In Silico Discovery of Novel Fusion Inhibitor Scaffolds Targeting a Membrane Proximal External Region of HIV-1 gp41

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Discovery of potent broadly neutralizing antibodies (bNAbs) isolated from HIV-1 long-term non-progressors gave hope for the possibility of overcoming the major challenges in the development of a globally safe and effective HIV-1 vaccine [1]. However, current HIV-1 vaccine candidates are unable to elicit neutralizing antibodies against most circulating virus strains, and thus the induction of a protective antibody response continues to be a major priority for HIV-1 vaccine development [1]. In this context, development of small-molecule HIV-1 entry inhibitors able to show structural and functional mimicry of anti-HIV-1 bNAbs paratopes may be of great interest.

In this work, an integrated computational approach to in *silico* drug design was used to identify novel HIV-1 fusion inhibitor scaffolds mimicking gp41-specific bNAb 10E8 that is one of the most potent and broad HIV-neutralizing antibodies isolated [2]. This computer-based approach included (i) generation of pharmacophore models representing 3D-arrangements of chemical functionalities that make bNAb 10E8 active towards the membrane proximal external region (MPER) of the HIV-1 gp41 protein, (ii) shape and pharmacophore-

based identification of 10E8-mimetic candidates by a web-oriented virtual screening platform pepMMsMIMIC (http://mms.dsfarm.unipd.it/MMsINC.html), (iii) high-throughput molecular docking of the identified compounds with the gp41 membrane proximal external region (MPER), and (iv) molecular dynamics (MD) simulations of the docked structures followed by binding free energy calculations and selection of the most probable 10E8 peptidomimetics.

Pharmacophore models for virtual screening of 10E8-mimetic candidates were generated in agreement with the first step of the pepMMsMIMIC strategy [3] consisting in the identification of amino-acid residues that play a key role in the protein-protein recognition process. These models generated based on the 10E8 binding hotspots [2] 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), allowing one to identify 4493 compounds. 3036 small molecules that satisfied Lipinski's rule of five were further screened by high-throughput docking to evaluate the efficacy of their binding to gp41. The X-ray crystal structure of bNAb 10E8 Fab in the complex with the gp41 MPER peptide [2] was used as the rigid receptor for flexible "blind docking" with compounds from the MMsINC database by AutoDock VINA (http://autodock.scripps.edu/resources/adt). 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. The MD simulations were performed using Amber 11 with the implementation of the Amber ff10 force field (http://ambermd.org/) [4]. 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 with a 2.0 fs time step and bonds with hydrogen atoms constrained by the SHAKE algorithm (http://ambermd.org/) [4]. The free energy of binding was calculated in AMBER 11 by the MM/PBSA method (http://ambermd.org/) [4].

Virtual screening of the MMsINC database in combination with high-throughput docking, MD simulations, and binding free energy calculations identified eight hits (Figure 1) that fully satisfied Lipinski's rule of five [5] and exposed a functional mimicry of bNAb 10E8

by targeting the gp41 MPER segment, allowing one to select these small molecules as the most promising 10E8-mimetic candidates.

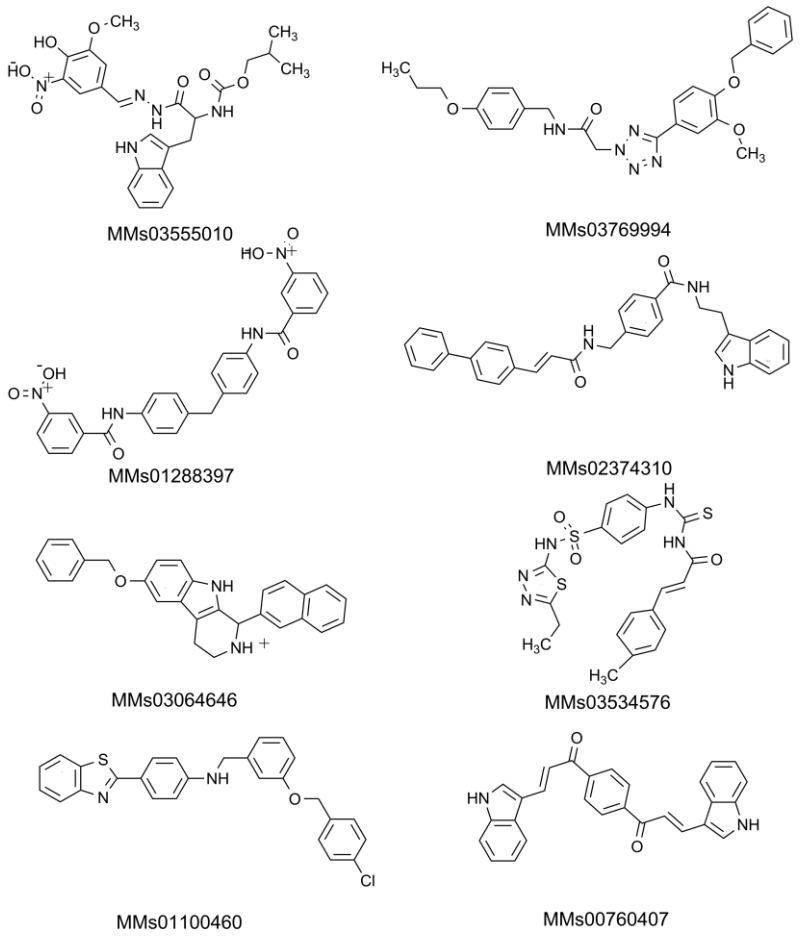

Figure 1. Chemical structures of the most probable 10E8-mimetic candidates (the molecule codes are taken from the MMsINC database [3]).

The data of molecular docking suggest that 10E8-mimetic candidates (Figure 1) may expose great neutralization potency inherent to bNAb 10E8. As this antibody [2], these compounds bind to a highly conserved linear epitope of the MPER hinge region and form a wide network of intermolecular contacts with the critically important residues of gp41, including π-stacking and/or T-shaped interactions, van der Waals forces, and hydrogen bonds. The findings of molecular docking are consistent with those of MD simulations and binding free energy calculations. The docked structures of the identified compounds with the gp41 MPER peptide are stable during the MD simulations, in agreement with the low values

of binding free energies, their enthalpy components, and corresponding standard deviations. Decomposition of the binding free energy into contributions 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 critical for virus-cell membrane fusion [2] are of great importance to specific interactions with the peptide mimetics. A similar conclusion also relates to Ile-675 of gp41 located in the central hinge region of the MPER segment that provides a conformational flexibility necessary for its functioning in the cell-virus membrane fusion process [2]. Finally, Arg-683 that plays an important role in specific interactions with bNAb 10E8 [2] makes the hotspot of the binding to the MMs03555010, MMs03769994, MMs01288397, MMs02374310, MMs01100460 and MMs00760407 compounds.

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.

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