Review Article

Anti-HIV Drug Development Through Computational Methods

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Abstract. Although highly active antiretroviral therapy (HAART) is effective in controlling the progression of AIDS, the emergence of drug-resistant strains increases the difficulty of successful treatment of patients with HIV infection. Increasing numbers of patients are facing the dilemma that comes with the running out of drug combinations for HAART. Computational methods play a key role in anti-HIV drug development. A substantial number of studies have been performed in anti-HIV drug development using various computational methods, such as virtual screening, QSAR, molecular docking, and homology modeling, *etc.* In this review, we summarize recent advances in the application of computational methods to anti-HIV drug development for five key targets as follows: reverse transcriptase, protease, integrase, CCR5, and CXCR4. We hope that this review will stimulate researchers from multiple disciplines to consider computational methods in the anti-HIV drug development process.

KEY WORDS: anti-HIV; computational methods; co-receptor; drug; enzyme.

INTRODUCTION

In the absence of a successful preventive vaccine, the spread of human immunodeficiency virus (HIV) infection has caused a worldwide pandemic. Approximately 33 million people are currently living with HIV-1, approximately 2.1 million people die every year of acquired immunodeficiency syndrome (AIDS) and associated complications, and there are 2.5 million new infections every year (www.unaids.org). The first anti-HIV drug, azidothymidine (AZT), was approved in 1987 (1), and more than 30 anti-HIV drugs are currently used for clinical treatment (Table I, www.fda.org). Although highly active antiretroviral therapy is effective in controlling the progression of AIDS, the combined use of multiple drugs is greatly hindered by the emergence of drug-resistant HIV strains. More and new anti-HIV drugs are therefore needed for clinical treatment.

Computational techniques are increasingly employed in drug discovery and optimization. Techniques applied to anti-HIV drug research are classified as (1) ligand methods based on known active compounds that can infer biological activity, such as classical quantitative structure–activity relationship (QSAR), (2) structure-based methods that rely on the 3D structure of protein receptors, such as molecular docking and molecular dynamics, and (3) universal methods, structure- or

ligand-based, such as 3D QSAR or 3D pharmacophore elucidation (2). Homology modeling is usually useful when an experimental 3D structure of protein receptor is not available. A review has provided the theoretical introduction and detailed protocols of the computational methods used in anti-viral agent development (2). Although multiple methods are applied to anti-HIV drug development, receptor structure-based molecular docking and ligand-based QSAR are the most frequently used methods. The HIV life cycle has multiple stages, including entry, reverse transcription, integration, protein translation, assembly, and release. Throughout the entire process, many viral proteins and host receptors can be targeted for drug development. In this review, we summarize the recent progresses of anti-HIV drug development *via* computational methods applied to five main targets: three key viral enzymes (reverse transcriptase, protease, integrase) and two common co-receptors.

REVERSE TRANSCRIPTASE

HIV is a retrovirus, and reverse transcriptase (RT) is its key enzyme; RT reverse transcribes the viral RNA into a provirus. RT plays a multifunctional role and is an essential component for HIV to complete the replication cycle. There are two types of reverse transcriptase inhibitors, namely non-nucleoside reverse transcriptase inhibitor (NNRTI) and nucleoside reverse transcriptase inhibitors (NRTI). As RT is the most important target for drug design, there are more than 240 crystal structures of HIV-1 RT and mutants available. Based on the vast number of crystal structures, numerous studies report the development of RT inhibitors using a computer-guided design. The structure-based molecular docking approach plays a key role in the computer-guided development of RT inhibitors. Although hundreds of

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Table I. Drugs Used in the Treatment of HIV Infection (www.fda.gov)

