REVIEW ARTICLE

Recent Advances in Computer-aided Antiviral Drug Design Targeting HIV-1 Integrase and Reverse Transcriptase Associated Ribonuclease H

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Abstract: Acquired immunodeficiency syndrome (AIDS) has been a chronic, life-threatening disease for a long time. Though, a broad range of antiretroviral drug regimens is applicable for the successful suppression of virus replication in human immunodeficiency virus type 1 (HIV-1) infected people. The mutation-induced drug resistance problems during the treatment of AIDS forced people to continuously look for new antiviral agents. HIV-1 integrase (IN) and reverse transcriptase associated ribonuclease (RT-RNase H), two pivotal enzymes in HIV-1 replication progress, have gained popularity as druggable targets for designing novel HIV-1 antiviral drugs. During the development of HIV-1 IN and/or RT-RNase H inhibitors, computer-aided drug design (CADD), including homology modeling, pharmacophore, docking, molecular dynamics (MD) simulation and binding free energy calculation, represent a significant tool to accelerate the discovery of new drug candidates and reduce costs in antiviral drug development. In this review, we summarized the recent advances in the design of single-and dual-target inhibitors against HIV-1 IN or/and RT-RNase H as well as the prediction of mutation-induced drug resistance based on computational methods. We highlighted the results of the reported literatures and proposed some perspectives on the design of novel and more effective antiviral drugs in the future.

1. INTRODUCTION

The acquired immunodeficiency syndrome (AIDS) is a chronic, life-threatening disease caused by human immunodeficiency virus type 1 (HIV-1). According to the latest statistical data, 38 million people in the world were found living with HIV-1 [1]. The life cycle of

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HIV-1 is complex, which needs the assistance of several enzymes such as integrase (IN) and reverse transcriptase associated ribonuclease H (RNase H) (Fig. **1**). Till now, six groups of antiviral drugs were approved for AIDS treatment, which have greatly expanded the lifespan of infected people and improved the quality of life [2]. Among the six groups of antiviral drugs, integrase strand transfer inhibitors (INSTIs) were considered as the cornerstone of the highly active anti-retroviral therapy (HAART) regimens against HIV-1/AIDS because of the great treatment effect and well tolerated property.

HIV-1 IN plays a key role in the process of integration of the dsDNA into the host genome, involving the

procedure of 3´-end process (3´-P) and strand transfer (ST) (Fig. **1**) [3, 4]. Focusing on different sites of IN, the inhibitors can be divided into two groups: INSTIs and allosteric integrase inhibitors (ALLINIs). INSTIs target the catalytic sites of HIV-1 IN, while ALLINIs target the site away from the catalytic site [2, 5]. Till now, four INSTIs have been approved, namely raltegravir (RAL), elvitegravir (EVG), dolutegravir (DTG), bictegravir (BIC) and Cabotegravir (CAB) (Fig. **2**), while no ALLINIs were approved. INSTIs were widely used for first-line therapy in the treatment of HIV-1-infected patients [6]. However, theHIV-1 IN drug-resistant mutations (DRMs) reduced the efficiency of INSTIs. The first generation INSTIs (RAL, EVG) showed obviously lower drug resistance barrier to DRMs and shared a high degree of cross-resistance with each other [7-11]. The second generation INSTIs (DTG, BIC and CAB) showed higher drug resistance barriers than RAL and EVG [12]. However, recent articles reported the drug resistance on DTG in treatment-naïve persons receiving triple combination DTG-containing regimens and in virologically suppressed patients receiving DTG monotherapy [13-16]. Thus, structure-based design of antiviral agents targeting HIV-1 IN with novel scaffolds or new mechanism of action and getting more information on drug resistance of INSTIs are urgently needed.

HIV-1RNase H, a functional domain of RT, shared a similar "DDE" motif in catalytic site with IN (Fig. **1**) [17, 18]. Viruses lacking RNase-H function are non-infectious, which make RNase-H a potential target for anti-HIV therapeutics. Large numbers of RNase H inhibitors (RNIs) have been reported till now [19, 20]; however, no RNIs have been approved due to the presence of a variety of host ribonucleases in the host cell that share similarities with RNase-H [19]. Moreover, the structure similarity of catalytic sites of HIV-1 IN and RNase H provides the opportunity to design inhibitors that target both the enzymes, hereby providing an opportunity for designing dual inhibitors with less side effects, pill burden and drug adherence [2, 21-23].

