Recent Advances in the Development of Small-Molecule Compounds Targeting HIV-1 gp41 as Membrane Fusion Inhibitors

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Abstract: Over the past few years, remarkable progress has been made in the development of human immunodeficiency virus (HIV) membrane fusion inhibitors. The focus has been on peptide inhibitors, which were developed by mimicking HIV sequences; however, these types of inhibitors generally lack oral bioavailability and are expensive. Therefore, development of small-molecule inhibitors has gained importance and recently progressed. This paper reviews the rapid advancements in the development of small-molecule HIV inhibitors over the last decade.

Keywords: Coiled-coil, docking simulation, gp41, heptad repeat, HIV, membrane fusion, quantitative structure-activity relationship, small-molecule inhibitors.

INTRODUCTION

The development of drugs targeting human immunodeficiency virus (HIV) has greatly accelerated after the approval of zidovudine [1], the first anti-HIV drug, by the U.S. Food and Drug Administration in 1987. Treatment of HIV infection has significantly improved since the establishment of highly active anti-retroviral therapy (HAART), resulting in HIV infection being transformed from a life-threatening disease to a chronic disease in developed countries. Despite these advances, eradication of HIV infection has not been achieved, and the emergence of multiple drug-resistant strains has become a serious problem. Novel drugs with different mechanisms of action are urgently needed. Consequently, several potent, new anti-HIV drugs such as the membrane fusion inhibitor enfuvirtide (also called T-20) [2], the CCR5 inhibitor maraviroc [3], and the integrase inhibitor raltegravir [4] have been approved for use in recent years.

This paper is a review of membrane fusion inhibitors that directly target the virus–cell fusion process mediated by the envelope glycoprotein gp41. In HIV infection, fusion is an initial functional step in which gp41 plays an important role. A complex of gp41 proteins forms a trimer. Each monomer consists of a fusion peptide region; 2 helical heptad repeats (HRs), N-terminal HR (N-HR) and C-terminal HR (C-HR), which is a transmembrane region; and a carboxyl-terminal region [5].

This fusion step has been widely studied [6–8]. Briefly, when the viral surface glycoprotein gp120 binds to CD4 and co-receptors on the surface of T-lymphocytes or macrophages, the gp41 complex undergoes a conformational change whereby its fusion peptides extend toward the membrane of the target cell. Subsequently, the N-HR and C-HR helices interact with each other to form a tight 6-helix bundle; this dramatic structural change brings the viral and cellular membranes into proximity and mediates membrane fusion. Therefore, inhibition of the 6-helix bundle formation is an attractive approach for preventing membrane fusion.

Currently, several C-HR mimic peptides including T-20 [9-17] (Fig. (**1**)), which is in clinical use in Europe and the United States, are interesting candidates for membrane fusion inhibitors. Unfortunately, T-20-resistant isolates have already appeared, and so, the development of more potent inhibitors is still necessary.

There has been progress in the development of peptide membrane fusion inhibitors (Fig. (**1**)). Otaka *et al.* developed SC36 peptides [15], and He *et al*. reported the development of CP32M [12] and sifuvirtide [16]. All of these peptides exhibited improved activities compared to T-20. Peptides that are effective against several HIV subtypes, including a T-20-resistant isolate, have been designed by our group [17]. Furthermore, peptide P5 (residues 628–683), which contains a calcium-binding site, exhibits inhibitory activity against T-20-resistant strains, as reported by Yu *et al*. [18].

However, peptide inhibitors generally lack oral bioavailability and are expensive. In order to overcome these problems, hallmark efforts have been directed towards the discovery of small-molecular-weight compounds using the following polyhedral approaches: (1) identifying candidates from available databases by *in silico* or *in vitro* screening and modifying these compounds to improve their activities; (2) mimicking peptides, especially tripeptides, using polycyclic compounds; (3) isolating compounds from Chinese herbs and other natural products; and (4) designing *de novo* molecules. Recently, small molecules with comparable inhibitory activities against gp41 6-helix bundle formation have been reported. The development of these small molecules and their activities are reviewed in this article.

