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Analysis of CYP3A4-HIV-1 protease drugs interactions by computational methods for Highly Active Antiretroviral Therapy in HIV/AIDS

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ABSTRACT

HIV infected patients often take at least three anti-HIV drugs together in Highly Active Antiretroviral Therapy (HAART) and/or Ritonavir-Boosted Protease Inhibitor Therapy (PI/r) to suppress the viral replications. The potential drug–drug interactions affect efficacy of anti-HIV treatment and major source of such interaction is competition for the drug metabolizing enzyme, cytochrome P450 (CYP). CYP3A4 isoform is the enzyme responsible for metabolism of currently available HIV-1 protease drugs. Hence administration of these drugs in HARRT or PI/r leads to increased toxicity and reduced efficacy in HIV treatment. We used computational molecular docking method to predict such interactions by which to compare experimentally measured metabolism of each HIV-1 protease drug. AutoDock 4.0 was used to carry out molecular docking of 10 HIV-protease drugs into CYP3A4 to explore sites of reaction and interaction energies (i.e., binding affinity) of the complexes. Arg105, Arg106, Ser119, Arg212, Ala370, Arg372, and Glu374 are identified as major drug binding residues, and consistent with previous data of site-directed mutagenesis, crystallography structure, modeling, and docking studies. In addition, our docking results suggested that phenylalanine clusters and heme are also participated in the binding to mediate drug oxidative metabolism. We have shown that HIV-1 protease drugs such as tipranavir, nelfinavir, lopinavir, and atazanavir differ in their binding modes on each other for metabolic clearance in CYP3A4, whereas ritonavir, amprenavir, indinavir, saquinavir, fosamprenavir, and darunavir share the same binding mode.

1. Introduction

Human immunodeficiency virus-1 (HIV-1) is one of the important targets for clinical research causes AIDS in human. This RNA virus replicates inside the host cell by integrating its genetic material with the host cell genome [1]. Several researches are happening worldwide to neutralize the activity of this virus for curing HIV-1 infected patients. There are no effective drugs yet to treat the patients because this virus is highly resistant to the drugs [2]. Hence a single HIV-drug administration is not advisable for AIDS treatment. Highly Active Antiretroviral Therapy (HAART) and/or Ritonavir-Boosted Protease Inhibitor Therapy (PI/r) and salvage therapy are widely in practice with the co-administration of three or more HIV drugs simultaneously to treat AIDS patients [3–5]. Researchers are mainly targeting the HIV-1 reverse transcriptase, HIV-1 protease and HIV-1 integrase to block the virus multiplication inside the host cell. HIV-1 protease enzyme is essential for the life-cycle of this virus [6,7]. The virus synthesizes its proteins as polyprotein precursor form, and should be cleaved to transform into mature, fully functional proteins to infect the cells [8]. HIV-1 protease is required for proteolytic cleavage of the viral polyprotein precursors to transform into the individual functional proteins [9,10]. Inactivation of HIV-1 protease by specific chemical compounds should render the virus noninfectious [11]. The compounds such as ritonavir (RTV) [12], amprenavir (APV) [13], tipranavir (TPV) [14], indinavir (IDV) [15], saquinavir (SQV) [15], nelfinavir (NFV) [15], lopinavir (LPV) [16], fosamprenavir (FOS-APV) [17], darunavir [18] and atazanavir (ATV) [19] are the US-FDA approved drugs available for HIV-1 protease inhibition (http://www.fda.gov/oashi/aids/virals.html).

In general, drugs are to be metabolized by enzymes to avoid toxic effects of drug accumulation. Cytochrome P450 (CYPs) is superfamily of heme-thiolate containing enzymes that are responsible for the oxidative metabolism of xenobiotic compounds [20]. Human CYP450 proteins CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 are the major drug metabolizing isoforms, which play an important role in the oxidative metabolism of 90% drugs in the

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current clinical use [21,22]. Of these isoforms, CYP3A4 is involved in the metabolism of more than 50% drugs and other xenobiotics [23]. In the present form, there are number of crystal structures for human CYP3A4 (PDB ID: 1TQN, 1WOE, 1WOF, 1WOG, 2JOD, and 2VOM) available in the protein structure database (http://www.rcsb.org/pdb). The most interesting features of the CYP3A4 structures are the shape and size of the active site cavity, which is adjacent to the heme iron. The two research groups Yano et al. [24] and Williams et al. [25] reported two different volumes of active site cavity 1386 and 520 Å respectively. This result indicates that CYP3A4 active site cavity can metabolize several bulky substrates [26] and also supporting binding of multiple compounds simultaneously for oxidative metabolism [27–29]. The methods of X-ray crystallography, site-directed mutagenesis, modeling and docking studies have revealed an important catalytic active site residues Val101, Asn104, Arg105, Met114, Ser119, Leu211, Arg212, Asp214, Asp217, Pro218, Glu374, Ile301, Ala305, Thr309, Ile369, Ala370, Leu373, Glu374, Ser478, Leu479, and 'Phe-cluster' (Phe108, Phe215, Phe219, Phe220, Phe241, Phe304 and Phe215) for interaction with substrates and inhibitors of CYP3A4 [24,25,30–32].

