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Selective docking of promising retroviral integrase inhibitors towards prototype foamy virus integrase

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Abstract
Integrase is a retroviral enzyme that inserts viral DNA (vDNA) into host DNA by performing two reactions: 3′-processing of the vDNA and strand transfer. Successful integration of retroviral DNA into the chromosomal host is an essential step for viral replication and it is known as promising target in antiretroviral drug development. In this study, the crystal structure of Prototype Foamy Virus (PDB: 3OYA) was used as a model protein in molecular docking to investigate its interaction with retroviral integrase inhibitors. The employed method shows roughly Mg^{2+} chelation motif in the active site if three parallel heteroatoms present in the ligand, while interactions with neighboring residues leave a lot to be desired. Docking-based virtual screening of potential inhibitors were used with modification of proper atom charge assignment or macromolecule reconstruction gave remarkable inhibition performed by integrase inhibitors.

Keywords: Retroviral integrase inhibitors, in silico docking, prototype foamy virus integrase, binding interaction

Introduction
Retroviral integrase (intasome) is an enzyme that inserts viral DNA reverse transcription into the host genome [1]. Integration happens in nucleus, irreversibly, and after integrating the host cell will produce components for new viruses [2]. Integration begins by processing (cleaving) of viral DNA (vDNA) 3' ends, which then attacks the host DNA (tDNA), and followed by repair of DNA by cellular enzymes [3-5]. Specifically, integrase binds to vDNA by recognizing the long terminal repeats (LTR), cutting GT dinucleotides from the 3' ends, leaving overhanging CA from 5' ends [4].
The 3' processing and DNA integration occur in an active site that requires the presence of two Mg^{2+} cofactors following 3' processing [6]. This active site becomes a target of the drug family integrase strand transfer inhibitor (INSTI) [4]. INSTI works by chelating the Mg^{2+} ions together with Asp64, Asp116 and Glu155 (HIV-1), or Asp128, Asp185 and Glu221 (PFV), preventing tDNA binding thus inhibiting strand transfer from vDNA to tDNA [4,7]. INSTI also forms pi-pi stacking with several residues like Tyr212 and aromatic rings of two terminal amino acids C and A [8].
Complete structure (full-length, complete with vDNA) of HIV-1 integrase had not been known until Passos *et al.* [9] successfully crystalized full-length HIV-1 strand transfer complex intasome (tDNA attacked, vDNA has been merged with tDNA). Integrase of a related retrovirus PFV has its full-length structure documented and so far is used as the model in studies about INSTI [8, 10]. Despite the low sequence similarity (22%), PFV integrase shares a high degree of structure similarity with HIV-1 integrase [11].
Some in silico studies of INSTI binding uses fine-tuning of PFV integrase, by manual corrections around the active site and rotation of several residues [12], completing/repairing crystal structure of HIV-1 integrase catalytic core domain (CCD) [13], and homology modelling from PFV integrase [14-15]. In this study, we used the crystal structure of PFV integrase in its holo conformation by raltegravir (a commercial INSTI (PDB: 3OYA) [16] without modifications to assess its viability in studying INSTI binding, compared with the other in silico methods used.
In this study, we analyzed the model's feasibility by using commercial drugs and then assessed 5 compounds known to be active against integrase and performed in silico analyses for their ability as HIV-1 INSTI.

Materials and Methods

Preparation of protein

Crystal structure of PFV integrase bound to raltegravir (PDB: 3OYA) [16] was used in this study. Receptor refinement was done by KobaMIN [22-24] and receptor validation was done by the protein structure validation software (PSVS) [25].

Preparation of ligands

Three-dimensional structures of eight molecules that show inhibitory effect on HIV-1 integrase were obtained from PDB, or alternatively converted from their respective 2D structures. Three were commercial INSTI; raltegravir (RAL), elvitegravir (EVG) and dolutegravir (DTG). The remaining five have limited in silico study against integrase (Figure 1). The 3D structure of ligands were converted into SMILES and analyzed by SwissADME [17].

