WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 10, 727-746. Research Article ISSN 2277– 7105

IN SILICO MOLECULAR DOCKING STUDIES ON NOVEL PEPTIDE ANALOGUES AS POTENT HIV PROTEASE INHIBITORS AGAINST HIV

Pavan P.[*1], Suvarnalakshmi G.[1], Habeela Jainab N.[1], Sriharsha S. N.[1], Sheshagiri R. Dixit[2] and Richa Shetty[2]

[1]Department of Pharmaceutical Chemistry, Hillside College of Pharmacy and Research Centre, Raghuvanahalli, Bengaluru-560062, Karnataka, India.

[2]Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru-570015, Karnataka, India.

Article Received on
22 April 2023,

Revised on 12 May 2023,
Accepted on 02 June 2023

DOI: 10.20959/wjpr202310-28464

***Corresponding Author**
Pavan P.
Department of Pharmaceutical Chemistry, Hillside College of Pharmacy and Research Centre, Raghuvanahalli, Bengaluru-560062, Karnataka, India.

ABSTRACT

AIDS is a sexually transmitted disease that is caused by the HIV. The HIV-1 protease enzyme plays a very important role in the life cycle of HIV. By understanding the importance of the HIV-1 protease enzyme, studies related to the inhibition of protease enzyme has increased in treatment of the AIDS. Structure-based drug design by use of structural biology remains one of the most logical and aesthetically pleasing approaches in drug discover paradigms. Managing AIDS, is the most challenging problems in the 21st century. The concept of structure-based drug discovery combines information from several fields, X-ray crystallography and/or NMR, molecular modeling, synthetic organic chemistry, qualitative structure-activity relationships (QSAR), and biological evaluation. We examined the protease inhibitory activities of the peptide compounds containing heteroatoms. The mechanism of inhibition can be related to the catalytic mechanism of protease action or include mechanism unrelated stearic blockage of the active site or its neighborhood. Protease inhibitor drugs block the action of protease enzymes. This prevents protease enzymes from doing their part in allowing HIV to multiply, interrupting the HIV life cycle as a result controls the disease. In the current study we planned to design some novel N-substituted pentacycloundecane peptide derivatives and identify the compounds with effective binding action on selected target HIV protease by using molecular docking studies. *In silico* docking studies were carried out using

BIOVIA Discovery Studio. The results showed that most of the chemical compounds binds effectively with HIV protease enzyme. Among the docked compounds, the compound 45, compound 9, compound 37, compound13, compound 41, and compound 6 shows higher –cdocker energy and -cdocker interaction energy than the standard drug whereas the other analogues showed comparatively lower scores.

KEYWORDS: HIV, HIV protease enzyme, peptide analogues, molecular docking.

INTRODUCTION

Human Immunodeficiency Virus (HIV) is a virus which causes Acquired Immune Deficiency Syndrome (AIDS).[1] HIV is an enveloped retrovirus which mainly targets and destroys the CD4 cells of T cells in immune system.[2] According to WHO, HIV continues to be a major global public health issue, having claimed 36.3 million [27.2–47.8 million] lives so far. There were an estimated 37.7 million [30.2–45.1 million] people living with HIV at the end of 2020. In 2020, 680 000 [480 000–1.0 million] people died from HIV-related causes and 1.5 million [1.0–2.0 million] people acquired HIV.[3]

Till date the main treatment for HIV infection is antiretroviral drugs. The main purpose of HIV is to copy itself as many times as it can. However, HIV lacks the machinery it needs to reproduce itself. Instead, it injects its genetic material into immune cells in the body called CD4 cells. It then uses these cells as a kind of HIV virus factory.[4] Protease is an enzyme in the body that is important for HIV replication. Protease inhibitor drugs block the action of protease enzymes. This prevents protease enzymes from doing their part in allowing HIV to multiply, interrupting the HIV life cycle as a result. This can stop the virus from multiplying.

HIV protease enzyme is a retroviral aspartyl protease which helps in hydrolysis of peptide bonds of polyprotein chains at nine cleavages to create mature protein components of HIV. HIV-1 protease cleaves Gag and Gag-Pol polyprotein precursor encoded by the HIV-1 virus genome at nine processing sites to produce mature active proteins.[5] A mature HIV protease exists as a 22ka homodimer, where each monomer is made up of 99 amino acids.[6] The active site is not fully exposed, being covered by two flexible β-hairpin flaps. The flaps need to open to allow the substrates to access the active site. The HIV-1 protease enzyme activity can be inhibited by blocking the active site of the protease.[5]

Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site. Characterization of the binding behavior plays an important role in rational design of drugs as well as to elucidate fundamental biochemical processes. Molecular docking research focuses on computationally simulating the molecular recognition process. It aims to achieve an optimized conformation for both the protein and ligand and relative orientation between protein and ligand such that the free energy of the overall system is minimized.[7,8] Docking combined with a scoring function can be used to quickly screen large databases of potential drugs in silico to identify molecules that are likely to bind to protein target of interest.[9,10] The aim of the current study is focused on determining the binding effectiveness and potent compounds of various peptide analogues against the HIV-1 protease enzyme.

MATERIALS AND METHODS

Molecular Docking Studies

Molecular docking studies were performed using BIOVIA Discovery Studio. The compounds were sketched on Perkin Elmer Chemdraw Professional. The proteins were selected from RCSB PDB. Docking was performed after protein and ligand preparation.

Protein Selection and Preparation

The objective is to find the interaction of the peptide analogue as protease inhibitor hence the substrate here is the HIV-1 Protease enzyme. The PDB I of the elected macromolecule is 1HXW. The X-ray structure of HIV-1 protease reveals that it is composed of two symmetrically related subunits, each consisting of 99 amino acid residues. The subunits come together in such a way as to form a tunnel where they meet. This tunnel is of critical importance because the active site of the protease is located in its interior. The active site consists of two Asp-Thr-Gly conserved sequences, making it a member of the aspartyl protease family. The two Asp's are essential catalytic residues either interact with the incoming water or protonate the carbonyl to make the carbon more electrophilic for the incoming water. You may be wondering how a polyprotein makes its way into the active-site tunnel, as the tunnel appears to be too narrow to admit it. The key is the two flexible flaps on the top of the tunnel that move to allow proteins to enter the tunnel. The flaps undergo a dramatic movement, shifting from an open to a closed conformation to bind the target in an appropriate conformation for cleavage. This is more clearly seen at Flaps Morph for HIV

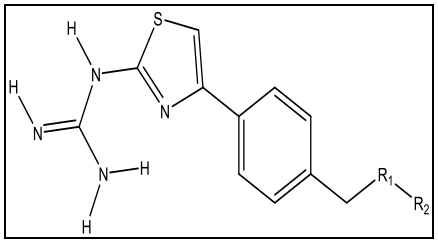
Protease.[9] The macromolecule was prepared by eliminating undesirable features like alternate chains, water molecules and co-crystal ligand. Thereafter, the proteins were subjected to energy minimization by applying the CHARMm force field.

Small Molecule Preparation

A total of 48 compounds and ritonavir were chosen to challenge against the protein target molecule. The ligands were sketched using 2D-Chemdraw and their 3D structures were generated using 3D-Chemdraw. Ligands were prepared by applying the CHARMm force field as a measure to minimize energy with the other factors like changing ionization, parallel processing, fixing bad valencies, generation of the tautomer, isomers, and coordinates as nil.

Molecular Docking

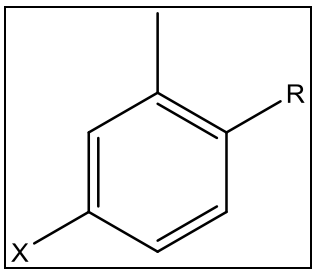
The location of the co-crystal present in the protein molecule was considered as the active site for docking. The maximum number of poses per ligand was set to 10 with no constraints to perform the molecular docking. For comparative studies of the designed ligands, ritonavir was considered the standard FDA drug for analysing the 2D and 3D binding interaction. Molecular docking was performed adopting the cdocker, which depends on CHARMm-based force field. The docking estimation was performed by the -cdocker energy, which was calculated, based upon the internal ligand strain energy and receptor-ligand interaction energy. Additionally, -cdocker interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. In both the cases, it has to be noted that greater -cdocker energy and -cdocker interaction energy value implies greater favorable binding between the protein and the ligand.



Pharmacophore 1 (P1)

R_1-Amino acids

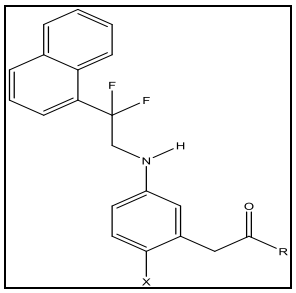
R_2-Five membered heterocyclic Group.