All currently available HIV-1 protease drugs of FDA approved (U.S. Food and Drug Administration) are metabolized by CYP3A4 [12–19]. As the HIV-1 treatment employs HAART practice, the co-administration of protease drugs leads to CYP3A4-related drug–drug interactions occur for metabolic clearance and for which elevated plasma concentrations are associated with serious and/or life-threatening events. The simple reason for this is to one drug modulates the metabolism of other (drug–drug interactions) by simple competition for the same active site, and/or by binding in an allosteric region of the same enzyme [33]. Hence for the effective treatment, it is important to have the co-administration of protease drugs having diverse binding modes and affinity into the CYP3A4 active site for metabolic clearance. In this present investigation, we used molecular docking method to predict such binding modes and affinity (i.e., interaction energies) of currently available 10 HIV-1 protease drugs into CYP3A4. In addition, understanding of how this complex CYP3A4 enzyme recognizes multiple, diverse chemical structures was investigated to rational design of potential drug candidates.

2. Methods

The atomic coordinates of ligand-free human microsomal cytochrome P450 3A4 (CYP3A4) was retrieved from Protein Data Bank [PDB ID: 1TQN] [34]. This structure was determined by X-ray crystallography to 2.05 Å resolutions [24]. A set of 10 drugs approved by FDA for HIV-1 protease were used for this docking study. These drugs are ritonavir [DrugBank: DB00503], amprenavir [DrugBank: DB00701], tipranavir [DrugBank: DB00932], indinavir [DrugBank: DB00224], saquinavir [DrugBank: DB01232], nelfinavir [DrugBank: DB00220], lopinavir [DrugBank: DB01601], fosamprenavir [DrugBank: DB01319], darunavir [DrugBank: DB01264] and atazanavir [DrugBank: DB01072] was retrieved from DrugBank database [35]. The two-dimensional structures of all these drugs are shown in Figs. 1 and 2. The geometry of drug molecules and CYP3A4 structure were optimized via Smart Minimizer protocol (Steepest Descent method followed by Conjugate Gradient method with 1000 steps) using Accelrys Discovery Studio (Version 1.7, Accelrys Software Inc.), the most comprehensive suite of modeling and simulation solutions for drug discovery available. Each of the minimization was carried out with CHARMm force field.

Docking studies for these drugs were carried out as per the protocol described by our previous work [36,37]. In brief, AutoDock 4.0 software package [38] was used for conducting docking studies on CYP3A4. The graphical user interface program "AutoDock Tools" was used to prepare, run, and analyze the docking simulations. The Kollman charges, solvation parameters and polar hydrogen were added into the water free CYP3A4 structure for the preparation of protein in docking simulation. The Gasteiger charge was assigned into ligands (i.e., HIV-1 protease inhibitors) and then non-polar hydrogen was merged. The rigid roots of each ligand were defined automatically instead of picking manually and amide bonds were made non-rotatable. AutoTors program was used to assign all rotatable dihedrals in the ligand. The number of rotatable bonds varied based on size of the ligand in the range 12–23 and were allowed to rotate freely. AutoDock requires pre-calculated three dimensional grid maps, one for each type of atom present in the ligand and its stores the interaction energy based on a macromolecular target using the AMBER force field. This grid must surround the region of interest in the macromolecule. AutoGrid 4.0 Program, supplied with AutoDock 4.0 was used to generate grid maps for the ligands. The grid box was fixed in the catalytic active region between heme moiety and SER119 of human CYP3A4. The box size in *x*-, *y*- and *z*-axis was normally set at 60 Å × 60 Å × 60 Å, though it was changed depending on the size of the ligands. The spacing between grid points was 0.375 Å. The GA-LS search algorithm (Lamarckian Genetic Algorithm) was chosen to search for the best conformers with 10 runs of each ligand. During the searching process, the enzyme was regarded as rigid, while ligands (i.e., drugs) were regarded as being flexible, to explore in any of the six degrees of freedom. The population size was set to 150 and the individuals were initialized randomly. The maximum number of energy evaluation was set to 250,000; maximum number of generations was to 1000. The maximum number of top individual that automatically survived was set to one. The rates of gene mutation and crossover were set at 0.02 and 0.80 respectively. All the AutoDock docking runs were performed in Intel Pentium PD-925 CPU at 3.0 GHz of HCL infosystem origin, with 2 GB DDR RAM. AutoDock 4.0 was compiled and run under Microsoft Windows XP operating system.

3. Results

The three dimensional structures of both CYP3A4 and HIV-1 protease drugs were optimized to have minimal potential energy using Smart Minimizer and values are shown in Table 1. After minimization, the drug molecules have the potential to change the initial states of it into a system of binding state in target CYP3A4 molecule when the energy is released. Docking simulation of 10 runs of GA-LA was performed for a set of 10 protease drugs into a catalytic active site of CYP3A4. The best docked conformation of each drug into CYP3A4 binding site was determined as the one which having the lowest interaction energies of both docking energy and binding free energy among the 10 different poses generated. The binding free energy, docking energy and binding site residues of CYP3A4 which involved in hydrogen bond and vdW interaction are given in Table 2. We were further analyzed the docked conformation for finding the binding mode of each protease drug into CYP3A4. We were compared our docking results with the experimentally measured metabolism of each HIV-1 protease drug to validate the drug orientation and position obtained likely to represent reasonable binding modes or conformations.