Brand name	Generic name	Manufacturer name ^a	Approval date	Type
Combivir	Lamivudine and zidovudine	GlaxoSmithKline	27-Sep-97	NRTI
Emtriva	Emtricitabine, FTC	Gilead Sciences	02-Jul-03	NRTI
Epivir	Lamivudine, 3TC	GlaxoSmithKline	17-Nov-95	NRTI
Epzicom	Abacavir and lamivudine	GlaxoSmithKline	02-Aug-04	NRTI
Hivid	Zalcitabine, dideoxycytidine, ddC (no longer marketed)	Hoffmann-La Roche	19-Jun-92	NRTI
Retrovir	Zidovudine, azidothymidine, AZT, ZDV	GlaxoSmithKline	19-Mar-87	NRTI
Trizivir	Abacavir, zidovudine, and lamivudine	GlaxoSmithKline	14-Nov-00	NRTI
Truvada	Tenofovir disoproxil fumarate and emtricitabine	Gilead Sciences, Inc.	02-Aug-04	NRTI
Videx EC	Enteric-coated didanosine, ddI EC	Bristol-Myers Squibb	31-Oct-00	NRTI
Videx	Didanosine, dideoxyinosine, ddI	Bristol-Myers Squibb	9-Oct-91	NRTI
Viread	Tenofovir disoproxil fumarate, TDF	Gilead	26-Oct-01	NRTI
Zerit	Stavudine, d4T	Bristol-Myers Squibb	24-Jun-94	NRTI
Ziagen	Abacavir sulfate, ABC	GlaxoSmithKline	17-Dec-98	NRTI
Eduvant	Rilpivirine	Tibotec Therapeutics	20-May-11	NNRTI
Intelence	Etravirine	Tibotec Therapeutics	18-Jan-08	NNRTI
Rescriptor	Delavirdine, DLV	Pfizer	4-Apr-97	NNRTI
Sustiva	Efavirenz, EFV	Bristol-Myers Squibb	17-Sep-98	NNRTI
Viramune	Nevirapine, NVP	Boehringer Ingelheim	21-Jun-96	NNRTI
Viramune	Nevirapine, NVP	Boehringer Ingelheim	25-Mar-11	NNRTI
Agenerase	Amprenavir, APV (no longer marketed)	GlaxoSmithKline	15-Apr-99	PI
Aptivus	Tipranavir, TPV	Boehringer Ingelheim	22-Jun-05	PI
Crixivan	Indinavir, IDV,	Merck	13-Mar-96	PI
Fortovase	Saquinavir (no longer marketed)	Hoffmann-La Roche	7-Nov-97	PI
Invirase	Saquinavir mesylate, SQV	Hoffmann-La Roche	6-Dec-95	PI
Kaletra	Lopinavir and ritonavir, LPV/RTV	Abbott Laboratories	15-Sep-00	PI
Lexiva	Fosamprenavir calcium, FOS-APV	GlaxoSmithKline	20-Oct-03	PI
Norvir	Ritonavir, RTV	Abbott Laboratories	1-Mar-96	PI
Prezista	Darunavir	Tibotec, Inc.	23-Jun-06	PI
Reyataz	Atazanavir sulfate, ATV	Bristol-Myers Squibb	20-Jun-03	PI
Viracept	Nelfinavir mesylate, NFV	Agouron Pharmaceuticals	14-Mar-97	PI
Fuzeon	Enfuvirtide, T-20	Hoffmann-La Roche & Trimeris	13-Mar-03	Fusion Inhibitor
Selzentry	Maraviroc	Pfizer	06-August-07	CCR5 inhibitor
Isentress	Raltegravir	Merck & Co., Inc.	12-Oct-07	INSTI
Stribild	Elvitegravir	Gilead Sciences	27-Aug-12	INSTI
Tivicay	Dolutegravir	GlaxoSmithKline	13-Aug-13	INSTI

NRTI nucleoside reverse transcriptase inhibitors, *NNRTI* non-nucleoside reverse transcriptase inhibitor, *PI* protease inhibitor, *CCR5* C-C chemokine receptor type 5, *INSTI* integrase strand transfer inhibitors, *RTV* ritonavir, *NFV* nelfinavir mesylate, *ATV* atazanavir sulfate, *LPV* lopinavir, *FOS-APV* fosamprenavir calcium, *IDV* indinavir, *APV* amprenavir, *SQV* saquinavir mesylate, *NVP* nevirapine, *TPV* tipranavir, *EFV* efavirenz, *DLV* delavirdine, *TDF* tenofovir disoproxil fumarate, *ABC* abacavir sulfate, *d4T* stavudine, *ddI* didanosine, dideoxyinosine, *ddI EC* enteric-coated didanosine, *ZDV* zidovudine, *AZT* azidothymidine, *FTC* emtricitabine, *3TC* lamivudine, *T-20* enfuvirtide