The computational methods such as homology modeling, pharmacophore, docking, molecular dynamics (MD) simulation and binding free energy calculationhave apparently accelerated the discovery of new drugs and reduced costs [24]. The computational methods, especially virtual screening (VS), played an important role in the development of novel HIV-1 integrase and/or RNase H inhibitors [25]. VS have been widely and successfully used in the discovery of inhibitors target HIV-1 IN and/or RNase H. In this review, the literatures published between 2015 and 2020, mainly focusing on VS of HIV-1 IN/RT-RNase H inhibitors, were collected and discussed. In addition, the recent reports of INSTI-resistance between 2015 and 2020, including the structural mechanism of drug resistance and drug resistance prediction through computational methods are also summarized in this review.
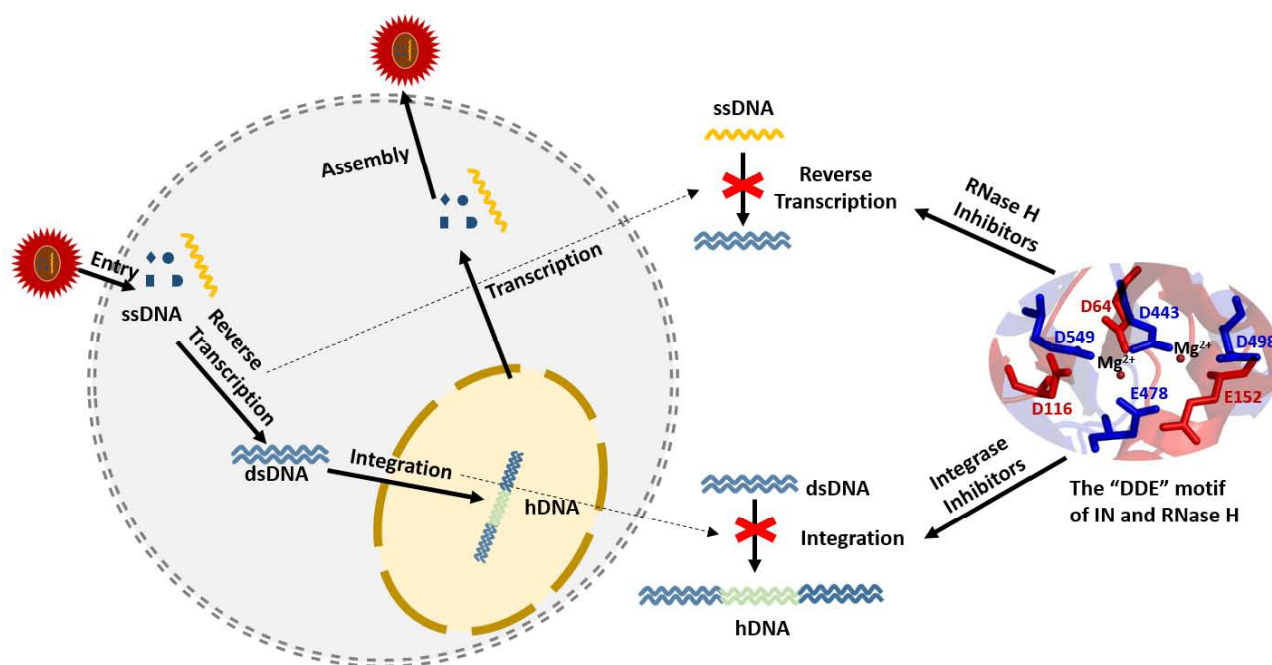

Fig. (1). The function of IN and RNase H in HIV-1 replication and the mechanism of IN and RNase H inhibitors *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*
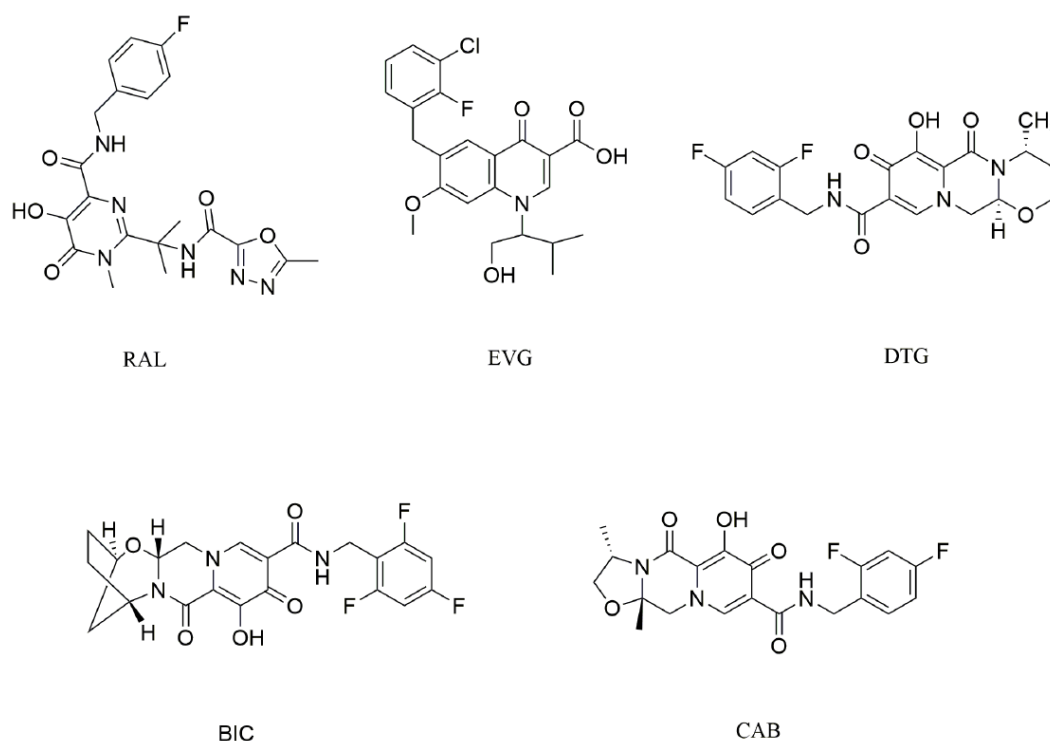
Fig. (2). The structures of the currently approved HIV-1 strand transfer integrase inhibitors.

We highlighted the results in the literatures and proposed some perspectives on the future directions on HIV-1 IN and RNase H drug design.

