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JSM Chemistry

Research Article

Identification of Novel HIV-1 Fusion Inhibitor Scaffolds by Virtual Screening, High-Throughput Docking and Molecular Dynamics Simulations

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Submitted: 16 March 2016

Accepted: 05 May 2016

Published: 11 May 2016

ISSN: 2333-6633

Keywords
- HIV-1
- gp41 protein
- Neutralizing antibody 10e8
- Peptidomimetics
- Virtual screening
- Molecular docking
- Molecular dynamics
- HIV-1 fusion inhibitors

Abstract

Virtual screening of entry inhibitor scaffolds mimicking anti-HIV-1 broadly neutralizing antibody 10e8 was carried out and evaluation of their potential inhibitory activity was performed using high-throughput docking and molecular dynamics simulations. The calculations identified eight small molecules exhibiting a high affinity to the membrane-proximal external region of the HIV-1 coat protein gp41 critical for cell-virus membrane fusion process. The identified compounds are considered as promising scaffolds for the design of novel, potent and broad anti-HIV-1 drugs.

INTRODUCTION

HIV-1 infection begins with virion entry into target cells through the interaction of viral envelope (Env) protein gp120 with its receptor CD4 (reviewed in [1,2]). The binding of gp120 to CD4 induces the exposure of a second binding site for its co receptor CCR5 or CXCR4 [1,2]. Following the binding, the gp41 transmembrane subunit of the envelope protein undergoes a dramatic conformational change to mediate virus-cell membrane fusion, enabling the virus capsid to enter the cell [1,2]. There are many HIV-1 entry inhibitors that target different epitopes on viral envelope critical for the virus attachment, co-receptor binding, and membrane fusion (reviewed in [3]). Among these inhibitors, only CCR5 antagonist maraviroc [4] and HIV-1 fusion inhibitor enfuvirtide [5] are currently used in highly active anti-retroviral therapy (HAART) (http://www.fda.gov). Maravirok binds to the chemokine co-receptor CCR5 and prevents the virus from entering $CD4^+$ cells [4]. In contrast to other anti-retroviral (ARV) drugs, Maravirok targets a host protein, rather than a viral target. This drug is therefore not used in standard HAART regimes and is recommended only for treatment of ARV-experienced persons infected with CCR5-tropic strains of HIV-1 [4]. Enfuvirtide (T20) is a 36 residue synthetic peptide that binds to the gp41 subunit of the viral envelope glycoprotein and prevents the conformational changes required for the fusion of viral and cellular membranes [5]. Enfuvirtide is employed in "salvage HAART" regimens [5]. However, its clinical application is limited by its relatively low potency, low genetic barrier to drug resistance and short half-life [6]. Therefore, it is essential to develop new HIV-1 entry inhibitors with improved antiviral efficacy, drug-resistance profile and pharmaceutical properties. During the past few decades, tremendous efforts have been made on developing inhibitors that can prevent each step in the HIV-1 entry process [3]. Many of these compounds including small molecule inhibitors, peptide inhibitors, vaccines and neutralizing antibodies are currently in clinical trials (reviewed in [3, 6-8]).

Discovery of anti-HIV-1 broadly neutralizing antibodies (bNAbs) blocking the initial steps of the virus entry provided a new strategy in vaccine and drug design (e.g., [9]). In particular, studies on the identification of small molecules able to mimic pharmacophoric properties of anti-HIV-1 bNAbs are of great interest. In this work, virtual screening of entry inhibitor scaffolds mimicking anti-HIV-1 bNAb 10e8 [10] was carried out and evaluation of their potential neutralizing activity was performed using high-throughput docking and molecular dynamics (MD) simulations. 10e8 is one of the most potent and broad HIV-neutralizing antibodies isolated and it neutralizes up

to 98% of diverse HIV-1 strains by specific interactions with the membrane-proximal external region (MPER) of the envelope protein gp41. This tryptophan-rich region of gp41 is critical for Env-mediated fusion and virus infectivity [10].

