A Comparative Study for the Accuracy of Three Molecular Docking Programs Using HIV-1 Protease Inhibitors as a Model

Twana Salih[1],*

*Department of Pharmacognosy & Pharmaceutical Chemistry, College of Pharmacy, University of Sulaimani, Iraq.

Abstract

Flexible molecular docking is a computational method of structure-based drug design. This method is used to evaluate binding interactions between receptor and ligand and identify the ligand conformation within the receptor pocket. Currently, various molecular docking programs are extensively applied; therefore, realizing the accuracy and performance of the various docking programs could have a significant value. In this comparative study, the performance and accuracy of three widely used non-commercial docking software (AutoDock Vina, 1-Click Docking, and UCSF DOCK) was evaluated through investigations of the predicted binding affinity and binding conformation of the same set of small molecules (Human Immunodeficiency Virus-1 protease inhibitors) and a protein target HIV-1 protease enzyme. The tested sets are composed of eight receptor-ligand complexes with high-resolution crystal structures downloaded from the Protein Data Bank website. Molecular dockings were applied between approved HIV-1 protease inhibitors and the HIV-1 protease using AutoDock Vina, 1-Click Docking, and DOCK6. Then, docking poses of the top-ranked solution were realized using UCSF Chimera. Furthermore, Pearson correlation coefficient (R) and coefficient of determination (R^2) between the experimental results and the top-scored docking results of each program were calculated using Graphpad prism V9.2. After comparing the saquinavir top-scored binding poses of each docking program with the crystal structure, various conformational changes were observed. Moreover, according to the relative comparison between the top-ranked calculated $\Delta G_{binding}$ values against the experimental results, the R^2 value of AutoDock Vina, 1-Click Docking, and DOCK6 were 0.65, 0.41, and 0.005, respectively. The outcome of this study shows that the top-scored binding free energy could not produce the best pose prediction. In addition, AutoDock Vina results have the highest correlation with the experimental results in comparison with the other programs.

Keywords: Molecular docking accuracy, Comparative study, 1-Click Docking, AutoDock Vina, UCSF DOCK, Binding free energy, HIV-1 protease inhibitors.

<div dir="rtl">

دراسة مقارنة لدقة ثلاثة برامج للالتحام الجزيئي باستخدام مثبطات البروتييز لفيروس نقص المناعة البشرية ـ1 كنموذج

توانا صالح*،١

*قسم العقاقير والكيمياء الصيدلانية، كلية الصيدلة، جامعة السليمانية، العراق

الخلاصة

الالتحام الجزيئي المرن هو طريقة حسابية لتصميم دواء قائم على المستقبلات لتقييم تفاعلات الربط بين المستقبل والجزيئة وتحديد شكل الترابط داخل جيب المستقبل. حاليًا ، يتم تطبيق العديد من برامج الالتحام الجزيئي على نطاق واسع ؛ لذلك ، يمكن أن يكون لتحقيق الدقة والأداء لمختلف برامج الالتحام قيمة كبيرة. الهدف من هذه الدراسة هو تقييم أداء ودقة ثلاثة برامج للالتحام الجزيئي غير تجارية مستخدمة على نطاق واسع (الالتحام الذاتي فينا و نقرة واحدة للالتحام و الالتحام جامعة كاليفورنيا سان فرانسيسكو). تتكون المجموعات المختبرة من ثمانية مجمعات مستقبلات ليجند مع هياكل بلورية عالية الدقة تم تنزيلها من موقع بنك بيانات البروتين. تم تطبيق الالتحام الجزيئي بين مثبطات الأنزيم البروتيني فيروس نقص المناعة البشرية ـ1 المعتمدة و البروتييز فيروس نقص المناعة البشرية ـ1 باستخدام الالتحام الذاتي فينا و نقرة واحدة للالتحام و الالتحام جامعة كاليفورنيا سان فرانسيسكو. بعد ذلك ، تم تحقيق وضعيات الالتحام للحل الأعلى مرتبة باستخدام برنامج كايميرا. علاوة على ذلك ، تم حساب معامل ارتباط بيرسون ومعامل التحديد بين النتائج التجريبية وأعلى نتائج الالتحام المسجلة لكل برنامج باستخدام منشور كرافباد. بعد مقارنة أوضاع الربط المسجلة بأعلى درجات ساكوينافير لكل برنامج الالتحام الجزيئى مع الهيكل البلوري ، لوحظت العديد من التغييرات التوافقية. علاوة على ذلك ، وفقًا للمقارنة النسبية بين قيم الربط المحسوبة الأعلى مرتبة مقابل النتائج التجريبية ، كانت قيمة معامل التحديد للالتحام الذاتي فينا ٠,٦٥ و نقرة واحدة للالتحام ٠,٤١ و الالتحام جامعة كاليفورنيا سان فرانسيسكو ٠,٠٠٥ . تظهر نتيجة هذه الدراسة أن الطاقة الحرة الملزمة ذات أعلى الدرجات لا يمكن أن تنتج أفضل تنبؤ للوضع. بالإضافة إلى ذلك ، فإن نتائج الالتحام الذاتي فينا لها أعلى ارتباط بالنتائج التجريبية.

