CHEMISTRY & BIODIVERSITY

REVIEW

HIV-1 Entry Inhibitors: A Review of Experimental and Computational Studies

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The HIV-1 life cycle consists of different events, such as cell entry and fusion, virus replication, assembly and release of the newly formed virions. The more logical way to inhibit HIV transmission among individuals is to inhibit its entry into the immune host cells rather than targeting the intracellular viral enzymes. Both viral and host cell surface receptors and co-receptors are regarded as potential targets in anti-HIV-1 drug design process. Because of the importance of this topic it was decided to summarize recent reports on small-molecule HIV-1 entry inhibitors that have not been considered in the latest released reviews. All the computational studies reported in the literature regarding HIV-1 entry inhibitors since 2014 was also considered in this review.

Keywords: HIV-1-entry, anti-HIV agents, CCR5, CXCR4, gp41, gp120.

Contents

1. Introduction

According to the latest released reports by WHO, HIV-1/AIDS was the cause of death of 1.0 million people worldwide in 2016 and the fifth cause of death in low-income economies. Taking the advantage of anti-HIV drugs, the rate of mortality by HIV-1/AIDS has been decreased compared to the year 2000, in which about 1.5 million people died of this disease.[1] Despite this decrease in the number of deaths, finding new anti-HIV-1 drugs still remains urgent because of the shortcomings of the common anti-HIV-1 regimens. Most of the American Food and Drug Association (FDA) approved anti-HIV drugs are nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors. In most cases, the 'highly active antiretroviral therapy' (HAART) that is the combination of three or fourt antiretroviral agents has improved the quality of life of the HIV-infected individuals.[2] Some of the most important weaknesses of the current anti-HIV-1 drug therapy are: drug toxicity, multi-drug-resistance of HIV-1 strains, and high costs, especially for the people in poor countries.[3 – 5] Most of the anti-HIV-1 medications target enzymes involved in the intracellular viral replication processes. Targeting more than one step in the HIV-1 life cycle would increase the chance of combating this fatal virus. Some of these targets lay inside the first step of the HIV-1 life cycle, namely, HIV-1 entry into the host cell.[6][7] HIV-1 entrance inhibitors are potentially more precious among anti-HIV medications, because these kinds of compounds can prevent transmission of HIV-1 among individuals and can be used for prophylaxis. A variety of small molecules and peptides with different mechanisms of action have been investigated for their HIV-1 entry inhibitory activity, among them Enfuvirtide (Fuzeon, also known as T20)

and Maraviroc (MVP) are approved by FDA.[8 – 11] Enfuvirtide an HIV-1 CHR-peptide-based compound is the first HIV-1 entry inhibitor that was approved as a drug in 2003 by FDA. Enfuvirtide is used in combination with other anti-HIV-1 drugs for the treatment of adults and children 6 – 16 years old, where all other treatments have failed.[12 – 14] It attaches to HIV-1 gp41 trans-membrane glycoprotein and disrupts its normal function in HIV-1 entry process. It can prevent the infection of uninfected host immune cells. Despite the benefits of this injectable drug, it causes some adverse reactions, such as injection site reactions, peripheral neuropathy, insomnia, depression, *etc*. HIV-1 resistance, high costs, and weak pharmacokinetic properties are some other problems of this drug.[15][16] Maraviroc is the second FDA-approved HIV-1 entry inhibitor drug. It is an antagonist of the CCR5, one of the surface co-receptors on the host immune system cells that helps the HIV-1 entry process.[17][18] Maraviroc has very useful effects on certain strains of HIV-1 and reduces the amount of HIV in the body when is used in combination with other anti-HIV-1 medicines. But, it has no effects when HIV uses the CXCR4 or both the CCR5 and CXCR4 co-receptors.[19] In addition to its limited clinical uses, it suffers from serious side effects, such as liver and heart problems and skin and allergic reactions. Such results make it necessary to find and improve new HIV-1 entry inhibitors with better efficacy, resistance profile, stability, biocompatibility, and bioavailability.

Nowadays computational methods play an important role in discovering new and effective drugs. They help researchers to speed up the drug design process and reduce high costs of experimental methods. Different computational techniques have been applied to HIV-1 entry inhibition drug research. These techniques are divided into three main groups: i) ligand-based methods based on the information extracted from the known biologically active compounds, such as quantitative structure–activity relationship (QSAR), ii) structure-based methods, such as molecular docking and molecular dynamics simulations based on the 3-dimentional (3D) structures of biological targets, and iii) universal methods, structure- or ligand-based, such as 3D QSAR or 3D pharmacophore modeling.

Different classes of HIV-1 entry inhibitors have been reviewed in several articles. The chemical structures and biological activity profiles of the inhibitors have been the subject of these reviews.[20 – 24] In a review articles belonging to 2014, anti-HIV-1 agents have been discussed from the computer-aided design point of view but, not all of the available HIV-1 entry-related targets were considered in that review article.[25] The present review provides updated information regarding the latest reported small-molecule HIV-1 entrance inhibitors considering all the computational studies reported in the literature regarding HIV-1 entry inhibitors since 2014.

2. Viral Entry into the Host Cell

The HIV-1 life cycle begins with the binding of the virus to the host cell and then its entry. This step is very important and is composed of several complicated stages. In the first step, the viral envelope glycoproteins attach to the CD4 T lymphocyte receptors. Then, the complex of viral envelope and CD4 receptor binds to one of the chemokine co-receptors, CCR5 or CXCR4. The last step is the fusion of viral and host cell membranes that is mediated by two viral envelope glycoproteins, gp120 and gp41, leading to the viral genome transformation into the host cell. Entry and fusion of HIV-1 can be stopped by inhibiting any of the proteins that have an important role in this process.

2.1. *Gp41 and Gp120 Functions*

Gp120 and gp41 are originated from a single polyprotein, gp160, which is cleaved in the Golgi apparatus by furin or furin-like proteases into two glycoproteins weighting 120 and 40 kilodaltons, respectively.[26] These two proteins are the only exterior proteins of HIV-1 lipid membrane, gp120 a surface protein and gp41, a trans-membrane one. The first steps of attachment begin with electrostatic interactions between the domains possessing positive charges on gp120 and negatively charged proteoglycan parts of CD4 receptors on the host cell surface.[26] At that point, a conformational change in gp120 exposes some parts of this glycoprotein for binding to co-receptors on the target cell, CCR5 or CXCR4. The Phe43 pocket on gp120 is a good target for designing HIV-1 attachment inhibitors. Other relative conserved regions in gp120, such as the $\beta 20 - \beta 21$ strands have also been mentioned as targets for drug design.

After binding of gp120 to the host cell receptors and co-receptors, gp41 undergoes some conformational changes. N-Heptad repeat (NHR) and C-heptad repeat (CHR), two important parts of gp41, join together to form a six-helix bundle (6-HB) structure. 6-HB formation can be inhibited following the attachment of a suitable compound to CHR or NHR regions. A conserved hydrophobic pocket on NHR trimer is a good target for developing new compounds as HIV-1 fusion inhibitors.

Chemicals that target gp120 seem to be more effective as HIV-1 entry inhibitors compared to gp41 inhibitors. The reason is that the CD4 binding site of gp120 is more accessible in comparison with gp41 hydrophobic pocket, which is exposed after the attachment of gp120 to CD4 just for a few minutes. However, gp41 inhibitors, targeting the hydrophobic pocket, have broader spectrum of activity against different HIV strains, since this part of gp41 is more conserved compared to CD4-binding site in gp120.[20]

2.2. *CCR5 and CXCR4 Functions*

The two co-receptors, CCR5 and CXCR4, on the host cells are also mentioned as targets for anti-HIV-1 drug design. They help gp120 and gp41 recognize the host cell and complete entry process. CXCR4 is involved in other physiological processes besides virus infection such as: chemotaxis, cell survival and proliferation, intracellular calcium flux, and gene transcription. This receptor has roles in some diseases such as cancer, inflammations, and different kinds of infections. Because of these various effects of CXCR4, its antagonists may have some side effects. However, no side effects of CXCR4 antagonists are reported and more long-term studies are needed.[8] CCR5, another co-receptor on the host cell, has a more important role in viral entry compared to CXCR4 and is a more attractive target in comparison with CXCR4 for anti-HIV-1 drug design. Presence of CCR5 is not essential for human immune system and its survival; thus, unlike CXCR4 inhibitors, CCR5 antagonists would not have significant adverse effects.[27]

3. HIV-1 Entry Inhibitors

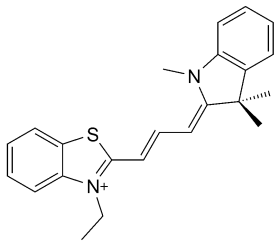
3.1. *Gp 120 Inhibitors and CD4 Mimetics*

Shibo Jiang et al. presented a very good review about small-molecule HIV-1 entry inhibitors that target gp120 and gp41 in 2016. They also prepared a patent review regarding the small molecule inhibitors of these glycoproteins in 2017. In this part, we summarize some newly reported inhibitors for gp41 and gp120 that have not been considered in the two recent reviews.