Molecular docking

Molecular docking was performed on crystal structure of PFV integrase (PDB: 3OYA) [16] using AutoDock 4.2 [18]. Bound raltegravir and water molecules were removed, polar hydrogens added and Kollman charges were assigned. Torsions in ligands were set by keeping amide (peptide) bonds rigid due to resonance of double bonds (C=O and C=N) that creates a planar O-C-N-H structure [19]. Preparation of macromolecule and ligands was done in AutoDock Tools 1.5.6 [18, 20]. Grid box was set with center x = -36.956; y = 31.989; z = -19.75 and size x = 40; y = 60; z = 50. Docking calculations used Lamarckian Genetic Algorithm with all parameters set to default. Visualization of protein-ligand complexes was done using DS Visualizer [21].

Results

Structure-guided of integrase inhibitors into PFV integrase

The search for the best docking method was done using PFV integrase, a homolog of HIV-1 integrase used in integrase inhibition studies [8, 16]. Crystal structure of PFV integrase bound to raltegravir (PDB: 3OYA was used in this study due to its good validation scores [16]. This structure contains vDNA that has its 3' ends cleaved and is already bound to an INSTI raltegravir. The other seven integrase drugs were also determined which the structures are shown in Fig.1.

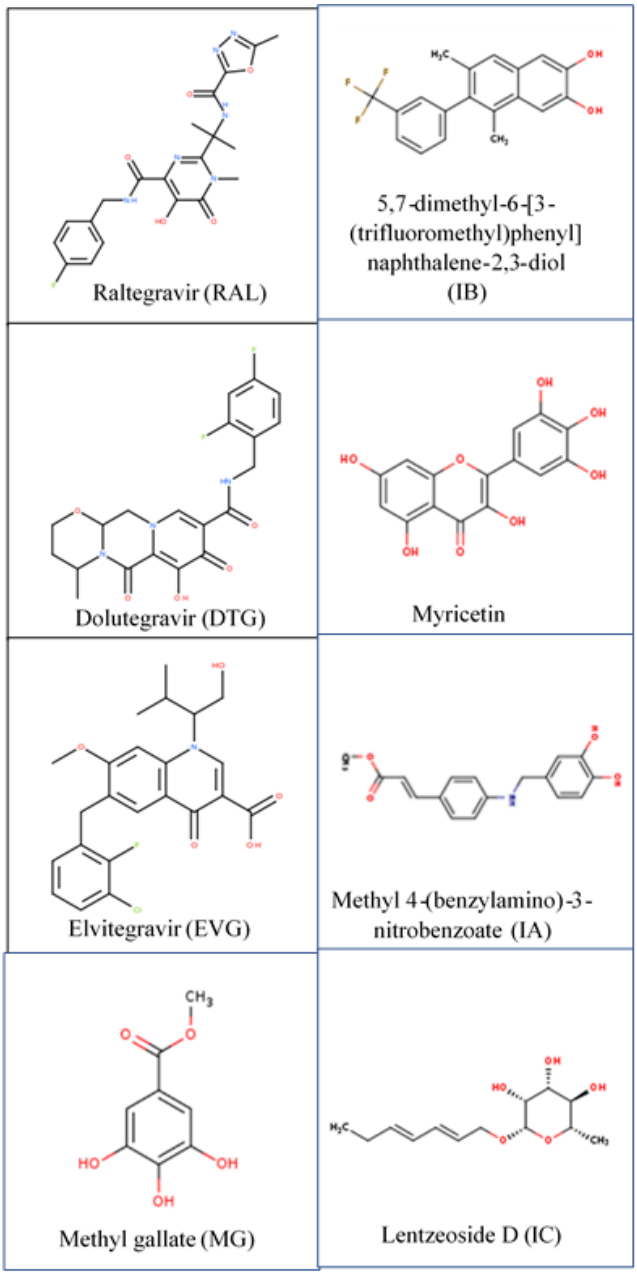


Fig 1: The structures of intregase inhibitors

AutoDock Tools does not assign electrostatic charge to magnesium ion [18]. Manual charge assignment of 1.5 on the two magnesium ions only resulted in little change of conformation in the best result (Figure 2), but gave lower binding energy (-11.53 kcal/mol vs -10.75 kcal/mol) and higher stability among top-10 conformations. The higher stability was seen from more results with similar conformations and lower number of significantly different conformations. The observed differences, however, lies within margin of error and coupled with very limited runs made us unable to say whether or not charge assignment makes a significant difference.