4. Discussion

4.1. Docking ritonavir into CYP3A4

Ritonavir, a potent HIV-1 protease inhibitor, is extensively metabolized by CYP3A4. The majority of currently available protease drugs are co-administered with low doses of RTV for

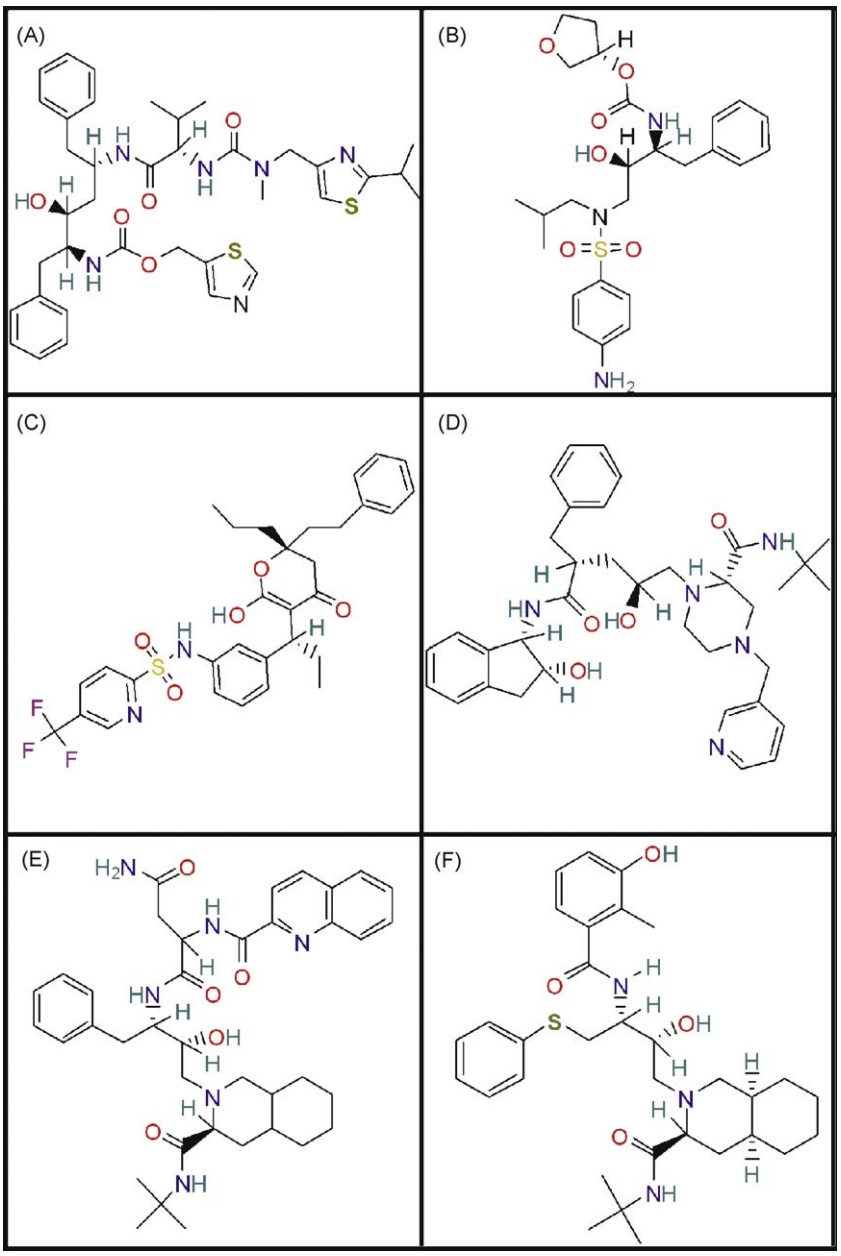

Fig. 1. Two-dimensional structures of HIV-protease drugs: (A) ritonavir, (B) amprenavir, (C) tipranavir, (D) indinavir, (E) saquinavir and (F) nelfinavir.

effective HIV treatment. Hence, first discussing the binding modes of RTV with CYP3A4 would boost in claiming docking results of drug–drug interactions further. In examining the position and orientation of the RTV in CYP3A4 active site predicted by our docking procedure, it was observed that side chain hydrogen atom of Arg212 and backbone-nitrogen atom of Glu374 acts as hydrogen bond donors to make multiple hydrogen bonds with oxygen and nitrogen group of RTV with maximum bond length of 1.9 Å (Table 2). In addition, the residues Phe57, Arg105, Arg106, Phe108, Ser119, Phe215, Ile301, Phe304, Ala305, Ile369, Ala370, Met371, Arg372, Leu373 and Leu482 were involved in van der Waals interactions in the scaling factor of 1.00 Å (Fig. 3A). In close assessment of this binding mode, maximum number of phenylalanine (i.e., Phe-cluster such as Phe57, Phe108, Phe215, and Phe304) and arginine residues participated in the interaction. The heme molecule has greater accessibility to the active site for making van der Waals contacts into RTV. The binding free energy and docking energy calculated by AutoDock for RTV was −7.74 and −15.31 kcal/mol respectively.