Pharmacophore 2 (P2)

R-Amino acids

X=Halogens (Br,Cl,I and F)



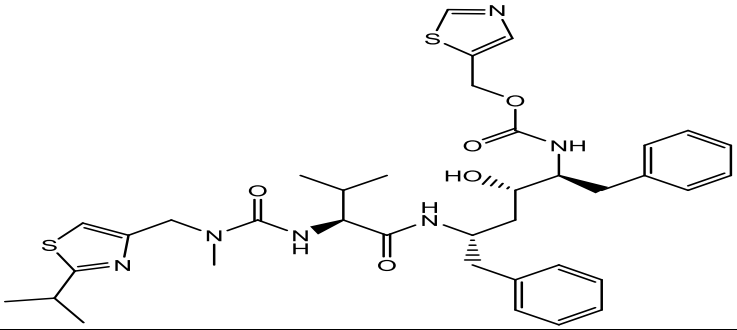
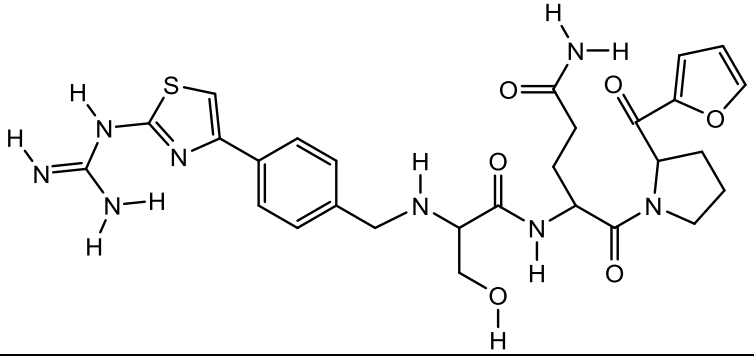
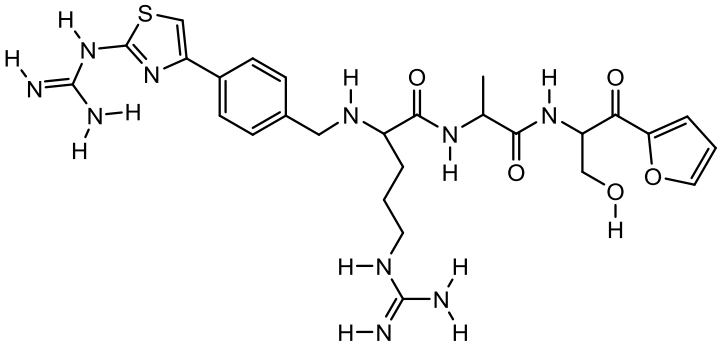
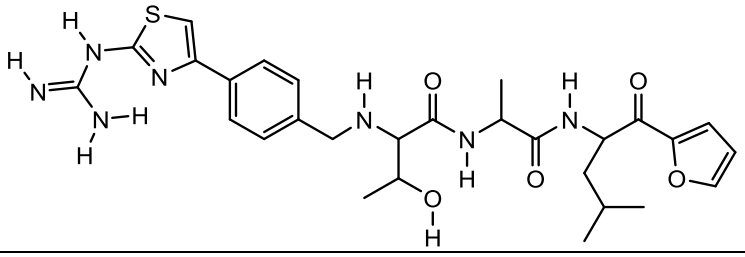
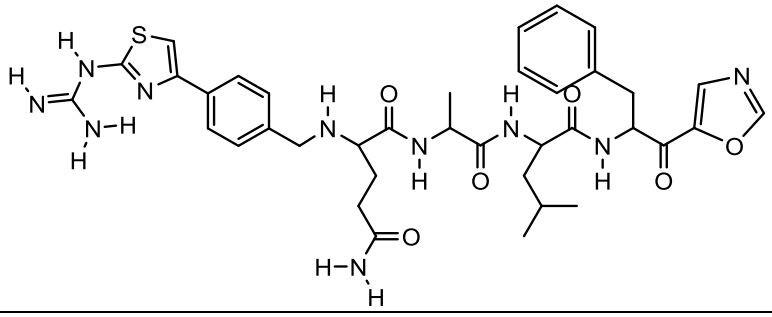
Pharmacophore 3 (P3)

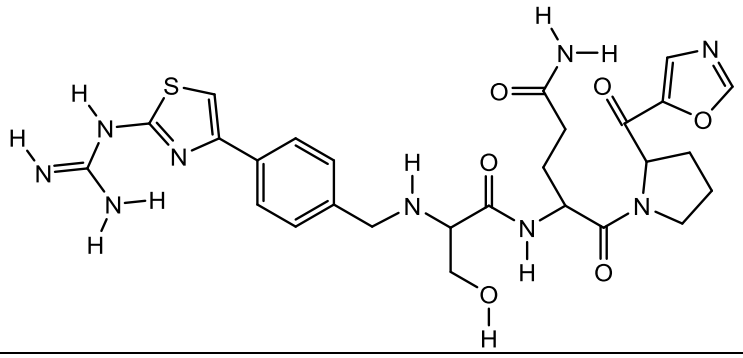
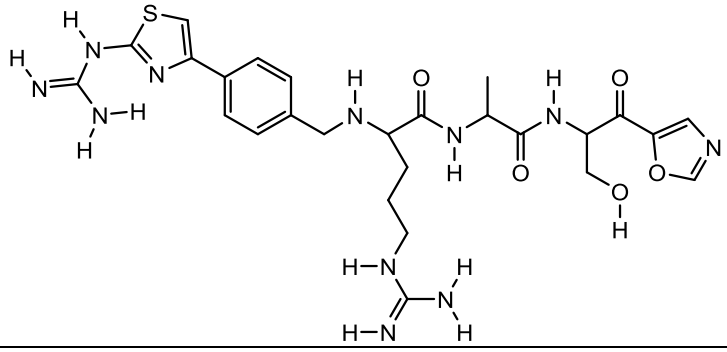
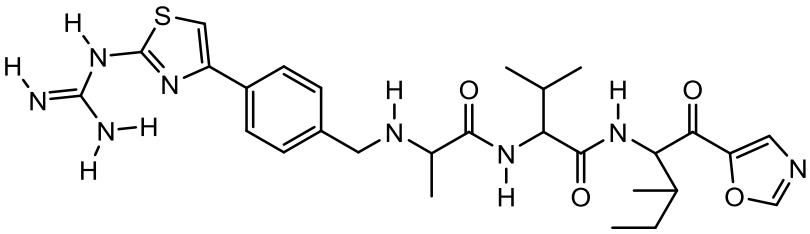
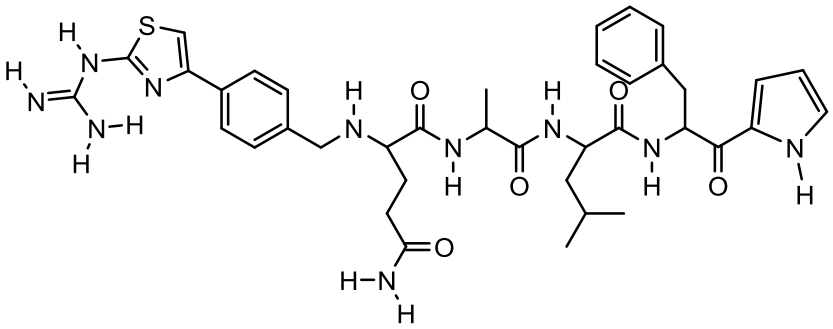
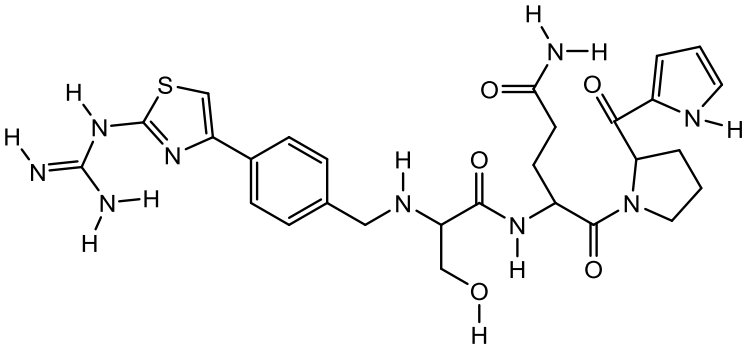
R-Amino acids,