الكلمات المفتاحية : دقة الالتحام الجزيئي، دراسة مقارنة، نقرة واحدة للالتحام، الالتحام الذاتي فينا، الالتحام جامعة كاليفورنيا سان فرانسيسكو، الطاقة الملزمة الحرة، مثبطات البروتييز لفيروس نقص المناعة البشرية ـ١ .

</div>

Iraqi Journal of Pharmaceutical Science

Introduction

One of the most crucial steps in the drug discovery process is lead identification; various approaches are available to identify a lead compound, such as reliable computational approaches [1]. *In silico* design of ligands based on the knowledge of the 3D chemical structures of receptors is known as Structure-Guided or Structure-Based Drug Design (SBDD). This method is predicting small-molecule ligands that are geometrically and physicochemically complementary to a receptor-binding site [2]. Regardless of the design method, prediction of complementarity between ligand and receptor involves two discrete but interdependent steps, firstly, docking of the 3D encoded ligand into a 3D model derived from the structural coordinates of the receptor structure (binding poses), and then, an assessment of the goodness of fit in terms of interaction energies ($\Delta G_{binding}$) [3, 4].

Receptor-ligand docking (molecular docking) is one of the most recommended approaches in SBDD of the drug discovery projects, as it could design new small molecule against the specified macromolecule receptors and screen the library of compounds to find the most appropriate molecule as a novel compound to activate or inhibit (as required) the target receptor [5]. This method can identify the ligand conformation within the receptor binding site (pocket) and predict the binding affinity between a ligand and a target protein. Hence, a successful docking experiment is the result of two piers, which are the right pose (sampling) and the binding affinity estimation (scoring function) [6]. Nowadays, the scientific community use diverse molecular docking programs, such as AutoDock, AutoDock Vina, UCSF DOCK, LigandFit, Glide, GOLD, MOE Dock [7], and Mcule [8, 9]; which are either commercial or free for academics [10]. According to Pagadala *et. al.* (2017), the most commonly used programs are AutoDock Vina, Gold, and MOE-Dock [11].

Each of the docking programs has advantages and limitations regarding their accuracy and time consumption due to applying diverse sampling approaches and scoring functions [12].

However, the issues related to sampling efficiency (pose prediction) and speed could be fixed to a large extent owing to a significant development of computer hardware and using supercomputers [13], while estimation of actual and comparative binding affinity of small molecules is still a critical concern. Despite much research on the efficiency and relative accuracy of the docking programs in the past three decades, it is still challenging to decide on certain software for a specific project. Therefore, investigations of the advantages and downsides of these programs are essential to select the most appropriate program and improve the drawbacks [14]. Various metric approaches can be implemented to define the accuracy of molecular docking programs and assist users to select the most appropriate program [15, 16].

One of the global human health threats is the suppression of immune functions due to human immunodeficiency virus (HIV). This disease is known as acquired immunodeficiency syndrome (AIDS). Currently several drug classes are available to treat HIV/AIDS, such as non-nucleoside reverse transcriptase inhibitors and HIV-1 protease inhibitors. In addition, many studies have been achieved to develop novel anti-HIV agents from the natural sources like coumarin-based compounds [17, 18]. The HIV-1 protease inhibitors are a class of antiviral drugs that are widely used to treat HIV/AIDS and hepatitis C. These protease inhibitor drugs prevent viral replication by selectively binding to HIV-1 protease and blocking proteolytic cleavage of protein precursors, which are necessary to produce infectious viral particles [19]. Numerous classes of substrate-based and symmetry-based inhibitors have been designed, synthesized, tested, and crystallized with the enzyme. The indispensable role of HIV-1 protease in viral maturation makes it a popular target for drug design [20]. Many solved HIV-1 protease structures can significantly facilitate the design of new and improved inhibitors. Presently, various HIV-1 protease inhibitors are approved by the medicinal regulatory authorities, which include amprenavir, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir (Fig. 1) [21].
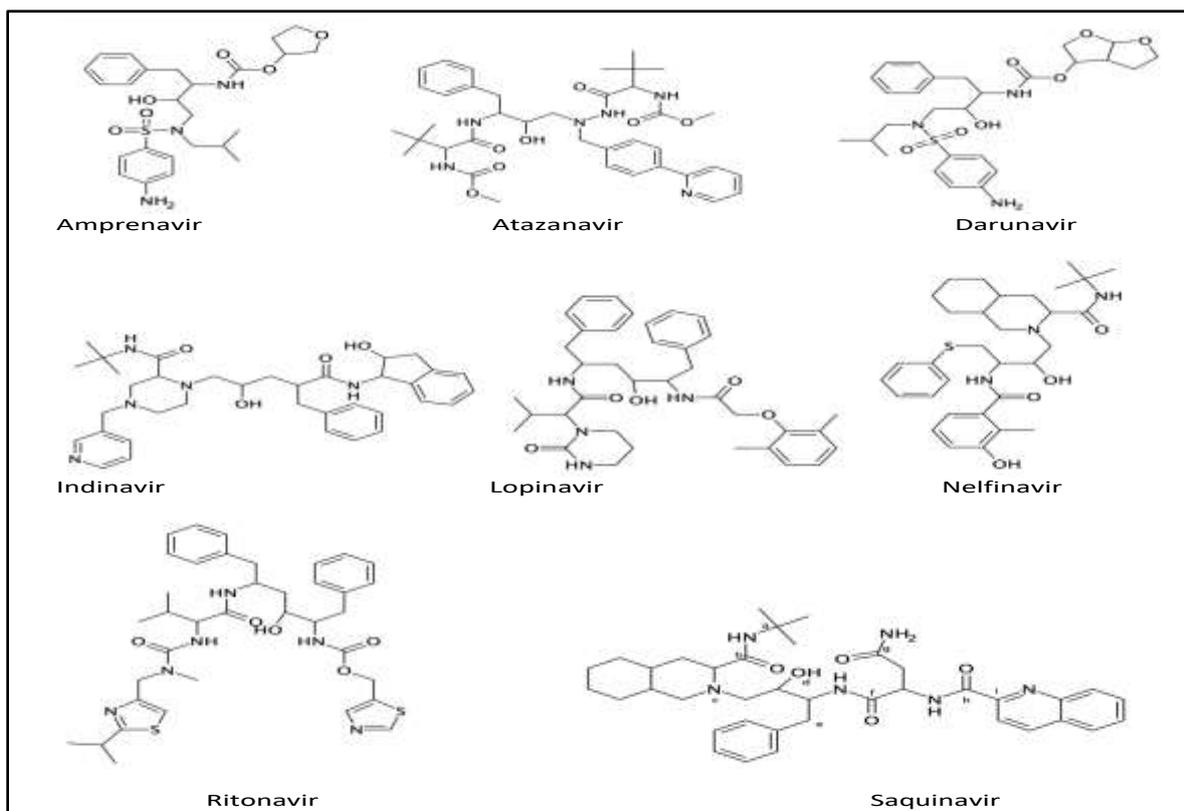
Figure 1. Structures of some of the HIV-1 protease inhibitors.