4.2. Docking amprenavir into CYP3A4

Docking simulation of APV into CYP3A4 resulted in the formation of single hydrogen bond with the bond distance of 1.7 Å (Fig. 3B) and it was observed that side chain hydrogen atom of Arg212 acts as hydrogen bond donor to interact with oxygen atom of the drug. The amino acid residues Phe57, Arg105, Phe108, Phe215, Phe304, Thr309, Aal370, Met371, Arg372, and Glu374 were also took part in van der Waals interaction with active participation of heme (Table 2). The binding free energy and docking energy of complex was observed with −6.18 and −10.96 kcal/mol respectively. When compared the binding site residues, both APV and RTV used the same residues for metabolic clearance, for which co-administration of these drugs might result in complex pharmacokinetics drug–drug interactions lead to elevated plasma drug concentration. These *in silico* results are well consistent with Arvieux et al. [39] experimental data, in which APV plasma concentrations increase 3- to 10-fold when using APV 450 or 600 mg combined with RTV 100 mg twice daily.

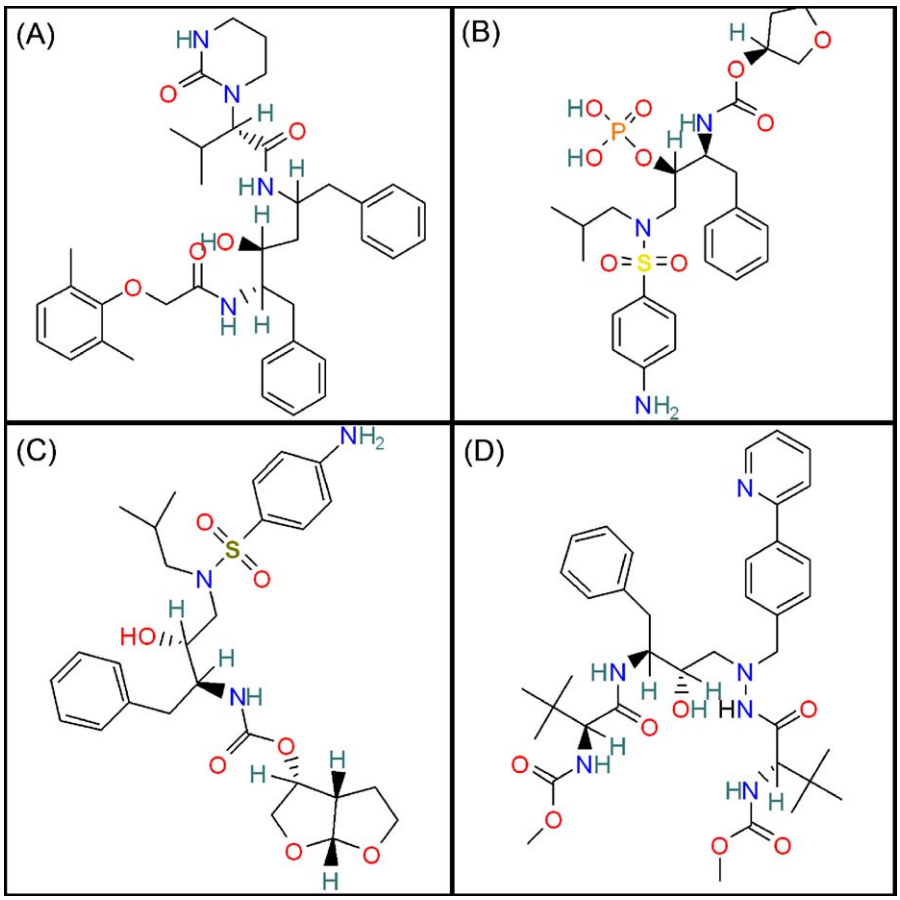

Fig. 2. Two-dimensional structures of HIV-protease drugs: (A) lopinavir, (B) fosamprenavir, (C) darunavir and (D) atazanavir.

4.3. Docking tipranavir into CYP3A4

The docked results of TPV into CYP3A4 are given in Table 2. It is important to note that the residue of Arg105 was held in the position of hydrogen bond formation, and in the case of van der Waals forces, Arg106, Arg212, Phe213, Leu373 and Leu482 were held in varying degree of binding modes except the conserved residues of Phe108, Ser119, Phe215, Phe304 and Arg372. It is different from RTV and APV in the mode of binding into CYP3A4 (Table 2). The hydrogen bond was formed with the side chain hydrogen atom of Arg105 into electro negative oxygen atom of the drug in the bond length of 1.829 Å. The interaction energies calculated for TPV are −10.07 kcal/mol of binding free energy and −15.94 kcal/mol of docking energy, and it is more favorable than that calculated for RTV and APV. Hence TPV should have high priority to interact with CYP3A4 instead of RTV or APV for metabolic clearance. As a whole, TPV was held in a different mode of binding conformation in concern with type of interactions and binding free energy. This result is well coincided with Phase II clinical trials of co-administration of TPV and RTV is very safe and well-tolerated in HIV-1 infected adults [40].

Table 1
Smart minimizer values (steepest descent followed by conjugate gradient method) of HIV-1 protease inhibitors and CYP3A4.