To reach the goal of the study, the amino-acid residues of bNAb 10e8 critical for specific binding to gp41 were selected based on the X-ray structure of this antibody Fab in the complex with the gp41 MPER peptide [10] Using these residues, pharmacophore models describing different combinations of the antibody binding hotspots were generated and used as the templates for identification of peptidomimetic candidates of bNAb 10e8 by a public web-oriented virtual screening platform (pepMMsMIMIC) [11] associated with the MMsINC database. Complexes of these candidates with gp41 were built by molecular docking and their stability was estimated by molecular dynamics simulations and binding free energy calculations.

The calculations identified eight small molecules exhibiting a high affinity to the membrane-proximal external region of the HIV-1 coat protein gp41 critical for cell-virus membrane fusion process. The identified compounds are considered as promising scaffolds for the design of novel, potent and broad anti-HIV-1 drugs.

MATERIALS AND METHODS

Generation of Pharmacophore Models

Pharmacophore models for virtual screenings of 10e8-mimetic candidates were generated in agreement with the first step of the pep MMsMIMIC strategy consisting in the identification of amino-acid residues that play a key role in the protein-protein recognition process [11]. In this step, all possible combinations of these residues exhibiting critical structural features in three-dimensional space may be used in generation of the templates to screen virtual compound libraries for novel ligands, which present the best similarity to the specific pharmacophore [11]. Based on this strategy, the hotspots of bNAb 10e8 for its interaction with gp41 were derived from the X-ray crystal structure of this antibody Fab in complex with the gp41 MPER peptide (code 4G6F in the Protein Data Bank) [10]. As follows from the study [10], residues Trp-33, Tyr-99, Asp-100, Phe-100a, Trp-100b and Gly-100d of the 10e8 heavy chain greatly contribute to the specific interactions of this antibody with gp41 and play important role in the formation of the complex of interest. According to the gp41–10e8 crystal structure [10], residues Trp-33 and Tyr-99 make van der Waals contacts with Trp-672 of gp41 presenting one of the key amino acids of linear epitope of 10e8 [10]. At the same time, residues Phe-100a and Gly-100d form hydrogen bonds with Arg-683 of gp41, and Trp-100b participates in van der Waals interactions with this gp41 residue that is also important for the binding to 10e8 [10]. These X-ray data are in line with those of the study [12] in which the hotspots of bNAb 10e8 for its interaction with gp41 were identified using MD simulations of the gp41–10e8 crystal structure [10] followed by binding free energy calculation and decomposition of the binding enthalpy into the contribution from each amino acid of the 10e8 Fab. With these calculations, the interactions of the 10e8 residues Trp-33, Tyr-99, Asp-100, Phe-100a, Trp-100b and Gly-100d dominate the binding [12]. These residues of bNAb 10e8 were therefore used as the basic pharmacophore model for search of its most probable peptidomimetics. To identify small-molecule peptidomimetic candidates, twelve different fragments of this model were generated and included in the input dataset for pepMMsMIMIC, allowing one to obtain a much wider collection of potential peptidomimetics of 10e8. When designing these fragments, the 10e8 residues Glu-53, Lys-97 and Tyr-98 that also make the direct contacts with gp41 [10] were taken into consideration. In the X-ray crystal structure [10], Glu-53 is involved in the H-bonding with Trp-672 of gp41, whereas Lys-97 and Tyr-98 make van der Waals contacts with this gp41 residue [10]. The final input dataset for pepMMsMIMIC comprised three-dimensional structures of the thirteen 10e8 segments targeting different regions of the gp41 MPER peptide (Table 1).

Shape and Pharmacophore-Based Virtual Screening

The pharmacophore models generated based on the 10e8 binding hotspots were screened against a library of 17 million conformers obtained from 3.9 million commercially available chemical structures present in the MMsINC database (http://mms.dsfarm.unipd.it/MMsINC.html). Screening of this virtual compound library was carried out by four scoring methods that are used in the current version of pepMMsMIMIC [11] to optimize the selection of the peptide mimetics. The tools of pepMMsMIMIC offer five search procedures including different combinations of two scoring approaches, such as ultrafast shape recognition and pharmacophore fingerprints similarity [11]. All these procedures were used for search of the 10e8 peptide mimetics, allowing one to identify 4493 compounds in the MMsINC database. 3036 small molecules that satisfied Lipinski's rule of five [13] were further screened by high-throughput docking to evaluate the efficacy of their binding to gp41.