2. HIV-1 IN AND/OR RT-RNASE H INHIBITORS DISCOVERY AND DESIGN BASED ON COMPUTATIONAL METHODS

2.1. Single HIV-1 IN or RNase H Inhibitors

Based on the crystal structure of HIV-1 integrase catalytic core domain(PDB code: 1QS4), Samorlu *et al.* [26] conducted a structure-based virtual screening to discover potential INSTIs. Molecular docking was conducted to screen compounds from 81,063 lead-like compounds extracted from OTAVA database. 3 hit compounds (107320240, 111150115 and 109750155) (Fig. **3**) were identified with high binding affinity and safe ADMET profile. Molecular dynamic (MD) simulations were then performed to examine the stability of interaction, the results suggested that the interaction between compound 107320240and HIV-1 IN was more stable than compounds 111150115 and 109750155.

Vora *et al.* [27] performed a structure-based virtual screening to find HIV-1 INSTIs from 22 plant-derived natural compounds. The crystal structure of HIV-1 integrase catalytic core domain (PDB code: 5EU7) and three different softwares were used for virtual screening. The compounds with high binding affinity in all the software were chosen for biological activities prediction through quantitative structure activity relation (QSAR). At last, the drug-like properties were analyzed and the results suggested that curcumin (Fig. **3**) possesses good binding activity with integrase and almost same pIC_{50} value with standard inhibitors (RAL, EVG and DTG).

Coupled with a combinatorial library design procedure, Sirous *et al.* [28] developed a step-filtering approach to identify novel HIV-1 INSTIs. A novel HIV-1 IN/DNA binary 3D-model was generated based on the (PFV) crystal structure of proto-type foamy virus (PFV) intasome (PDB code: 3L2T). Two investigated 3-hydroxyl-pyran-4-one-2-carboxamide derivatives (HPCAR) were used as main core to generate hit compounds for screening. Then, 37,000 hit compounds were generated and screened through combinatorial docking, molecular properties prediction, and Quantum Polarized Ligand Docking Simulation (QPLD). Finally, 3 hit compounds (HPCAR-28, HPCAR-89 and HPCAR-142) were synthesized for biological assays and MD simulation. The results demonstrated that compound HPCAR-28 (Fig. **3**) was the most potent inhibitor with lower nanomolar activity (Table **1**) and appreciable therapeutic index.

Based on the structure of tetrameric HIV-1 strand transfer complex intasome (PDB code: 5U1C),
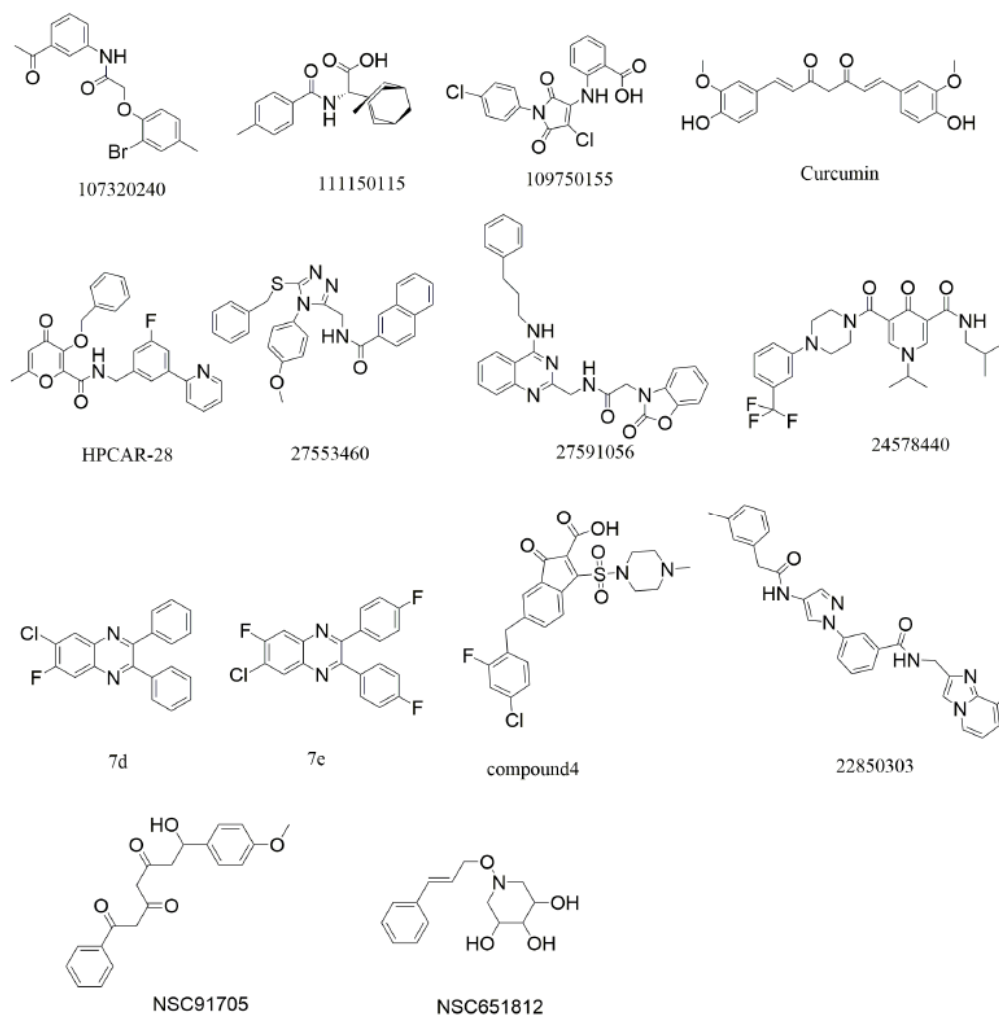
Fig. (3). The structures of the recently reported HIV-1 IN inhibitors.