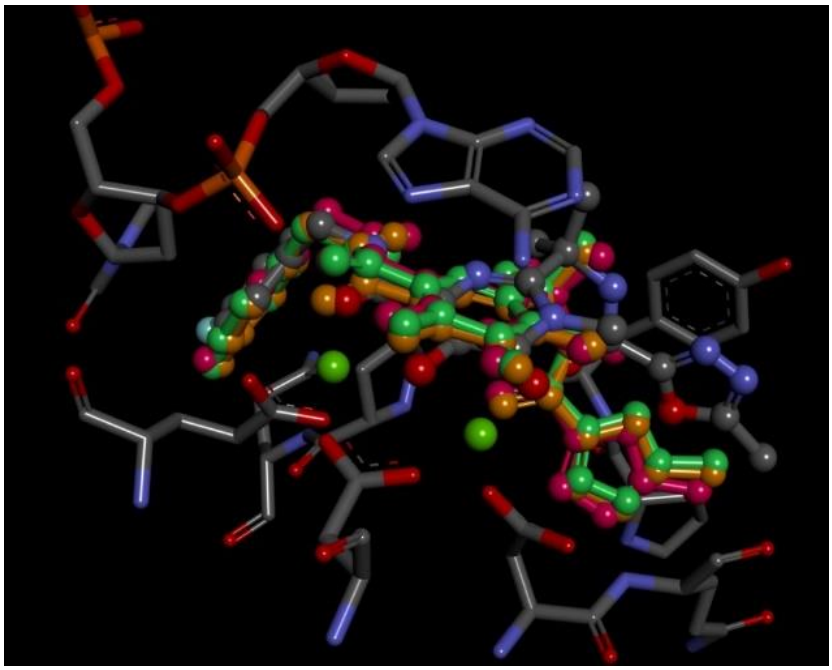


Fig 2: Redocking results on PFV integrase (PDB: 3OYA). Colors of receptor and ligand from crystalized structure follow atom type (grey: carbon, red: oxygen, blue: nitrogen). Magnesium ions shown as two green spheres. For ligand, green: uncharged Mg, orange: charged Mg (+1.5), red: refined, charged Mg (+1.5)

Physiochemical properties of experimental inhibitors

In this study five compounds known to have inhibitory effect against HIV-1 integrase (Myr, Mg, IA, IB, IC; Figure 1), but with no/limited *in silico* study were used. These compounds are either naturally produced or synthetized and collectively we call them experimental inhibitors.

To estimate the characteristics and feasibilities of these experimental inhibitors physiochemical property were performed using SwissADME [17]. All five compounds are hydrophilic with one only moderately soluble (LogS -5.85 = $10^{-5.85}$ mol/L). All five cannot permeate through the blood brain barrier (cannot enter the brain) and can be easily absorbed by gastrointestinal tract except for Myricetin. Such characteristics are also possessed by commercial INSTIs, e.g. raltegravir [4], as shown on Table 1.

Table 1: Physiochemical property results of experimental inhibitors

Compound	ESOL Log S	ESOL Class	GI Absorption	BBB Permeant	Lipinski #violations	Bioavailability Score
	Water solubility		Drug absorption by gastrointestinal tract	Permeation through blood brain barrier	Criteria for orally administrable drug	Probability of F (unchanged drug in blood) > 10% in rat
RAL	-3.95	Soluble	High	No	0	0.55
EVG	-7.10	Moderate	High	No	0	0.55
DTG	-3.78	Soluble	High	No	0	0.55
Myr	-3.01	Soluble	Low	No	1; H-donor > 5	0.55
MG	-1.73	Soluble	High	No	0	0.55
IA	-3.57	Soluble	High	No	0	0.55
IB	-5.85	Moderate	High	No	1; MlogP > 4.15	0.55
IC	-1.36	Very	High	No	0	0.55

Molecular Docking of experimental inhibitors

Docking results of integrase drugs, including raltegravir to ligand-removed and prepared PFV integrase returns a rather different conformation from the original crystalized structure. The main differences are in directions of the three magnesium-binding heteroatoms (O) and the end with 5-membered ring (Figure 3).