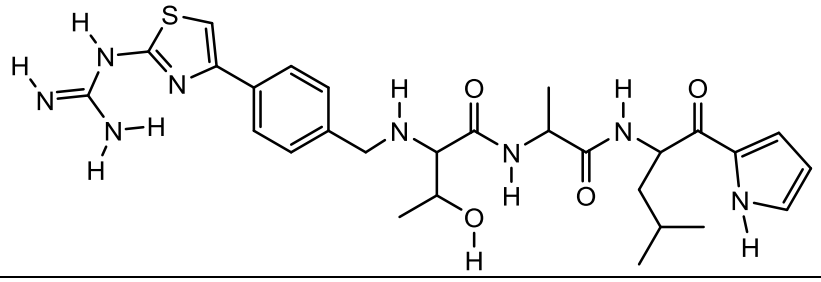
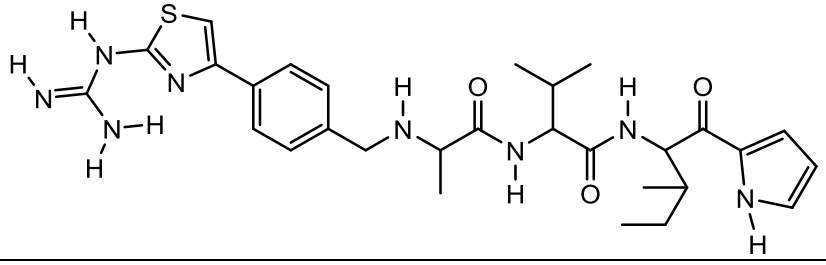
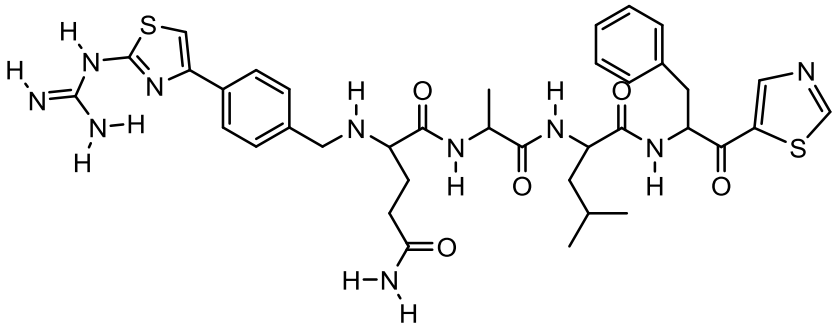
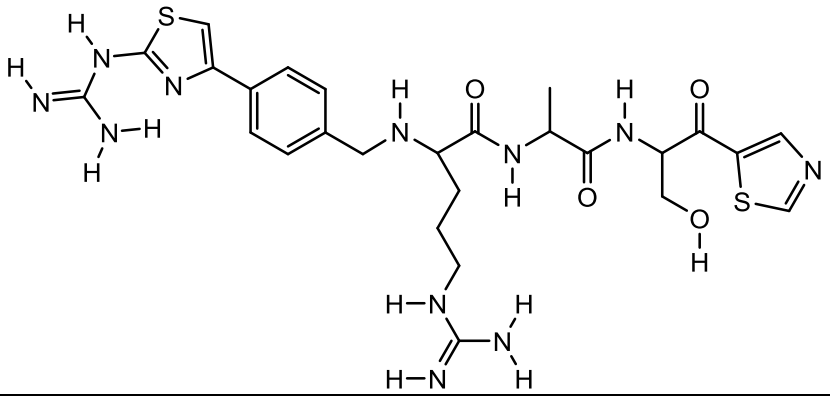
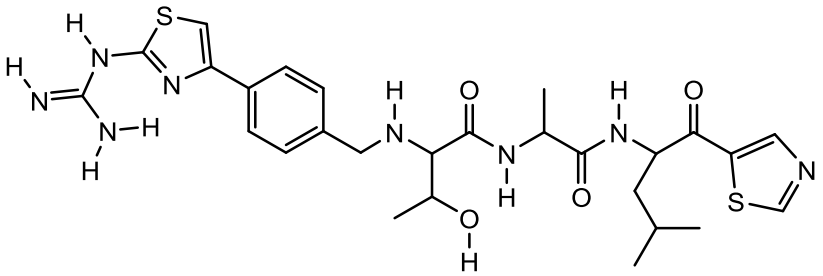
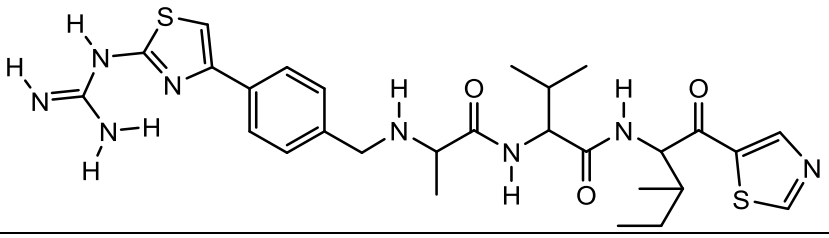
X=Halogens(Br,Cl,I and F)

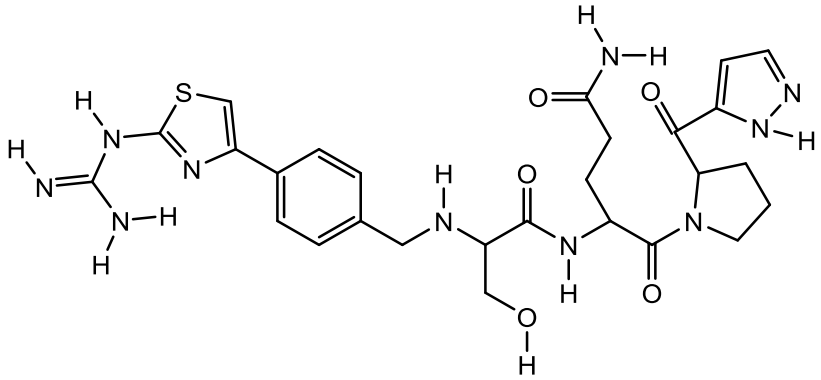
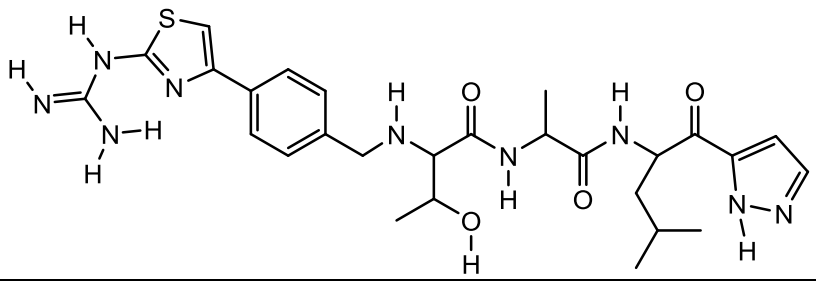
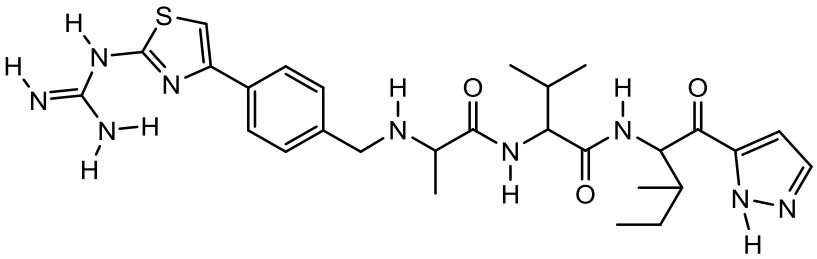
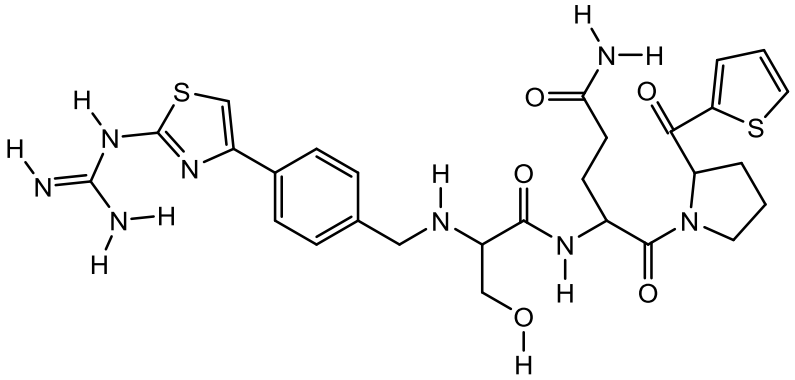
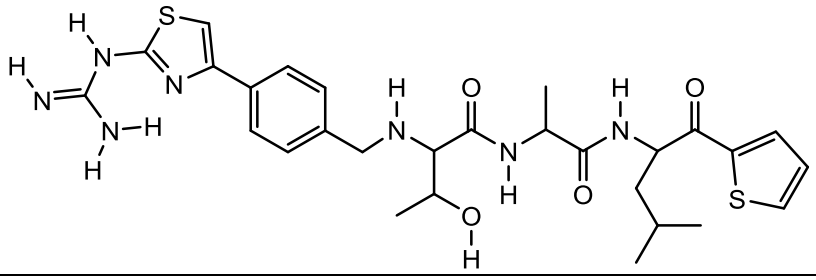
Fig 1: Structure of the Pharmacopores 1, 2 and 3 (P1, P2 and P3).