Human neutrophil peptide-1 (HNP-1) is a member of human innate immunity system, having broad antimicrobial, cytotoxic, and anti-adenoviral activities. Detailed studies on the anti-HIV-1 activity of HNP-1 identified that it can inhibit HIV-1 entry into the immune host cells by inhibiting the binding of viral envelope to CD4 and co-receptors. Some screening processes have been performed on the compounds having the structural properties of the critical residues responsible in antimicrobial activities of HNP-1. The benzothiazolium derivative **1** (5660386; *Figure 1*), extracted from screening has been reported in 2015 as a novel HIV-1 entry inhibitor. This compound exerts its activity by inhibiting the interaction of gp120 to CD4. A broad-range activity against primary HIV-1 isolates from different geographical areas and subtypes was observed for this compound.[28]

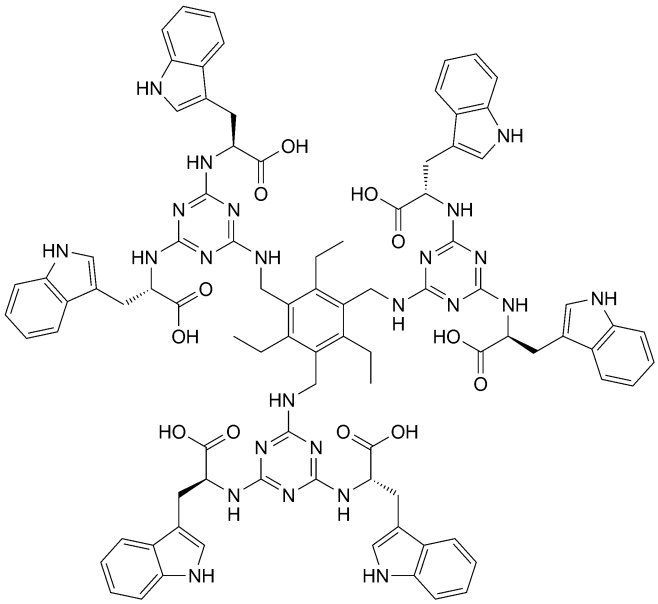
It has been proven that lectins (carbohydrate-binding proteins) have anti-HIV-1 activity by blocking virus-to-cell fusion. They bind to the carbohydrates present on gp120 and prevent normal conformational changes of gp120 and gp41 essential for HIV-1 entry process. Because of their high molecular weight and peptidic nature, nonpeptidic lower molecular weight structures behaving in a similar way as lectins do, are preferred. As a result of some rational investigations, dendrimers with 1,3,5-triazine structures and aromatic amino acids such as tryptophan in trimeric forms have shown good anti-HIV-1 activities. More modifications on this class of compounds showed that dendrimers with more than six tryptophan residues (*i.e.*, 9, 12, 15, and 18) on the periphery had more anti-HIV-1 activity than the lead compound **2** (EC_{50} 16 μM; *Figure 2*). Anti-HIV evaluations showed that the presence of at least nine tryptophan residues is necessary for their activity. The triazine spacer arms are not crucial for the antiviral activity and can be replaced by other moieties. Time-of-addition experiments on some of these compounds revealed HIV-1 entry inhibition activity of these compounds. Binding of the studied compounds to gp120 and gp41 was proven by surface plasmon resonance studies. The compound with 15 tryptophans on the periphery had the best anti-HIV-1 activity with EC_{50} of 2.2 μM.[29]

Cinnamon is a well-known spice that is also used broadly in traditional medications. Anti-inflammatory, antimicrobial, antioxidant, immunomodulatory, and some other effects have been attributed to cinnamon



1 (5660386)

Figure 1. Chemical structure of compound **1** (5660386).



2

Figure 2. Compound **2** with six tryptophan residues and triazine spacer arms.

extracts. Some compounds in these extracts with chemical structures like tannins have shown anti-HIV-1 and anti-HIV-2 effects. IND02, a type A procyanidin polyphenol which appears in trimeric and pentameric forms, has been reported in 2016 as an anti-HIV-1 agent against both R5- and X4-tropic viruses. This compound exerts its anti-HIV-1 activity by inhibiting the binding of envelope to CD4 and heparan sulfate. IND02-trimer (*Figure 3*) can limit T cell exhaustion by down-modulation of the inhibitory receptors Tim-3 (T-cell immunoglobulin and mucin-domain containing-3) and PD-1 (programmed cell death protein 1) on $CD4^+$ and $CD8^+$ cells. These natural compounds could be regarded as cost-effective entry inhibitor candidates for more investigations.[30]

One of the interesting sites on gp120 for drug design is a conserved part located at the base of the V3 loop. This site binds to the sulfated N-terminus of CCR5 in HIV-1 entry process. Some small-molecule hydrazonothiazolyl pyrazolinones with naphthyl di- and tri-sulfonic acids (*Figure 4*) were introduced as anti-HIV-1 compounds that mimic the natural sulfated CCR5 N-terminus. The disulfonic acids were the most potent compounds. Surface plasmon resonance tests showed that these compounds bind to trimeric gp120 and docking studies also verified the structure–activity relationship results.[31]

Curreli et al. in a continuous work reported two potent compounds, NBD-14088 and NBD-14107 (**7** and **8**, respectively; *Figure 5*), as CD4 mimic entry inhibitors that target the Phe43 cavity of HIV gp120. These compounds had better anti-HIV-1 effects and ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties than the potent HIV-1 attachment inhibitor Temsavir **9** (BMS-626529; *Figure 5*). Fostemsavir, the prodrug of Temsaviris is under phase III clinical trials.[32] Sixteen clinical studies on pharmacodynamics, safety, pharmacokinetics, and drug-drug interactions of BMS-663068 and BMS-626529 have been completed but no study results were posted until now.[33]

NBD-11021A2 (**10**; *Figure 6*) has been previously reported as a viral entry antagonist targeted for the Phe43 cavity of gp120. This compound has been used as a template to design some new compounds. Among the compounds evaluated for viral entry inhibition activity, the ones with thiazole ring and a simple primary amine ($–CH_2NH_2$) had the most potent antiviral activity and were the least cytotoxic agents. Compound **11** (NBD-14010; *Figure 6*) had the best antiviral activity against a wide variety of HIV-1 subtypes and also cell-to-cell HIV-1 transmission inhibition activity.[34]

In a study based on NBD compounds, **12** (YYA-021; *Figure 7*) with CD4 mimicking properties, was introduced with anti-HIV-1 activity and low cytotoxicity. As a result of its hydrophobicity, wide tissue distribution and relatively high distribution volumes in rats and rhesus macaques was observed in pharmacokinetic tests. By more investigations, a more lipophilic

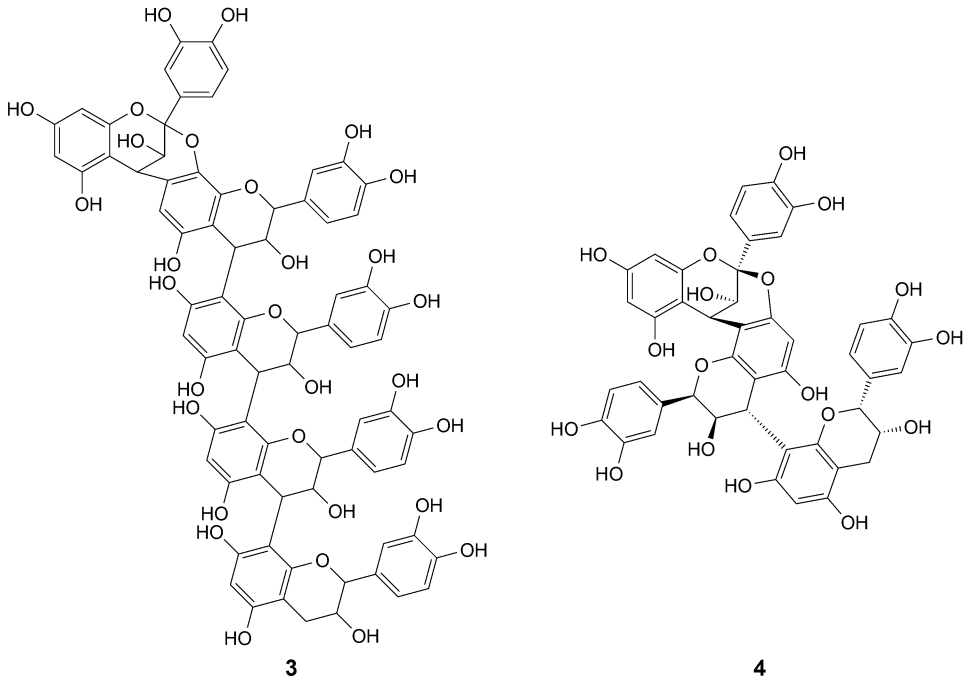


Figure 3. Structures of the IND02, a type A procyanidin polyphenol in pentameric and trimeric forms.