STRUCTURE OF GP41 AND IDENTIFICATION OF DRUG TARGETS

Crystallographic studies [19,20] have shown that the 6-helix bundle of the gp41 complex comprises 3 N-HR and 3 C-HR peptides Fig. (**2**). A side view of the 6-helix bundle with a hydrophobic pocket at the C-termini of the N-HR peptides is shown in Fig. (**3**). Small-molecule inhibitors are designed to bind to this hydrophobic pocket to inhibit membrane fusion.

In natural complexes, 3 conserved, large side-chain residues (Trp628, Trp631, and Ile635) in the C-HR bind the hydrophobic pocket, and synthetic C-HR peptides can inhibit the formation of helix bundles. Chan *et al*. synthesized C-HR peptides with single Ala mutations at each conserved residue and found that the inhibitory activities of all mutated peptides decreased. The cell–to–cell fusion inhibitory activity of the Trp631 mutant was approximately 30-fold lowered, suggesting that Trp631 is critical for helix bundle formation [6]. This information is useful for optimizing small-molecule inhibitor design.

Zhou *et al.* tested a truncated, wild-type C-HR peptide containing *p*-(*N*-carboxyethyl)aminomethyl benzoic acid-linked cyclopropyl propionic acid and e-glutamic acid at its N-terminus, which is thought to bind the hydrophobic pocket. This mimic peptide inhibited HIV-1 cell–to–cell fusion with an EC_{50} of 300

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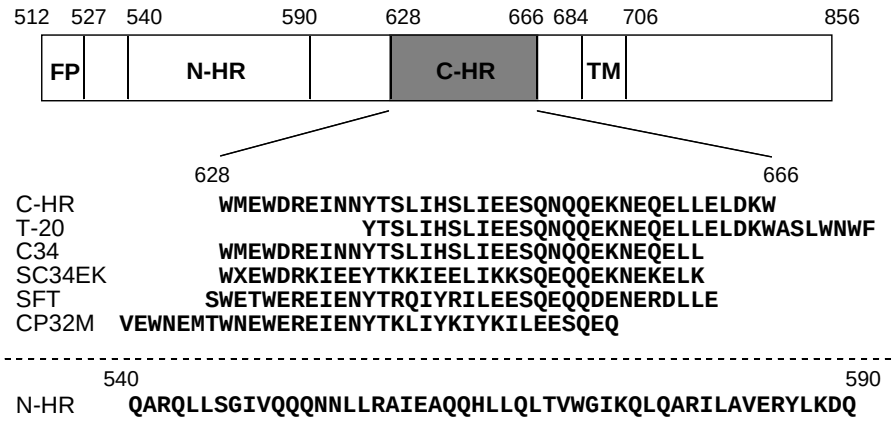

Fig. (1). Schematic representation of gp41 domains and amino acid sequences of N-HR and C-HR. FP: fusion peptide, HR: heptad repeat, TM: transmembrane, SFT: sifuvirtide.

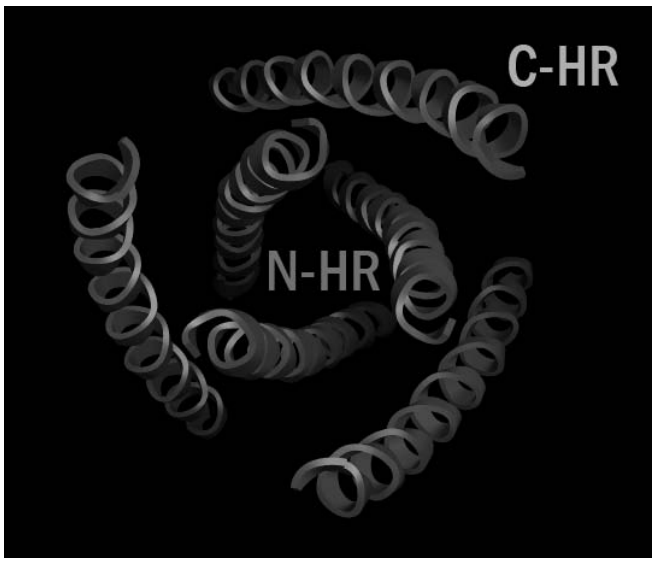

Fig. (2). Crystal structure of gp41. The inner 3 helices are N-HRs and outer 3 helices are C-HR.