The aim of this study is to realize conformations of the best energy scores and identify the most accurate and promising program through docking various approved HIV-1 protease inhibitor molecules against the HIV-1 protease enzyme with a known experimental binding affinity of the relevant protein-ligand complexes.

Methods

The HIV-1 protease enzyme as a target protein of this study dockings is an essential element for viral maturation in the HIV life cycle. It is a homodimeric aspartyl protease; each monomer is composed of 99 amino acid residues with a catalytic Asp25 residue. 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. Blocking the active site can inhibit the HIV-1 protease enzyme activity. Inhibition of this key enzyme proved highly effective at reducing viral burden, specifically (Fig. 2) [22].
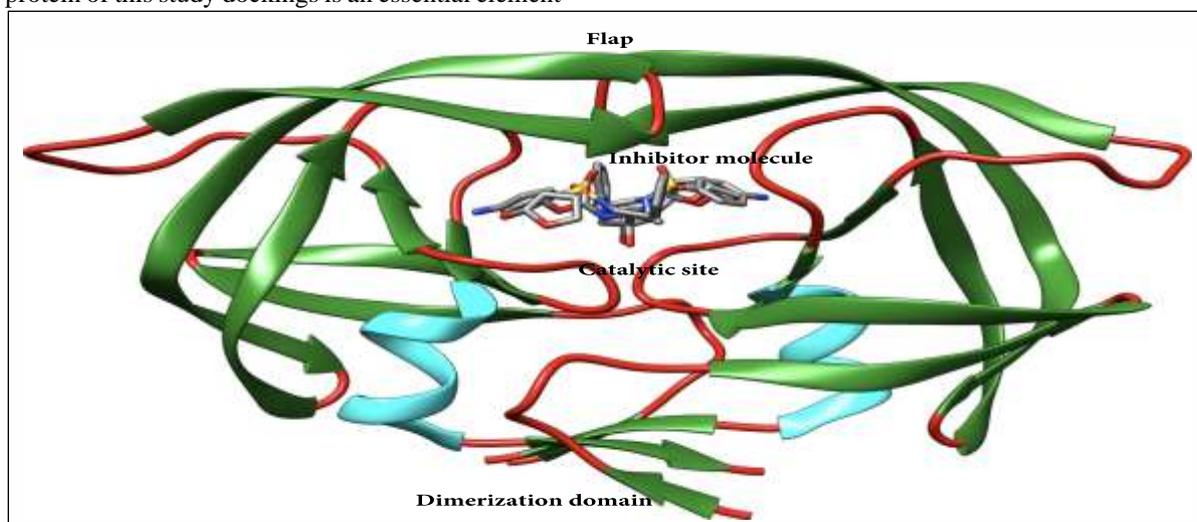
Figure 2. The crystal structure of the HIV-1 protease-inhibitor ligand complex [23].

This project was performed to compare the relative accuracy of predicted binding free energy of three non-commercial docking software (AutoDock Vina, UCSF DOCK, and 1-Click Docking) by testing eight of the FDA-approved protease inhibitor drugs against the target HIV-1 protease enzyme. The details of the applied programs are summarized in Table 1.

Table 1. The outlines of the applied docking programs.