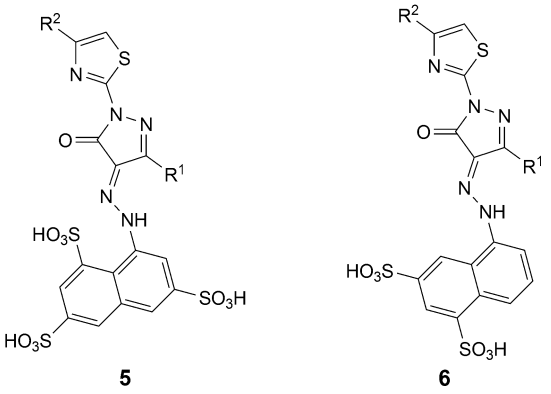


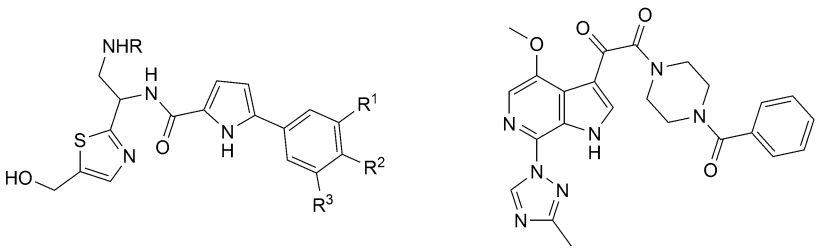
Figure 4. Two hydrazonothiazolyl pyrazolinones with naphthyl di- and tri-sulfonic acid derivatives as gp120 inhibitors.

compound **13** with an 1,3-benzodioxolyl moiety (*Figure 7*) was found that had lower tissue distribution. This compound had relatively high anti-HIV activity, low cytotoxicity, and an appropriate pharmacokinetic profile in a rhesus macaque, and can be regarded as a good lead for HIV-1 entry inhibitors.[11]

Thiol/disulfide exchange processes both on the surface of CD4 receptors on the host cells and in the gp120 Env proteins of HIV are required for successful HIV entry. Compounds that inhibit this exchange process can be classified as a new generation of HIV entry inhibitors. *Szilvia Kanizsai et al.* introduced some thiolated pyrimidine derivatives that interact with redox active SH groups required for successful HIV-1 entry. *In vitro* experimental data show that these compounds can effectively inhibit infectivity of primary HIV-1_{IIIB} strain and both HIV-1 R5- and X4-tropic pseudovirions and virally induced cell fusion.[35]

Signal peptides (SP) are attached to some of the newly synthesized type 1 transmembrane proteins such as cell-surface CD4 receptors. They help translocation of the nascent proteins from endoplasmic reticulum during translation to the lumen and then to the cell membrane. After this translocation SP cleaves, some compounds can bind to signal peptides and inhibit their function in the displacement of the transmembrane proteins. Some new unsymmetrical compounds were designed, synthesized, and evaluated *in vitro* for CD4 down-modulation and anti-HIV-1 effects. The most potent compound was **14** (IC_{50} = 63 nM; *Figure 8*).

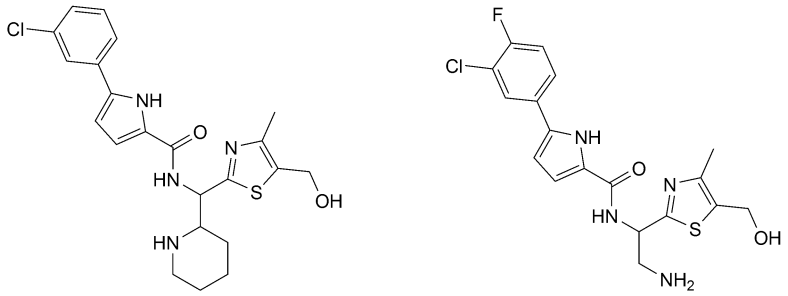
For these compounds with cyclotriazadisulfonamide structure, there was a significant correlation between the pIC_{50} values of the compounds for CD4 down-modulation and the component of the electric dipole moment. This correlation shows that the electron distribution in the non-tosyl side arms has the most important role in the stabilization of the CD4 SP-ligand complex. The electric dipole moment lies in the aromatic ring plane and along the C–S bond of the arenesulfonamide group. It also indicates a π–stack interaction between one side arm and a key functional group in the CD4 SP. This interaction stabilizes the complex of molecule and the CD4 SP target. This



7 (NBD-14088) R^1 = F, R^2 = Me, R^3 = H
8 (NBD-14107) R^1 = F, R^2 = Cl, R^3 = F

9 (BMS - 626529)

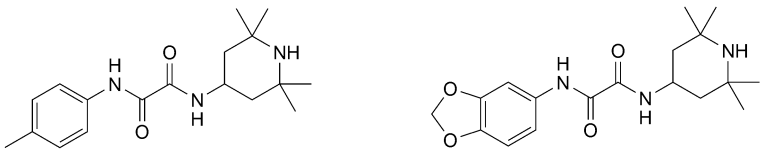
Figure 5. Chemical structures of compounds **7** – **9** with CD4 mimetic activities.



10 (NBD-11021A2)

11 (NBD-14010)

Figure 6. NBD-11021A2 (**10**) and NBD-14010 (**11**), two gp120 inhibitors.



12 (YYA-021)

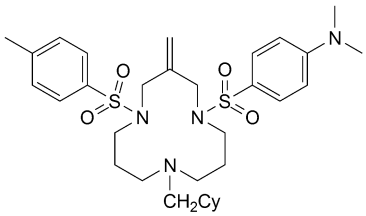
13

Figure 7. Chemical structures of compounds **12** and **13**.

information can help to find more potent analogs of this scaffold as anti-HIV-1 compounds.[36]

Aloperine and its derivatives belong to a new class of HIV-1-entry inhibitors with chemical structures different from other HIV-1 entry inhibitors. More than 30 derivatives of this compound were synthesized and their anti-HIV-1 activity was evaluated. The length of amine linkers and the cyclical terminal groups and their substitutions had important effects on their anti-HIV-1 activity. **16** (*Figure 9*) was the best compound with the EC_{50} value of 0.69 μM. Aloperine, as a new lead compound, inhibits both R5 and X4 viruses with a mechanism of entry inhibition different from that of Maraviroc.[37] More investigations on the optimization of the anti-HIV-1 activity of this group of compounds led to a 15-fold increase in anti-HIV-1 activity for compound **17** (*Figure 9*). Studies on the mechanism of anti-HIV-1 activity of this compound proved its binding to gp120 receptor.[38]

A new group of spiro mono-cyclohexyl-type compounds with a guanidine group as a substituent on the nitrogen atom was introduced as CD4 mimetic ligands. Molecular modeling results from the docking of the most potent compounds into the gp120 binding site (PDB ID:3TGS) using the MOE software package, showed hydrophobic interactions with the isopropyl group of Val430 and electrostatic interactions with the carboxy group of either Asp368 or Asp474 of gp120. Remarkable synergistic anti-HIV-1 activity was observed with co-administration of the most potent compounds YIR-821 and YIR-819 (**18** and **19**, respectively; *Figure 10*) with the neutralizing antibody KD-247. Assay results and molecular modeling of **18** and **19** showed that the length of the linker between the piperidine nitrogen atom and the guanidine moiety is important for appropriate interactions with Asp368 and Asp474. The guanidino moiety in the structure of this series of compounds, in which the



14

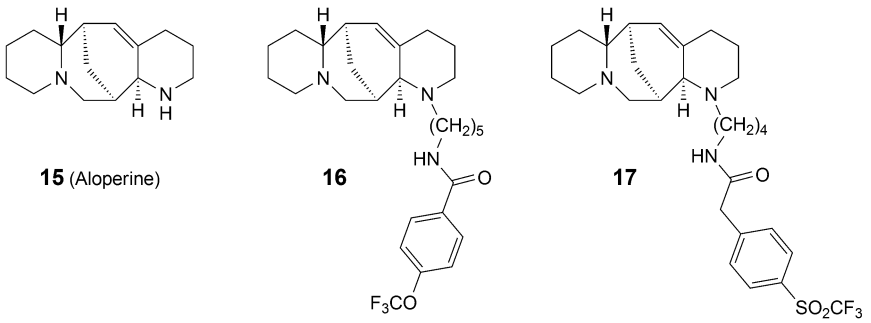
Figure 8. Compound **14**, a signal peptide inhibitor with CD4 down-modulation activity.