Molecular Docking

The X-ray crystal structure of bNAb 10e8 Fab in the complex

Table 1: Pharmacophore models used as the templates for virtual screening of the 10e8-mimetic candidates in the MMsINC database.

N	Pharmacophore model [a,b]
1	Trp-33^{H} Tyr-99^{H} Asp-100^{H} Phe-100a^{H} Trp-100b^{H} Gly-100d^{H}
2	Trp-33^{H} Glu53^{H} Lys97^{H} Tyr98^{H} Tyr99^{H}
3	Tyr-99^{H} Asp-100^{H} Phe-100a^{H} Trp-100b^{H}
4	Lys-97^{H} Tyr-98^{H} Tyr-99^{H}
5	Trp-33^{H} Tyr-98^{H} Tyr-99^{H}
6	Glu-53^{H} Tyr-98^{H} Tyr-99^{H}
7	Trp-33^{H} Lys-97^{H} Tyr-99^{H}
8	Glu-53^{H} Lys-97^{H} Tyr-99^{H}
9	Trp-33^{H} Glu-53^{H} Tyr-99^{H}
10	Trp-33^{H} Lys-97^{H} Tyr-98^{H}
11	Glu-53^{H} Lys-97^{H} Tyr-98^{H}
12	Trp-33^{H} Glu-53^{H} Tyr-98^{H}
13	Trp-33^{H} Glu-53^{H} Lys-97^{H}

Footnotes: [a]Superscript H indicates amino-acid residues associated with the 10e8 heavy chain.
[b]Designations of the 10e8 residues correspond to those given in the PDB file of the gp41–10e8 crystal structure (code 4G6F in the Protein Data Bank.

with the gp41 MPER peptide (code 4G6F in the Protein Data Bank; was used as the rigid receptor for flexible "blind docking" with compounds from the MMsINC database by Autodock Vina. The gp41 MPER peptide structure was prepared by adding hydrogen atoms with the Auto Dock Tools software. For all compounds, the docked structures with the highest scores were analyzed to identify the molecules that, similarly to 10e8, target the antibody-binding site of gp41. As a result, the complexes of 35 top-ranking compounds with gp41 were selected based on the values of scoring function to be exposed to MD simulations and binding free energy calculations.

Molecular Dynamics Simulations

The MD simulations for the docked structures of 35 top compounds with gp41 were performed using Amber 11 with the implementation of the Amber ff10 force field. The ANTECHAMBER module was employed to use the Gasteiger atomic partial charges individually for each of the compounds, and the general AMBER GAFF force field was used to prepare the force field parameters. Hydrogen atoms were added to gp41 by the tleap program of the AMBER 11 package. The system was solvated using TIP3P water as an explicit solvent and simulated in an octahedron box with periodic boundary conditions. The structure was first energy minimized by 500 steps of the steepest descent algorithm followed by 1000 steps of the conjugate gradient method. The atoms of the complex assembly were then restrained by an additional harmonic potential with the force constant equal to 1.0 kcal/mol and then heated from 0 to 310 K over 1 ns using a constant volume of the unit cell. Additional equilibration was performed over 1 ns by setting the system pressure to 1.0 atm and by using a weak coupling of the system temperature to a 310 K bath with 2.0 ps characteristic time. Finally, the constraints on the complex assembly were removed and the system was equilibrated again at 310 K over 2 ns under constant volume conditions. After equilibration, the isothermal-isobaric MD simulation (T = 310 K, P = 1.0 atm) generated 30 ns trajectory using a Berendsen barostat with 2.0 ps characteristic time, a Langevin thermostat with collision frequency 2.0 ps^{-1}, a non-bonded cut-off distance of 8 Å, and a simple leapfrog integrator [9] with a 2.0 fs time step and bonds with hydrogen atoms constrained by the SHAKE algorithm.