Program	Outline	URL
AutoDock Vina	Designed and implemented by Dr Oleg Trott in the Molecular Graphic Lab at The Scripps Research Institute. The current version is v.1.2.0. (2021) [24].	http://vina.scripps.edu/
UCSF DOCK	Developed by the Kuntz group in the department of pharmaceutical chemistry, University of California San Francisco. The current version is DOCK6 [25].	http://dock.compbio.ucsf.edu/
1-Click Docking	A web-based server developed by Robert Kiss group in Gedeon Richter Plc [8].	https://mcule.com/apps/1-click-docking/

Protein preparation

The started coordinate of the wild-type HIV-1 protease enzyme bound with Saquinavir (solved at 1.16 Å resolution) was downloaded from Protein Data Bank (PDB) (PDB ID: 3OXC) [26] website (https://www.rcsb.org) [27]. After downloading the three-dimensional structure of the complex, all water molecules, formic acid, sulfate ions, and the ligand (Saquinavir) were removed, then, hydrogen atoms were added using PyMOL molecular graphic system [28] to prepare the protein for molecular docking. Furthermore, partial charges were added by Chimera using AMBER ff14SB [14].

Ligand preparation

All the eight ligands were produced for both AutoDock Vina and UCSF DOCK6 through using a suite of applications known as Marvin (http://www.chemaxon.com/products/marvin) [29]. All the Marvin tools were accessible from the MarvinSketch application. The two-dimensional structures of the ligands were generated using the import name of MarvinSketch. After that, the structures were modified to the three-dimensional models of the molecules using molecular dynamics (MD) and energy minimization algorithm to calculate a new position of the ligand's atoms. Lastly, hydrogen atoms were added. All the compounds were saved in Tripos Mol2 file format. Besides, UCSF Chimera was used to assign partial charges of each ligand by adding gasteiger charges for non-standard residues [30]. Contrarily, production of the 3D ligands for 1-Click Docking was achieved using InChIKey formulae obtained from PubChem substance and compound database (https://pubchem.ncbi.nlm.nih.gov) [31]. 1-Click Docking software as a part of MCULE platform were assigned both hydrogen atoms and gasteiger charges to the ligands automatically through using AutoDock tools [32].

Molecular dockings

After the preparation of the target protein and the ligands, docking of the HIV inhibitor ligands over HIV protease enzyme achieved using AutoDock Vina (http://vina.scripps.edu) [33], UCSF DOCK (http://dock.compbio.ucsf.edu) [25] and 1-Click Docking (https://mcule.com/apps/1-click-docking) [8]. All the dockings performed were automated computational algorithms (virtual screening) to select and score the potential molecules matching the shape and chemical complementarity of the HIV-1 protease enzyme and the inhibitor molecules according to the poses. The magnitude of the search space was determined for each of the applied programs with the HIV-1 protease binding centre was X= 20.568, Y= -0.979, Z=15.127, and the dimension was X= 21.033, Y= 16.539, Z= 16.445 in angstrom (Å) [34, 35]. The output files were ranked according to their binding mode from the highest to the lowest binding free energy.

Visualization and analysing programs

PyMOL Molecular Graphics System was implemented to remove water and other non-required molecules and add hydrogen atoms to the target protein [28]. Both PyMOL and UCSF Chimera 1.6 were used to visualize interactions, measure distances between the ligands and the HIV-1 protease enzyme for the selected atoms, and investigate the conformational changes [36]. Graphpad prism V9.2 (GraphPad Software Inc., San Diego, CA; www.graphpad.com) was applied to evaluate the binding affinity results of the various programs [37].

Results and Discussion

Nowadays, the field of molecular docking as a part of SBDD is a fundamental technique in the drug discovery and development process [38]. The components of docking programs are considered as search algorithms to identify the poses of the protein-ligand complexes and scoring function

(binding affinity) based on the generated poses [39]. In this study, the prime focus was on the performance and challenges of three non-commercial programs (AutoDock Vina, UCSF DOCK, and 1-Click Docking) by examining the accuracies of binding pose estimation (power of sampling) and the binding free energy prediction (power of scoring). The test set was composed of eight globally approved inhibitors of HIV-1 protease. Each molecule bound with HIV-1 protease enzyme. All of them have a high-resolution crystal

structure (< 2.5 angstroms) and reliable binding free energy or dissociation constant (*Kd*) (Table 2). The original ligand-receptor binding conformation in the X-ray crystal structures was compared with the top-ranked solutions produced by each of AutoDock Vina, UCSF DOCK, and 1-Click Docking programs. Moreover, the predicted scoring functions of each program were compared with the experimental values to reveal the relative accuracy of each platform.

Table 2. Experimental and calculated binding free energy using Autodock Vina, 1-Click Docking, and UCSF DOCK6 programs [40-42].