piperidine nitrogen atom is directly amidinated or have a linker which is shorter than the linker in the structure of **19**, cannot interact efficiently with Asp368. Compound **18** with the longest linker had the best anti-HIV-1 activity (IC_{50} = 2.6 μM).[39]

A variety of applications of structure-based computational procedures in designing and finding new gp120 inhibitors and CD4 mimics have been reported. For example, *Teoh et al.* proposed L-biphenylalanine as a gp120 attachment inhibitor using molecular modelling and docking studies in which the crystal structure of gp120-CD4 protein complex was the target for the ligand attachment. The HIV-1 entry inhibition activity of L-biphenylalanine was verified by CD4 capture enzyme-linked immunosorbent assay (ELISA) experiments with a submicromolar IC_{50}. It can be a good drug candidate because of its good anti-HIV-1 activity, low cytotoxicity, and fulfillment of 'the *Lipinski*'s rule of five' criteria.[40]

In another study, five new compounds were introduced as possible CD4-mimetics and HIV-1 entry antagonists by virtual screening and molecular modelling tools. In the first step, amino acids which have the most critical roles in the binding of CD4 to gp120 were selected from CD4–gp120 crystal structure. Then, some pharmacophore models showing the hotspots of primary CD4 receptor for binding to gp120 were created. These models were used as templates for finding new CD4 mimetic compounds by the pepMMsMIMIC virtual screening platform and the MMsINC database. In the next steps, docking-based virtual screening, molecular dynamics simulations (MD), and binding free energy calculations were used to find the best candidates. Compound **20** (MMs03927209) had the best properties, *i.e.*, fulfillment of 'the *Lipinski*'s rule of five' criteria and highest binding energy to the key hotspots of the CD4-binding site, for a proposed hit as a CD4-mimetic and HIV-1 entry inhibitor (*Figure 11*).[41]

In a work by *Bruno Melillo et al.*, some new small-molecule CD4-mimetics that target the Phe43 cavity of the envelope glycoprotein gp120 of HIV-1 were introduced based on computational, thermodynamic, and crystallographic data of a previously reported anti-HIV-1 entry inhibitor (compound **21**; *Figure 12*). The protein-ligand crystal structure of **21** showed some regions as potential binding hotspots near the aromatic portion of the indane. According to this information, some modifications were carried out in the indane moiety. The best spacer between the indane skeleton and the guanidinium moiety in **21** was proven to be a methylene (one carbon) structure. A computational analysis including a virtual fragment growing algorithm and docking showed the contacts between the new designed ligands and 'hotspot' Gly472. According to the computational results, it was suggested that a (methylamino)methyl unit at position 6 of the indane skeleton will result in additional hydrogen bonding with the backbone carbonyl of Gly472. These compounds were synthesized and their anti-HIV-1 activity and binding affinity to gp120 were evaluated. The newly designed compound **22** (*Figure 12*) showed an improved inhibitory activity versus compound **21**. The X-ray diffraction data of (+)-(*R*,*R*)-**22**:gp120 complex showed two additional hydrogen-bonding interactions, in agreement with the computational prediction. Compound (+)-(*R*,*R*)-**23** (*Figure 12*), the other newly designed molecule showed good



15 (Aloperine) **16** **17**

Figure 9. Chemical structures of aloperine and its derivatives.

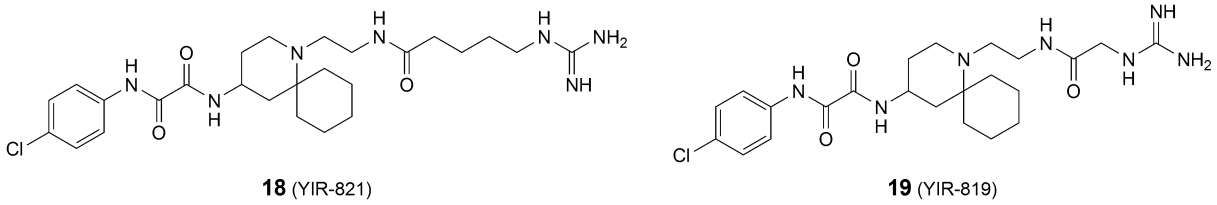


Figure 10. YIR-821(**18**) and YIR-819 (**19**), two CD4 mimetic ligands.

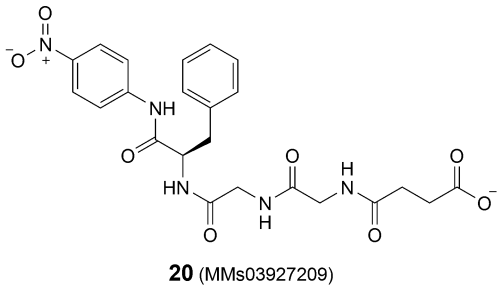


Figure 11. Chemical structure of compound **20** (MMs03927209), an anti-HIV-1 compound with CD4-mimetic activity.

inhibitory potency against the neutralization-resistant HIV-1 strain JR-FL.[42]

The information extracted from structure-based methods such as molecular docking and MD simulations have been used to find out the mechanism of inhibitory activity of some gp120–CD4 binding inhibitors. For example in 2003, *Kawai et al.* introduced (−)-epigallocatechingallate (EGCG; *Figure 13*) from green tea, as one of the most potent inhibitors of gp120–CD4 binding. In 2016, *Adel Hamza* and *Chang-Guo Zhan* reported some mechanistic insights gained from computational methods including molecular docking, molecular dynamics (MD) simulations, and binding free energy calculations. These computations showed the most stable structures of solvated glycoprotein gp120, glycoprotein CD4, and EGCG. For each of the three complexes of CD4 -EGCG, gp120-CD4 and gp120-CD4–EGCG solvated in water, stable MD trajectories have been obtained. It was understood that in the most favorable simulated complexes of both CD4–EGCG and gp120-CD4–EGCG, the studied inhibitor (EGCG) was more stable in the gp120 binding site of CD4. It shows that because of the placement of EGCG in gp120 binding site of CD4, gp120 could not be placed in this site and inhibition of HIV-1 entry into the host cell happens. The binding free energy calculations were done using the molecular mechanics-*Poisson–Boltzmann* surface area (MM-PBSA) method.[43] The free energy of the inhibitor binding, ΔG_{bind}, in MM-PBSA method is calculated by the following equation:

$$\Delta G_{bind} = G_{cpx} - (G_{rec} + G_{lig}) \quad (1)$$

where G_{cpx} is the free energy of the receptor-ligand complex, G_{rec} is the free energy of the unbound receptor and G_{lig} is the free energy for the unbound ligand. The calculated free binding energies (ΔG_{bind}) were −5.5, −9.9, and −2.9 kcal/mol for EGCG binding with CD4, gp120 binding with CD4, and gp120 binding with the CD4-EGCG complex, respectively. These values showed that the binding of EGCG with CD4 can effectively inhibit gp120–CD4 binding. The calculated results are in agreement with the observed experimental results that EGCG can inhibit HIV-1 entry into the immune host cells by blocking the gp120–CD4 binding site of CD4.[44]

Pharmacophore models as another technique in structure-based drug design have also been used to find more suitable BMS piperazine-based gp120 inhibitors. This group of entry inhibitors consists of the most broadly acting and potent anti-HIV-1 compounds. Their anti-HIV-1 activity on a broad range of viruses shows that their binding site on the target protein is well conserved. In a scientific work for finding compounds which can be regarded more drug-like than the BMS group, a combination of field-based three-dimensional similarity virtual screening experiments using Blaze (Cresset, Litlington, UK) with a high-content field-based pharmacophore template derived from BMS-626529 (*Figure 5*) and Field Templater (Forge) were used. A template of the three-dimensional conformation of the lead compound in binding to the target was created. The field point pattern was used to screen a dataset of six million commercially available compounds using Blaze. Fifty compounds with more similarities to BMS-626529 as the template structure were purchased to evaluate their biological effects by the single-round infection assay. Compound **25** (*Figure 14*) was the most potent one with an IC_{50} value of half of the lead compound. In order to improve the ADMET properties of this compound, some *in silico* bioisosteric replacements (scaffold-hopping) experiments using Spark (Cresset, UK) were performed. The final compound was SC11 (**26**; *Figure 14*) with a nanomolar potency that

