLY48:O-D10:H44	-C=O------H-O-C-	2.223
D11	HIVs1	B:GLY148:HN - :D11:O23	-NH------OH-C-	2.212
	HIVs2	A:ASP30:HN - :D11:O26	-NH------O=C-	2.172
		A:GLY48:HN - :D11:O25	-NH------O=C-	2.118
		A:ASP30:OD2-D11:H43	-C=O------H-O-C-	2.206
CrystDrug	HIVs1	A:ARG8:HH22 - :Drg:O36	-NH------OH-C-	2.090
		B:ILE150:HN - :Drg:O27	-NH------O=C-	2.073
		B:ASP125:OD2-Drg:H67	-C=O------H-O-C-	2.398

Table 5.3 Interaction distances (Å) of Drugs: D1, D2, D3, D4, D5, D6, D7 and D8 with the nearest amino acid residues of HIV(1EBZ) in the complex in the Docked (*Liganfit*) Structures.

Drug	Complex/site	Interacting Atoms	Type of Hydrogen Bonds	Distance (Å)
D1	HIVs1	A:ARG8:HH2 – D1:O32	-NH------O=C-	2.271
	HIVs2	A:ASP29:HT1 – D1:O33	-NH------O=C-	1.734
D2	HIVs1	B:ASP130:HN – D2:O30	-NH------O=C-	2.444
		B:GLY148:HN1 – D2:O26	-NH------OH-C-	1.737
		B:GLY148:O - D2:H43	-C=O------H-O-C-	1.347
	HIVs2	B:GLU134:H9 – D2:O26	-C-O-H------OH-C-	2.448
		B:GLU134:OE1 - D2:H43	-C=O------H-O-C-	2.371
D3	HIVs1	B:GLY148:HN1 – D3:O30	-NH------O=C-	2.383
		B:ASP129:OD2 - D3:H48	-C=O------H-O-C-	1.981
	HIVs2	A:ASP30:HT1 – D3:O30	-NH------O=C-	2.022
D4	HIVs1	A:ARG8:HH22 – D4:O30	-NH------O=C-	1.460
	HIVs2	A:ASP29:HT1 – D4:O30	-NH------O=C-	2.042
D5	HIVs1	A:ARG8:HH22 – D5:O27	--NH-----O=C-	2.295
	HIVs2	A:ASP29:HT1 – D5:O28	-NH------O=C-	2.422
		A:GLY48:O - D5:H37	-C=O------H-O-C-	1.554
D6	HIVs1	A:ARG8:HH21 – D6:O28	-NH------O=C-	2.405
		A:ARG8:HH22 – D6:O28	-NH------O=C-	1.946
		B:ASP129:OD2 - D6:H45	-C=O------H-O-C-	1.389
	HIVs2	A:ASP25:OD2 - D6:H45	-C=O------H-O-C-	1.580
D7	HIVs1	B:ASP130:OD2 - D7:H45	-C=O------H-O-C-	2.491
	HIVs2	A:ASP25:OD2 - D7:H45	-C=O------H-O-C-	1.162
D8	HIVs1	B:ASP129:HN – D8:O31	-NH------O=C-	2.461
	HIVs2	A:ASP30:HN – D8:O26	-NH------OH-C-	2.493
		A:GLY48:HN – D8:O30	-NH------O=C-	2.446

Table 5.4 Interaction distances (Å) of Drugs: D9, D10, D11 and Crystal Drug with the nearest amino acid residues of HIV(1EBZ) in the complex in the Docked (*Liganfit*) Structures