Eurtivong *et al.* [29] performed a structure-based virtual screening to discover potential INSTIs. 4 diverse compounds from 25,132 small molecule compounds in Chembridge were filtered through molecular docking. Then, taking RAL as a reference, the physicochemical properties and ADME and toxicity of the 4 compounds (22850303, 27553460, 27591056 and 24578440) (Fig. 3) were further calculated and predicted. The results suggested that the pharmacokinetic and toxicity profiles of the 4 compounds were better than or equal to RAL.

Patel *et al.* [30] combined pharmacophore modelling and 3D-QSAR together to design novel INSTIs. Pharmacophore models containing features of two donor sites, one acceptor atom and one hydrophobic region were considered as the best model for screening. 6 compounds from National Cancer Institute (NCI) database were obtained and selected as a core moiety to design novel INSTIs through 3D-QSAR and contour maps. Based on the best pharmacophore and contour maps, 32 quinoxaline derivatives were designed and docked into HIV-1 IN. 7 molecules (7a, 7b, 7c, 7d, 7e, 7f and 7g) with high docking score and satisfactory interactions with receptor were selected for biological assays. 2 quinoxaline derivatives (Fig. 3) were found with good anti-HIV-1 activity (Table 1).

Based on a series of HIV-1 INSTIs from reported literatures, Guasch *et al.* [31] used QSAR to construct pharmacophore models for HIV-1 INSTIs virtual screening. These models were validated by 5-fold cross-validation and the reasonable models were then used for screening. A small combinatorial library of potential synthetic candidates was prepared for screening. Further molecular docking was applied to filter out ineligible compounds. As a result, 236 compounds with good drug-likeness properties and correct docking pose were identified as potential candidates and 6 compounds (compound 1, compound 2, compound 3, compound 4, compound 5 and compound 6) (Fig. 3) were

Table 1. VS studies in the discovery of inhibitors targeting IN and RNase H published between 2015 and 2020.

Year	Target	Screening Method	Filtering Criterion	Database	Hits	Bioassay	Refs.
2016	IN	LBVS	Lipinski's rule of five; Pharmacophore fit value ≥98%; Pharmacophore fit value ≥80%; good GOLD-score; number of hydrogen bonds formed; stable interactions with key residues.	NCI database	2	7d: IC_{50}> 11.78 µg/ml 7e: IC_{50}> 15.45 µg/ml	[30]
2016	IN	LBVS	pIC_{50}>7.5; few stars in QikProp; stable interactions with key residues;	BindingDB	1	compound 4: IC_{50} =37 µM	[31]
2016	IN	LBVS	Estimated activity < 7.453 nM, Pharmacophore fit value ≥13.210; Lipinski's rule of five; Veber's rule; dock score >185.25.	NCI database	2	- [a]	[32]
2017	IN	SBVS	Highest binding affinity; Lipinski's rule of five; potential interaction with hERG K+ channel (QPlog-HERG);	Pubchem database	1	-	[27]
2018	IN	SBVS	Lipinski's rule of five; good physicochemical properties; Auto Dock Vina(PyRx) binding affinity ≤-8.00 kcal/mol; Autodock 4.2 binding energy > -8.00 Kcal/mol.	OTAVA database	3	-	[26]
2019	IN	SBVS	GlideScore values < -0.6 kcal/mol; stable interactions with key residues; membrane permeability, Lipinski's rule of five; XP GlideScore values <− 6 kcal/mol; estimated binding energy values <− 25 kcal/mol.	Combinatorial library	1	HPCAR-28: IC_{50} = 65 nM	[28]
2019	IN	SBVS	Docking score >60, hydrogen bond score > 1; stable interactions with key residues; chemical scaffold diversity.	Chembridge	4	-	[29]
2017	RNase H	Combined	Ligands with highest binding affinity in all the software (Discovery studio 4.0, Schrodinger and Molegrow virtual docker); 3D-QSAR; Lipinski's rule of five; potential interaction with hERG K+ channel (QPlog-HERG);	Pubchem database	1	-	[26]
2018	RNase H	SBVS	Lipinski's rule of five; reactive group filters; docking score; QM-based refinement calculation; chemical scaffold diversity.	Specs database	4	A: IC_{50} =9.35 µM AA: 5.17 AB: 12.82 AC: 8.73	[33]
2017	Dual	SBVS	Lipinski's rule of five; QikProp; reactive filter; Glide high throughput virtual screening (top 5% hits); standard mode of Glide (top 5% hits); XP Glide (top 30 hits); XP Glide (G-score < -10).	Asinex database	7	-	[36]

[a]Studies lacking bioassays.

synthesized for bioactivity assays. Finally, compound 4 experimentally confirmed to inhibit the strand transfer reactions which were found with good anti-HIV-1 activity (Table **1**).

Islam *et al.* [32] constructed a group of 3D-QSAR pharmacophore models to discover potential HIV-1 INSTIs. 540 compounds with integrase inhibition ac-

tivity from BindingDB were selected for constructing 3D-QSAR pharmacophore models. The models were validated by five methods: internal validation, cost function analysis, Fischer's randomization test, test set prediction and decoy set. The best pharmacophore models and NCI database were chosen for virtual screening. Finally, 2 compounds (NSC91705 and NSC-

651812) (Fig. **3**) from a library of 265,242 compounds were considered as promising HIV-1 INSTIs. The results of MD simulation suggested that the interaction between NSC651812 and HIV-1 IN was more stable than NSC91705. Further comparison of drug-likeness with FDA approved HIV-1 integrase inhibitors suggested that NSC91705 and NSC651812 were promising HIV-1 INSTIs.