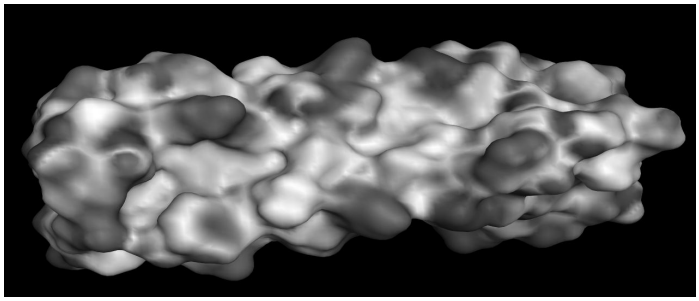

Fig. (3). Molecular surface of N-HR. Left side: C-terminus; right side: N-terminus. The ligand-binding cavities are located at the C-terminus of N-HR.

nM, indicating that this pocket is an effective target for smaller molecules [21].

Recently, computer-based techniques, such as docking simulation, have been widely used to find small-molecular-weight inhibitors. Docking simulation is a virtual screening method commonly used to find potential ligand–receptor complexes by assessing their binding affinity and predicting the activities of candidate molecules. The pockets located at the C-terminus of the N-HR, which interact with the Trp628 residues of C-HR *per se*, have been selected as drug targets in most studies. Jiang *et al.* have made great efforts using this approach and achieved compelling results. Their work has been reviewed in several published papers [22-24].

DEVELOPMENT OF SMALL-MOLECULE INHIBITORS OF GP41

Debnath *et al.* developed ADS-J1–ADS-J16 by screening 20,000 compounds from a chemical database using the Dock program (University of California, San Francisco). Only ADS-J1 and ADS-J2 (Fig. (**4**)) showed inhibitory activity against HIV-1