^aManufacturer/sponsor at time of approval

HIV-1 RT structures were determined, only one structure was shown to contain an RNA/DNA hybrid before 2013. Recently, three structures of HIV-1 RT in complex with a non-nucleotide RT inhibitor (NVP) and an RNA/DNA hybrid were reported (3). These three structures differ from all previously reported RT–DNA complexes. These findings indicate that a RT–nucleic acid complex may adopt two structural states, one suited to DNA polymerization and the other suited to RNA degradation (3). Researchers also speculate that RT mutations that confer drug resistance, but that are distant from the inhibitor-binding sites, often map to the unique RT-hybrid interface that undergoes conformational changes between the two catalytic states (3). The structure–activity relationship (SAR) of three RT inhibitors of marine origin (THD, HDD, and ADD) was approached with molecular modeling (4). Molecular docking studies of THD

into HIV-1 RT wildtype and 12 different mutants showed that mutations have little influence in the positioning and interactions of THD (4). Following a rational drug design approach, a modification of THD was suggested to improve its biological activity (4). Five docking programs (Glide, FlexX, Molegro Virtual Docker, AutoDock Vina, and Hyde) were evaluated for their ability to predict the relative biological activity of 111 known 1,2,4-triazole and 76 other azole type HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs) (5). The results show that after proper validation and optimization, molecular docking programs can help predict the relative biological activity of azole NNRTIs and facilitate the identification of novel triazole NNRTIs (5). Computational methods provide insights into the detailed interaction between compounds and targets, providing a comprehensive understanding of the pharmacological

activities of compounds and information after modification of the drug. Computational methods are convenient, especially when large-scale experiments are difficult to conduct. Other studies have focused on the discovery of potential RT inhibitors *via* molecular docking. The unliganded HIV-1 RT (1DLO) was used for the virtual screening of 4-thiazolidinone and its derivatives (ChemBank database) by using AutoDock4 (6). One derivative, (5E)-3-(2-aminoethyl)-5-(2-thienylmethylene)-1,3-thiazolidine-2,4-dione (CID 3087795), was discovered to be a promising inhibitor for HIV-1 RT with a minimum energy score and the highest number of interactions with active site residues (6). Molecular docking is also widely used in SAR studies, as a way to evaluate the anti-viral activity of newly discovered or synthesized compounds (7–16).

PROTEASE

Protease (PR), as one of the three key enzymes, cleaves the viral polyprotein after its translation to release functionally mature proteins. After the protease is inactivated, the HIV virion becomes non-infectious. Two copies of 99 amino acid protein chains are non-covalently associated to form the long and symmetrical tunnel of the binding sites of HIV protease. Recently, a room-temperature joint X-ray/neutron structure of the HIV-1 protease in complex with the clinical drug amprenavir was reported, providing a direct determination of the hydrogen atom positions in the enzyme's active site (17). This structure may provide insight for the design of improved protease inhibitors. Eleven PR inhibitors are FDA-approved at present (Table I). These PR inhibitors (PIs) are essential components of highly active antiretroviral therapy. Our review focuses on the discussion of recent innovative examples of PR inhibitor development *via* computer-guided drug design. Danuravir is the most potent HIV PR inhibitor that is known. A series of danuravir derivatives was evaluated with 3D-QSAR and docking methods (18), demonstrating that the ligand-based and receptor-based results were in agreement with the experimental results (18). Five QSAR models were generated on the basis of available experimental IC₅₀ values (HIV-I- and HIV-IIIB-infected MT4 and CEMSS cells and HIV-I-infected C8166 cells) (19). The sixth model was generated by combining the inhibitors of all five models, critically examining the role of conceptual DFT descriptors and docking scores on 156 HIV PR inhibitors (19). Previous molecular dynamics simulations revealed some opening pathways and conformations in the opening process of HIV PR (20). Recent studies evaluated the possibility of the existence of alternative opening pathways to the semi-open conformation, by performing a molecular dynamics simulation of the early opening process of an inhibitor-free HIV-1 protease with explicit solvent (20). An X-ray diffraction structure of HIV-1 protease was used in this study as a template. The mutant residues in 1D4L were restored to the original residues *via* model building (20). This made the results suitable for comparison with experimental findings. Consistent with former experimental studies, the closed form of the HIV-1 protease structure (inhibitor-free) converts to a semi-open conformation spontaneously (20). However, the opening motion to the semi-open conformation happens in a manner that differs from both the previously reported modes. In