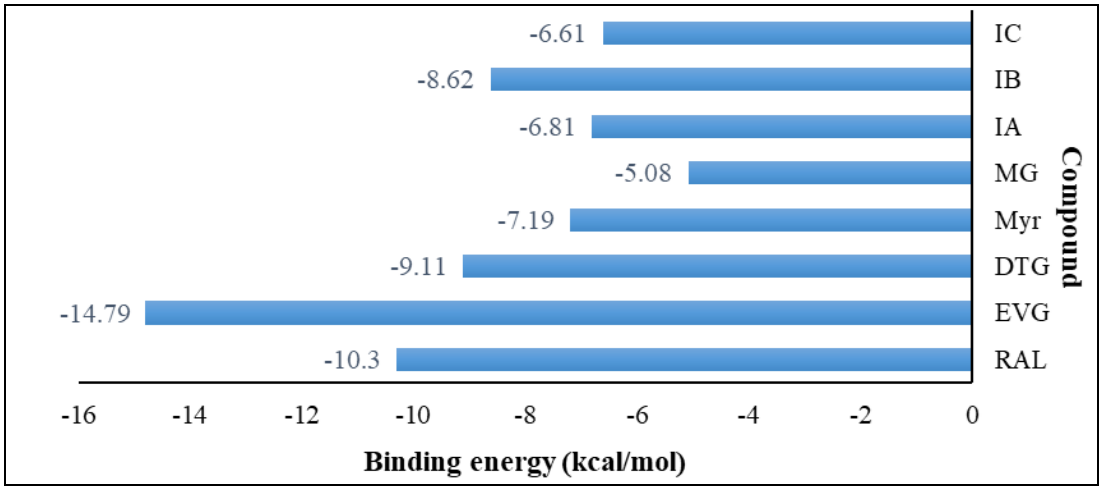


Fig 3: Binding energy values from eight integrase inhibitors

Discussion

In optimizing the ligand structures, KobaMIN was used to removes all non-standard residues (amino acids) prior to refinement [22-24], while magnesium ion and 3' vDNA ends play a crucial role in the docking of INSTI [4,7-8]. Redocking of raltegravir to refined structure requires adding back vDNA and both magnesium ions to the active site. The coordinates used were obtained from the original structure and magnesium ions were assigned charges of +1.5. Docked raltegravir conformation is slightly worse than its pre-refinement counterpart (Figure 2) where shift of heteroatoms is more pronounced, and there is a considerable drop in binding energy and conformation stability. Considering vDNA and magnesium ions in refinement should yield different result, since the positions of magnesium ions and vDNA will fit with the refined protein. Accordingly, refined protein was not used and for further docking 3OYA with +1.5 charge on both magnesium ions was used. To find out the effect ligand removal has on the crystal structure, the protein structure was then optimized and compared to the unoptimized structure. An increase of residues in most favorable conformation (89.7% to 93.5%) can be observed. Unfavorable residual conformations remain low (0.8% and 0.4%) showing little overlap between residues.

Visible differences in visualized ligand binding conformations appear to be largely caused by hydrogen bonds (e.g. O/N with H). In raltegravir, the middle O atom that is supposed to take part in magnesium ion chelation appears to form a hydrogen bond with O from Asp128 and N from DA17 (vDNA adenine) (Figure 3). Exocyclic N and O of DA17 and RAL are also bound by hydrogen bond. Consequently, raltegravir is positioned 'deeper' into the formed pocket (the 'inside' part of the pocket is raltegravir fluorobenzene) (Figure 4). The angle of 6-membered ring also changes from hydrogen bond, which facilitates hydrogen bond between the outer ring and N of Gln186, which in turn removes pi-pi stacking with Tyr212. Both redocked and original structure show pi-pi force between both DA17 rings and the center ring, and between inner ring and ring of DC16. Redocking of raltegravir gives a binding energy of -11.53kcal/mol. Predictably a similar model of interactions, particularly around the active site and inner pocket (raltegravir fluorobenzene) will be observed in other docked inhibitors.