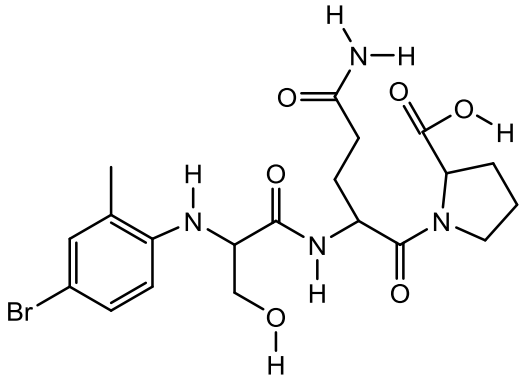
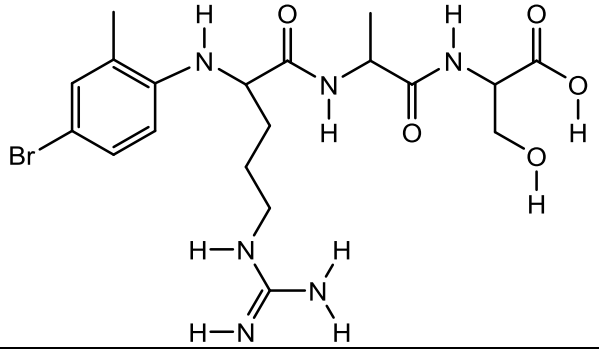
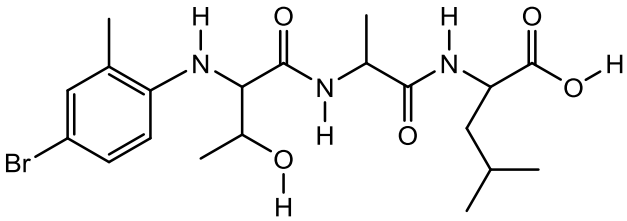
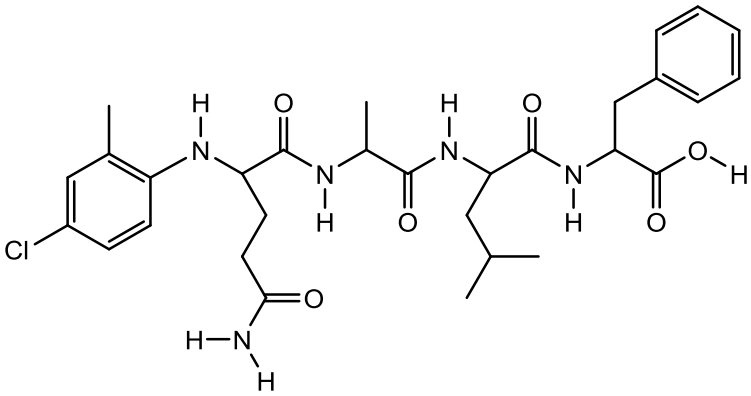
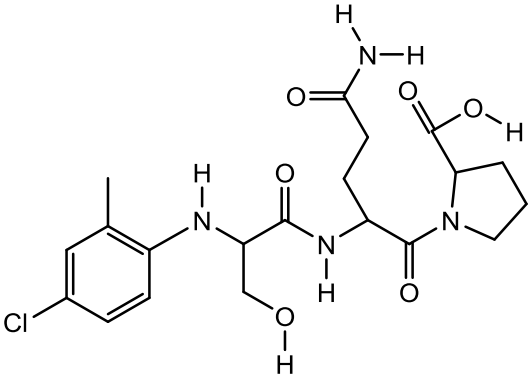
Table 1: Structures of the peptide analogues of the pharmacophores containing various amino acids.

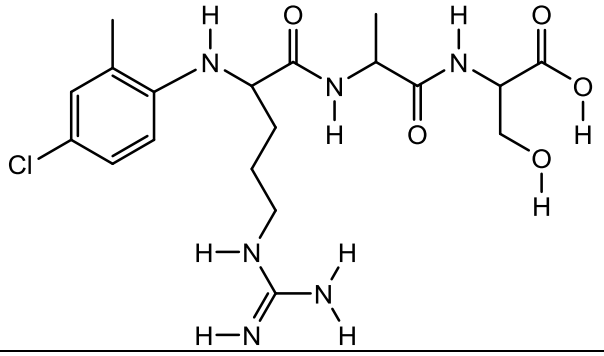
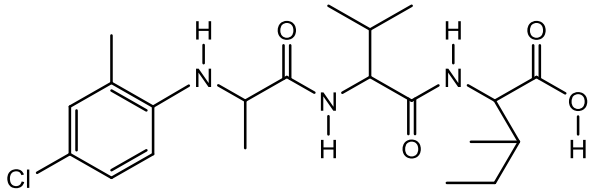
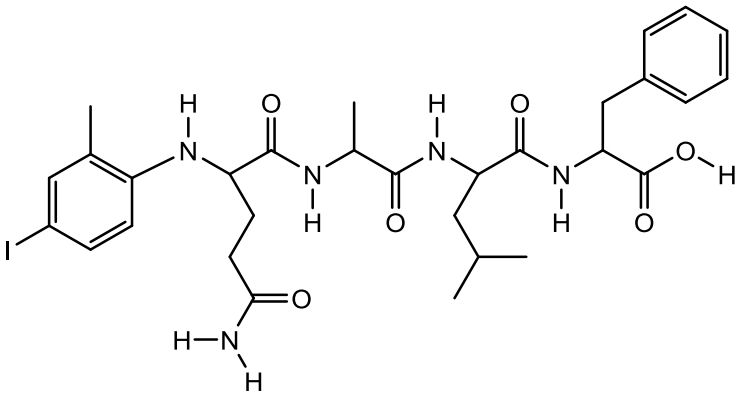
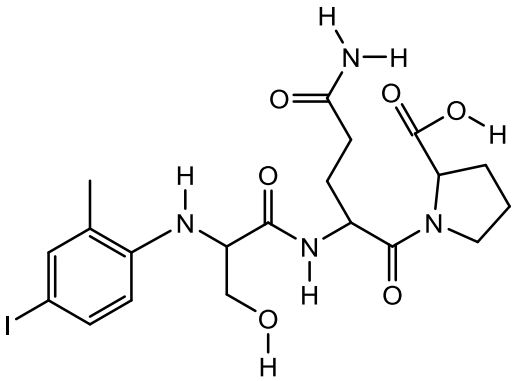
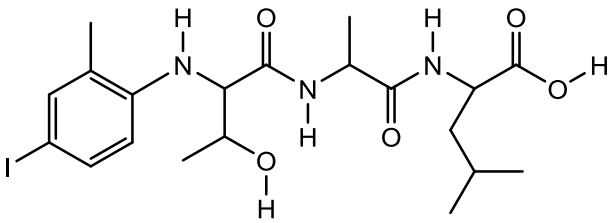
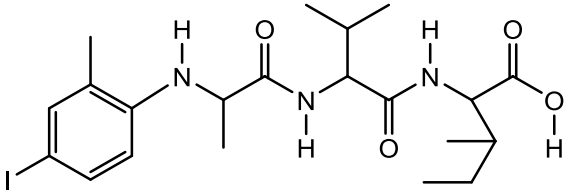
S.No	Name used in docking	Name	Structure
1	Standard Molecule	Ritonavir	
2	Compound 2	P1-Gln-Ala-Leu-Phe-Furan	
3	Compound3	P1-Ser-Gln-Pro-Furan	
4	Compound4	P1-Thr-Ala-Leu-Furan	
5	Compound5	P1-Gln-Ala-Leu-Phe-Oxazole	

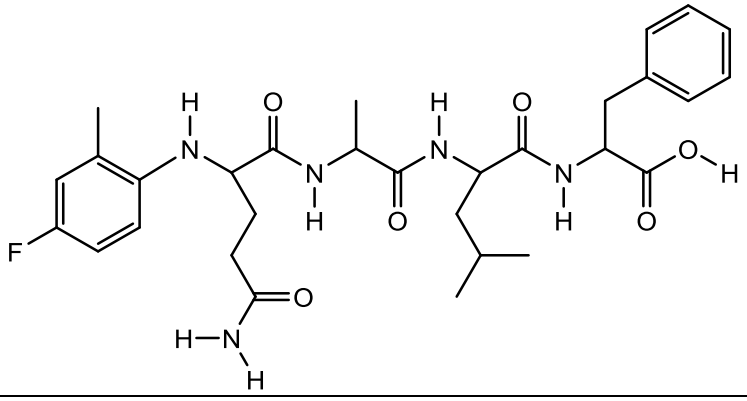
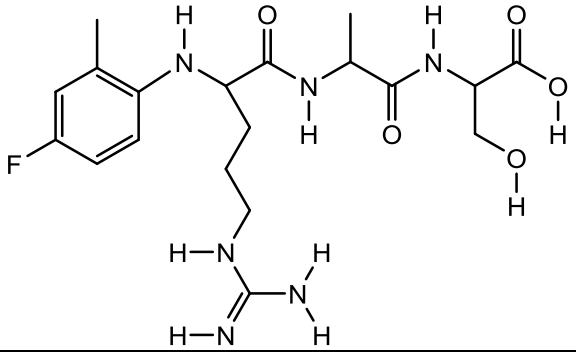
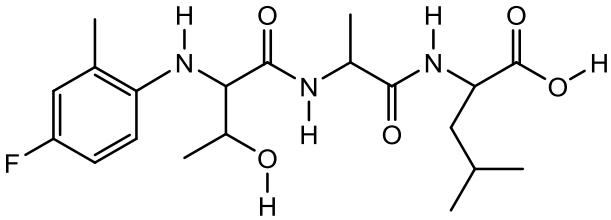
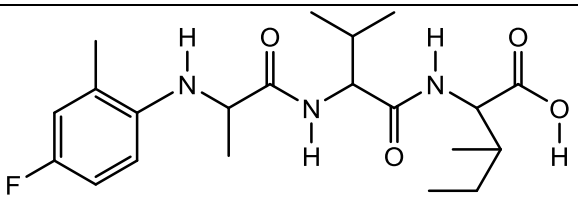
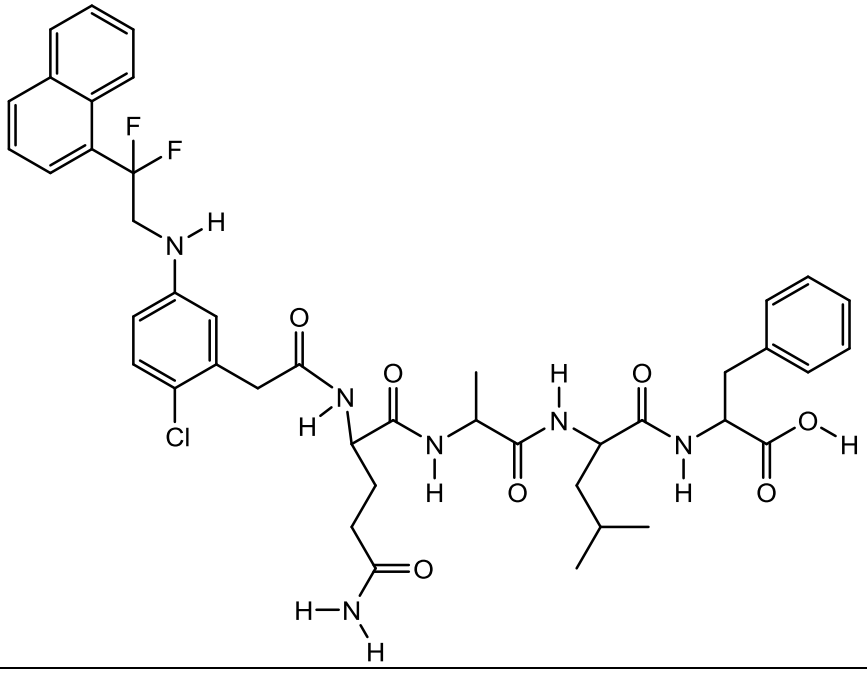
6	Compound6	P1-Ser-Gln-Pro-Oxazole	
7	Compound7	P1-Arg-Ala-Ser-Oxazole	
8	Compound8	P1-Ala-Val-Ile-Oxazole	
9	Compound9	P1-Gln-Ala-Leu-Phe-Pyrrole	
10	Compound10	P1-Ser-Gln-Pro-Pyrrole	