Drug	Complex/site	Interacting Atoms	Type of Hydrogen Bonds	Distance (Å)
D9	HIVs1	B:ASP129:OD2 - D9:H48	-C=O------H-O-C-	1.370
	HIVs2	A:ASP30:OD2 - D9:H48	-C=O------H-O-C-	2.258
D10	HIVs1	B:ASP129:HN1 – D10:O28	-NH------O=C-	2.224
		B:ASP129:OD2 - D10:H44	-C=O------H-O-C-	1.713
		B:ASP125:OD2 - D10:H48	-C=O------HN-	2.428
	HIVs2	A:ASP30:HN – D10:O29	-NH------O=C-	2.269
D11	HIVs1	B:GLY148:HN1 – D11:O23	-NH------OH-C-	1.970
		B:ASP125:OD2 - D11:H49	-C=O------HN-	2.494
	HIVs2	A:GLY48:HN1 – D11:O25	-NH------O=C-	2.371
		A:ASP30:OD2 - D11:H43	-C=O------H-O-C-	2.459
Cryt Drug	HIVs1	A:GLY27:O - Drug-1:H63	-C=O------HN-	2.494
		A:ASP25:OD2 - Drug-1:H66	-C=O------H-O-C-	2.166
		B:ASP125:OD2 - Drug-1:H67	-C=O------H-O-C-	2.148
		B:GLY127:O - Drug-1:H68	-C=O------HN-	2.133
		B:ASP129:N - Drug-1:H74	-C-N------H-O-C-	2.409
		A:GLY48:O - Drug-1:H86	-C=O------HN-	1.744

A: HIV Sequence A, B: HIV Sequence B, ASP: Aspartic Acid, GLY: Glycine,GLU: Glutamic Acid, ARG: Argenine, ILE: Isoleucine

conformation at the active site are not equal. It should be noted that some of the amino acids are much closer towards the ligand and tight interaction through hydrogen bonds is expected. The types of hydrogen bonds with various amino acids are different. Here, the binding modes of the class of anti-HIV agent have been analysed using molecular docking protocols, and further minimised the structures with CHARMM minimization. The types of hydrogen bonds with the amino acid residues of ligand-protein are examined. Contrasting structural features of molecules at the active sites are observed while the ligand binding is distinctive with respect to the receptor subtype residues i.e. the amino acids. It should be noted that the cavity of the active site and molecular volume of the ligands should be compatible for entering the ligand inside the active region. Also, in most structures of receptor – ligand structures, the hydrophobic group of the ligand is appeared oriented towards the cavity if other steric hindrance does not occur from the amino acid residue. In some cases, additional hydrogen bonds are also observed between ligand and amino acid residues.

The molecule is found slightly outward from the active site. However, relative binding mode of these ligands can be assured from docking studies. So, there may be several interactions like hydrophobic, hydrophilic, electrostatic and hydrogen bonding between the ligand and receptor. For all these molecules, the selection of binding site is common except specific changes in the conformation of ligands and pattern of interactions with amino acids. The docking studies have certain advantage over modelled structures because it gives the precise features of the receptor binding sites and that may be useful for understanding the mode of interactions with the ligands. With these comparative results of several molecules, it is possible to give additional information on effort of ligand design. The only disadvantages of this class of method are that the technique cannot be progressed without crystal structures of receptor-ligand. So, the docking structures can give some idea of predicting what would inhibit a receptor that is very relevant in drug design.

Conclusion: In this study, it has been found that the molecules bind within the cavity of active sites. The binding specificity within the cavity of active site varies significantly. It may be due to the types and sizes of substituents present in the ligand. These ligands preferably bind within the HIV sequences A and B. In the minimised structure distinct hydrogen bonds are seen between the molecule and amino acids residues. The internal energy values for the most favourable ligand-protein structures are ~ -10 kcal/mol.

REFERENCES

(a) Cecchetti, V., Clementi, S., Cruciani, G., Fravolini, A., Pagella, P., Savino, A. and Tabarrini, O. 1995. 6-Aminoquinolones: A New Class of Quinolones Antibacterials? *J. Med. Chem., 38*, 973-982.

(a) Mahmood, N., Moore, P., De Tommasi, N., De Simone, F., Colman, S., Hay, A. and Pizza, C. 1993. Inhibition of HIV-1 Infection by Caffeoylquinic Derivatives. *Antiviral Chem., Chemother. 4*, 235-240.

(a) Musmuca, I., Simeoni, S., Caroli, A. and Ragno, R. 2009. Small-Molecule Interferon Inducers. Toward the Comprehension of the Molecular Determinants through Ligand-Based Approaches. *J. Chem. Inf. Model*., *49* (7), 1777–1786.

(b) Cecchetti, V., Fravolini, A., Lorenzini, M. C., Tabarrini, O., Terni, P. and Xin, T. 1996. Studies on 6-aminoquinolones: Synthesis and antibacterial evaluation of 6-amino-8-methylquinolones. *J. Med. Chem., 39*, 436-445.