HIV-1 inhibitor drug	Experimental $\Delta G_{binding}$ (kcal/mol)	Autodock Vina $\Delta G_{binding}$ (kcal/mol)	1-Click Dock $\Delta G_{binding}$ (kcal/mol)	DOCK6 grid score (kcal/mol)
Amprenavir	-12.10	-8.90	-8.50	-90.03
Atazanavir	-12.81	-10.00	-9.60	-90.22
Darunavir	-12.00	-8.30	-9.70	-93.21
Indinavir	-11.90	-10.10	-7.70	-89.28
Lopinavir	-13.63	-10.20	-10.40	-90.60
Nelfinavir	-13.05	-10.40	-10.30	-92.11
Ritonavir	-12.37	-8.60	-8.80	-106.77
Saquinavir	-12.98	-10.50	-10.40	-106.30

As illustrated in Table 2, the highest binding free energy of the experimental results is Lopinavir (-13.63 kcal/mol). The binding free energy rank of saquinavir is considered as one of the highest ones (-12.98 kcal/mol) after lopinavir and nelfinavir. However, the observed results were showed that the highest binding free energy of AutoDock Vina is saquinavir (-10.5 kcal/mol), 1-Click Docking is lopinavir and saquinavir (-10.4 kcal/mol) and UCSF DOCK is ritonavir and saquinavir (-106.77, and -106.3 kcal/mol, respectively). Ostensibly, all three programs were confirmed that saquinavir has the highest or one of the highest binding free energies. In addition, this ligand is the first discovered HIV-1 protease inhibitor [43]. Therefore, the crystal structure conformation and the molecular docking program's top-scored poses were analyzed. A model of saquinavir-HIV-1 protease complex, determined by X-ray crystallography to a resolution of 2.30 Å as described by Krhon *et al.* in 1991, where saquinavir is referred to as Ro 31-8959 [43]. The structure has been taken from the Protein Data Bank (PDB) website (http://www.rcsb.org, access code 1HXB.pdb) [27]. To investigate this crystal structure and saquinavir-HIV-1 protease enzyme docking results, UCSF Chimera was used [36].

The top-scored binding poses of saquinavir with HIV-1 protease enzyme in each docking program and the X-ray crystal structure [26] are illustrated in Fig. 3. Both AutoDock Vina and 1-Click docking poses have a significant similarity and a moderate difference with the crystal structure conformation, nevertheless, all the conformations are occupying the substrate-binding cavity of HIV-1 protease.

However, the UCSF DOCK pose is shown a significant change; the ligand's conformation in the enzyme's binding site is upturned and the quinoline group of Saquinavir is positioned further towards out of the pocket. As shown in Fig. 4, the essential binding interactions between saquinavir and HIV-1 protease enzyme in the crystal structure and the docking results were realized. X-ray crystal structure of the ligand-protein complex was revealed four binding interactions between saquinavir and HIV-1 protease. The hydroxyl group of saquinavir (**d**) (Fig. 2) is an essential functional group to interact with the receptor. It was produced a hydrogen bond (H-bond) with both carboxylic acid side-chains of Asp25A (2.9 Å) and Asp125B (2.7 Å) in the active site of HIV-1 protease. In addition, the ligand's carbonyl oxygen of both amide groups (**g** and **h** groups) were interacted with Asp129B through the main-chain N-H group, with distances 3.4 Å and 3.1 Å, respectively. Lastly, the NH_2 group of the amide at **g** position was produced H-bond with the carboxylic acid side-chain of Asp130B (Fig. 4A).

Then, the docking pose of the highest calculated $\Delta G_{binding}$ using AutoDock Vina was investigated. As shown in Fig. 4B, four binding interactions are available between saquinavir and HIV-1 protease. The ligand's hydroxyl (**d**) and NH_2 (**g**) groups H-bonding interactions were conserved, however, with a weaker binding interaction of the hydroxyl with Asp25A (4 Å) and the stronger interaction of the NH_2 group with the main-chain carbonyl of Gly48B (2.9 Å) rather than Asp130B. Moreover, interactions between the ligand and both Asp125B and Asp129B were abolished. On the other hand, two new weaker

hydrophilic interactions were formed. The first was between the main-chain N-H group of Asp29A and the ligand's oxygen of amide carbonyl attached to the decahydroisoquinoline group (**b**) (4.1 Å). The

second interaction was between the carboxylic acid side-chain of Asp29B and the carbonyl oxygen of an amide group adjacent to the quinoline group (**h**) (4.5 Å).

Figure 3. Saquinavir-HIV-1 complex conformation of the top-scored docking results and the crystal structure. A) X-ray crystal structure of the HIV-1 protease active binding site bound with saquinavir. The enzyme shows in grey when the ligand is entirely in its pocket, B) AutoDock Vina conformation of the enzyme pocket (blue) and saquinavir (pink), C) 1-Click Docking conformation of the HIV-1 protease binding site, which is in violet and the ligand, D) UCSF DOCK6 conformation of the HIV-1 protease binding site and upturned saquinavir in the receptor's pocket.

Figure 4. Binding interactions between Saquinavir and HIV-1 protease in the crystal structure and the top-scored docking solutions. A) X-ray crystal structure of Saquinavir-HIV-1 protease, B) AutoDock Vina top-score docking solution, B) 1-Click Docking top-score docking solution, D) UCSF DOCK6 top-score docking solution.

After realizing the conformation of the top-scored 1-Click Docking results, five interactions were identified between the ligand and receptor. Despite maintaining a H-bond between the ligands' hydroxyl group and the carboxylic acid side-chain of Asp25A (3.1 Å), the NH_2 group at **g** position was interacted with the same functional group of Asp25A (2.8 Å). Moreover, the amide carbonyl of the **b** group was created H-bond with the backbone N-H group of Gly48B (2.9 Å). Furthermore, two new favourable hydrophilic interactions were observed; carbonyl oxygen atom of **h** group interacted with guanidine side-chain of Arg8A (3.0 Å) and the nitrogen of quinoline group (**i**) interacted with the hydroxylic side-chain of Asp29B (3.9 Å) (Fig. 4C).