this simulation, the gap between the flap tips in the closed conformation was 15 Å thinner than in the semi-open conformation (20). Additionally, the alternative opening route discovered in this study was consistent with those observed by some nuclear magnetic resonance (NMR) or electron paramagnetic resonance (EPR) experiments that failed in identifying a large-amplitude curling motion as reported previously (20). Characterizing the dynamics of these opening and closing events led to a more comprehensive understanding of the binding of inhibitors. This is helpful for understanding the functional mechanism and substrate-/inhibitor-binding dynamics of PRs. Such findings provide new directions for the design of more effective inhibitors for the treatment of HIV infection.

INTEGRASE

Integrase (IN) is the viral enzyme that catalyzes the reaction of integration. The integration of viral DNA into the host genome is critical for permanent infection by the virus, which is achieved through 3'-terminal processing (3'-P) and strand transfer (ST) (21). The first integrase inhibitor, raltegravir, was FDA-approved in 2007 for the clinical treatment of AIDS (22–24). Three integrase inhibitors have been FDA-approved for the treatment of HIV infection at present (Table I). Many studies were reported on the design and screening of new integrase inhibitors, targeting both strand transfer and lens epithelium-derived growth factor (IN-LEDGF)/p75 interaction *via* virtual screening, molecular dynamic simulation, and 3D-QSAR. Pharmacophore modeling and atom-based 3D-QSAR studies were performed for 3-hydroxypyrimidine-2,4-diones, of which three compounds were identified as potential HIV-1 IN strand transfer inhibitors (INSTIs) (25). Translocation of preintegration complex (PIC) (26) into the nucleus is a precondition for successful integration, which is one process involved in protein–protein interactions (PPI) (27). PIC is composed of integrase, viral DNA, and a bunch of host factors. Although studies of PIC are attractive to virologists, and significant progress has been made in recent years, the current understanding of PIC is incomplete. Lens epithelium-derived growth factor (LEDGF/p75) is the first reported cellular factor to interact with integrase (28, 29). The interaction details were disclosed with the report of the crystal structure of IN-LEDGF/p75 complex (30–32). Molecular dynamics simulations were also carried out to study the detailed interactions between IN and LEDGF/p75. Residues Gln168, Glu170, and Thr174 in chain A of IN, Thr125, and Trp131 in chain B of IN as well as Ile365, Asp366, Phe406, and Val408 in LEDGF/p75 were identified as hotspots at the binding interface (33). The crystal structure of the LEDGF/p75 integrase-binding domain (IBD) in complex with a dimer of the integrase catalytic core domain was reported (34). Disrupting the LEDGF/p75-integrase interaction is an attractive target for the development of novel integrase inhibitors. Against the LEDGF/p75 binding pocket of IN, 26 known drugs from DrugBank were screened *via* molecular docking, and eight drugs were identified as potential IN inhibitors that target IN-LEDGF/p75 interactions (35). A series of azaindole carboxylic acid derivatives, previously reported as promising HIV-1 integrase inhibitors, were studied through a three-dimensional quantitative structure–activity relationship (3D-QSAR) (36). Binding