Molecules	Energy levels with minimization max of 400 steps (kcal/mol)			
	Initial potential energy	Potential energy	Van der Waals energy	Electrostatic energy
Ritonavir	31.20435	−89.81911	−17.98885	−98.74673
Amprenavir	139.80224	−33.51425	−11.19059	−62.37323
Tipranavir	161.28703	27.41732	−7.43604	1.57638
Indinavir	135.79255	63.39831	−6.67096	−26.00454
Saquinavir	40.64444	−46.89278	−13.34890	−76.20760
Nelfinavir	82.07763	1.70377	−15.93508	−26.72349
Lopinavir	40.90840	−44.46649	−12.82773	−54.94629
Fosamprenavir	77.08153	−131.92838	−15.79051	−153.65444
Darunavir	216.94528	47.48450	−9.86367	−61.43771
Atazanavir	25.18861	−121.07763	−14.41369	−151.75300
CYP3A4	6622.30902	30,761.90824	−3387.30974	−31,588.29899

4.4. Docking indinavir into CYP3A4

The 'CYP3A4–IDV' docked complex adopts in the formation of two hydrogen bonds between Arg212 (i.e., HE and HH22 atoms) and oxygen atom in the drug, in addition to the active participation of Arg105, Arg106, Ser119, Phe215, Thr309, Ile369, Ala370, Met371, Arg372, and Glu374 for van der Waals contacts (Table 2). The observed binding free energy and docking energy for IDV was −3.92 and −8.76 kcal/mol respectively. When compared the docked positions of the complexes such as 'CYP3A4–IDV', 'CYP3A4–RTV', and 'CYP3A4–APV', it shows the same binding site residues involved in the interaction (i.e., Arg212 for hydrogen bond and Arg105, Ser119, Phe215, Ala370, Met371, and Arg372 for van der Waals (Fig. 3D)). In addition, CYP3A4–IDV shows higher interaction energies compared to all other HIV-1 protease drug, which shows that RTV and APV should have high preference to interact with a target metabolizing enzyme CYP3A4 instead of IDV. These docking results are consistent with in vitro metabolic studies using rat liver microsomal fractions and in vivo oral administration studies, which shows that varying a degree of metabolic clearance for APV, RTV and IDV [41]. It is remarkable to note that IDV and TPV

Table 2
Molecular interactions of HIV-1 protease inhibitors into CYP3A4 isoenzyme.

Drugs	H-bond donor	H-bond acceptor	H-bond length (Å)	vdW interaction residues (scaling factor = 1.00 Å)	ΔG (kcal/mol)	Docking energy (kcal/mol)
Ritonavir	CYP:A:ARG212:HH22 CYP:A:GLU374:HN	DRUG::O DRUG::N	1.767 1.956	PHE57, ARG105, ARG106 PHE108, SER119, PHE215, ILE301, PHE304, ALA305, ILE369, ALA370, MET371, ARG372, LEU373, LEU482, HEM508	−7.74	−15.31
Amprenavir	CYP:A:ARG212:HH22	DRUG::O	1.723	PHE57, ARG105, PHE108, PHE215, PHE304, THR309, ALA370, MET371, ARG372, GLU374, HEM508	−6.18	−10.96
Tipranavir	CYP:A:ARG105:HE	DRUG::O	1.829	ARG106, PHE108, SER119, ARG212, PHE213, PHE215, PHE304, ARG372, LEU373, LEU482, HEM508	−10.07	−15.94
Indinavir	CYP:A:ARG212:HE CYP:A:ARG212:HH22	DRUG::O DRUG::O	2.14 1.877	ARG105, ARG106, SER119 PHE215, THR309, ILE369, ALA370, MET371, ARG372, GLU374, HEM508	−3.92	−8.76
Saquinavir	CYP:A:ARG212:HH22	DRUG::N	1.882	PHE57, ASP76, ARG105, ARG106, PHE108, SER119, PHE213, PHE215, PHE304, ILE369, ALA370, MET371, ARG372, GLU374, LEU482, HEM508	−6.29	−11.26
Nelfinavir	DRUG::H DRUG::H DRUG::H	CYP:A:HEM508:NA CYP:A:ARG372:O CYP:A:ARG372:O	1.993 1.92 1.946	ARG105, SER119, ARG212 PHE215, ALA305, THR309 ILE369, ALA370, GLU374, LEU482	−10.06	−13.89
Lopinavir	CYP:A:ARG106:HN	DRUG::O	1.906	ARG105, PHE108, SER119, ILE120, ARG212, PHE215, ALA305, THR309, ILE369, ALA370, MET371, GLU374, ILE482, HEM508	−12.42	−17.43
Fosamprenavir	DRUG::H	CYP:A:GLU374:OE1	2.075	PHE57, ARG106, PHE108, SER119, ARG212, PHE215, ALA305, THR309, ALA370, HEM508	−8.55	−16.89
Darunavir	CYP:A:ARG212:HH22	DRUG::O	1.794	ARG105, ARG106, PHE108, PHE213, PHE215, PHE304, ALA370, MET371, ARG372, LEU373, HEM508	−8.97	−13.69
Atazanavir	DRUG::H CYP:A:SER119:HG	CYP:A:ALA370:O DRUG::O	1.99 2.037	PHE57, ARG105, ARG106 PRO107, PHE108, ILE120, ARG212, PHE215, THR224, PHE304, ALA305, ARG372, GLU374, HEM508	−7.40	−13.08

were differ in their binding site residues for hydrogen bond interactions and shows maximum variation in van der Waals forces with CYP3A4 enzyme.