The last docking experiment was UCSF DOCK6. In this pose, four bonding interactions were explored. This conformation is remarkably different from the crystal and other programs docking poses. Hydroxyl group of the ligand produced an interaction with the backbone N-H group of ILE50A (3.1 Å). The next interaction was between the amide oxygen atom of the **h** group with

both the main-chain N-H group (2.8 Å) and the side-chain carboxylic group (3.3 Å) of Asp29A. The last H-bond interaction was between the amide NH_2 **g** group and the backbone N-H group of Asp30A (3.0 Å) (Fig. 4D). Due to the invisibility of hydrogen atoms in the crystal structure, hydrogen atoms were removed from the docking results to be consistent. Hence, approximately 1.0 Å should be reduced from each of the measured distances. For example, if the distance between the hydroxyl group (**d**) and Asp30A side-chain is 3.0 Å, after subtracting 1.0 Å, the distance will be 2.0 Å.

This study denoted diverse predicted binding free energies of each HIV-1 protease inhibitor for each program owing to the differences in the docking poses. The top-ranked predicted binding modes were compared relatively with the experimental observed results. What can be highlighted in Fig. 5 is the differences in accuracy of the docking programs using top-scored values, when the most accurate results were produced by AutoDock Vina, the coefficient of determination (R^2) is 0.65 and the Pearson correlation coefficient (R) is 0.80.
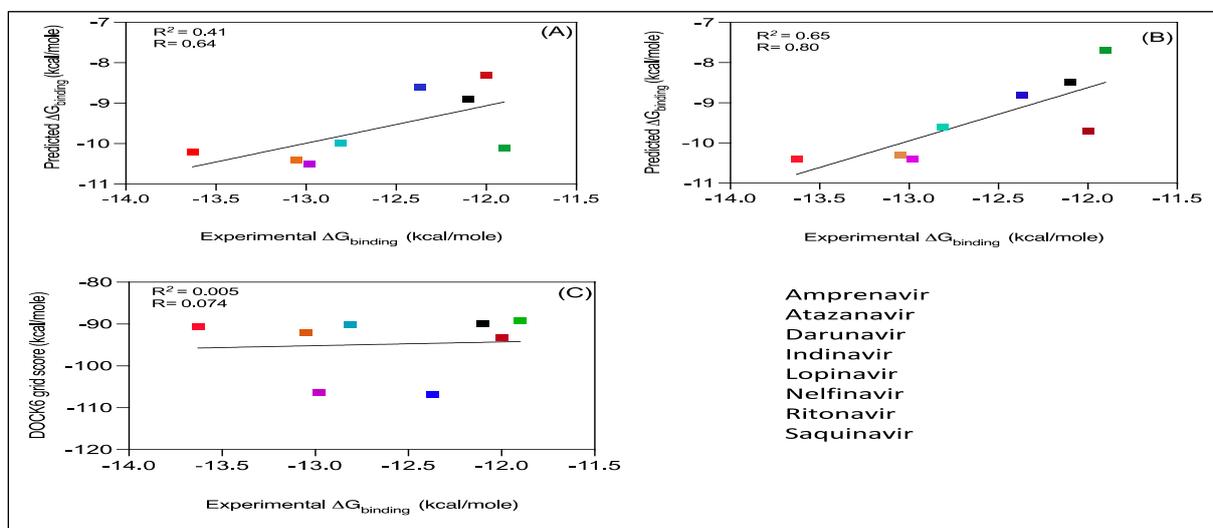
Figure. 5. Correlation between ΔG$_{binding}$ results and experimental results of HIV-1 protease inhibitor drugs. (A) Correlation between experimental results and ΔG$_{binding}$ results using 1-Click Docking. (B) Correlation between experimental results and ΔG$_{binding}$ results using AutoDock Vina. (C) Correlation between experimental results and ΔG$_{binding}$ results using UCSF DOCK6.

Although the AutoDock Vina correlation was moderate (R^2 is 0.65), the program was considered as the most accurate one in this study owing to the highest correlation between the predicted and experimental values compared with the other two programs. After that, 1-Click Docking was ranked as the second program in accuracy, as the R^2 value is 0.41(weak correlation) [44]. However, the DOCK6 program's correlation between the calculated binding free energies and the experimental values was insignificant i.e., no correlation (R^2 is 0.005). The issues of correlation between the predicted and experimental results

could not only be due to the conformational changes but also be related to the preferences of non-covalent interactions between the ligand and the receptor molecule [45].

Conclusion

The best-scored energy of the molecular docking programs could not correspond to the preferred pose of the ligands within the biological macromolecules binding site. Furthermore, AutoDock Vina presented better pose prediction for saquinavir and scoring function for all the inhibitor

molecules compared with 1-Click Docking and DOCK6 programs.