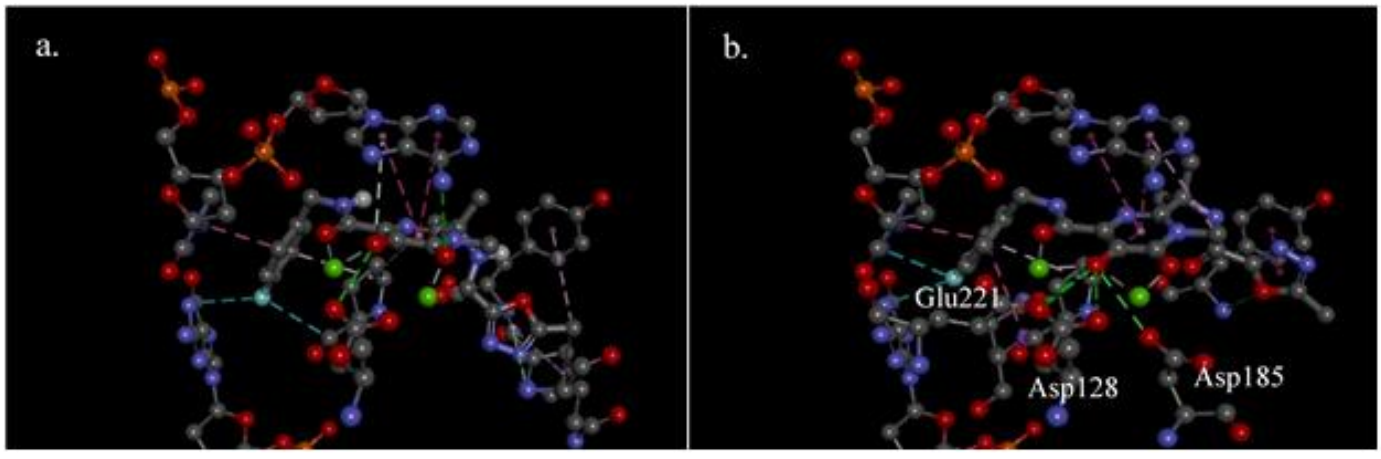


Fig. 4: Raltegravir-3OYA complex. Colors follow atom type (grey: carbon, red: oxygen, blue: nitrogen). a. initial docking mode, b. redocking mode of crystallized conformation

Compared to raltegravir, heteroatoms in dolutegravir and elvitegravir (Figure 5) did not form hydrogen bonds with DA17 but instead they form it with residues 'below' like Asp128 and Asp185. The chelation motif is more visible in these two ligands. All three inner halobenzene form pi-pi stacking with DC16. Despite dolutegravir showing s remarkably similar conformation to previous docking result [26], elvitegravir only shows good results in the three Mg^{2+} chelating heteroatoms. In elvitegravir halobenzene faces the wrong way and the two chains jutting out from the center 6-membered rings are also in the wrong conformation [26].