Vora *et al.* [27] performed a structure-based virtual screening to find potential RNIs from 22 plant-derived natural compounds. HIV-1 RT-RNase H (PDB code: 3QIN) was selected as target for virtual screening. Three different softwares were used to predict binding affinity of receptor-ligand. The docking scores of the three software suggested that chebulic acid (Fig. **4**) has potential activity against RT-RNase H.

Vasanthanathan *et al.* [33] performed a structure-based virtual screening combining molecular docking and QM-based refinement calculations together to search potential RNIs. A library of 1205 compounds from ZINC database was obtained after the docking-based virtual screening and these compounds were subsequently applied for QM-based refinement calculation. 25 structurally diverse compounds with the best scores were chosen for the enzymatic assays. 3 compounds inhibiting the RNase H activity below an IC_{50} value of 100 μM were used as query molecules for new chemo-types RNIs by similarity-based search. Finally, 4 scaffolds (A, AA, AB and AC) (Fig. **4**) were identified to inhibit the RNase H activity in lower micromolar activity (Table **1**), while the most active compound AA inhibits HIV-1 RT-RNase H with an $IC_{50} = 5.1$ μM.

Based on the crystal structures of the incomplete HIV-1 IN, structure-based virtual screening was widely used for discovering HIV-1 INSTIs in these years [24]. Molecular dockings were frequently used in screening. To improve the performance of virtual screening, Samorlu *et al.* [26] and Vora *et al.* [27] tended to use more than one kind of docking tools for screening. Besides, some teams tried to take the stable interactions between compounds and HIV-1 IN/RNase H as a filtering criterion for virtual screening, as shown in Table **1**. However, the structure of full length HIV-1 integrase has not been resolved yet. The crystal structures of prototype foamy virus (PFV) intasomes on provide opportunity for well understanding the detailed binding mechanism of HIV-1 INSTIs [34, 35]. However, the structure of PFV and HIV-1 IN only shares limited similarity of sequence identity. The recently reported crystal structures of simian immunodeficiency virus (SIV) and HIV-1 integrase strand transfer complexes

(STCs) have been used to clarify the INSTI binding modes within the intasome active sites for screening potential INSTIs [29]. In Eurtivong's work, [29].the structure of tetrameric HIV-1 strand transfer complex intasome (PDB code: 5U1C) was used to screen potential INSTIs, providing more information of the binding modes of compounds that target HIV-1 integrase STC.

Along with the growing numbers of potential integrase inhibitors, ligand-based virtual screening (LBVS) was frequently used for discovering HIV-1 integrase inhibitors in recent years. It is worth thinking about how to construct reliable ligand-based models for LBVS, like QSAR or pharmacophore models. The influencing factors including data set, molecular descriptors, statistical methods and model validation etc. should be carefully considered when construct models [24]. Patel *et al.* [30] take the typical features (two donor sites, one acceptor atom and one hydrophobic region) of the known INSTIs as a criterion for evaluating the quality of pharmacophore models. The typical features mentioned above were recognized as the key components of stable interaction between the metal cofactors and three "DDE" motifs in the active site of HIV-1 IN [34, 35]. The two hydrogen bond acceptors and one aromatic ring feature were also recognized as good models constructed by 3D-QSAR in Islam' work [32]. Besides, model validation is considerably needed to check the productivity and applicability as well as robustness of the models. Till now, many different methods have been developed for model validation [24]. Combining different methods together for model validation was widely used in the recently studies. Islam *et al.* [32] combined five methods (internal validation, cost function analysis, Fischer's randomization test, test set prediction and decoy set) together for models validation.

Due to the presence of a wide array of host ribonucleases which are important for the proper functioning of the host cell share similarities with RT RNase H, there is no RNIs approved till now. With the availability of a larger number of high-resolution 3D crystal structures of HIV-1 RNase H, SBVS was used to discover HIV-1 RNIs. To improve the performance of screening, Vora *et al.* [27] used three different software to perform molecular docking for filtering potential RNIs. While, Vasanthanathan *et al.* [33] combined molecular docking and QM-based refinement calculations together to improve the performance of screening. Meanwhile, similarity-based search was used for seeking new chemo-types RNIs.
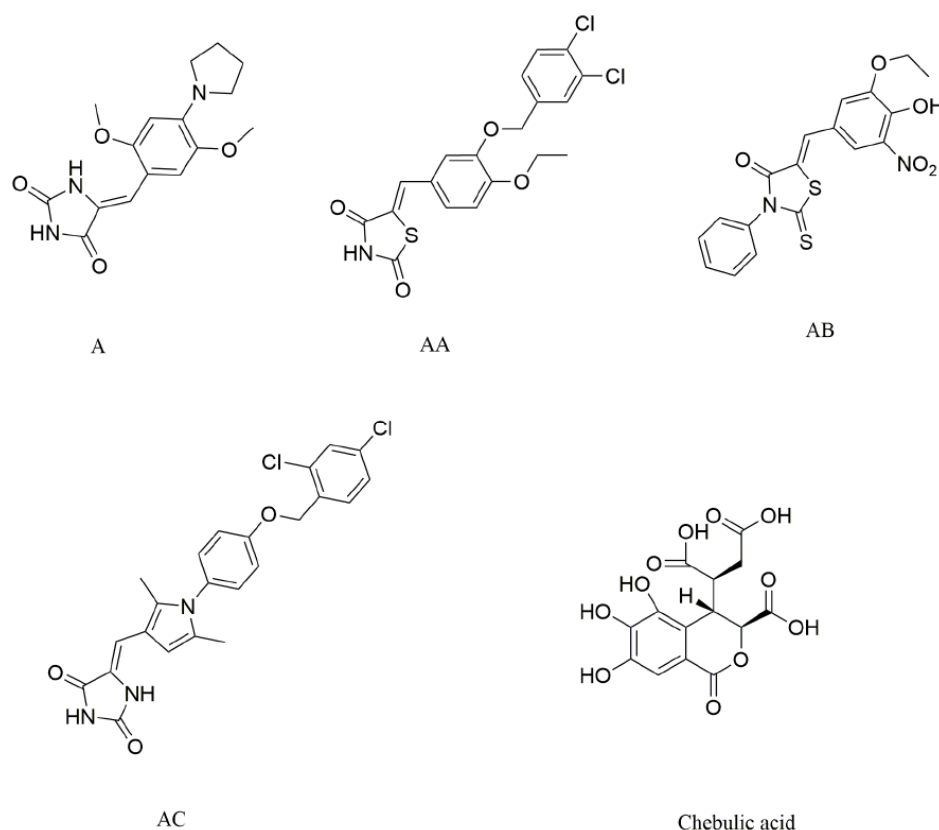
Fig. (4). The structures of the recently reported HIV-1 RNase H inhibitors.