References

1. Sinha S, Vohora D. Chapter 2 - Drug Discovery and Development: An Overview. In: Vohora D, Singh G, editors. Pharmaceutical Medicine and Translational Clinical Research. Boston: Academic Press; 2018. p. 19-32. DOI: https://doi.org/10.1016/B978-0-12-802103-3.00002-X.
2. Batool M, Ahmad B, Choi S. A Structure-Based Drug Discovery Paradigm. Int J Mol Sci. 2019;20(11):1-18. DOI: 10.3390/ijms20112783.
3. Fan J, Fu A, Zhang L. Progress in molecular docking. Quantitative Biology. 2019:1-7. DOI: https://doi.org/10.1007/s40484-019-0172-y.
4. Sulimov VB, Kutov DC, Sulimov AV. Advances in docking. Current medicinal chemistry. 2019;26(42):7555-7580. DOI: https://doi.org/10.2174/0929867325666180904115000.
5. Dong D, Xu Z, Zhong W, Peng S. Parallelization of Molecular Docking: A Review. Curr Top Med Chem. 2018;18(12):1015-1028. DOI: 10.2174/1568026618666180821145215.
6. Torres PHM, Sodero ACR, Jofily P, Silva-Jr FP. Key Topics in Molecular Docking for Drug Design. Int J Mol Sci. 2019;20(18):1-29. DOI: 10.3390/ijms20184574.
7. Salman MM, Al-Obaidi Z, Kitchen P, Loreto A, Bill RM, Wade-Martins R. Advances in Applying Computer-Aided Drug Design for Neurodegenerative Diseases. International journal of molecular sciences. 2021;22(9):4688. DOI: 10.3390/ijms22094688.
8. Kiss R, Sandor M, Szalai FA. http://Mcule.com: a public web service for drug discovery. Journal of Cheminformatics. 2012;4(S1). DOI: 10.1186/1758-2946-4-s1-p17.
9. Di Filippo JI, Cavasotto CN. Guided structure-based ligand identification and design via artificial intelligence modeling. Expert opinion on drug discovery. 2021:1-8. DOI: 10.1080/17460441.2021.1979514.
10. Shaker B, Tran MK, Jung C, Na D. Introduction of Advanced Methods for Structure-based Drug Discovery. Current Bioinformatics. 2021;16(3):351-363. DOI: http://dx.doi.org/10.2174/1574893615999200703113200.
11. Pagadala NS, Syed K, Tuszynski J. Software for molecular docking: a review. Biophys Rev. 2017;9(2):91-102. DOI: 10.1007/s12551-016-0247-1.
12. Saikia SaB, M. Molecular Docking: Challgenges, Advances and its Use in Drug Discovery Perspective. Current Drug Targets.

2019;20(5):1389-4501. DOI: https://doi.org/10.2174/1389450119666181022153016.
13. Amaro RE, Baudry J, Chodera J, Demir O, McCammon JA, Miao Y, et al. Ensemble Docking in Drug Discovery. Biophys J. 2018;114(10):2271-2278. DOI: 10.1016/j.bpj.2018.02.038.
14. Wang Z, Sun H, Yao X, Li D, Xu L, Li Y, et al. Comprehensive evaluation of ten docking programs on a diverse set of protein-ligand complexes: the prediction accuracy of sampling power and scoring power. Physical chemistry chemical physics : PCCP. 2016;18(18):12964-12975. DOI: 10.1039/c6cp01555g.
15. Maia EHB, Assis LC, de Oliveira TA, da Silva AM, Taranto AG. Structure-Based Virtual Screening: From Classical to Artificial Intelligence. Front Chem. 2020;8:343. DOI: 10.3389/fchem.2020.00343.
16. Caballero J. The latest automated docking technologies for novel drug discovery. Expert opinion on drug discovery. 2021;16(6):625-645. DOI: 10.1080/17460441.2021.1858793.
17. K. Hadi M, T. Abduljabbar T. Synthesis, Characterization and Antibacterial Evaluation of Some Coumarin Derivatives. Iraqi Journal of Pharmaceutical Sciences. 2021;30(1):249-257. DOI: 10.31351/vol30iss1pp249-257.
18. Xu Z, Chen Q, Zhang Y, Liang C. Coumarin-based derivatives with potential anti-HIV activity. Fitoterapia. 2021;150:104863. DOI: https://doi.org/10.1016/j.fitote.2021.104863.
19. Ghosh AK, Osswald HL, Prato G. Recent Progress in the Development of HIV-1 Protease Inhibitors for the Treatment of HIV/AIDS. J Med Chem. 2016;59(11):5172-5208. DOI: 10.1021/acs.jmedchem.5b01697.
20. Palese LL. Analysis of the conformations of the HIV-1 protease from a large crystallographic data set. Data Brief. 2017;15:696-700. DOI: 10.1016/j.dib.2017.09.076.
21. Lv Z, Chu Y, Wang Y. HIV protease inhibitors: a review of molecular selectivity and toxicity. HIV/AIDS (Auckland, NZ). 2015;7:95-104. DOI: 10.2147/HIV.S79956.
22. Piliero PJ. Atazanavir: a novel HIV-1 protease inhibitor. Expert Opin Investig Drugs. 2002;11(9):1295-1301. DOI: 10.1517/13543784.11.9.1295.
23. Kozisek M, Lepsik M, Grantz Saskova K, Brynda J, Konvalinka J, Rezacova P. Thermodynamic and structural analysis of HIV protease resistance to darunavir - analysis of heavily mutated patient-derived HIV-1 proteases. FEBS J. 2014;281(7):1834-1847. DOI: 10.1111/febs.12743.
24. Eberhardt J, Santos-Martins D, Tillack A, Forli S. AutoDock Vina 1.2. 0: new docking methods, expanded force field, and Python

bindings. 2021. DOI: 10.26434/ chemrxiv. 14774223.v1.