4.5. Docking saquinavir into CYP3A4

Docking simulation of SQV into CYP3A4 resulted in formation of single hydrogen bond by the conserved atom of residue Arg212 (i.e., HH22) as like RTV, APV and IDV. The binding free energy calculated by AutoDock was −6.26 kcal/mol. This drug was made additional van der Waals interactions with the residues of Phe57, Asp76, Arg105, Arg106, Phe108, Ser119, Phe213, Phe215, Phe304, Ile369, Ala370, Met371, Arg372, Glu374 and Leu482 in the scaling factor of 1.00 Å to further stabilize the interactions. The calculated docking energy was −11.26 kcal/mol. The conclusion drawn from this docking result was SQV, RTV, APV and IDV shares the same binding residues or binding modes in CYP3A4 for metabolic clearance (Table 2) [40], whereas SQV and IDV show different modes of binding to CYP3A4.

4.6. Docking nelfinavir into CYP3A4

In the assessment of position and orientation of CYP3A4–NFV docked complex, backbone oxygen atom of Arg372 interacts with two hydrogen atoms in the drug to form multiple hydrogen bonds. In addition, cyclic nitrogen atom of heme was involved in hydrogen bond formation with hydrogen atom of NFV. The bond distance between donor and acceptor atoms was approximately 1.9 Å (Table 2). The van der Waals interactions between CYP3A4 and NFV was observed in the residues Arg105, Ser119, Arg212, Phe215, Ala305, Thr309, Ile369, Ala370, Glu374, and Leu482. The binding free energy of this complex was −10.06 kcal/mol, which is similar to be docked complex of TPV into CYP3A4. The docking energy of the complex was calculated as −13.89 kcal/mol. When compared the binding site residues of CYP3A4–NFV complex to each HIV-1 protease drug, observed that all were differ in their binding modes or binding site residues for hydrogen bond formation.

4.7. Docking lopinavir into CYP3A4

Lopinavir is usually co-formulated with ritonavir for ease of administration as part of combination therapy with other antiretroviral drugs [16]. Both drugs undergo extensive and rapid first pass metabolism by CYP3A4 isoenzyme. Thus, by comparing CYP3A4 interaction residues of LPV to other HIV-1 protease drugs would produce valuable suggestions for combination therapy. The binding free energy and docking energy calculated by AutoDock for LPV are more favored than calculated for other HIV-1 protease drugs (Table 2). The lowest interaction energies of this complex show its ability of first pass metabolism by CYP3A4. The backbone-nitrogen atom of Arg106 interacts with oxygen atom of the drug to form hydrogen bond. This drug interacts significantly with Arg105, Phe108, Ser119, Ile120, Arg212, Phe215, Ala305, Thr309, Ile369, Ala370, Met371, Glu374, and Ile482 residues for van der Waals