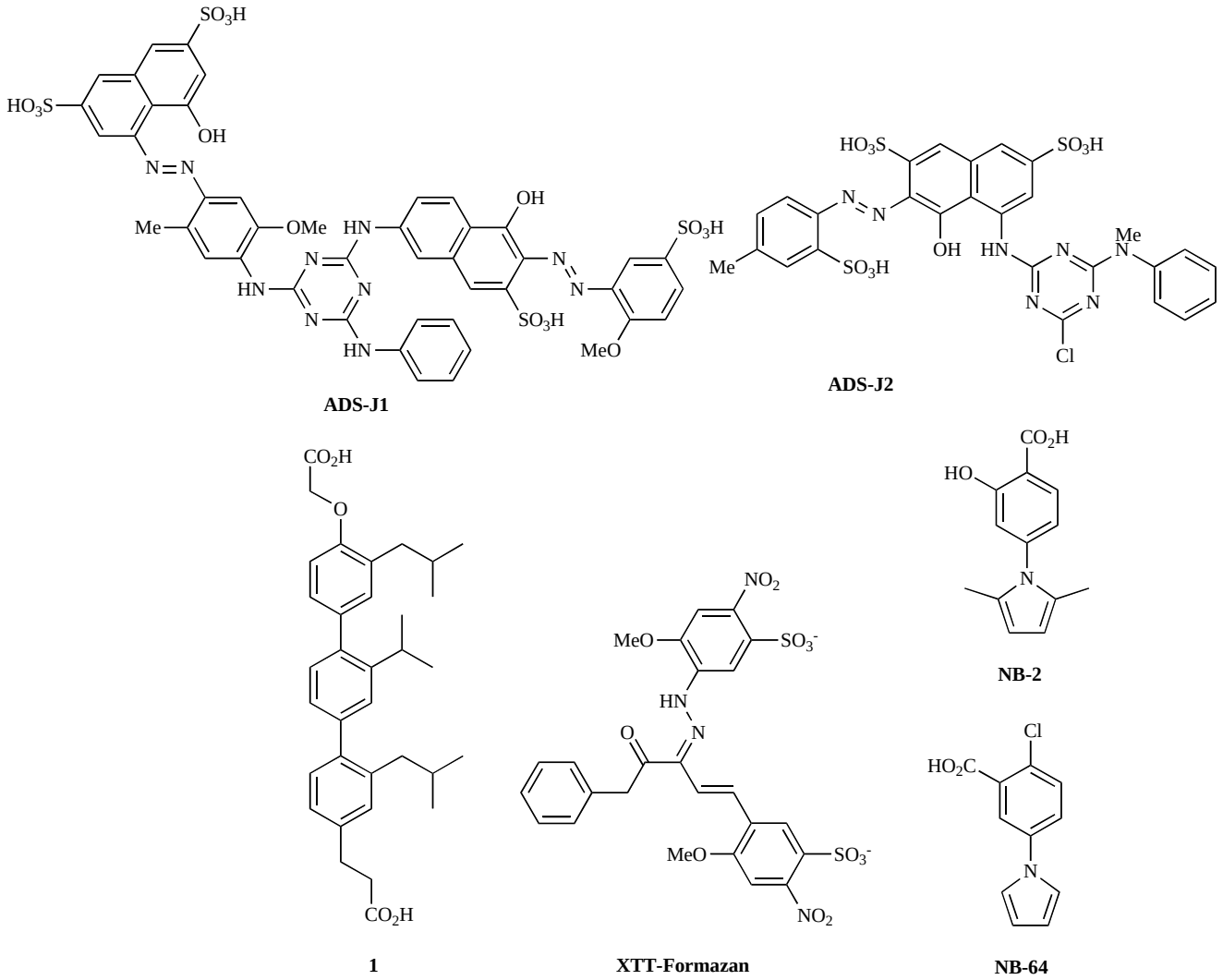

Fig. (4). Structures of ADS-J1, ADS-J2, **1**, XTT formazan, NB-2, and NB-64.

cell-to-cell transfer (IC_{50} values: 4.95 μg/mL and 21.85 μg/mL, respectively) [25]. Using a docking study, the investigators showed that the compounds bind to the coiled-coil pocket of gp41, and this observation was supported by the measured anti-HIV activities of the compounds. However, González-Ortega *et al.* recently reported that ADS-J1 interacted with the V3 loop of gp120 and did not bind gp41 [26]. This interesting finding suggests that the mechanism of action of potential compounds should be identified without bias. Docking studies only provide possible binding modes around a site (pocket) defined by the user. This may occasionally result in errors when a compound exhibits activity at sites other than the one investigated. In the case of gp41 inhibitors, circular dichroism spectroscopy and surface plasmon resonance can be used to detect the inhibition of helix bundle formation more clearly. Another noteworthy point is that González-Ortega *et al.* did not discuss ADS-J2 or the other compounds, probably because these molecules exhibit less anti-HIV activity than J1. A re-analysis of current inhibitors may lead to the discovery of new structural data, which may be useful in rational designs.

Ernst *et al*. developed compound **1**, a small-molecule inhibitor (Fig. (**4**)) that mimics 3 key C-HR residues (Trp628, Trp631, and Ile635) located at the interface between N-HR and C-HR. Compound **1** inhibited HIV-1 cell-to-cell transfer with an IC_{50} of 15.70 μg/mL [27]. This is the first mimicry of C-HR by small-molecular-weight compounds. Links between the aromatic rings make this molecule less rotatable, which may allow a better fit into the pocket. Presentation of the possible binding modes of compound **1** by Tan *et al*. using docking studies and calculations of molecular dynamics [28] supported the experimental observation of Ernst *et al.*

Zhao *et al*. found that HIV entry was effectively inhibited by a metabolite called XTT formazan or sodium 3-[1-(phenyl-amino)carbonyl]-3,4-tetrazolium-bis(4-methoxy-6-nitro)benzene-sulfonic acid hydrate (Fig. (**4**)) [29]. The interaction model that was constructed using the molecular program Dock [30] suggested that after the methoxy group fits into the pocket, one of the phenyl groups interacts with Trp571, and 1 sulfonic acid group forms a salt bridge with the conserved Lys575 in N-HR. Especially, the salt bridge with Lys575 may play an important role in ligand–receptor interaction. This hypothesis is in agreement with the observed interaction between C-HR and N-HR, and Lys575 may be a key interaction point.