modes were explored by molecular docking based on the molecule with the highest activity (36). New azaindole carboxylic acid derivatives were designed, and their inhibitory activities on HIV-1 integrase were predicted based on the computational models constructed in these studies (36). Molecular docking is also used to simulate the interaction modes between IN and newly synthesized compounds. Halogen-substituted phenanthrene beta-diketo acids were synthesized and identified as new HIV-1 integrase inhibitors (37). The most active compound, 4-(6-chlorophenanthren-2-yl)-2,4-dioxobutanoic acid (18), inhibited both 3'-terminal processing and strand transfer (37). Two predominant binding modes that were distinct from the binding modes of raltegravir and elvitegravir were revealed *via* docking, and docking modes suggested a novel binding region in the IN active site (37). The rapid emergence of cross-resistance to strand transfer inhibitors is a serious issue in the therapy of AIDS. Molecular dynamics simulation is also used to study the detailed molecular mechanism of the cross-resistance mutation E138K/Q148K to raltegravir, elvitegravir, and dolutegravir, which are three important INSTIs (38). *Via* structure-based virtual screening, 38 compounds were selected from an in-house library containing 1,430 natural products and their derivatives. The virtual screening was performed using an induced fit model to discover inhibitors that target IN-LEDGF/p75 interactions (39). Eighteen hits were discovered through AlphaScreen bioassays. One compound, (E)-3-(2-chlorophenyl)-1-(2,4-dihydroxyphenyl)prop-2-en-1-one (NPD170), showed the highest anti-viral activity ($EC_{50}=1.81\text{ }\mu\text{M}$) (39). Although the therapeutic index (TI) of NPD170 was not very high (4.88), this was a good case for the discovery of an anti-HIV compound *via* virtual screening.

CXCR4

HIV infection is initiated by the binding of viral gp120 to human CD4 on the cell surface. A conformational change of gp120 induced by binding exposes the co-receptor binding domain. The fusion of gp41 with the cell membrane after co-receptor binding is the final step of entry. CXCR4 (C-X-C chemokine receptor type 4) and CCR5 (C-C chemokine receptor type 5) are two major co-receptors of HIV, expressed on T cells and macrophages, respectively. CXCR4 is a seven trans-membrane G-protein-coupled receptor (GPCR) of stromal cell-derived factor-1 α (SDF-1 α). SDF-1 α inhibits cell fusion and infection of syncytium-inducing phenotype HIV strains. CXCR4 is an appealing target for anti-viral drug development. AMD3100 was the first promising CXCR4 antagonist, but it proved to be unsuitable for the treatment of AIDS in later studies (40). Before 2011, the CXCR4 antagonist development depended on traditional chemical synthesis and drug design to mimic the available part of SDF-1 α /CXCR4 structures. The first low molecular weight antagonist, AMD3100, is a representative example (40). T22 is a representative polypeptide antagonist of CXCR4, which was synthesized with chemical modification from the horseshoe crab hemocytic polypeptides (41–43). T134 is a small-sized analog of T22 with reduced positive charges, highly potent activity, and significantly less cytotoxicity (41). Two other CXCR4 antagonists, ALX40-4C and vMIP II, are active against the AMD3100 resistant strains (41, 44, 45). An excellent review on CXCR4 antagonist development before 2006 is available (46).