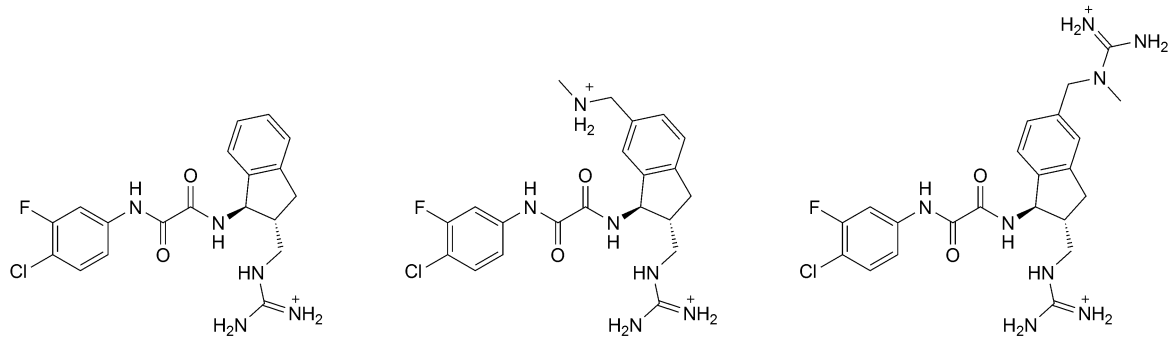


Figure 12. Chemical structures of compounds **21**, **22**, and **23**.

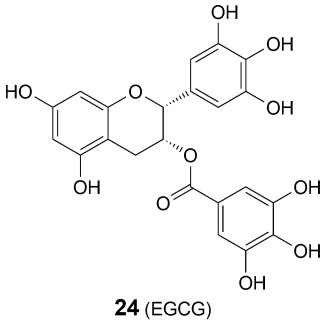


Figure 13. EGCG from green tea.

prevents HIV-1 entry. The new dipyrrolidine scaffold showed varying degrees of potency against different subtypes of HIV-1 isolates.[45]

QSAR studies as ligand-based methods have also assisted medicinal chemists to get more information about the most important properties of gp120 inhibitors for better anti-HIV-1 effects. Two following paragraphs consider the recently reported articles which have used QSAR models for designing new gp120 inhibitors based on some existing HIV-1 entry inhibitors.

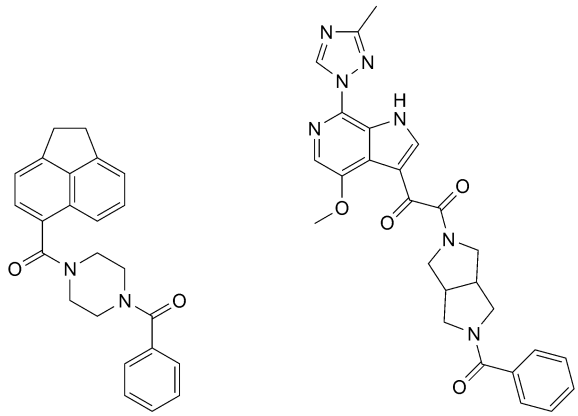
As it is shown, the attachment of gp120 to CD4 receptors on the T-cells is one of the most important stages in HIV-1 entry process. Before this attachment, a 15-residue fragment within the gp120 undergoes a conformational switch from β-sheet to α-helix. There are some anti-HIV-1 compounds that are introduced as switch inhibitors. These compounds have some common chemical characteristics. The presence of at least two-six-membered rings, often but not always aromatic, with a certain distance is essential. The presence of potential negative charges in a resonance form of the carbonyl groups is the second necessary factor. *Reed* and *Kinzel* found a number of exogenous inhibitors. Subsequently by the help of QSAR method, their most important common chemical characteristics were determined for a better design of anti-HIV-1 compounds with gp120 switch inhibitory activity.[46]

A three-dimensional QSAR model by the use of comparative molecular field analysis (CoMFA) was presented by *Asim Kumar Debnath et al.*, based on some porphyrin-type compounds with anti-HIV-1 activity, targeted to the V3 loop of the gp120 envelope glycoprotein of HIV-1. This model had a high cross-validated r^2_{cv} (0.590), a high correlation coefficient of the 'fitted model' and a low standard error of estimate, suggesting a good predicting tool for designing better porphyrin-type anti-HIV-1 compounds with gp120 inhibitory activities. CoMFA analysis showed some necessary structural elements of the porphyrin derivatives required for anti-HIV-1 activity. The electrostatic contour plots showed that meso-tetraphenylporphine type compounds need at least three carboxylic acid groups on the phenyl ring for a significant anti-HIV-1 activity. These three negatively charged carboxylic acid groups were proven to be able to interact with positively charged interaction sites on the receptor. Both favorable and unfavorable steric effects were indicated by steric contour plots. These electrostatic and some hydrophobic interactions can lead to distortion of the conformation of the V3 loop of the gp120 and inhibition of its binding to CD4 receptor and virus entry.[47]

3.2. *CCR5 Inhibitors*

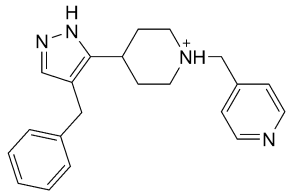
After the review article of *Liu et al.* in 2014,[48] some novel researches have been made both experimentally and theoretically on CCR5 inhibitors. A conclusion on the compounds prepared for this purpose can be made as follows.

A series of pyrazolo-piperidines were reported to target three distinct proteins to exert the anti-HIV-1



25 **26** (SC11)

Figure 14. Chemical structures of compounds **25** and **26** (SC11) as gp120 inhibitors extracted from pharmacophore modeling and virtual screening methods.

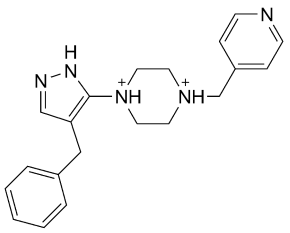


27

Figure 15. Chemical structure of compound **27** as a CCR5, CXCR4, and reverse transcriptase inhibitor.

activity: reverse transcriptase, CCR5, and CXCR4. The most potent compound **27** (*Figure 15*) was identified through GPCR-guided screen (G-protein coupled receptor) method. The (non)nucleoside reverse transcriptase (NNRTIs) inhibitory activity effect of compound **27** was more than its CCR5 and CXCR4 inhibition. This dual chemokine activity can cause a delay in drug resistance in comparison with other NNRTIs.[49]

Using computer-aided methods, *Parish et al.* designed a new synthetically-accessible molecule according to compound **27** with dual inhibition of CCR5 and CXCR4. Experimental data from the previous research had shown higher affinity of **27** for CCR5 than CXCR4. *Parish* and his colleagues evaluated the binding affinity of this compound for CCR5 and CXCR4 by molecular dynamics simulation studies. The results of the computational studies were in a good agreement with the experimental data. Then, they determined the atomistic interactions of **27** in both receptors. The most important interactions were π-stacking of the three aromatic rings, and the interactions between the positively-charged hydrogen bond donor group and the negatively-charged glutamates



28

Figure 16. Compound **28** designed on the basis of compound **27** by the addition of a second protonated nitrogen atom in the piperidine ring of **27**.

and aspartates. But the solvent exposure of those negatively-charged amino-acids decreased because of the presence of **27** in the active site resulting in a destabilizing effect. They decided to change the protonated piperidine of **27** to a doubly protonated piperazine ring to increase the electrostatic interactions and decrease the destabilization effects of the polar solvation energy term and maintain the π-stacking interactions. After all this, they suggested compound **28** (*Figure 16*) as a possible dual inhibitor of CCR5 and CXCR4. Computational studies showed almost a 20% increase in the binding affinity of **28** for the two receptors.[50]

Wang et al. designed a series of (*E*)-3,4-dihydroxystyryl 4-acylaminophenethyl sulfone and sulfoxide derivatives as dual inhibitors of HIV-1 CCR5 and integrase. The crystal structures of CCR5-maraviroc and integrase-raltegravir complexes showed the most important interactions needed for their inhibitory activities. They merged the critical structural elements of some benzanilides as CCR5 inhibitors and caffeic acid phenethyl ester as an integrase inhibitor (*Figure 17*), prepared them, and evaluated their anti-HIV-1 activity. After the successful results of anti-HIV-1 tests, proprietary experiments for CCR5 and integrase inhibitory activities were carried out. In anti-HIV-1 tests, sulfoxides showed better activities than sulfones. The compounds with 3,5-difluorophenyl, cyclohexyl, 4-nitrophenyl, 1-adamantaneformyl, and 4-fluorophenyl groups had the best CCR5 inhibitory activities. Biological assays proved binding affinity toward HIV-1 integrase for the (*E*)-3,4-dihydroxystyryl sulfone and sulfoxide derivatives. Finally, compound **29** with potent CCR5 inhibitory activity and moderate binding affinity with integrase (KD = 141.8 μM) was introduced as a new lead compound with a dual inhibitory activity.[51]