2.2. Dual HIV-1 IN and RT-RNase H Inhibitors

Chander *et al.* [36] performed a structure-based virtual screening approach to search compounds targeting both IN and RNase H. Molecular docking was used to screen Asinex database to find potential molecule against HIV-1 IN. The top 30 ranked hits were then evaluated against RT-RNase H domain using Glide docking. A total of 11 common potential hits were obtained and subjected to in-silico prediction of drug-likeness properties. Finally, 7 compounds (AS3, AS5, AS6, AS15, AS17, AS18, and AS20) (Fig. **5**) were found showing potential inhibitory activity against IN and RT-RNase H.

The structure similarity of the catalytic sites of HIV-1 IN and RT-RNase H provides the possibility to design inhibitors that are effective on both targets [2, 22]. However, many IN and RNase H dual inhibitors discovered before were found hardly to meet the requisite features as a drug candidate. In Chander's work [36], molecular docking was used to screen potential dual IN and RNase H inhibitors. The filtering criteria were shown in Table **1**. *In-silico* physiochemical and AD-MET parameters against both the selected targets were evaluated to make sure the hits meeting the requisite features as a drug candidate.

3. COMPUTATIONAL STUDIES ON HIV-1 IN MUTATION-INDUCED DRUG RESISTANCE

3.1. Revealing the Drug Resistance Mechanism of HIV-1 IN Inhibitors

Investigating the drug resistance mechanism is important for the development of drug resistance prediction. MD simulation, a powerful tool for delineating motions of proteins and the process, has been applied for describing the drug resistance mechanism of IN-STIs. Chen *et al.* [37] constructed a series of HIV-1 IN homology models using the crystal structure of the full length PFV (PDB code: 3L2U) and HIV-1 integrase sequence [38]. Then, they performed MD simulation and multi-perspective analysis (cross correlation and clustering methods) to reveal the drug resistance mechanism of EVG in mutant system (E92Q/N155H). The results showed that the conformational changes of the 140's loop eventually caused the drug resistance of EVG.

HIV-1 subtype C (HIV-1C) accounts for nearly 50% of all global HIV-1 infections, while HIV-1 subtype B (HIV-1B) accounts for only approximately 12%. Recently, several studies have identified subtype specific differences in DTG cross-resistance pattern in
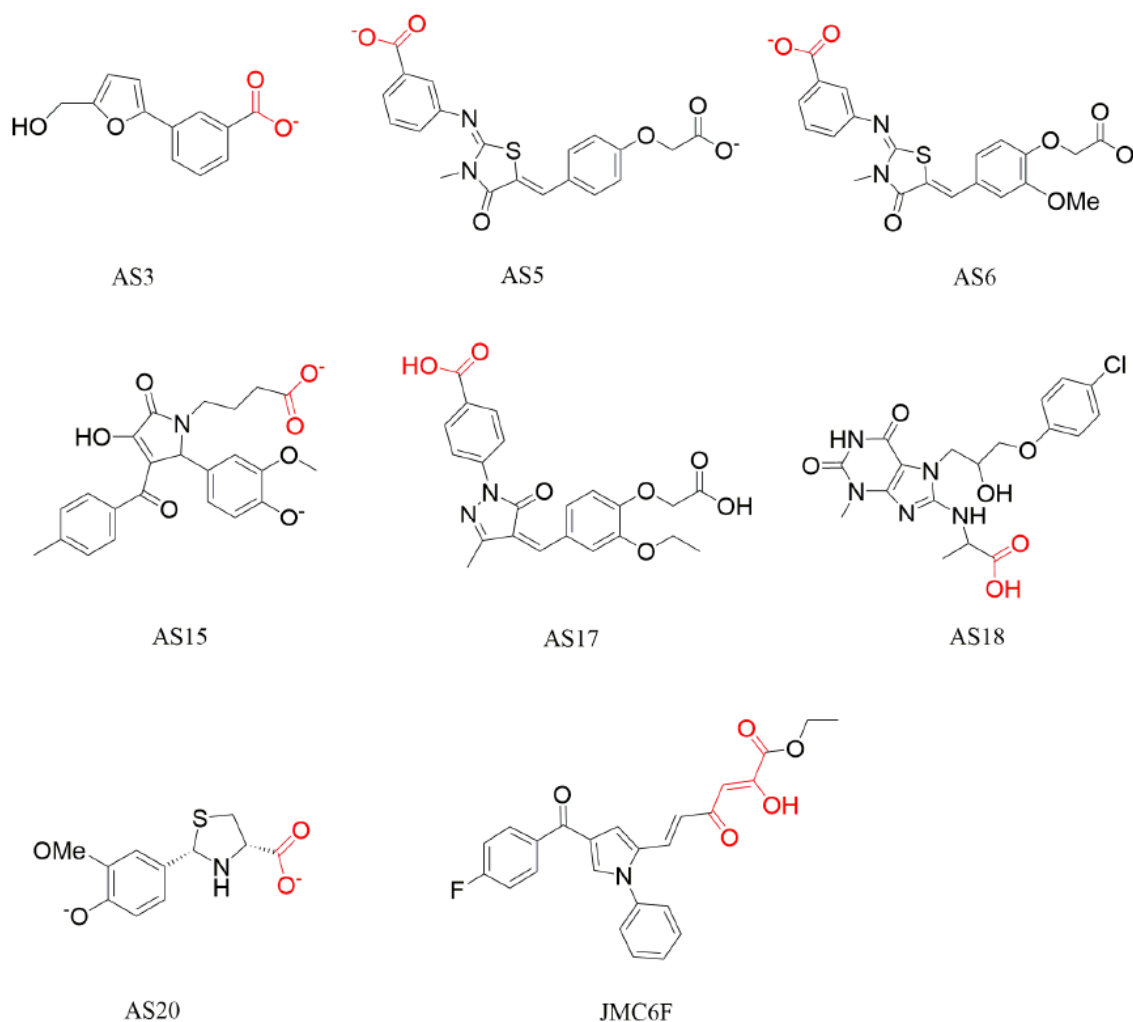
Fig. (5). The structures of the recently reported dual HIV-1 IN and RNase H inhibitors.