Lacking CXCR4 crystal structure, the usage of computational methods in antagonist development was limited. However, there may still be some potential research directions for future studies in this field. A three-dimensional model of human CXCR4 was constructed *via* homology modeling according to the high-resolution bovine rhodopsin structure (47). Interactions between the constructed CXCR4 and flavonoids were investigated with *in silico* docking (47). A similar homology modeling study was also performed to construct homology models of CXCR4 and CCR5. The models were used for virtually screening a library consisting of 602 known CXCR4 and CCR5 inhibitors and some 4,700 similar, presumed inactive, molecules for comparison of ligand-based shape-matching searches and receptor-based docking methods (48). Although these studies described valuable trials in developing CXCR4 antagonists through computational methods, the revealed CXCR4 crystal structures are much different from the structures constructed by homology modeling. Five independent crystal structures of CXCR4 bound to an antagonist small-molecule IT1t and a cyclic peptide CVX15 at 2.5–3.2 Å resolution were reported in 2010 (49). All structures reveal a consistent homodimer with an interface including helices V and VI, and this interface may be involved in regulating signaling (49). The ligand-binding sites are closer to the extracellular surface, the location and shape of which are much different from other G-protein-coupled receptors (49). The structures provide new clues about the interactions between CXCR4 and SDF-1 α and with gp120 (49). Thereafter, a receptor-based virtual screening performance of the five crystallized CXCR4 structures along with a CXCR4 rhodopsin-based homology model was carried out using 248 known CXCR4 inhibitors from four different chemotype families and 4,696 different presumed inactives (50). The results showed that the 3OE6 structure achieved the highest docking-based performance and was proposed as the best for CXCR4 antagonist discovery *via* virtual screening (50). The 3OE6 structure of CXCR4 in complex with antagonist IT1t has been reported (49).

Recently, a highly specific and sensitive pharmacophore model was reported for identifying CXCR4 antagonists that could potentially serve as HIV entry inhibitors (51). It achieved the best performance when compared with docking and shape-matching virtual screening approaches using the 3OE6 CXCR4 crystal structure and high-affinity ligands as query molecules, respectively (51). Although no CXCR4 antagonist has been approved for the clinical treatment of HIV infection, numerous candidates are under investigation, and several are in clinical trials (41–43, 46, 52, 53). The disclosure of CXCR4 crystal structures will greatly improve the progress of development of HIV entry inhibitors through CXCR4.

CCR5

CCR5 is also a member of seven trans-membrane G-protein-coupled receptors. In contrast to CXCR4, CCR5 has three natural ligands: RANTES, MIP-1 α , and MIP-1 β (54, 55). Maraviroc is the first CCR5 co-receptor FDA-approved antagonist for the treatment of CCR5-tropic HIV infection (56, 57). The detailed usage of maraviroc in the treatment of HIV infection was reviewed by Perry (58). Vicriviroc is a small-molecule CCR5 antagonist that has entered

phase III clinical trials (59). More information can be found in a review article on CCR5 antagonists, which also provides information on the integrase inhibitor and fusion inhibitor (60). Homology modeling was the main computational method for CCR5 antagonist development before 2013. Several studies were performed on the human CCR5 structure construction *via* homology modeling by using the X-ray structure of the bovine rhodopsin receptor (61–64). Additionally, a homology model of human CCR5 was developed based on the reported CXCR4 structure as a template (65). The 2.7 Å-resolution crystal structure of human CCR5 bound to the marketed HIV drug maraviroc was reported recently (66). The reported structure revealed a ligand-binding site that was distinct from the proposed major recognition sites for chemokines and gp120, providing insights into the mechanism of the allosteric inhibition of chemokine signaling and HIV entry (67). The high-resolution crystal structure of CCR5 enables structure-based drug discovery for the treatment of HIV-1 infection (67).

SUMMARY

This review emphasizes the advances of anti-HIV drug development *via* computational methods applied to five key targets. Although the above examples are representative, the computational methods described are also helpful in anti-viral drug development targeting other HIV proteins, such as gag (68), gp41 (69–71), and gp120. The interaction between viral gp120 and human CD4 not only initiates HIV infection but is also an important and attractive target for anti-AIDS drug development. Crystal structures of HIV gp120-CD4 complexes reveal a close interaction of the virus receptor with CD4 Phe43, which was embedded in a pocket of gp120 (72). Molecular docking is used widely in anti-AIDS drug studies targeting gp120-CD4 interactions (72–77). The high mutation rate of HIV leads to the emergence of drug-resistant strains. Computational methods play an important role in modern anti-HIV drug development. To characterize the step of virus mutation, virtual screening and QSAR significantly reduce the time needed for drug discovery. Considering the enormous number of currently available compounds from sources, such as plants, marine organisms, and bacteria, computational methods are clearly promising and low cost.

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