Binding Free Energy Calculations

The free energy of binding was calculated in AMBER 11 by the MM/PBSA method. Five hundred snapshots were selected from the last 25 ns to estimate the binding free energy, by keeping the snapshots every 50 ps. The polar solvation energies were computed in continuum solvent using Poisson-Boltzmann and ionic strength of 0.1. The non-polar terms were estimated using solvent accessible surface areas.

Based on the MM/PBSA analyses of the MD trajectories, chemical compounds that showed negative free energies of the binding to the MPER peptide of gp41 were selected for the final discussion.

RESULTS AND DISCUSSION

Virtual screening of the MMsINC database combined with molecular docking and MD simulations identified eight top hits that exposed the high-affinity binding to gp41 by targeting the MPER segment of this HIV-1 protein, allowing one to consider these molecules as promising peptidomimetic candidates of bNAb 10e8. Chemical structures of these compounds are shown in (Figure 1).

Figure 2 casts light on the docked structures of the identified compounds (Figure 1) with the gp41 MPER peptide. In particular, analysis of the MMs03555010–gp41 docked structure indicates (Figure 2a) that, similarly to bNAb 10e8, this molecule targets the central hinge region of the MPER peptide providing the conformational flexibility necessary for the Env-mediated hemi fusion and fusion processes [10,14]. At the same time, MMs03555010 participates in π-π interaction with Trp-672 of the MPER peptide (Figure 2a) presenting one of the key residues of linear epitope of 10e8 [10]. The MMs03555010 compound is involved in van der Waals interactions with the gp41 residues Trp-666, Ala-667, Ser-668, Leu-669, Trp-670, Asn-671, Trp-672, and Ile-675, resulting in the formation of sixty seven hydrophobic contacts. Among these residues, one needs to note Trp-666, Trp-670, and Trp-672 participating in the cell-virus membrane fusion [15] as well as Asn-671 promoting the conformational flexibility of the MPER segment [14,16]. The MMs03555010 compound also forms two hydrogen bonds with Trp-670 and Trp-672 of gp41 (Figure 2, a). The mechanism of interactions between the other identified compounds and gp41 (Figure 2, b–h) is close to the one appearing in the docked structure of MMs03555010 with the MPER peptide (Figure 2, a). This mechanism is generally provided by intermolecular π-π interactions and van der Waals contacts, leading to the blockade of the MPER residues critical for the fusion of viral and cellular membranes (Figure 2). Comparative analysis of the images a–h of (Figure 2) shows that all the identified compounds are involved in π-π interaction with the highly conserved residue Trp-672 of gp41 [14], allowing one to suppose that this interaction may be important for their specific binding to the central hinge region of the gp41 MPER peptide. Excepting MMs03769994, these molecules also make the direct interatomic contacts with Trp-666 and Ile-675 of gp41 (Figure 2). These observations are of great interest in conjunction with the findings of the study [17], whereby the HIV-1 entry defects are associated with alanine replacement of the gp41 Trp-666, Trp-672, Phe-673, and Ile-675. In this study, alanine replacement of individual MPER residues, Trp-666, Trp-672, Phe-673, and Ile675, was shown to result in ~ 8–120-fold reductions in viral entry, suggesting that a full complement of these aromatic and hydrophobic residues is required for efficient MPER-mediated destabilization of the viral envelope [17].