patients failing the first-generation RAL treatment. To interrogate the effect of known drug resistance associated mutations (RAMs) on the protein structure, Chitongo *et al.* [39] modeled the structure of HIV-1 subtype C IN based on the crystal structure of HIV-1B intasome (PDB code: 5U1C).Three different mutant systems (E92Q, G140S and Y143R) were prepared based on the model and all systems were subjected to 300 ns MD simulation by Gromacs version 5.1.The results suggested that the G140S mutant system was more flexible than other systems. The flexible region forced DTG moving away from the binding pocket. Malet *et al.* [40] employed MD simulations to investigate the impact of mutations (N155H, K156N, K211R and E212T)on the binding of DTG. The homology model was constructed based on the crystal structure of PFV intasome (PDB codes: 3S3M and 3S3O). All the mutated systems were submitted to 100 ns of MD simulation. The results indicated that DTG lost the stacking interaction with viral 3'-deoxyadenosine (A17) and

shifted from the catalytic binding site, which resulting DTG less stable in the pre-integration complex. Chitongo *et al.* [39] and Malet *et al.* [40] demonstrated that the stability of DTG in the pre-integration complexes appears to be an important parameter that obviously contributes to its efficiency and could explain data obtained from the clinic in some patients failing DTG treatment.

3.2. Drug Resistance Prediction of HIV-1IN Inhibitors

Common approaches for predicting resistance is time-consuming and high costing, e.g. phenotypic assays. Along with genome sequencing became widely available than traditional phenotypic test, automatic prediction based on algorithms has gain popularity for drug resistance prediction [41-43]. From the suboptimal rule-based approaches that are widely used in publicly available software (Stanford HIVdb, Rega or ANRS) to the computational models based on classifi-

cation and statistical methods leveraging sequence, automatic prediction became a popular methods in HIV-1 drug resistance prediction [41]. In recent years, machine learning was used for constructing computational models. Masso *et al.* [44] developed a series of predictive statistical learning models to quantify the effects of complex mutational patterns on drug resistance of RAL and EVG. N-grams was employed to characterize each IN variant as a feature vector of input attributes. Based on this, the performance of models developed by implementing two supervised classification and two regression statistical learning algorithms was improved, suggesting that these models were good enough as supplementary tools for making treatment decisions. Ramon *et al.* [45] used weighted categorical kernel functions to predict drug resistance from virus sequence data. 21 drugs of four classes: protease inhibitors (PI), integrase strand transfer inhibitors (INSTIs), nucleoside reverse transcriptase inhibitors (NRTI) and non-nucleoside reverse transcriptase inhibitors (NNRTI) were collected and analyzed. Results showed that categorical kernel functions that consider both the categorical nature of the data and the presence of mixtures consistently are a good method for constructing prediction model.