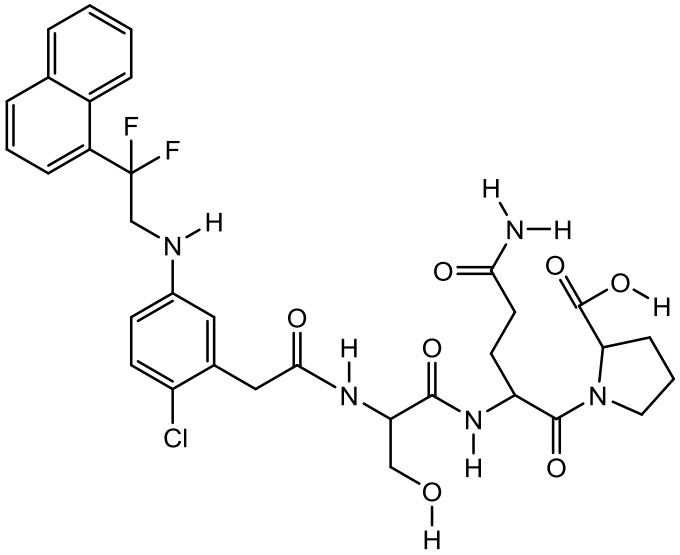
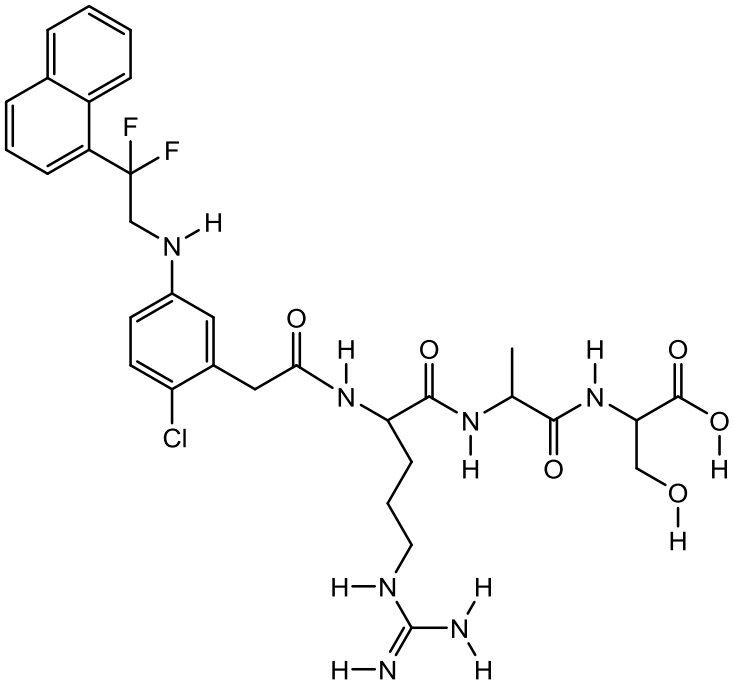
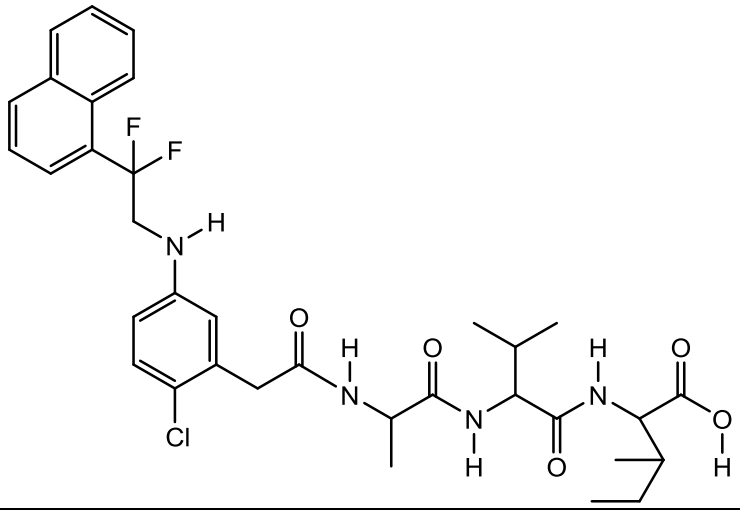
11	Compound11	P1-Thr-Ala-Leu-pyrrole	
12	Compound12	P1-Ala-Val-Ile-Pyrrole	
13	Compound13	P1-Gln-Ala-Leu-Phe-Thiazole	
14	Compound14	P1-Arg-Ala-Ser-Thiazole	
15	Compound15	P1-Thr-Ala-Leu-Thiazole	
16	Compound16	P1-Ala-Val-Ile-Thiazole	

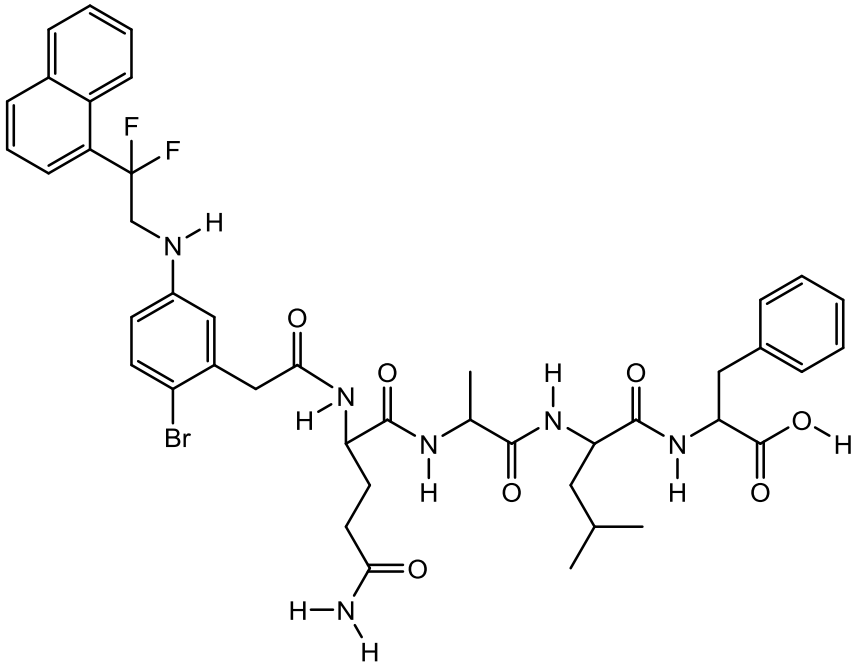
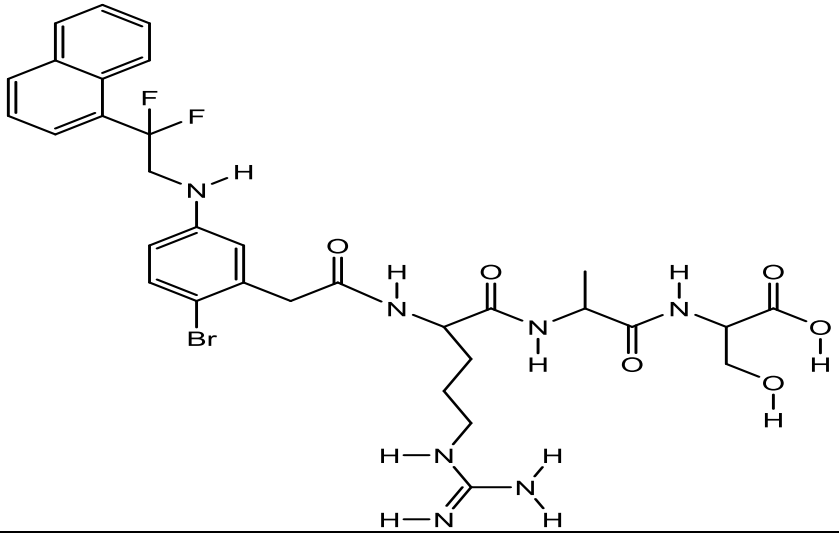
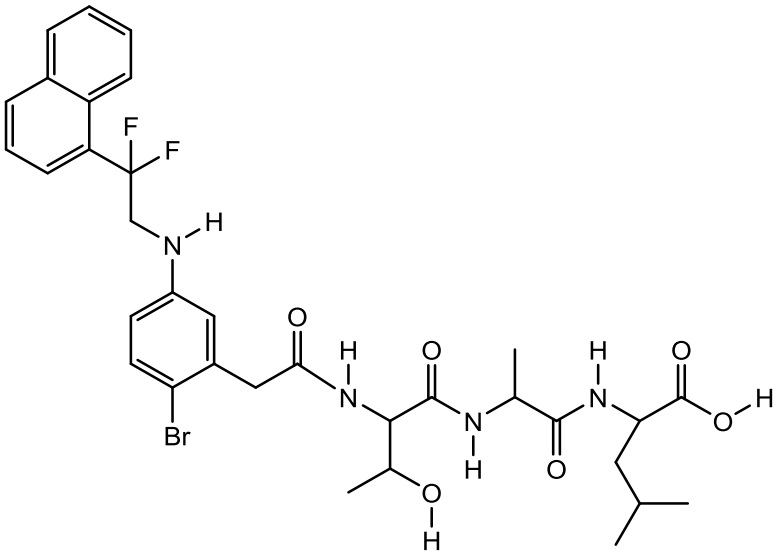
17	Compound17	P1-Ser-Gln-Pro-Pyrazole	
18	Compound18	P1-Thr-Ala-Leu-Pyrazole	
19	Compound19	P1-Ala-Val-Ile-Pyrazole	
20	Compound20	P1-Ser-Gln-Pro-Thiophene	
21	Compound21	P1-Thr-Ala-Leu-Thiophene	