The docked structures of the identified compounds with gp41 do not undergo substantial rearrangements during the MD simulations, in agreement with the low averages of free energy of their formation that are −16.1±3.4 kcal/mol (MMs03555010), −12.0±4.8 kcal/mol (MMs03769994), −7.3±4.8 kcal/mol (MMs01288397), −7.2±3.9 kcal/mol (MMs02374310), −6.6±4.4 kcal/mol (MMs03064646), −5.7±4.0 kcal/mol (MMs03534576), −5.4±3.6 kcal/mol (MMs01100460), and −5.3±4.5 kcal/mol (MMs00760407). Decomposition of the binding free energy into the contribution from each amino acid of the MPER peptide shows that, in all of the cases of interest, the gp41 residues Trp-666, Trp-670 and Trp-672 are of great importance to specific

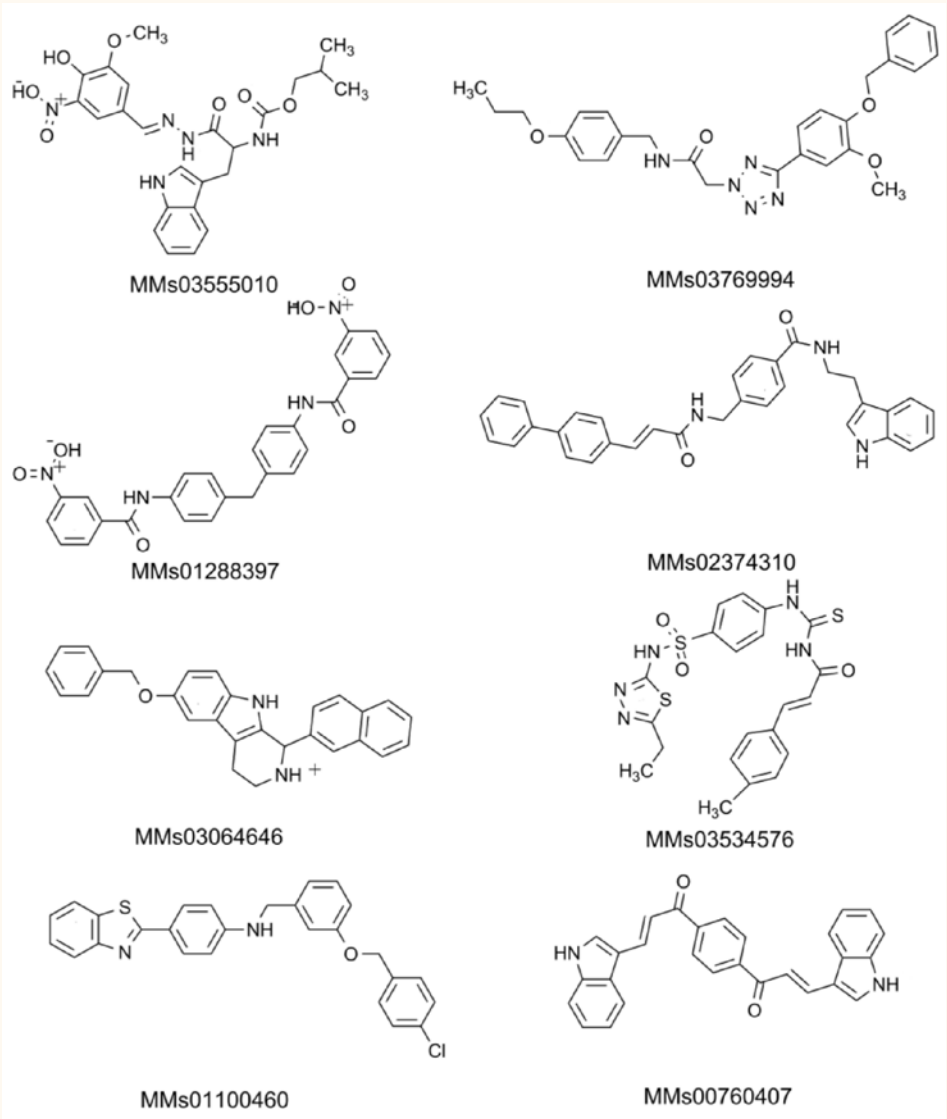

Figure 1 Chemical structures of the most probable peptidomimetics of bNAb 10e8. The molecule codes are from the MMsINC database. For these molecules, the four Lipinski's parameters – molecular weight, lipophilicity (LogP), number of H-bond donors and acceptors [13] – are respectively: 497.5 Da, 3.97, 4 and 5 (MMs03555010); 487.6 Da, 3.15, 1 and 7 (MMs03769994); 496.5 Da, 2.36, 2 and 2 (MMs01288397); 499.6 Da, 4.52, 3 and 2 (MMs02374310); 405.5 Da, 0.0, 1 and 1 (MMs03064646); 487.6 Da, 3.62, 3 and 6 (MMs03534576); 457.0 Da, 3.0, 1 and 2 (MMs01100460); 416.5 Da, 2.94, 2 and 2 (MMs00760407).