Before 2013, there was no crystallographic structure of human chemokine receptor. For this lack of data, homology modeling methodology was used to predict the structure of CCR5. The X-ray structure of

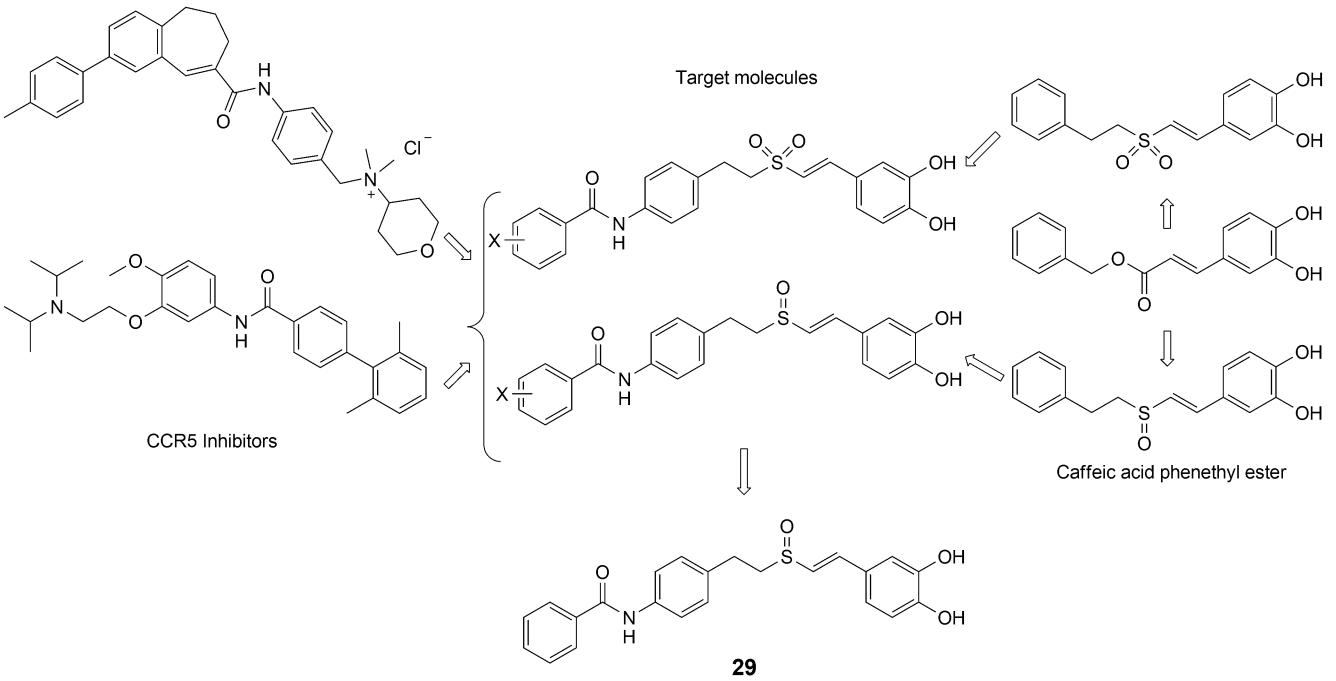


Figure 17. Design of dual inhibitors based on potent CCR5 antagonists and derivatives of caffeic acid phenethyl ester.

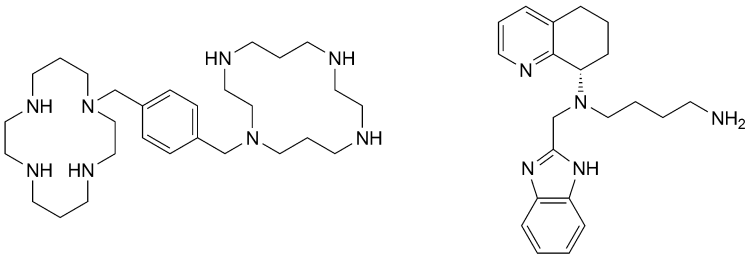
the bovine rhodopsin receptor has been used as a template in many homology modeling studies of CCR5 receptor.[52 – 54] In the obtained 3D structures, different binding sites within the CCR5 extracellular pocket were suggested. In a study, this hypothesis was developed more by a novel spherical harmonic-based consensus shape clustering approach and strongly verified that there are three main binding sites within the CCR5 extracellular cavity.[55] After 2013, several crystallographic structures of CCR5 chemokine receptor were released.[56][57] The reported ligand-binding sites in these reports were different from the computationally predicted sites.

Some extensive quantitative structure–activity relationships investigations have been performed on CCR5 antagonists of different scaffolds by *Shahlaei et al.* Both linear and non-linear statistical models have been considered in their studies. In a study, between principal component-radial basis function neural network (PC-RBFNN) and principal component-generalized regression neural network (PC-GRNN) non-linear regression models, PC-RBFNN model have shown priority for predicting the CCR5 inhibitory activities of a set of CCR5 inhibitors.[58] For 1-amino-2-phenyl-4-(piperidin-1-yl)butane derivatives, both linear: genetic algorithm-multiple linear regression (GA-MLR) and factor analysis in combination with multiple linear regression (FA-MLR) and non-linear: genetic algorithm-least squares support vector machine (GA-LS-SVM) and factor analysis-least squares support vector machine (FA-LS-SVM) models have been studied with FA-LS-SVM as the best predicting model.[59] Statistical models based on neuro-fuzzy inference system with genetic algorithm as the descriptor selection method (GA-ANFIS) have also shown high statistical quality and low prediction errors for some CCR5 antagonists with the general structure of 4-hydroxypiperidine.[60]

Different uses of principle component analysis (PCA), as a helpful mean for data reduction in multivariate data analysis, have also been the subject of study using a dataset of CCR5 inhibitors by the same research group in 2018. The authors have listed various benefits and proved the usefulness of PCA in QSAR studies.[61]

3.3. *CXCR4 Inhibitors*

CXCR4 antagonists are divided into three groups: bicyclams, monocyclam, and non-cyclams. AMD3100 and AMD070 (**30** and **31**, respectively; *Figure 18*) are two reported CXCR4 antagonists. AMD3100 (**30**), the first specific antagonist of CXCR4 was discovered by *Schols et al.* Different modifications on AMD3100 (**30**) were performed to improve its anti-HIV-1 potency.[62] This compound failed clinical tests because of poor bioavailability and adverse effects (such as stem cell mobilization from the bone marrow). This adverse effect was used for transplant patients.[63] AMD3100 (Mozobil) was approved by FDA as a drug with immunostimulant activity to mobilize hematopoietic



30 (AMD3100) **31** (AMD070)

Figure 18. AMD3100 (**33**) and AMD070 (**34**), as CXCR4 inhibitors.

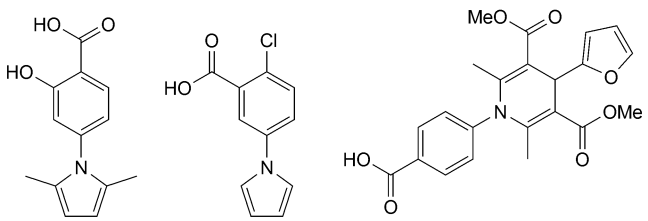
stem cells from the bone marrow into the circulating blood of hematological malignancies patients. Unlike cyclam compounds, non-cyclam antagonists have better bioavailability. AMD070 (**31**) was tested in human cases which have shown X4-tropic virus in their plasma. It had good bioavailability and anti-HIV-1 potency. It was well tolerated and had mixed-order pharmacokinetics. This study was stopped in phase II clinical tests, because of non-clinical reports of hepatotoxicity (cytochrome P450 2D6 inhibition) and histologic findings. Some other CXCR4 inhibitors have been introduced with non-anti-HIV-1 effects that are not related to the present review.[64 – 68]

The first crystal structure of CXCR4 receptor was reported in 2010 with a resolution of 2.5 Å.[69] Although the revealed crystal structures of CXCR4 receptor were different from the computationally constructed structures, many valuable trials were designed and proposed based on the computationally-derived models.