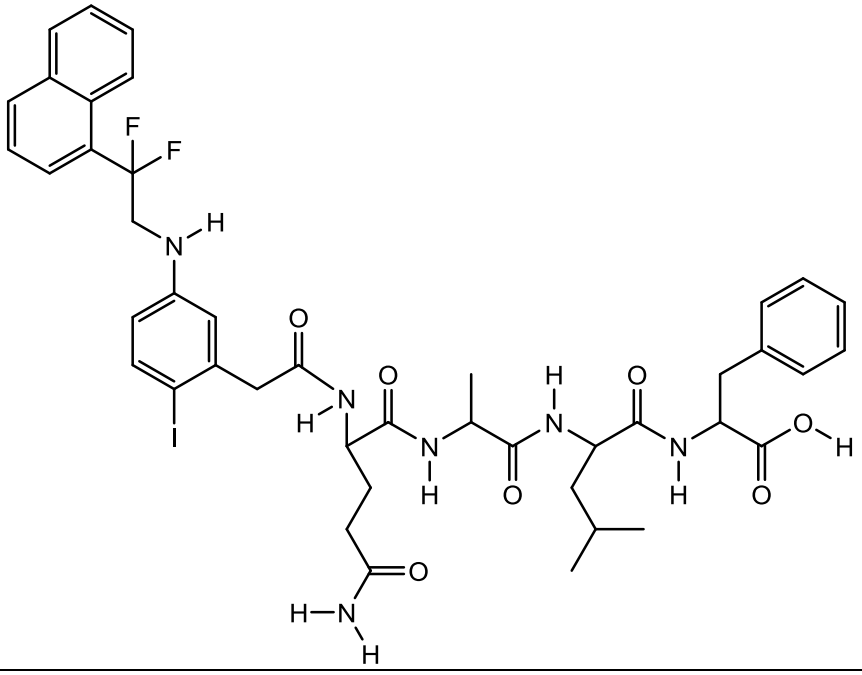
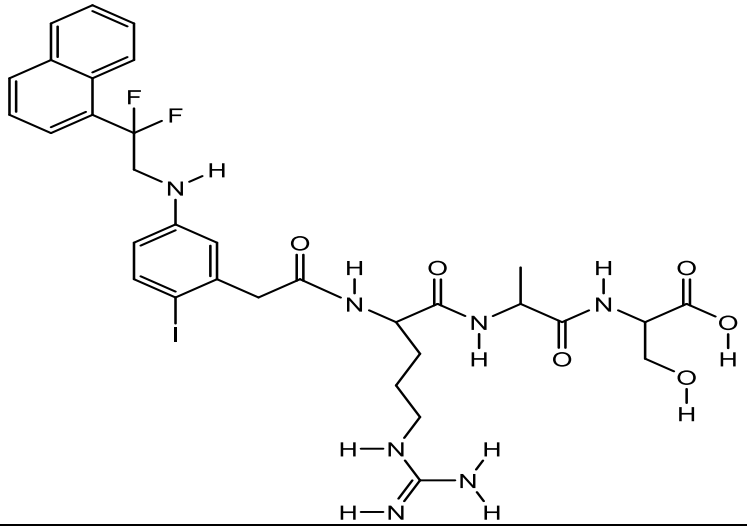
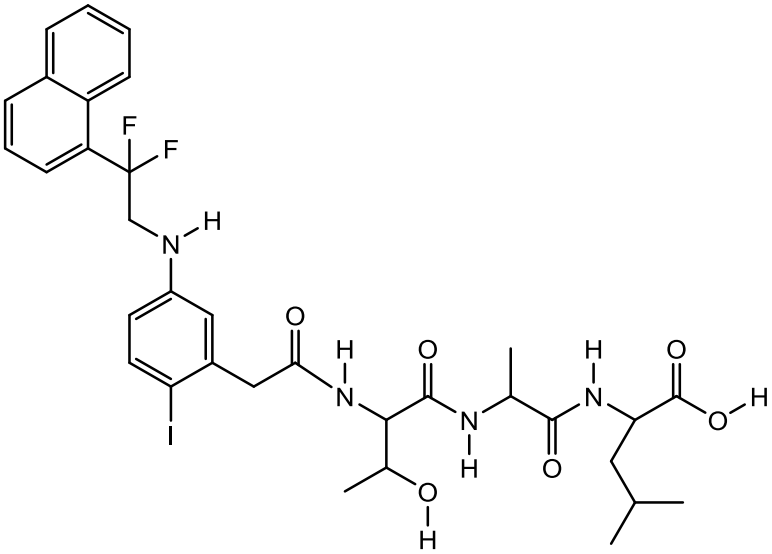
22	Compound22	P2-Ser-Gln-Pro-Br	
23	Compound23	P2-Arg-Ala-Ser-Br	
24	Compound24	P2-Thr-Ala-Leu-Br	
25	Compound25	P2-Gln-Ala-Leu-Phe-Cl	
26	Compound26	P2-Ser-Gln-Pro-Cl	

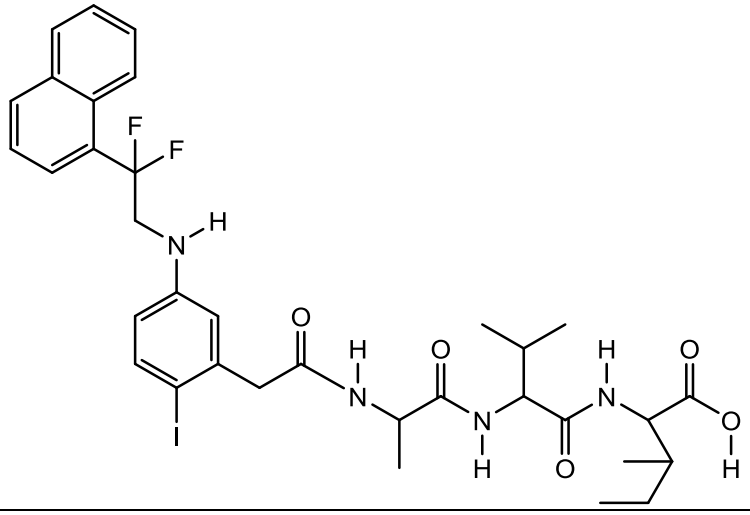
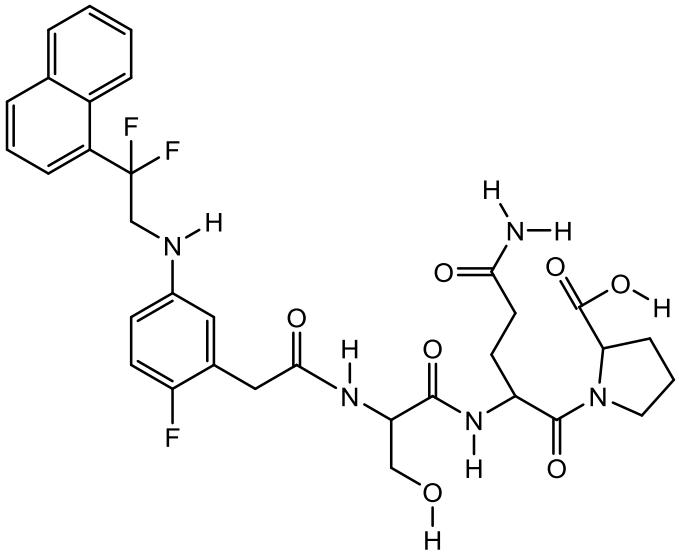
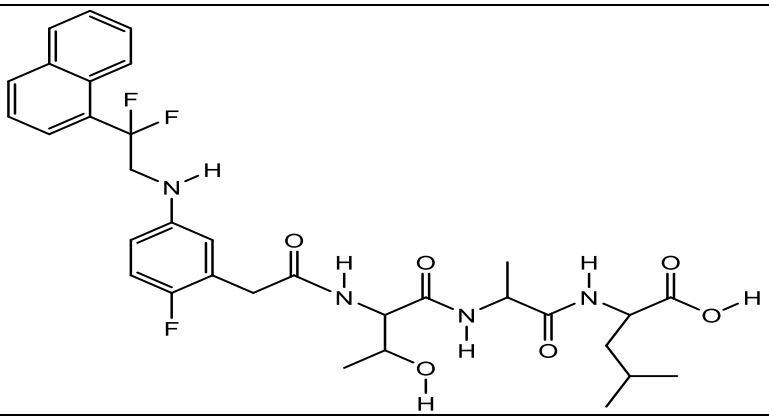
27	Compound27	P2-Arg-Ala-Ser-Cl	
28	Compound28	P2- Ala-Val-Ile-Cl	
29	Compound29	P2- Gln-Ala-Leu-Phe-I	
30	Compound30	P2- Ser-Gln-Pro-I	
31	Compound31	P2- Thr-Ala-Leu-I	
32	Compound32	P2- Ala-Val-Ile-I	