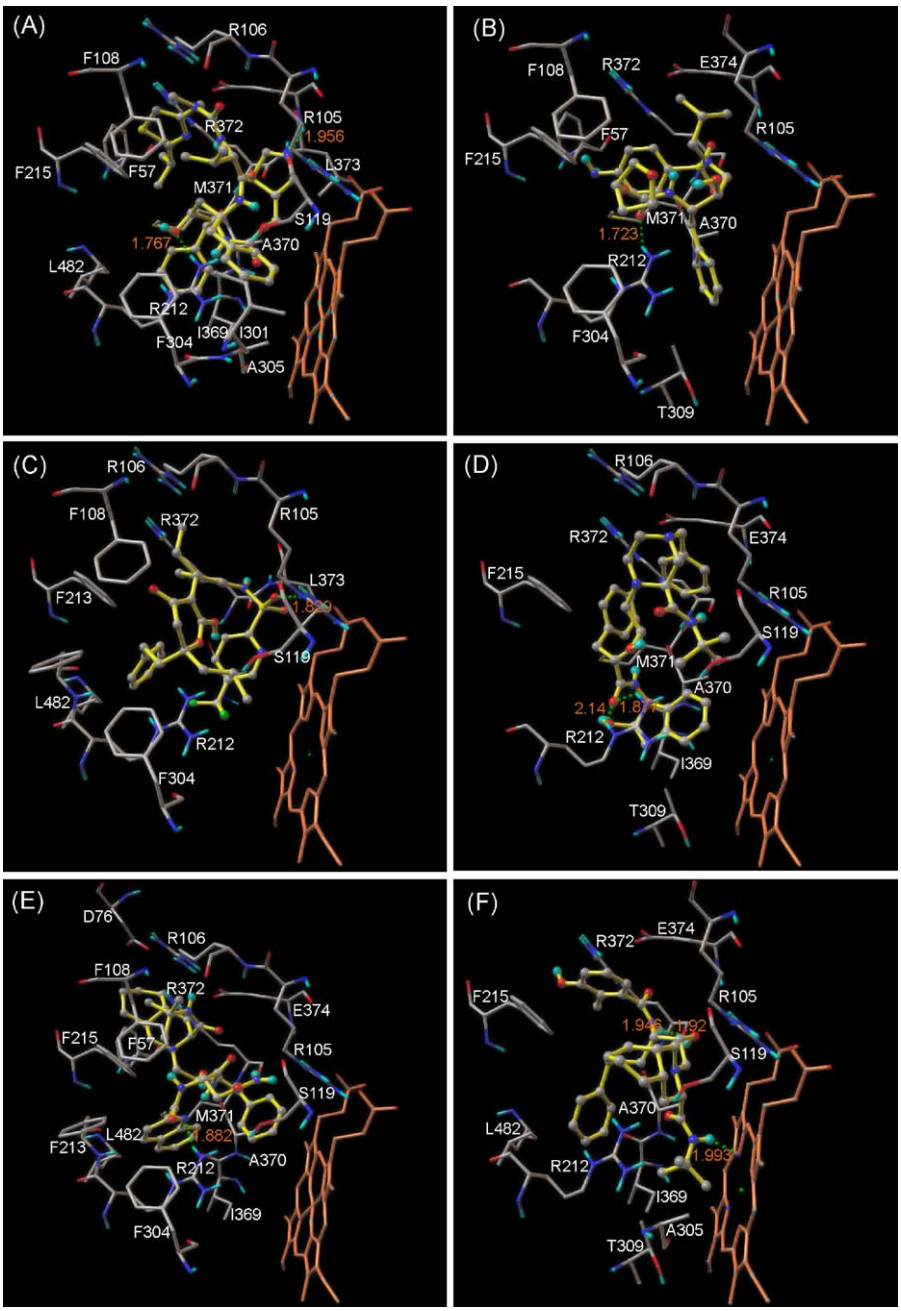

Fig. 3. Illustration of docked complex for CYP3A4 into: (A) ritonavir, (B) amprenavir, (C) tipranavir, (D) indinavir, (E) saquinavir and (F) nelfinavir. CYP3A4 interaction residues and ligands are shown in ball and stick model and colored by atom types. Carbon colored in gray, oxygen in red, nitrogen in blue, hydrogen in cyan and sulfur in yellow. The sticks of ligands (HIV-protease drugs) are colored in yellow. Heme is represented in orange color. The green dot lines denote hydrogen bond.

forces to stabilize the complex (Fig. 4A). It is interesting to note that CYP3A4-LPV docked complex is differing in their binding mode to each of HIV-1 protease drug for metabolic clearance.

4.8. Docking fosamprenavir into CYP3A4

Fosamprenavir is a HIV-1 protease inhibitor of APV prodrug. This drug will rapidly and extensively convert to APV after oral intake. Thus extensive analysis of docked results of both FOS-APV and APV could provides guidance on how to co-administer FOS-APV and/or APV with other commonly co-prescribed HIV medications. We have already discussed about docked result of CYP3A4–APV in above, whereas FOS-APV shows hydrogen bond formation by side chain Glu374 oxygen atom acts as a hydrogen bond acceptor to interact with hydrogen atom of the drug in hepatic metabolism by CYP3A4 (Table 2). The amino acid residues Phe57, Arg106, Phe108, Ser119, Arg212, Phe215, Ala305, Thr309, and Ala370 were involved in van der Waals forces with the binding free energy of −8.55 kcal/mol and docking energy of −16.89 kcal/mol. Since, the residues Glu374 (i.e., used for FOS-APV) and Arg212 (i.e., used for APV) are involved in hydrogen bond formation, the co-administration of other protease drugs may not share the same residues in CYP3A4 for metabolic clearance. The binding site residues calculated for FOS-APV, RTV, APV, IDV, and SQV are shares the same residues for metabolic clearance and it is in agreement with the experimental data of combination therapy for FOS-APV, APV, and RTV to elevated plasma concentration of the drugs

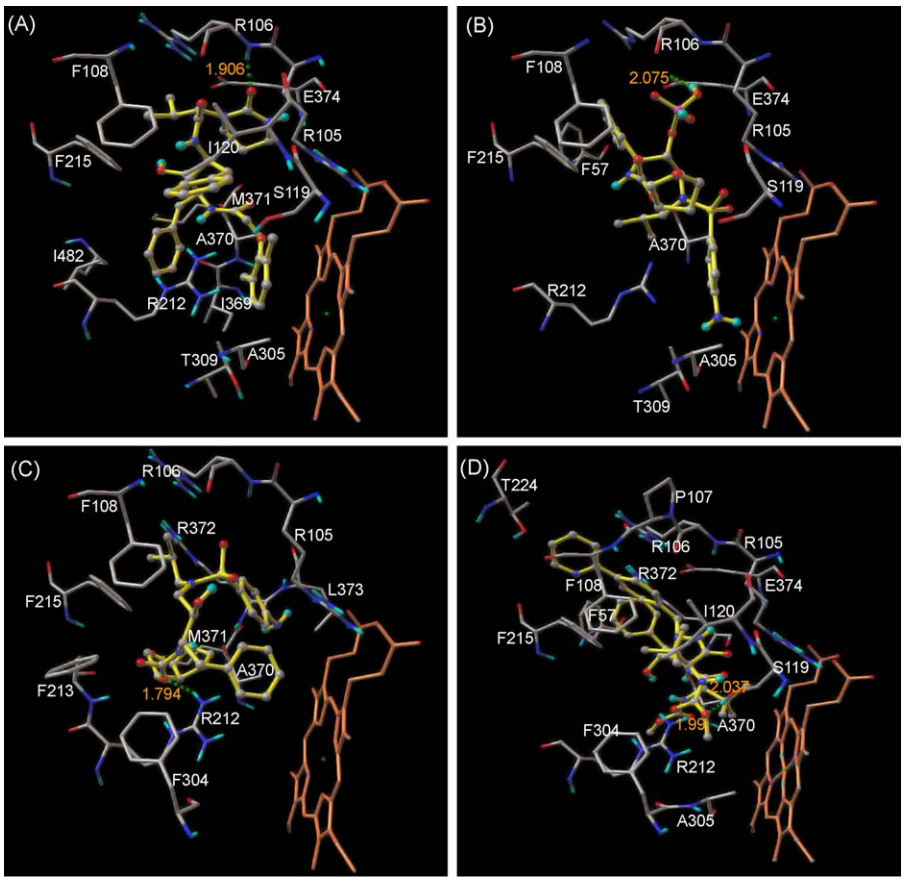

Fig. 4. Illustration of docked complex for CYP3A4 into: (A) lopinavir, (B) fosamprenavir, (C) darunavir and (D) atazanavir.