In 2008, *Nueno et al.* reported a comparative study between ligand-based and receptor-based virtual screening of HIV entry inhibitors for the CXCR4 and CCR5 receptors. They wanted to know which way was better for finding potential CXCR4 and CCR5 antagonists. They built homology models of CXCR4 and CCR5, using bovine rhodopsin (PDB code 1HZX) as the template. Then, a large database of CXCR4 and CCR5 antagonists from the literature was prepared. A set of 4696 inactive compounds with several similar 1D properties to the active compounds was collected. The docking results of these compounds were compared to the reference ligands of CXCR4 and CCR5. This large database was used to perform retrospective virtual screening of antagonists against these two targets and also to compare docking-based and ligand-based virtual screening methods. The results of dockings were used to rank the ligands databases. The final results showed the superiority of the ligand-based searches to the docking-based approaches, especially for CXCR4 inhibitors.[55]

3.4. *Gp41 Inhibitors*

Fassihi et al. presented a series of novel 4-arylpyridin-1(4*H*)-yl)benzoic acid derivatives designed based on their structural similarities with the known gp41 inhibitors, NB-2 and NB-64 (**32** and **33**; *Figure 19*). The other necessity for an effective gp-41 inhibitor, *i.e.*, the proper length to occupy the hydrophobic pocket of the gp-41 active site was also considered in the structures that they designed. These compounds showed moderate to good inhibitory activity against HIV-1 growth without any significant cytotoxicity on MT-2 cell line. Compound **34** (*Figure 19*) was the most potent one in inhibition of P24 expression at 100 μM with inhibition percentage of 84.00%. The authors believed that the presence of 2-furyl and 2-thienyl hetero-aromatic substituents at the C4 position of 1,4-dihydropyridine helps improve the anti-HIV-1 activity and decrease the cytotoxic effect on MT-2 cell line.



32 (NB-2) **33** (NB-64) **34**

Figure 19. Chemical structures of compounds **32** – **34**.

Docking studies performed in this report showed good electrostatic and hydrophobic interactions with the gp41 binding site.[9]

In continuation of the recent reported 4-arylpyridin-1(4*H*)-yl)benzoic acid derivatives, *Fassihi et al.* introduced some novel tetrahydropyrimidines as HIV-1 entry inhibitors as well. These compounds possessed the necessary structural elements of HIV-1 gp41 inhibitors NB-2 and NB-64 (**32** and **33**; *Figure 19*), such as a carboxylic group for electrostatic interactions with Lys574 or Arg579, some hydrophobic moieties to interact with Val570, Trp571, Leu568, and Trp631 and some hydrophilic groups for hydrogen bond interactions with the amino-acids in the active site of gp41. Evaluation of the anti-HIV-1 activity of these compounds showed moderate to good activities. Using *in silico* methods, they showed that all of these compounds had proper pharmacodynamics, pharmacokinetics, and drug-likeness properties. Docking studies also verified the biological effects and showed almost all the predicted interactions of the designed compounds with the critical amino acids of the lipophilic pocket of gp41. Biological results showed the best anti-HIV-1 activity for the compounds with 4-chlorophenyl substituent and 4-methylphenyl group at the C4 position of the tetrahydropyrimidine ring (*Figure 20*).[70]

So far, no review regarding the *in silico* reports on the gp41 inhibitors has been published yet. Most of the computational studies on this class of inhibitors have used structure-based methods for designing new ligands or proposing mechanistic information about the inhibition processes. In this part, we present a summary of these computational works.

In 1999, a database containing 20,000 organic compounds, commercially available from Com Genex, Inc., Budapest, Hungary, was screened by docking into the gp41 core structure with the help of the DOCK suit of programs. Sixteen compounds with the best binding energies were selected to be tested in enzyme-linked immunosorbent and virus inhibition assays. ADS-J1 and ADS-J2 (*Figure 21*) were introduced by this structure-based strategy for more investigations.[71]

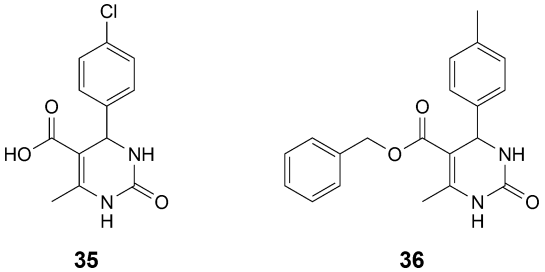


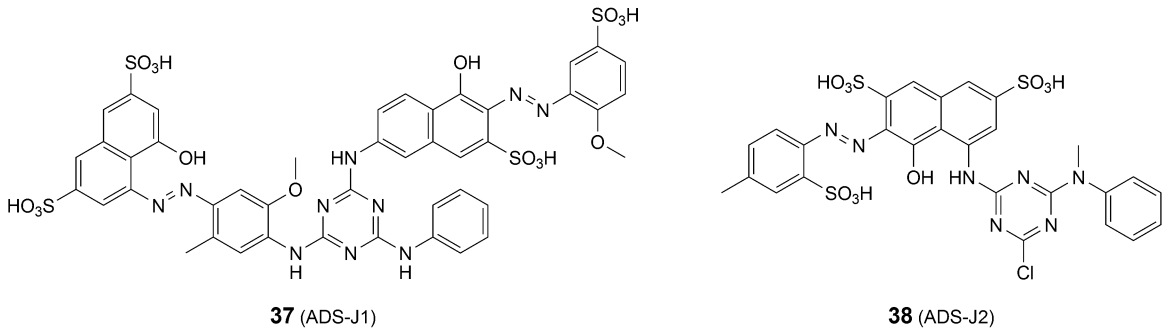
Figure 20. The most potent derivatives of tetrahydropyrimidines with anti-HIV-1 activities.

Pandey et al. reported a very interesting use of molecular dynamics (MD) simulations study in recognizing the most important amino acids responsible for 6-HB formation. The high decomposition energies of Gln, Ser, and Ile showed their critical role in the formation of 6-HB structure in HIV-1 entry process. Electrostatic interactions were also proven to be very necessary for a good binding between NHR and CHR regions of gp41 for the formation of 6-HB structure. Thus, effective inhibitors of 6-HB formation should have the ability to connect to these critical residues by proper interactions and inhibit the CHR-NHR binding.[72]

In another structure-based study, the binding modes between a small molecule gp41 inhibitor and the large groove of gp41 were explored. Molecular docking and molecular dynamics simulations presented five energetically favorable models in this report. According to the data from other experimental studies such as the binding modes determined from crystallography of inhibitors bound to C-peptide, mutation data for N-peptides and viral entry inhibition by C-peptides, one binding mode was selected as the most favorable one. By the help of the interactions shown in this binding model, new ligands can be designed and compound databases can be screened more rapidly.[73]

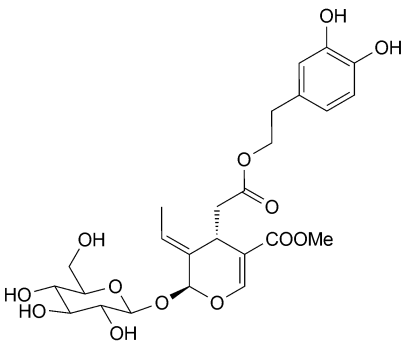
Oleuropein (*Figure 22*) and its metabolites from olive leaf extract have anti-HIV-1 effects by inhibition of gp41. In a computational study, docking, molecular dynamics simulation, and molecular mechanics energies/*Poisson–Boltzmann* surface area (MM-PBSA) calculation were used to explore detailed interactions of these compounds with lipophilic pocket of gp41. Conserved hydrophobic cavity located at the N-terminal of gp41 core N36 trimer structure was considered to be the most possible binding site. The dihydroxyphenol ring was determined as the most important group for fusion inhibitory activity in these compounds. Glucoside moiety in the oleuropein molecule was shown to decrease anti-HIV-1 activity. Polyacrylamide gel electrophoresis (PAGE) and circular dichroism spectroscopy were finally used to evaluate the anti-HIV-1 activity of these compounds. The experimentally achieved data were in agreement with the computational predictions.[74]

Some ligands with probable gp41 inhibitory activity were designed by exploring the mode of interaction of 62 inhibitors of 6-HB formation reported in the



37 (ADS-J1) **38** (ADS-J2)

Figure 21. Chemical structures of ADS-J1 and ADS-J2.