25. Allen WJ, Balius TE, Mukherjee S, Brozell SR, Moustakas DT, Lang PT, et al. DOCK 6: Impact of new features and current docking performance. J Comput Chem. 2015 ;36(15):1132-1156. DOI: 10. 1002 /jcc. 23905.

26. Tie Y, Kovalevsky AY, Boross P, Wang YF, Ghosh AK, Tozser J, et al. Atomic resolution crystal structures of HIV-1 protease and mutants V82A and I84V with saquinavir. Proteins. 2007;67(1):232-242. DOI: 10.1002/prot.21304.

27. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, et al. The Protein Data Bank. Nucleic acids research. 2000;28(1):235-242. DOI: https://doi.org/10.1093/nar/28.1.235.

28. Schrodinger, LLC. The PyMOL Molecular Graphics System, Version 1.3r1. 2010.

29. ChemAxon M. www.chemaxon.com/products/marvin. Budapest: Jan; 2016.

30. Butt SS, Badshah Y, Shabbir M, Rafiq M. Molecular Docking Using Chimera and Autodock Vina Software for Nonbioinformaticians. JMIR Bioinformatics Biotechnol. 2020;1(1):e14232. DOI: 10.2196/14232.

31. Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, et al. PubChem Substance and Compound databases. Nucleic acids research. 2016;44(D1):D1202-1213. DOI: 10.1093/nar/gkv951.

32. Odhar HA, Ahjel SW, Albeer A, Hashim AF, Rayshan AM, Humadi SS. Molecular docking and dynamics simulation of FDA approved drugs with the main protease from 2019 novel coronavirus. Bioinformation. 2020;16(3):236-244. DOI: 10.6026/97320630016236.

33. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010;31(2):455-461. DOI: 10.1002/jcc.21334.

34. Hornak V, Simmerling C. Targeting structural flexibility in HIV-1 protease inhibitor binding. Drug discovery today. 2007;12(3-4):132-138. DOI: 10.1016/j.drudis.2006.12.011.

35. Ahsan M, Pindi C, Senapati S. Electrostatics Plays a Crucial Role in HIV-1 Protease Substrate Binding, Drugs Fail to Take Advantage. Biochemistry. 2020;59(36):3316-3331. DOI: 10.1021/acs.biochem.0c00341.

36. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF chimera - A visualization system for exploratory research and analysis. J Comput

Chem. 2004;25(13):1605-1612. DOI: 10.1002/jcc.20084.

37. Swift ML. GraphPad Prism, Data Analysis, and Scientific Graphing. Journal of Chemical Information and Computer Sciences. 1997;37(2):411-412. DOI: 10.1021/ci960402j.

38. Kaneria M, Parmar J, Rakholiya K. Molecular docking and drug design of phytoconstituents from Couroupita guianensis – An in silico perspective. Journal of Pharmacognosy and Phytochemistry. 2019;8(6):53-60.

39. Li J, Fu A, Zhang L. An Overview of Scoring Functions Used for Protein–Ligand Interactions in Molecular Docking. Interdisciplinary Sciences: Computational Life Sciences. 2019;11(2):320-328. DOI: 10.1007/s12539-019-00327-w.

40. Kozisek M, Bray J, Rezacova P, Saskova K, Brynda J, Pokorna J, et al. Molecular analysis of the HIV-1 resistance development: enzymatic activities, crystal structures, and thermodynamics of nelfinavir-resistant HIV protease mutants. J Mol Biol. 2007;374(4):1005-1016. DOI: 10.1016/j.jmb.2007.09.083.

41. King NM, Prabu-Jeyabalan M, Bandaranayake RM, Nalam MN, Nalivaika EA, Ozen A, et al. Extreme entropy-enthalpy compensation in a drug-resistant variant of HIV-1 protease. ACS chemical biology. 2012;7(9):1536-1546. DOI: 10.1021/cb300191k.

42. Dierynck I, De Wit M, Gustin E, Keuleers I, Vandersmissen J, Hallenberger S, et al. Binding kinetics of darunavir to human immunodeficiency virus type 1 protease explain the potent antiviral activity and high genetic barrier. J Virol. 2007;81(24):13845-13851. DOI: 10.1128/JVI.01184-07.

43. Krohn A, Redshaw S, Ritchie JC, Graves BJ, Hatada MH. Novel binding mode of highly potent HIV-proteinase inhibitors incorporating the (R)-hydroxyethylamine isostere. Journal of Medicinal Chemistry. 1991;34(11):3340-3342. DOI: 10.1021/jm00115a028.

44. Moore DS, Notz W, Fligner MA. The Basic Practice of Statistics. 2013. W.H. Freeman and Company; [15-24]. Available from: https://books.google.iq/books?id=aw61ygAAC AAJ.

45. Li X, Li Y, Cheng T, Liu Z, Wang R. Evaluation of the performance of four molecular docking programs on a diverse set of protein-ligand complexes. J Comput Chem. 2010;31(11):2109-2125. DOI: 10.1002/jcc.21498.