(b) Ghosh, A. K. and Brindisi, M. 2015. Organic Carbamates in Drug Design and Medicinal Chemistry. *J. Med. Chem*., *58*, 2895−2940.

(b) Palu`, G., Palumbo, M., Cusinato, R., Meloni, G. A. and Marciani-Magno, S. 1984. Antiviral properties of psoralen derivatives: a biological and physicochemical investigation. *Biochem. Pharmacol*., *33*, 3451-3456.

Cappelli, A., Bini, G., Valenti, S., Giuliani, G., Paolino, M., Anzini, M., Vomero, S., Giorgi, G., Giordani, A., Stasi, L. P., Makovec, F., Ghelardini, C., Mannelli, L. D. C., Concas, A., Porcu, P. and Biggio, G. 2011. Synthesis and Structure-Activity Relationship Studies in Translocator Protein Ligands Based on a Pyrazolo (3,4-b)quinoline Scaffold. *J. Med. Chem*., *54*, 7165–7175.

Cecchetti, V., Parolin, C., Moro, S., Pecere, T., Filipponi, E., Calistri, A., Tabarrini, O., Gatto, B., Palumbo, M., Fravolini, A. and Palu, G. 2000. 6-Aminoquinolones as New Potential Anti-HIV Agents. *J. Med. Chem*., 43, 3799-3802.

Cruciani, G., Fravolini, A., Cecchetti, V., Filipponi, E., Tabarrini, O., Palu′, G., Del Pup, L. and Parolin, C. 1996. Quinolones as anti-HIV-1 agents. *Atti XII Conv. Naz. Div. Chim. Farm. Abstr. C-M5* 71.

Discovery Studio, Accelyrs

Frisch, M. J., Trucks, G. W., Schlegel, H. B., Gill, P. M. W., Johnson, B. G., Robb, M. A., Cheeseman, J. R., Keith, T., Petersson, G. A., Montgomery, J. A., Raghavachari, K., Al-Laham, M. A., Zakrzewaki, V. G., Ortiz, J. V., Foresmann, J. B., Ciolowski, J., Stefanov, B. B., Namayakkara, A., Challacombe, M., Peng, C. Y., Ayala, P. Y., Chen, W., Wong, M. W., Andres, J. L., Replogle, E. S., Gomperts, R., Martin, R. L., Fox, D. J., Binkley, J. S., Defrees, D. J., Baker, J., Stewart, J. P., Head-Gordon, M., Gonzalez, C. and Pople, J. A. 2003. GAUSSIAN, Gaussian Inc., Pittsburgh, PA.

García-Sosa, A. T., Sild, S., Takkis, K. and Maran, U. 2011. Combined Approach Using Ligand Efficiency, Cross-Docking, and Antitarget Hits for Wild-Type and Drug-Resistant Y181C HIV-1 Reverse Transcriptase; *J. Chem. Inf. Model*., *51*, 2595–2611.

Garg, R., Gupta, S. P., Gao, H., Babu, M. S., Debnath, A. K. and Hansch, C. 1999. Comparative Quantitative Structure−Activity Relationship Studies on Anti-HIV Drugs. *Chem. Rev*., *99* (12), 3525−360.

Matsuyama, S., Aydan, A., Ode, H., Hata, M., Sugiura, W. and Hoshino, T. 2010. Structural and Energetic Analysis on the Complexes of Clinically Isolated Subtype C HIV-1 Proteases and Approved Inhibitors by Molecular Dynamics Simulation. *J. Phys. Chem. B., 114* (1), 521–530.

Tzoupis, H., Leonis, G., Avramopoulos, A., Mavromoustakos, T. and Papadopoulo, M. G. 2014. Systematic Molecular Dynamics, MM−PBSA, and Ab Initio Approaches to the Saquinavir Resistance Mechanism in HIV-1 PR Due to 11 Double and Multiple Mutations. *J. Phys. Chem. B., 118*, 9538−9552.

Zhou, G., Sofiyev, V., Kaur, H., Snyder, B. A., Mankowski, M. K., Hogan, P. A., Ptak, R. G. and Gochin, M. 2014. Structure−Activity Relationship Studies of Indole-Based Compounds as Small Molecule HIV-1 Fusion Inhibitors Targeting Glycoprotein-41. *J. Med. Chem.*, *57*, 5270−5281.

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