Oleuropein (**39**)

Figure 22. Oleuropein from olive leaf extract inhibits gp41of HIV-1.

literature. Molecular docking and molecular mechanics/generalized born surface area calculations (MM/GBSA) were used in this study. Extra precision (XP) docking, induced fit docking, and quantum mechanics-polarized ligand docking were used to increase the accuracy of docking. MM/GBSA calculations were then carried out for the approximation of binding affinities of the ligands in gp41 lipophilic pocket. Finally, good correlation was obtained between the experimental pIC_{50} and computational results. By the help of the obtained results, two groups of new ligands with dipeptide, and small molecule structures with the essential structural framework were designed and their pIC_{50}s were predicted. The pIC_{50} values of them were better than the experimentally active reported ligands.[75]

As it was shown, the highly conserved hydrophobic cavity at the N-terminal of NHR trimer of gp41 is a good target for anti-HIV-1 drug design. In a non-experimental study, computational solvent mapping method (CS-Map) was used to search the hydrophobic cavity of gp41 NHR trimer to find potential hot spots for inhibitor design. CS-Map algorithm can find major contributors to the binding energy of a protein which are the most important binding sites on it. This method can identify the most energetically favorable binding positions by moving small organic functional probes all over the protein surface. The places where the highest number of different probe clusters overlap can be the hot spots of the protein. Two cavities on NHR region, occupied by Trp628 and Trp631 on CHR peptides during the formation of 6-HB structure, were recognized as the best druggable sites for small-molecule inhibitor design. Besides lipophilic amino acids in these cavities, two positively charged amino acids, Lys574 and Arg579, were identified as potential hot spots on gp41 NHR trimer. Molecular docking and MD simulations studies showed that NB-2 and NB-64, two pyrrole derivatives with 6-HB formation inhibitory activity,[76] with similar chemical structures, interact with different amino acids in NHR region. Some low-molecular weight molecules in agreement with the structural properties for a potent lead compound as HIV-1 fusion inhibitors were proposed by a virtual screening method.[77] In another study, the binding complexes of N-substituted pyrrole derivatives with gp41 core structure were explored in more details by docking and MD simulations and MM-PBSA calculation. The detailed interactions between molecules and the protein and the free energies of binding could help find out more information about the binding mechanisms and propose new ligands with better inhibitory activities.[78]

A creative docking-rescoring method using molecular footprint comparisons was invented by *Rizzo et al.* in 2011. In this method by per-residue breaking down of *Van der Waals* and electrostatic interactions for a ligand with its target, the most important groups of residues for binding can be identified. In virtual screening studies, footprint-based methods can help find the best molecules for a certain target in a quantitative and logical way. In a virtual screening study, footprint similarity (FPS) method was used to help a better selection among 500,000 publically available compounds for HIV-1 gp41 target. After docking of these molecules, groups of rank-ordered ligands

passed two filters of FPS method: i) ligand pose properties which included DCE (DOCK Cartesian energy) and FPS score distributions, number of rotatable bonds, molecular weight, ligand formal charge, ligand efficiency, volume overlap, and footprint comparisons between experimentally tested active and inactive compounds and ii) ligand pose stability which included root-mean-square deviation (rmsd)-stability and footprint-stability. In this way, the molecules that best mimic properties of a reference molecule will be selected. In the case of gp41, a native peptide substrate was used as the reference molecule. At the end of this study, seven active compounds, tested experimentally previously, showed high footprint overlap to the reference relative to the inactive group.[79]

In a virtual screening study on a dataset of 7174 compounds collected from available databases such as ZINC, PubChem, and Binding DB, certain stages and filters have been applied to find some new compounds with the probable ability of binding to gp41. Compounds were selected based on the known key contacts of the reported inhibitors with gp41 binding site residues. Then, by docking of these compounds inside gp41 binding site, those with the best binding energies were selected. In order to pick the compounds with proper oral pharmacokinetic profiles (*Lipinski*'s rule of five) and ADMET properties, some filters were used. Docking of the compounds predicted as active ones resulted in a small series of potent compounds. Three compounds with the best pharmacokinetic and pharmacodynamic profiles were finally suggested after molecular dynamics simulation studies on the complex of the inhibitor inside with gp41 binding site.[80]

In several research articles, the MD simulations of gp41 have disclosed the detailed interactions of NHR/CHR regions of gp41 together and with their ligands. This information provides important clues for rational anti-HIV-1 drug designs. In the following paragraphs, some of these studies are presented.

In a computational study by *Tan et al.*, MD simulations were used to identify the effect of a unique hook-like structure (M-T hook) in C-terminal heptad repeat of gp41 on the efficacy of anti-HIV-1 activity of inhibitors. The results of this study showed more hydrophobic and hydrogen bond interactions of inhibitors with the M-T hook structure which caused an improvement in anti-HIV-1 activity of ligands.[81]

In 2017, *Unissa et al.* presented an interesting use of MD simulations in investigating the effects of mutations on drug resistance of HIV-1 gp41 and fusion inhibitors such as Enfuvirtide (EVT). The binding affinity of EVT with a wild-type (WT) and modeled mutants (MTs) form of gp41 was investigated. The binding affinity of EVT with the WT was higher than MTs types because of more favorable bonds with WT form.[81] *Pandey et al.* carried out an MD simulation study of trimeric form of gp-41 to identify the most important amino acids for inner core binding. The amino acids with highest decomposition energies (Gln, Ser, Ile) assumed to be the most responsible residues for the formation of gp41 N/C terminal six helical core structure.[82]

In 2017, *Shibo Jiang et al.* reported some N-carboxyphenylpyrrole derivatives as potent HIV-1 fusion inhibitors targeting gp41. In order to model the gp41 inhibitory activities of these compounds, 2D-QSAR, 3D-QSAR, and molecular docking studies were performed. In docking studies, the represented interactions between ligands and protein were complementary with CoMFA maps in 3D-SAR and the 2D-QSAR models.[83]

4. Conclusions

In spite of the improvements in quality of life for HIV-1/AIDS patients by the help of current anti-HIV-1 regimens, the rate of HIV-1 transmission among people is still high. Thus, beside the efforts for finding new medications to cure and reduce HIV-1/AIDS symptoms, discovering new drugs to prevent HIV-1 entry and its transmission between people is very important. The most important targets considered in designing HIV-1 entry inhibitors are: gp41 and gp120, viral surface glycoproteins, and CD4 receptor, CXCR4 and CCR5 co-receptors located on the host cells.

In addition to considering the CD4 binding site, Phe43 pocket, $\beta 20 - \beta 21$ strands and variable regions V1/V2 on gp120, some different strategies have been used for designing gp120 inhibitors. For example, considering the structural properties of Human Neutrophil Peptide-1 in human immune system, which has HIV-1 entry inhibitory activity by blocking the attachment of gp120 to CD4 receptors, compound **1** with a broad spectrum of anti-HIV-1 activity was discovered. Other useful approaches include targeting the carbohydrates present on gp120 and signal peptides attached to the newly generated CD4 receptors. Inhibition of thiol/disulfide exchange in HIV-1 entry processes is another attractive plan for designing new anti-HIV-1 compounds. IND02, a type A procyanidin polyphenol from cinnamon extracts have also been introduced as a cost-effective entry inhibitor that can block the attachment of the envelope to CD4 and heparan sulfate. The hydrazonothiazolyl pyrazolinones with naphthyl di- and tri-sulfonic acids, NBD-14088, NBD-14107, NBD-11021A2, NBD-14010, YYA-021, derivatives of aloperine, YIR-821 and YIR-819 are other gp120 inhibitors reported in recent articles. Some new possible

gp120 inhibitors and CD4 mimetics were designed and introduced based on different computational methods, such as L-biphenylalanine, MMs03927209, compounds **22**, **23**, **25**, and **26**. The anti-HIV-1 activity of some of these computationally-designed compounds was verified by experimental tests.

Design of compounds with dual antagonist activity on chemokine co-receptors (CCR5 and CXCR4) and intracellular viral enzymes can postpone the inevitable drug resistances. This approach has been exploited successfully in the design of compounds **27** and **29**. Atomistic investigations of the interactions between **27** and CXCR4 and CCR5 resulted in designing a new ligand (**28**) with *in silico* binding affinities more than the parent compound. This compound can be synthesized and evaluated in experimental tests.

Considering gp41 as a target for HIV-1 entry inhibitor drug design, peptide compounds have shown more potent anti-HIV-1 activities compared to small molecule inhibitors, because they can occupy more space of the long lipophilic pocket of gp41. But, because of the pharmacokinetic problems associated with peptide drugs, researchers have focused on finding effective gp41 inhibitors with small molecule structures. 4-Arylpyridin-1(4*H*)-yl)benzoic acid and tetrahydropyrimidine derivatives are some of these small molecule inhibitors that are reported in the current review.

High amounts of computational studies have been reported in the field of HIV-1 entry inhibitors. In this review, we reported a summary of different structure and ligand-based computational studies about HIV-1 entry inhibitors that have not been considered in the latest review published in 2014. These studies have provided the researchers with useful information about the interactions between HIV-1 entry related targets and their inhibitors, elucidating the most critical residues in these targets and achieving better insights about the mechanism of their action. All these information helped to find potent inhibitors and there is the hope for discovering more effective HIV-1 entry inhibitors in the future.

Author Contribution Statement

Tahereh Mostashari Rad conducted a comprehensive literature review and wrote the article along with *Lotfollah Saghaie* and *Afshin Fassihi*.

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