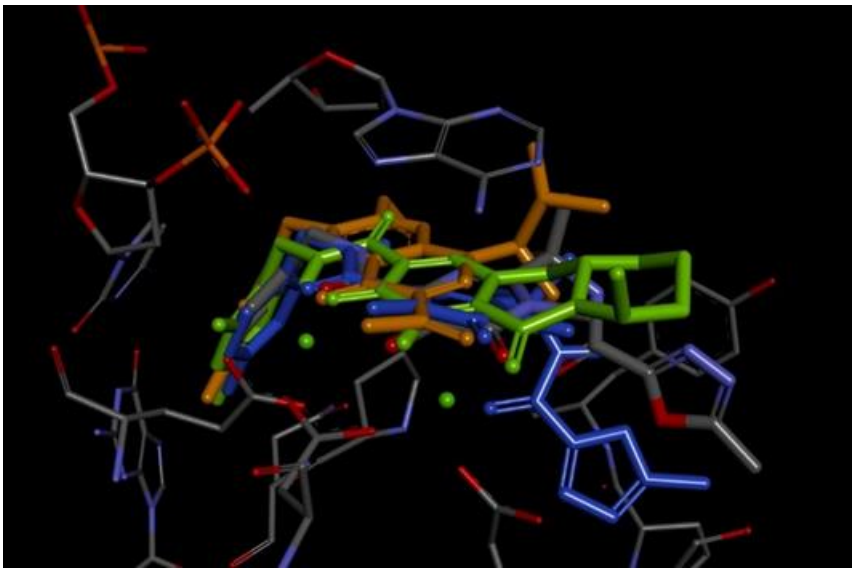
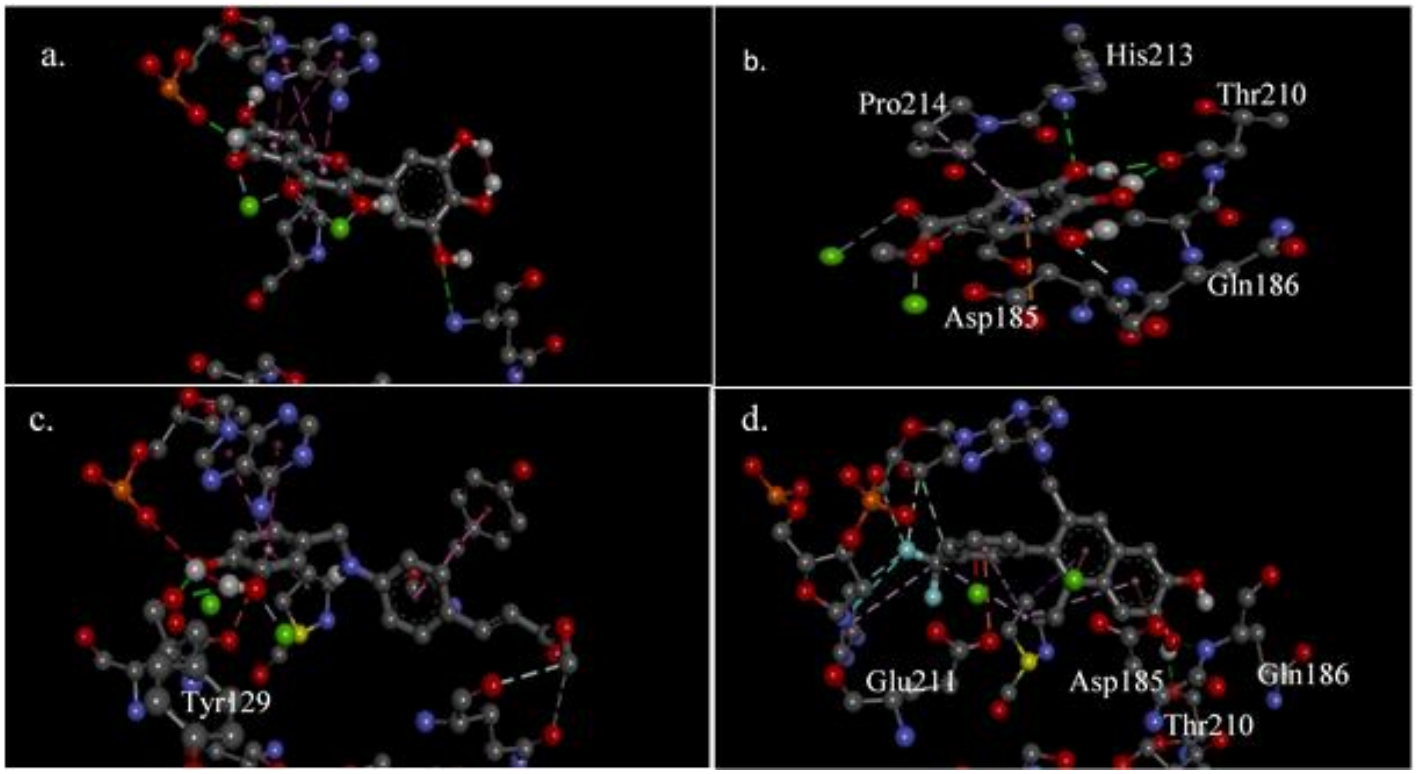


Fig 5: Docked raltegravir (blur), elvitegravir (orange) and dolutegravir (green) on PFV integrase (PDB: 3OYA). Colors of receptor and ligand from crystal structure following atom type (grey: carbon, red: oxygen, blue: nitrogen). Magnesium ions shown as two green spheres.