interactions with the peptide mimetics. A similar conclusion also relates to Ile-675 of gp41 located in the central hinge region of the MPER peptide. In addition, Trp-678 and Leu-679 of gp41 greatly contribute to the energy stabilization of the ligand–gp41 structures, excepting MMs03555010 and MMs03064646 respectively. Finally, Arg-683 that play important role in specific interactions with bNAb 10e8 [10] makes the hotspot of the binding to the MMs03555010, MMs03769994, MMs01288397, MMs02374310, MMs01100460 and MMs00760407 compounds.

Analysis of the superimposed complexes of the MPER peptide with the 10e8 Fab and peptidomimetic candidates indicates that the identified compounds partially mask the region of gp41 that is targeted by bNAb 10e8. These small molecules bind to the vulnerable spots of this gp41 region and may therefore exhibit the functional mimicry of 10e8. The molecules of interest mimic segment Trp-33, Gly-52c, Pro-52b, Glu-53, Lys-97 of the 10e8 heavy chain that forms the direct intermolecular contacts with the functionally important residues of gp41. According to the X-ray data [10], Trp-33, Glu-53 and Lys-97 of 10e8 make van der Waals contacts with Trp-672 of gp41, and residue Gly-52c of this antibody participates in van der Waals interactions with the gp41 amino acid Trp-670. As noted above, the identified compounds also interact with these residues of gp41 critical for destabilization of the viral membrane during the fusion process [15]. Moreover, Glu-53 and Trp-33 of bNAb 10e8 contact Phe-673 of the MPER peptide, and Gly-52c interacts with the gp41 residues Ser-668 and Leu-669 [10].

CONCLUSIONS

The data of molecular modeling show that eight chemical compounds from the MMsINC database (Figure 1) may be able