Moreover, Jiang *et al*. discovered pyrrole derivatives NB-2 and NB-64 using a computational method designed to search for potent lead compounds (Fig. (**4**)). These derivatives inhibited cell-to-cell transfer of different HIV subtypes with an EC_{50} value of less than 100 μM in various cells; they also exhibited low cellular toxicity [31]. Both NB-2 and NB-64 contain carboxylic acid groups (-COOH), which can form salt bridges with Lys575 due to their proximity. Later, Jiang and coworkers also developed furan and pyrrole derivatives of NB-2 and NB-64, which occupy a larger

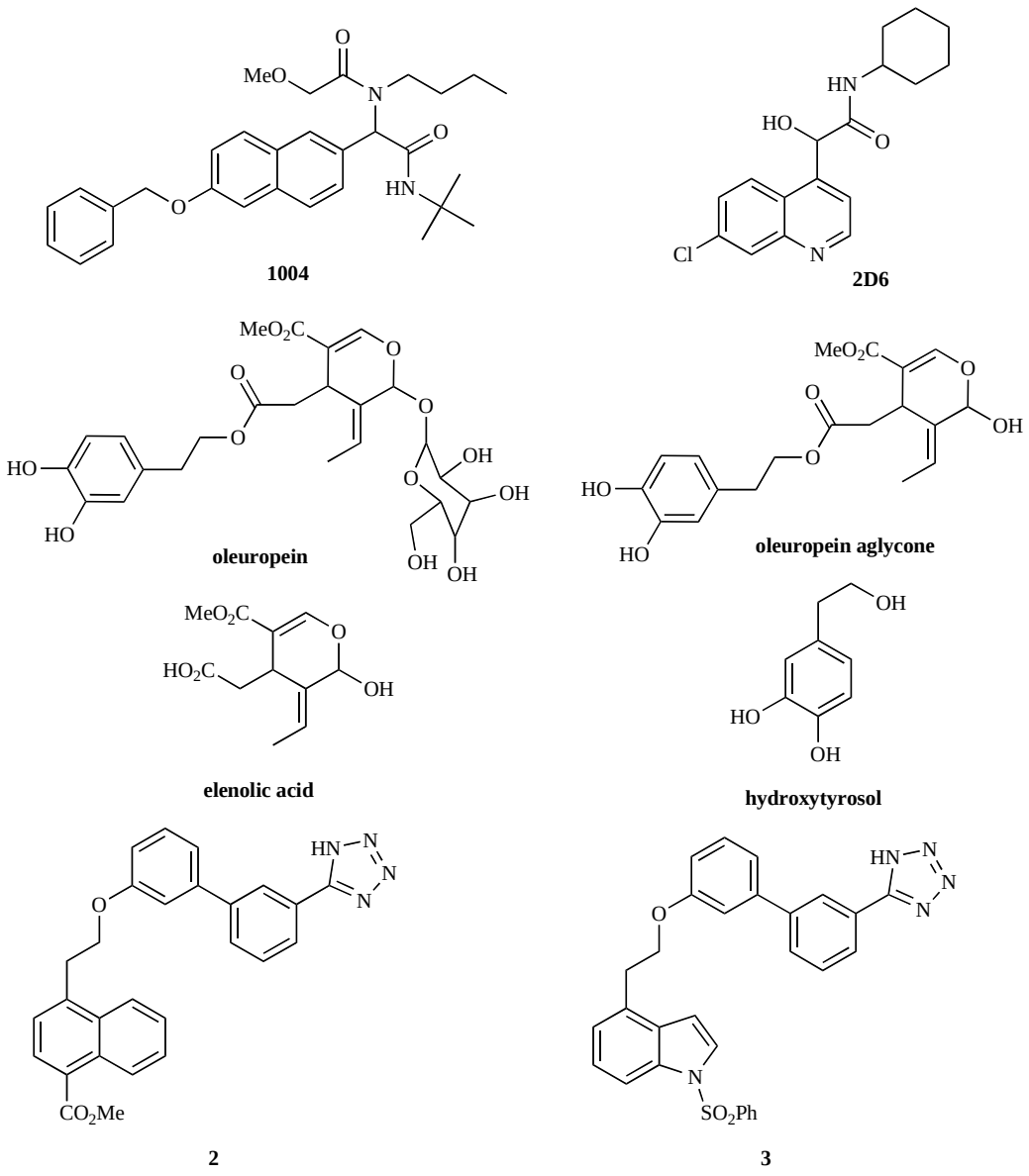

Fig. (5). Structures of 1004, 2D6, oleuropein, oleuropein aglycone, elenolic acid, hydroxytyrosol, **2**, and **3**.

space in the hydrophobic pocket [32, 33]. All compounds in the new series contain carboxylic acid and exhibit better occupation of the empty regions in the pocket. These efforts led to improved antiviral activity.

Using Ugi 4-component condensation, Xu *et al*. synthesized several compounds that have naphthalene and quinoline scaffolds [34]. Of these, 1004 and 2D6 (Fig. (**5**)) inhibited HIV-1 cell-to-cell transfer with IC_{50} values of 40 μM and 15 μM, respectively [35]. Although a low selective index (SI or CC_{50}/IC_{50}) prevented these compounds from becoming drug candidates, the efforts directed towards the discovery of ligands from small, planar, aromatic scaffolds worked well in gp41 inhibitor identification.