33	Compound33	P2- Gln-Ala-Leu-Phe-F	
34	Compound34	P2- Arg-Ala-Ser-F	
35	Compound35	P2- Thr-Ala-Leu-F	
36	Compound36	P2- Ala-Val-Ile-F	
37	Compound37	P3-Gln-Ala-Leu-Phe-Cl	

38	Compound38	P3- Ser-Gln-Pro-Cl	
39	Compound39	P3-Arg-Ala-Ser-Cl	
40	Compound40	P3- Ala-Val-Ile-Cl	

41	Compound41	P3-Gln-Ala-Leu-Phel-Br	
42	Compound42	P3-Arg-Ala-Ser-Br	
43	Compound43	P3-Thr-Ala-Leu-Br	

44	Compound44	P3-I-Gln-Ala-Leu-Phe-I	
45	Compound45	P3- I-Arg-Ala-Ser-I	
46	Compound46	P3-I-Thr-Ala-Leu-I	

47	Compound47	P3- I-Ala-Val-Ile-I	
48	Compound48	P3-F-Ser-Gln-Pro-F	
49	Compound49	P3-F-Thr-Ala-Leu-F	

RESULTS

Table 2: The -cdocker and -cdocker interaction energy of the peptide analogues of the pharmacophores against HIV-1 PR target.

S.No	Compound	-cdocker energy	-cdocker interaction energy
1	Ritonavir	50.851	57.1088
2	Compound2	48.8887	56.0948
3	Compound3	31.8071	53.797
4	Compound4	48.8663	52.4523
5	Compound5	51.769	54.7893
6	**Compound6**	**60.5778**	**57.7368**
7	Compound7	41.9484	52.7743
8	Compound8	48.6895	54.5869
9	**Compound9**	**65.6897**	**62.5367**
10	Compound10	40.4988	51.2285
11	Compound11	47.7411	54.813
12	Compound12	31.7601	55.9551
13	**Compound13**	**54.9076**	**59.807**
14	Compound14	45.4426	41.3052
15	Compound15	43.9397	37.4821
16	Compound16	46.4134	39.9951
17	Compound17	61.0472	49.6736
18	Compound18	44.9027	35.0013
19	Compound19	45.0088	35.506
20	Compound20	33.7701	39.4559
21	Compound21	62.597	52.1643
22	Compound22	42.5939	34.2674
23	Compound23	48.4764	40.2901
24	Compound24	30.3959	37.6282
25	Compound25	62.2293	49.3434
26	Compound26	47.2076	38.9989
27	Compound27	47.6952	36.147
28	Compound28	33.0975	34.7566
29	Compound29	49.083	51.9892
30	Compound30	32.6892	47.7179
31	Compound31	42.8073	50.9404
32	Compound32	35.4577	44.1136
33	Compound33	29.9614	53.0063
34	Compound34	39.3295	51.5595
35	Compound35	39.4106	44.5214
36	Compound36	50.9485	52.3553
37	**Compound37**	**60.1587**	**60.3027**
38	Compound38	45.6434	52.0253
39	Compound39	48.7188	49.2881
40	Compound40	33.7902	47.4784
41	**Compound41**	**65.7424**	**58.9997**
42	Compound42	49.6203	44.9253
43	Compound43	56.9485	48.6511

44	Compound44	36.4595	46.5359
45	**Compound45**	**72.061**	**63.7013**
46	Compound46	44.9578	42.1848
47	Compound47	57.0892	517597
48	Compound48	34.2119	47.1454
49	Compound49	65.2424	54.5983

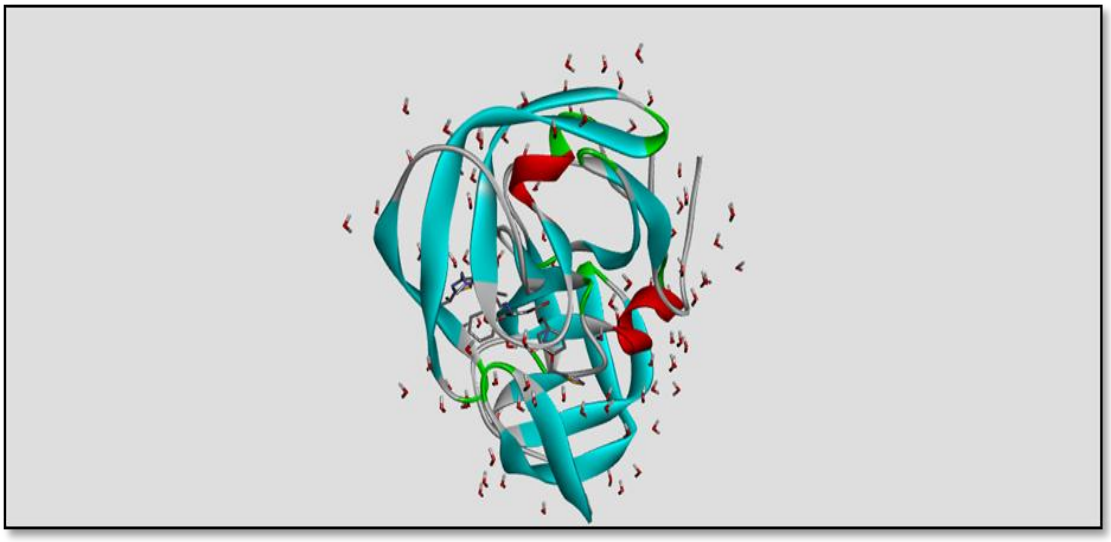


Fig 2: Structure of the HIV-1 PR target (PDB ID 1HXW).

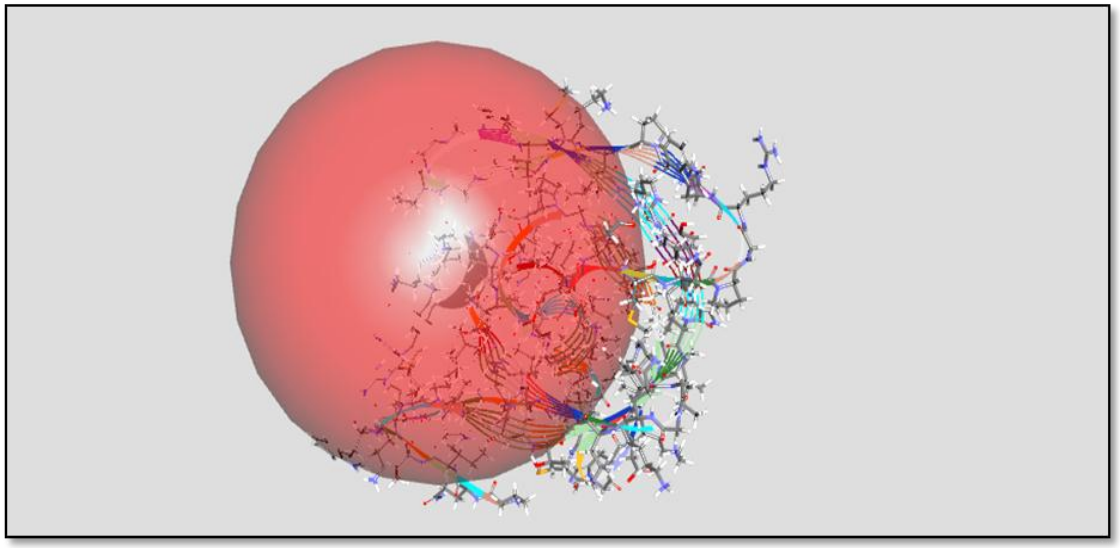


Fig 3: Interaction of compound 45 with the active domain of the HIV-1 PR target (PDB ID 1HXW).