We were still able to obtain low docked energies from EVG (-14.79 kcal/mol) and DTG (-9.11 kcal/mol). Elvitegravir also shows reasonably good conformation stability (uniformity), but not so in dolutegravir (we had to use the second-best result). The presence of 3 heteroatoms around the magnesium ions and fluorobenzene 'inside' the pocket can be maintained across the three molecules, although the chelation effect is is till questionable because the three heteroatoms don't always form a plane with the 2 magnesium ions and the seemingly insignificant effect of charging magnesium in raltegravir. This also shows the docking software used still has some limitations where charges for atoms around the active site need to be manually assigned to reproduce a good chelation effect while not trivializing other atom-atom interactions in the active site [27]. On the other hand, with magnesium charge not considered during preparation by AutoDock Tools the surrounding atomic charges may also deviate from the correct numbers. From these results, it is expected that we can get to see the binding conformations of experimental ligands, particularly around the active site.

Drug-likeness was assessed using Lipinski's Rule of Five (RO5). In RO5, an orally administrable drug should not violate more than one of the following circumstance: 1) no more than 5 hydrogen bond donors, 2) no more than 10 hydrogen bond acceptors, 3) no more than 500 dalton in weight and 4) its octanol/water distribution coefficient (LogP) does not exceed 5 (= MLpgP > 4.15) (hydrophobic) [28]. All compounds pass RO5 with two compounds having one violation; Myr has more than 5 donors and IB has MLogP above 4.15 (Table 1). Drug delivery ability is estimated using bioavailability score, which looks at the amount of drug that makes it to blood unchanged/undegraded [29]. All 5 compounds have 55% probability for >10% unchanged drug in blood. All in all these five compounds are still feasible as oral drug.



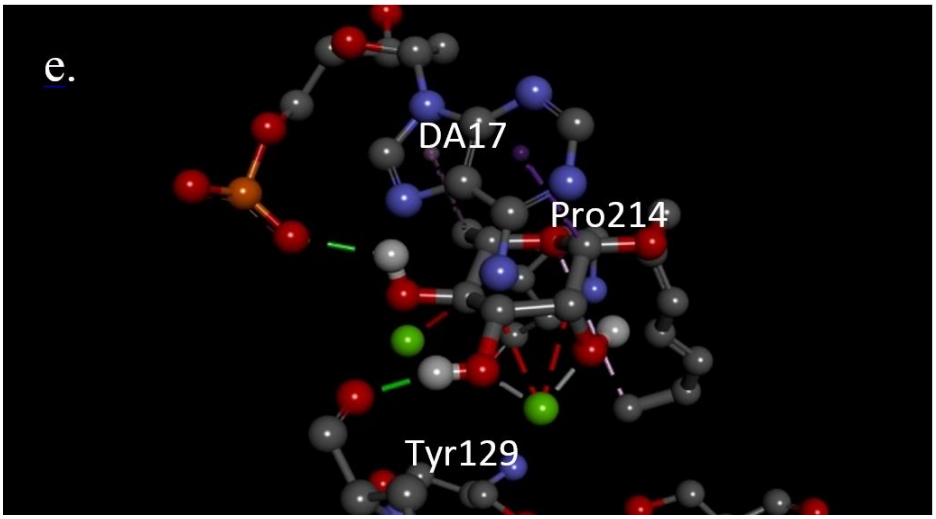


Fig 6: Ligand interaction with surrounding residues. Colors follow atom type (grey: carbon, red: oxygen, blue: nitrogen, white: hydrogen, orange: phosphorous). Dashed line represents bonds; green: hydrogen, purple: pi force, silver: metal-acceptor, red: steric bump, orange: electrostatic, cyan: halogen. a. Myr, b. MG, c. IA, d. IB, e. IC

Myricetin is a compound found in the medicinal plant *Dioscorea bulbifera* and shows inhibitory activity against HIV-1 integrase [13]. Previous study also performed docking of Myr to HIV-1 CCD which residues complemented and given magnesium ions by using 3OYA positions [13]. From docking of the three molecules before, it appears vDNA holds an important role in ligand-receptor binding so we wanted to see how Myr binds to 3OYA, which contains vDNA.