Bao *et al*. measured anti-HIV activities of olive extracts such as oleuropein, oleuropein aglycone, elenolic acid, and hydroxytyrosol (Fig. (**5**)). Among these, oleuropein and hydroxytyrosol were effective against HIV fusion. Using molecular dynamics, Bao *et al.* showed that the activities of these compounds were attributable to an interaction with Gln577. Common features of these extracts include rapid adsorption and bioavailability [36]. This study showed the potential of natural products in anti-HIV activities via gp41 inhibition. Several other groups have made efforts to screen and extract active compounds from Chinese medicinal herbs, such as *Balanophora japonica* Makino [37], and Tannine [38]. Since medicinal herbs and natural products in general are safer, these studies should open a novel avenue for inhibiting gp41.

Liu *et al*. carried out *de novo* design with a fragment-based discovery technology [39]. In their research, the region around Lys574 in the N-HR was identified as a high-affinity binding site. The simulation also predicted that the small groove consisting of Trp571, Gln577, and Arg579 would be a good binding region. On the basis of these results, Liu *et al.* synthesized several compounds and discovered an active compound, **2** (Fig. (**5**)). Then, they derived a novel compound **3**, whose activity was comparable to that of compound **2** and which contained an indole ring instead of a naphthalene ring. The association of different parts of the molecule with the neighboring space in the pocket worked well in this case.

Using nuclear magnetic resonance (NMR)-based screening, Stewart *et al*. identified compound **4**. The compound inhibited HIV-1 cell–to–cell fusion with an EC_{50} value of 14 μM. Then, they developed tricarboxylic acid derivatives (**5** and **6**) and monocarboxylic acid derivatives (**7** and **8**) (Fig. (**6**)) by conducting a structure–activity relationship study of compound **4**. In these