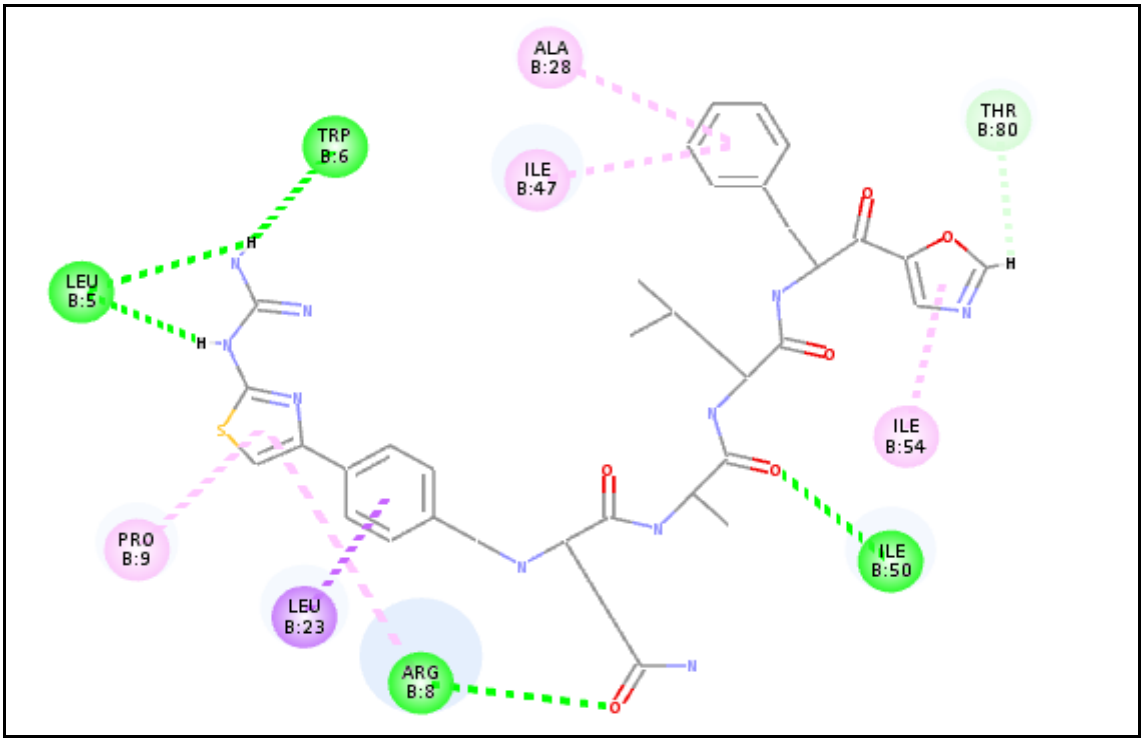


Fig 4: Interaction of compound 45 with the active domain of the HIV-1 PR target (PDB ID 1HXW).

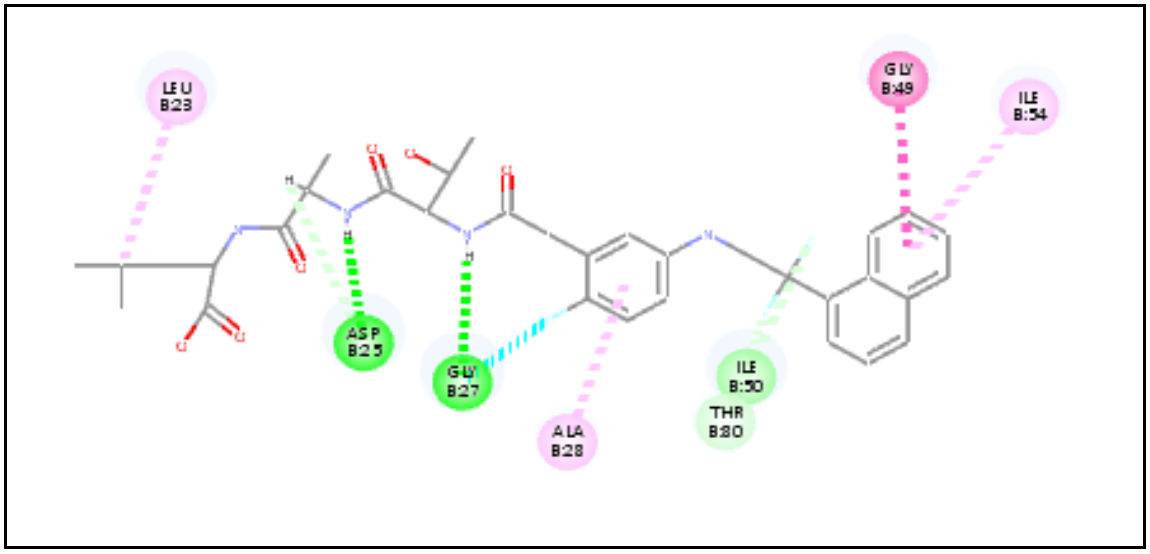


Fig 5: Interaction of compound 2 with the active domain of the HIV-1 PR target (PDB ID 1HXW).

DISCUSSION

Protease is an enzyme which helps in hydrolysis of peptide bonds and converts the long nonfunctional amino acid sequences into functional proteins, enzymes and other organelles of the cell. Hence protease helps to create mature form of proteins which is helpful in creating

the virulent form of a virion cell. Apart from this, proteases are involved in many biological functions, including digestion of ingested proteins, protein catabolism, and cell signaling. The HIV-1 protease (HIV-1 PR) enzyme is responsible for the maturation of the cell during its replication process. The fully functional form of HIV-1 protease that is needed for the virus to survive is the homodimer form. Mature HIV protease exists as a 22 kDa homodimer, with each subunit made up of 99 amino acids. A single active site lies between the identical subunits and has the characteristic Asp-Thr-Gly (Asp25, Thr26 and Gly27) catalytic triad sequence common to aspartic proteases. As HIV-1 PR can only function as a dimer, the mature protease contains two Asp25 amino acids, one from each monomer, that act in conjunction with each other as the catalytic residues. Additionally, HIV protease has two molecular "flaps" which move a distance of up to 7 Å when the enzyme becomes associated with a substrate. There are three domains present in the HIV-1 PR, active site cavity as core domain, terminal domain as dimerization domain and the flap domain.[11] Molecular docking was performed adopting the CDOCKER, which depends on CHARMm-based force field. The docking estimation was performed by the -CDOCKER energy, which was calculated, based upon the internal ligand strain energy and receptor-ligand interaction energy. Additionally, -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. In both the cases, it has to be noted that greater -CDOCKER energy and -CDOCKER interaction energy value implies greater favorable binding between the protein and the ligand. The Compound 45, Compound 9, Compound 37, Compound13, Compound 41, and Compound 6 shows higher -cdocker energy and -cdocker interaction energy than the standard drug where other analogues show comparatively low scores.

CONCLUSION

With the help of extensive literature studies, we have selected HIV-1 protease enzyme as target to control the virulence of HIV in human body. By performing the molecular docking studies of different peptide analogues, we have understood that few analogues have good binding affinity towards the active site of the enzyme. The knowledge obtained here will be useful in the synthesis of antiviral drug that acts by inhibiting the protease enzyme.

This study will provide basement support for performing research work of peptide analogues for the treatment of AIDS.

ACKNOWLEDGMENTS

Our sincere thanks to Rajiv Gandhi University of Health Sciences-Bangalore for providing the research fund for students research project.

REFERENCES

1. HIV and AIDS. What is HIV. [Updated 2023 Jan 13];Available from: https://www.hiv.gov/hiv-basics/overview/about-hiv-and-aids/what-are-hiv-and-aids.
2. Vidya VKK, Karthigeyan KP et. al. Pathophysiology of CD4 and T-cell depletion in HIV-1 and HIV-2 infections. Front Immunol, 2017; (8): 580.
3. HIV Statistics. Statistics of HIV in 2020. [Cited 2023 April 19]; Available from: https://www.who.int/news-room/fact-sheets/detail/hiv-aids.
4. HIV Guide to protease inhibitors. [Cited 2020 April 25]; Available from https://www.healthline.com/health/hiv-aids/protease-inhibitors#interactions.
5. Zhengtong LV, Yuan Chu and Yong Wang. HIV protease inhibitors; a review of molecular selectivity and toxicity. Dove press journal, 2015; (7): 95-104.
6. Davies DR. The structure and function of the aspartic proteinases. Annual Review of Biophysics and Biophysical Chemistry, 1990; 19(1): 189–215.
7. Kitchen DB, Decornez H, Furr JR, Bajorath J. Docking and scoring in virtual screening for drug discovery: methods and applications. Nature Reviews, 2004; 3(11): 935–49.
8. Mostashari-Rad T, Arian R, Mehridehnavi A, Fassihi A, Ghasemi F. Study of CXCR4 chemokine receptor inhibitors using QSPR and molecular docking methodologies. Journal of Theoretical and Computational Chemistry, 2019; 178(4).
9. Suresh PS, Kumar A, Kumar R, Singh VP. An in silico [correction of insilico] approach to bioremediation: laccase as a case study. Journal of Molecular Graphics & Modelling, 2008; 26(5): 845–9.
10. Basharat Z, Yasmin A, Bibi M. Implications of Molecular Docking Assay for Bioremediation. Data Analytics in Medicine, 2020; 1556–1577.
11. Hodis E, Sussman JL, Martz E, Canner D, Harel M, Prilusky j, Berchasky A. HIV-1 protease. Proteopedia; 2013, DOI: https://dx.doi.org/10.14576/315240.1864715.