Myr shows three heteroatoms forming metal bond with both magnesium ions and the two rings form pi-pi stacking with both rings of DA17 (Figure 6a), similar to the previous three ligands. *Trihydroxybenzene* appears to be placed 'outside' because of the narrow inner pocket. Overall Myr shows the same binding motif as reported in [13], even though using a different receptor. Binding energy is higher than control ligand at -7.19 kcal/mol which appears to come from the lack of interactions (bonds) on the inner pocket. This number is however still higher than previous report (-5.68 kcal/mol) which partially comes from difference in used macromolecule [13].

MG (methyl gallate) was isolated from the mushroom *Pholiota adiposa* by [30]. Wet lab testing found that MG is an antioxidant capable of inhibiting reverse transcription and HIV integrase. The presence of 3 oxygen atoms on one side made us expect these atoms to play in magnesium chelation, however they are shown facing the opposite way and the other side makes interactions with the magnesium ions. The hydroxyl groups form hydrogen bonds with Thr210 and His213, while benzene pi interacts with Gln186, Asp185 and Pro214 (Figure 6b). Binding energy of MG is even higher at -5.08 kcal/mol but this is most likely caused by the small molecule size (and therefore lack of atomic interactions). However, since we don't know yet the binding site of MG (no docking study has been performed on MG with integrase), there is a possibility that MG uses another active site to bind.

IA is an ester of cinnamic acid synthetized by [31] to be an anticancer agent, and as with how cinnamic acid derivates have integrase inhibitory activity, also shows inhibitory activity against HIV-1 integrase albeit not very potent (43.2% vs. 0.67% residual activity at 10 μM, *vs.* raltegravir) [31]. Docking of IA to integrase (Figure 4c) returns very irregular results where no two conformations are similar. The absence of three adjacent heteroatoms means IA cannot form magnesium chelation like the previous ligands. The result with the lowest energy (-6.81 kcal/mol) shows both hydroxyl groups 'entering' the active site to form hydrogen bonds with O of Tyr129 (Figure 6c). The rest of the molecule faces 'outside.' Pi-pi stacking is still visible with DA17 and Tyr212. The absence of chelation motif, relatively high binding energy (considering the large molecule size), and highly irregular results explains IA's lack of potency. Another possibility is that Is has another binding site.

IB is one among the compounds synthetized from catechol and bis-catechol by [32]. In their study, IB is the best drug candidate and is rather effective thanks to its non-toxicity unlike most bis-catechols [32]. The docking result (Figure 6d) shows IB's lack of heteroatoms to be electron donors to magnesium ions. Instead it relies on forces from its three aromatic rings, and forms electrostatic interactions with one magnesium ion, O of Asp185 and O of Glu221. Three F atoms give halogen forces inside and outside there are hydrogen bonds with O of Gln186 and Thr 210.

IC is a glycoside isolated from the fungi *Lentzia sp.* by [33]. IC works against HIV integrase with IC_{50} of 16 μM [33]. Docking of IC to integrase (Figure 6e) is similar to MG, which returns very irregular results, with the lowest binding energy of -6.61 kcal/mol. The three hydroxyl groups cannot form bond with the magnesium ions because they are not parallel. Two O atoms still form interactions with a magnesium ion, 2 hydrogens from the hydroxy groups form hydrogen bonds with DA17 and Tyr19 while the long chain doesn't make any interactions except at the terminal C which forms interaction with the ring of Pro214. Like MG, the relatively small molecule size and lack of atomic interactions makes it difficult to get the correct conformation, and IC might use another binding site as well.

Conclusion

Crystal structure of PFV integrase that used in this study was employed to roughly reproduce chelation motifs of two magnesium ions in the active site in compounds with a 'classic' structure (three parallel heteroatoms with ring that interacts with DA17, such as: raltegravir, elvitegravir and dolutegravir). This kind of molecule has the tendency to be placed 'deeply' which changes the atomic interactions on the 'inside', and on the 'outside' there were huge variabilities which make the results less reliable. Thus, molecules with the 'classic' shape in this method has limitation to depict molecular interactions. All five experimental inhibitors are suitable for oral drugs as the physiochemical properties

revealed. However, issue may rise in docking analysis which most notably in in overly small or flexible molecules which make the conformations have changed. These molecules were suggested to perform additional steps including manual charge assignment or homology modelling and usage of alternative docking software that has a better calculation when involving metal ions.

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