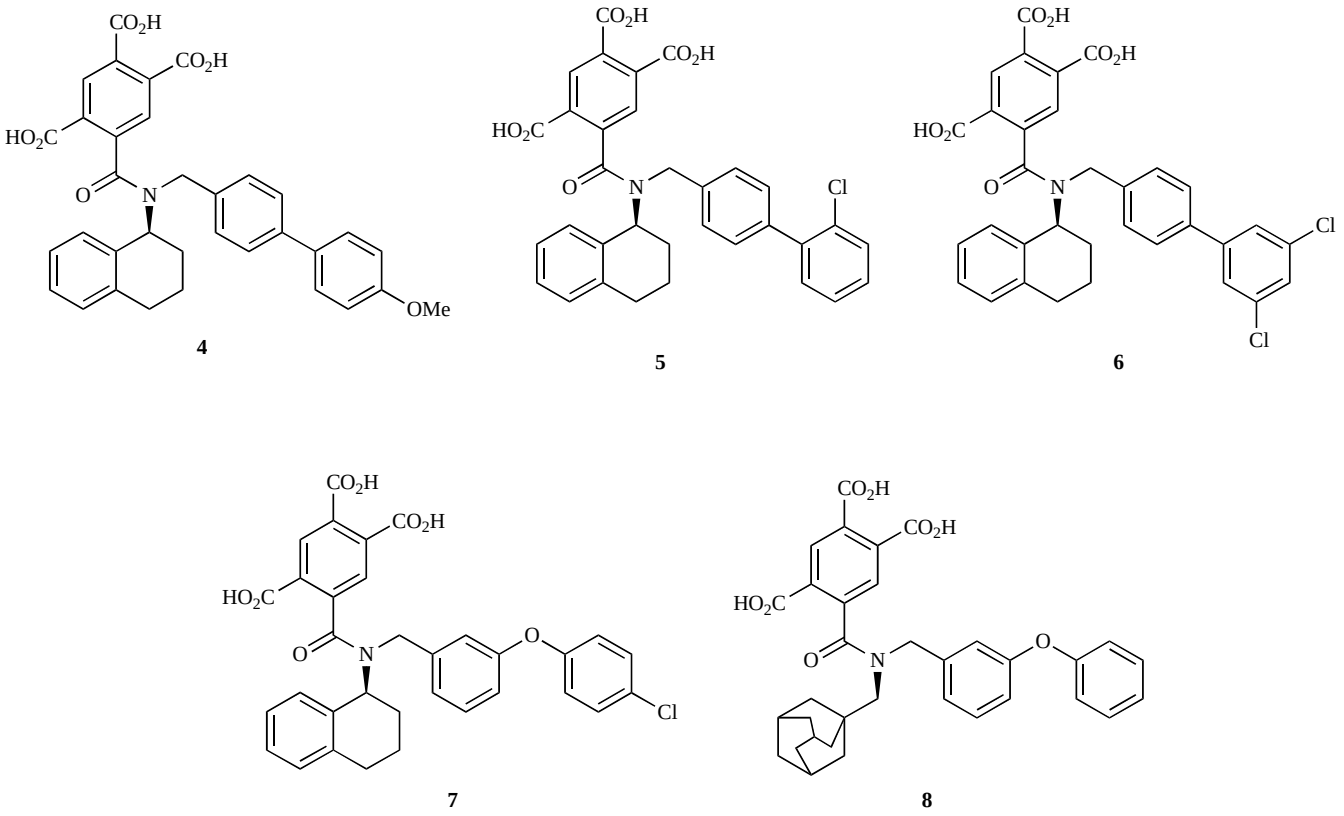

Fig. (6). Structures of compounds **4**–**8**.

studies, compounds **5** and **6** inhibited HIV-1 cell–to–cell fusion with EC_{50} values of 9 μM and 4 μM, respectively, and compounds **7** and **8** had EC_{50} values of 3 μM and 7 μM, respectively [40].

Wang *et al*. reviewed the works of Jiang *et al.* by docking ligands into the hydrophobic pocket of gp41 by using a different docking software, AutoDock [41], and found a potentially distinct interaction mode of A12, which was previously reported by Jiang *et al*. by using GeometryFit [42]. According to the novel docking mode, modifications were carried out on A12 and, fortunately, more active compounds, GLS22 and GLS23, (Fig. (**7**)) were developed *in silico*. Thereafter, 2 molecules were synthesized using short steps from *m*-amino benzoic acid. This method can be used to synthesize a number of derivatives.