Besides the genotypic testing, molecular modelling methods (e.g. docking and MD simulation) were other considered methods to predict potential drug resistance mutants. In recent years, some teams attempted to perform structure-based prediction using molecular docking and/or MD simulation to predict drug resistance [41]. The binding affinity of ligand-receptor complex was recognized as a typical criterion to predict drug resistance. Sachithanandham *et al.* [46] performed molecular docking to predict the drug resistance of known integrase inhibitors (RAL, EVG and DTG) when accessory mutations exist. The model of HIV-1 IN was constructed based on the sequences generated from treatment naïve HIV-1 infected individuals (n=102) and the crystal structure of HIV-1 IN (PDB code: 1WKN). Then, molecular docking was performed to estimate binding affinity. The results demonstrated that the binding affinity was decreased with either a single accessory or major mutation. The reduction of binding affinity was increased when accessory mutations combined with major mutations. Based on the structure of tetrameric HIV-1 strand transfer complex intasome (PDB code: 5U1C) by SwissModel, Silva *et al.* [47] established a framework to predict novel HIV-1 mutations related to DTG resistance. Molecular docking was used to construct the structure of wide type HIV-1 intasome and DTG complex. Then, the point mutation

scanning through MAESTROweb server was performed to find novel possible resistance mutations. Depending on the binding affinity ($\Delta\Delta G$), the Y226K mutation with a higher $\Delta\Delta G$ and high allele frequency was filtered out from the point mutation scanning mutation list. Further molecular docking and MD simulation of Y226K-INT-DTG showed an even more accentuated decrease in the binding affinity ($\Delta\Delta G = 104.88$), indicating Y226K a possible resistance mutation. Yang *et al.* [22] employed MD simulation to predict the drug resistance profile of a dual inhibitor (JMC6F) (Fig. **5**) targeting HIV-1 IN and RT-RNase H. The full-length homology model of the HIV-1 IN was constructed based on the crystal structure of PFV IN (PDB code: 3OY9 and 3S3M). 10ns long simulation was taken for 16 mutant systems (T66A/I/K, E92Q, E138A/K, G140C/S, Y143C/H/R, S147G, and Q148H/K/R, N155H) to predict drug resistance of JMC6F. Based on the binding affinity calculated by MM-PBSA, three mutations (Y143C, Q148R and N155H) in HIV-1 IN resulted in the reduction of the binding affinity, which indicated their potential role in resulting drug resistance to JMC6F.

CONCLUSION AND FUTURE PERSPECTIVES

The development of novel HIV-1 IN and/or RNase H inhibitors was recognized as an attractive strategy for the development of anti-HIV-1 therapy due to the emergence of HIV-1 antiviral drug resistance. Here, we collected the recent literatures about computational approaches used for the drug design of HIV-1 IN and/or RNase H inhibitors,as well as the drug resistance studies of HIV-1 INSTIs. Summarized from the results, we can find that a proper model of HIV intasomes in complex with INSTIs is important for designing new HIV-1 IN and resolving drug resistance problems. In the past years, crystal structures of PFV intasome complexes were widely used in the discovery of HIV-1 integrase inhibitors. However, PFV and HIV-1 integrases shared lower sequence identity with each other. Recently, Passos *et al.* [48] presented a series of high-resolution cryo-electron microscopy structures of HIV intasomes bound to the latest generation of INSTIs. These structures suggested that the regions near the active sites of the PFV and HIV-1 intasomes are similar, but diverge in the region away from the active sites. The clinically relevant drug resistance mutations were also located in regions with different amino acid sequences. The water molecules surrounding INSTIs especially the three tightly bound water molecules underneath the "DDE" motif played important role in guiding the development of new INSTIs with high drug resistance barrier. These

crystal structures could be used for designing novel HIV-1 INSTIs in the future.

Virtual screening (VS), including SBVS and LBVS, was widely used in the development of INSTIs and RNIs. The SBVS approach is highly dependent on the structures of HIV intasomes and the methods for screening [24]. Molecular docking, a key method frequently used in virtual screening, was influenced by many factors, like appropriate receptor structure, docking tools and scoring functions. Combining several docking tools together could somehow improve the accuracy of docking. For LBVS, constructing more accurate pharmacophore models were important. Recently, multi-conformation dynamic pharmacophore modeling (MCDPM), a receptor-based pharmacophore modeling method (RPM) based on the different ligand-bound conformations from MD simulation, was applied for virtual screening. The MCDPM could reflect many conformations of the protein, and is able to play an important role in screening drug-like molecules [49].The compounds obtained from virtual screening need further prediction of the molecular properties and drug-like properties before taking bioassays, especially the stability of interaction between ligand and receptor.

Molecular dynamics (MD) simulation has been a powerful computational method for delineating motions of proteins on an atomic scale. Comparative stability of the complexes is important not only for designing INSTIs but also for drug resistance studies of HIV-1 INSTIs. Molecular modelling methods have been successfully applied for drug resistance study; however, accurate prediction of resistance is still a challenge due to the complicated drug resistance mechanism. We proposed that the improvement of existing computational methods should be one of the major goals in HIV-1 INSTIs drug resistance research. While, machine learning in combined with molecular modelling studies may be another future direction of drug resistance prediction. Much of drug discovery today is predicated on the concept of selective targeting of particular bioactive macromolecules by low-molecular-mass drugs. The binding of drugs to their macromolecular targets is therefore seen as a paramount for pharmacological activity. Recently, an article presents an alternative perspective on drug optimization in terms of drug-target complex residence time, namely inhibitors with good efficacy have long residence times [50]. Studies suggested that dissociation kinetics and residence time of compound are likely to be important for INSTIs. The dissociation data presented differential binding of INSTIs to wild-type and mutant HIV-1 in-

tasome complexes, suggesting that prolonged INI binding may contribute to efficacy [51]. Garvey *et al.* [52] found that long dissociation half-life of DTG than RAL and EVG, may contribute to its distinct resistance profile and highlight the potential for improved activity against wild-type HIV-1 and clinically relevant INI-resistant viruses. Based on this, Non-equilibrium simulations, like steered molecular dynamics simulations (SMD) will provide opportunities for HIV-1 INSTIs and RNIs as well as drug resistance studies. Furthermore, we believe that computational methods will provide more information about the discovery of novel HIV-1 integrase and/or RNase H inhibitors.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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