Table 3
The binding modes of HIV-1 protease drugs into CYP3A4 isoenzyme.

Drugs	Drugs share the same binding mode	Drugs share the different binding modes
Ritonavir	Amprenavir, indinavir, saquinavir, fosamprenavir, darunavir	Tipranavir, nelfinavir, lopinavir, atazanavir
Amprenavir	Ritonavir, indinavir, saquinavir, fosamprenavir, darunavir	Tipranavir, nelfinavir, lopinavir, atazanavir
Tipranavir	–	Ritonavir, amprenavir, indinavir, saquinavir, nelfinavir, lopinavir, fosamprenavir, darunavir, atazanavir
Indinavir	Ritonavir, amprenavir, saquinavir, fosamprenavir, darunavir	Tipranavir, nelfinavir, lopinavir, atazanavir
Saquinavir	Ritonavir, amprenavir, indinavir, fosamprenavir, darunavir	Tipranavir, nelfinavir, lopinavir, atazanavir
Nelfinavir	–	Ritonavir, amprenavir, tipranavir, indinavir, saquinavir, lopinavir, fosamprenavir, darunavir, atazanavir
Lopinavir	–	Ritonavir, amprenavir, tipranavir, indinavir, saquinavir, nelfinavir, fosamprenavir, darunavir, atazanavir
Fosamprenavir	Ritonavir, amprenavir, indinavir, saquinavir, darunavir	Tipranavir, nelfinavir, lopinavir, atazanavir
Darunavir	Ritonavir, amprenavir, indinavir, saquinavir, fosamprenavir	Tipranavir, nelfinavir, lopinavir, atazanavir
Atazanavir	–	Ritonavir, amprenavir, tipranavir, indinavir, saquinavir, nelfinavir, lopinavir, fosamprenavir, darunavir

[17]. On the other hand FOS-APV, TPV, NFV, and LPV show different binding modes for CYP3A4 interaction.

4.9. Docking darunavir into CYP3A4

The docking simulation of darunavir into CYP3A4 produced with the similar results as like we observed in RTV, APV, IDV, SQV, and FOS-APV in means that these drugs sharing the same binding mode for metabolic clearance. Darunavir interacts with CYP3A4 through the hydrogen bond between the side chain hydrogen atom of Arg212 pack against the oxygen atom in the drug. Fig. 4C shows several amino acid residues that contact docked darunavir molecule through van der Waals interactions. The interaction energies of this complex were −8.97 kcal/mol for binding free energy and −13.69 for docked energy.

4.10. Docking atazanavir into CYP3A4

The docked results of ATV into CYP3A4 are given in Table 2. It is interesting to note that the catalytic active site residues of Ser119

and Ala370 participated in the hydrogen bond formation against the drug, which is entirely different from other HIV-1 protease drug interactions. This complex was formed with the binding free energy of −7.4 kcal/mol and docking energy of −13.08 kcal/mol. The amino acid residues Phe57, Arg105, Arg106, Pro107, Phe108, Ile120, Arg212, Phe215, Thr224, Phe304, Ala305, Arg372, and Glu374 contributed in van der Waals contacts for metabolic clearance of this drug in addition to the hydrogen bonds.

In summary, the docked structures of CYP3A4 exhibit relatively a large number of amino acid residues involved in drug interaction and it is consistent with the cavity size of the enzyme for oxidative metabolism of bulky substances [25,29]. The heme of CYP3A4 was also observed in each HIV-1 protease interaction. As shown in Table 3, it is obvious that TPV, NFV, LPV, and ATV share the different binding modes for metabolic clearance in CYP3A4 enzyme.

5. Conclusion

In this work, molecular docking studies were performed to explore possible binding modes of HIV-1 protease drugs into CYP3A4. AutoDock 4.0 software was used to dock protease drugs into CYP3A4. The specific binding pattern of amino acid residues participating in metabolic clearance was identified reasonably and validated by site of the reaction and interaction energies of the docked complexes. Our docking results showed that Arg212 made an important contribution in hydrogen bond formation, which implicated in the drug interactions and Arg105, Arg106, Glu374, Ser119, Ala370, and Arg372 also took part in hydrogen bonding with comparatively higher frequency. The possible binding mode of each HIV-1 protease drugs was found to have good correlation with the experimental data of inhibitory activities [16,17,25,29,39–41]. Our docking results suggested that HIV-1 protease drugs TPV, NFV, LPV, and ATV are differed in their binding conformations into CYP3A4, whereas RTV, APV, IDV, SQV, FOS-APV, and darunavir share the same binding mode. As well as, the identified favored biding modes of CYP3A4 into drugs will be useful for the development of new HIV-1 protease drugs and also provides valuable insights into the metabolism of drugs.

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