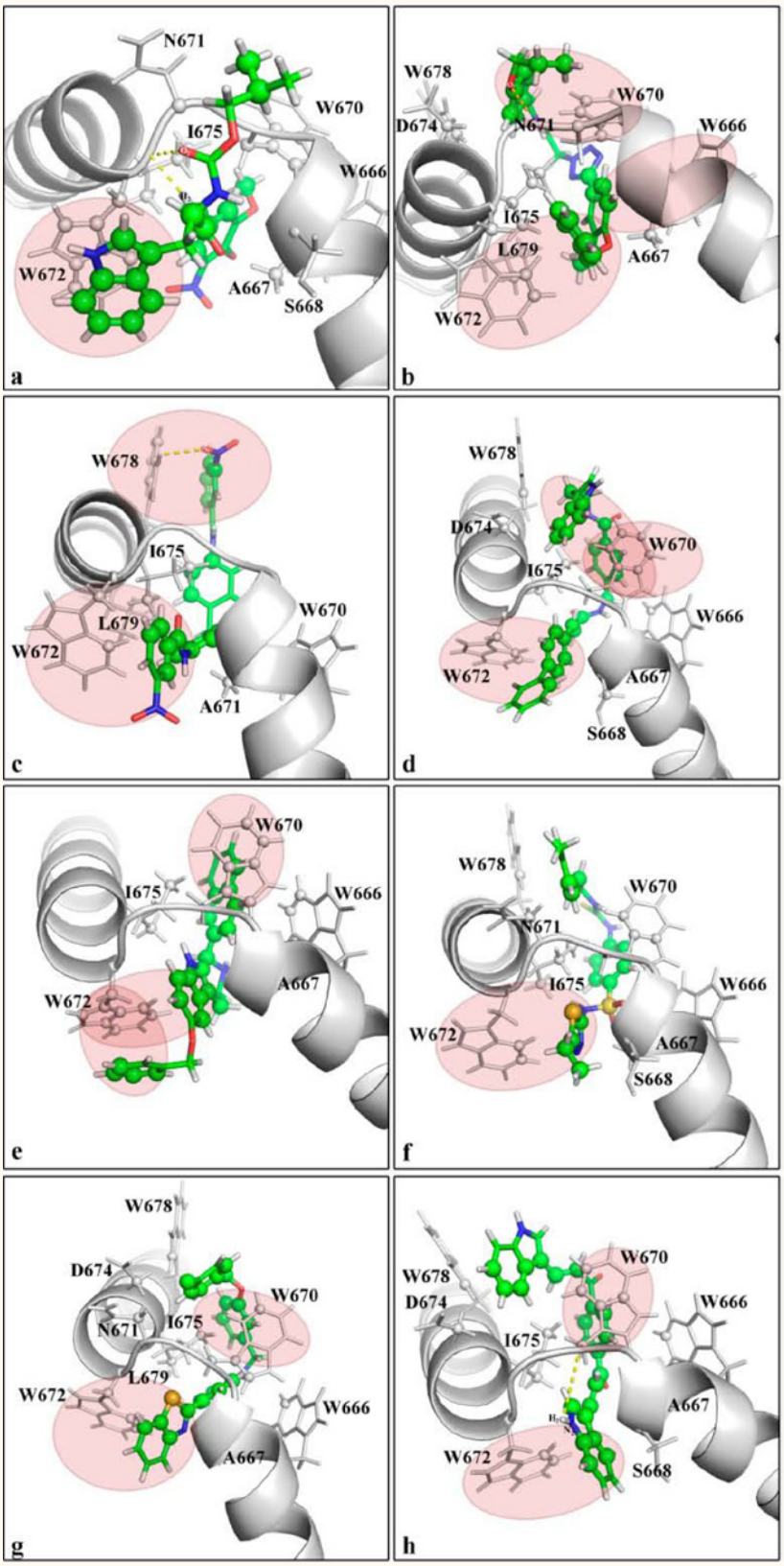

Figure 2 The docked structures of the gp41 MPER peptide with the MMs03555010, MMs03769994, MMs01288397, MMs02374310, MMs03064646, MMs03534576, MMs01100460, and MMs00760407 compounds. Structures of these compounds are represented by a stick-ball-stick model. The residues of gp41 forming hydrogen bonds, π-π stacking and van der Waals contacts with the 10e8-mimetic candidates are indicated. Structural elements of gp41 and ligands involved in specific π-π interactions are located inside the circles. Hydrogen bonds are shown by dotted lines. Hydrogen bonds and π-π interactions were identified by the BINANA program. Van der Waals contacts were determined with the program Ligplot.

to mimic pharmacophoric properties of bNAb 10e8 by specific and effective interactions with the MPER region of the HIV-1 protein gp41. Important role in the binding may belong to π-π interactions between aromatic rings of these molecules (Figure 1) and π-conjugated system of the gp41 residue Trp-672 (Figure 2) presenting one of the key epitope residues for 10e8 [10]. In a mechanism similar to that of bNAb 10e8, the identified compounds target the central hinge region of the MPER peptide (Figure 2) that provides a conformational flexibility necessary for its functioning in the cell-virus membrane fusion process [14].

In light of the findings obtained, the identified compounds are considered as promising scaffolds for the design of novel, potent and broad anti-HIV-1 drugs that inhibit the cell-virus membrane fusion by targeting the MPER segment of the HIV-1 coat protein gp41.

ACKNOWLEDGEMENTS

This study was supported by grant from the Belarusian Foundation for Basic Research (project X15-022).

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Cite this article

Andrianov AM, Kashyn IA, Tuzikov AV (2016) Identification of Novel HIV-1 Fusion Inhibitor Scaffolds by Virtual Screening, High-Throughput Docking and Molecular Dynamics Simulations. JSM Chem 4(2): 1022.