Zhou *et al*. developed compound **9** containing an indole ring designed from the structure of gp41 and a compound (M1) complex determined by NMR (Fig. (**7**)). Since tryptophan residues have an indole ring and play critical roles in the 6-helix bundle formation in natural viruses, the substitution of the fluorophenyl group of M1 with an indole ring was tested. This modification increased hydrophobicity while maintaining solubility. Furthermore, they developed a more active compound, **10**, by optimizing the substituents on the indole ring [43]. Subsequently, Gochin *et al.* examined the binding modes of similar indole compounds using NMR [44].

Quantitative structure-activity relationship (QSAR) is a drug development strategy that explains the relationship between physicochemical parameters (descriptors) and biological activity using statistical analysis. Teixeira *et al*. reported 2D- and 3D-QSAR analyses of 23 pyrrole derivatives of compounds reported by Jiang *et al*. [31, 45]. Comparative molecular field analysis (CoMFA), a

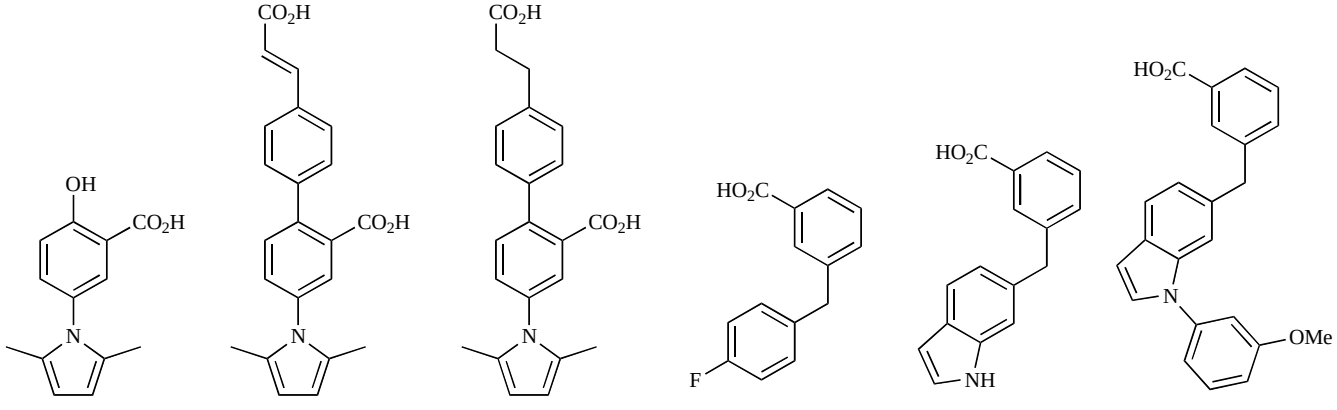

Fig. (7). Structures of A12, GLS-22, GLS-23, M1, **9**, and **10**.

method that uses steric or electrostatic contour maps (called CoMFA field), showed a high correlation coefficient between CoMFA field and biological activity.

CONCLUSION

Peptides and small molecules have been developed as novel membrane fusion inhibitors of HIV. Currently, T-20, a peptide inhibitor, is the only membrane fusion inhibitor used clinically. However, peptide inhibitors are not orally available and have a short half-life in blood. In contrast, small-molecule inhibitors have lower total stabilization energy associated with binding to the gp41 target than peptide inhibitors; therefore, small-molecule inhibitors are less potent than peptide inhibitors. Most small-molecular-weight compounds that have been developed interact with a conserved Lys575 and exhibit enhanced binding activities. The possibility of mutations by drug-dependent selective pressure may become a problem in future investigations. However, potent compounds targeting the gp41 helix bundle may be used in cocktails with enfuvirtide and/or other anti-HIV drugs. The development of next-generation small-molecule inhibitors possessing high activity is a challenge, and we expect growth in these fields.

CONFLICT OF INTEREST

None.

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