IDENTIFYING POTENTIAL DRUGS FOR INHIBITION THE M2 PROTEIN CHANNEL OF INFLUENZA A BY STEERED MOLECULAR DYNAMICS

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Abstract

Combining Lipinski's rule and docking method were used as a virtual screening tool to find out top hits from the large data base CHEMSPIDER with more than 1,4 million compounds. The lowest binding energy ΔE_{bind} obtained in the best docking mode was chosen as a scoring function for selecting top ligands. Virtual screening has obtained top-leads compounds with binding energy less than -11.0 kcal.mol^{-1} for inhibition the M2 protein channels of influenza A virus H5N1. Since the predictive power of the docking method is limited, top-leads were selected for further study by the more precise steered molecular dynamics method. The main idea of this method is that instead of the binding free energy, the rupture force needed to unbind a ligand from a receptor used as a measure of binding affinity. The higher is rupture force, and the stronger is binding.

Keywords: *Binding free energy, docking method, M2 protein, SMD, virus H5N1.*

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XÁC ĐỊNH NHỮNG THUỐC TIỀM NĂNG NHẰM ỨC CHẾ KÊNH M2 CỦA VIRUS CÚM A BẰNG PHƯƠNG PHÁP KÉO ĐỘNG HỌC PHÂN TỬ

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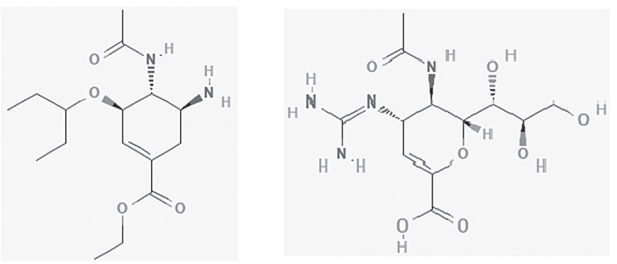
Tóm tắt

Kết hợp qui tắc Lipinski và phương pháp docking được sử dụng cho sàng lọc thô để tìm các hợp chất tiềm năng nhất từ ngân hàng hợp chất CHEMSPIDER, ngân hàng này có khoảng 1,4 triệu hợp chất (2013). Năng lượng liên kết ΔE_{bind} thấp nhất thu được bằng phương pháp docking được xem như một hàm chấm điểm cho việc chọn các phối tử tiềm năng. Sàng lọc thô thu được các hợp chất tiềm năng với năng lượng thấp hơn -11.0 kcalmol^{-1} cho khả năng ức chế kênh M2 protein của virus cúm A H5N1. Bởi vì khả năng sàng lọc của phương pháp docking bị hạn chế nên các hợp chất tiềm năng được nghiên cứu chi tiết hơn bằng phương pháp SMD. Sử dụng phương pháp SMD là thay vì xác định năng lượng liên kết tự do, lực bứt ra (F_{max}) để tách phối tử khỏi thụ thể được xem như là năng lượng liên kết. Lực bứt ra cao hơn điều đó có nghĩa phối tử bám vào thụ thể tốt hơn.

Từ khóa: *Năng lượng liên kết tự do, phương pháp docking, pro-tê-in M2, SMD, vi-rút H5N1.*

1. Introduction

Target in anti-influenza drug design has been the influenza A M2 channels protein due to its importance in viral infection. The M2 protein as the tetrameric structure forms a pH-dependent channel across the viral membrane for control of proton conductance (Pielak & Chou, 2011). The primary strategy for prevention influenza A viruses is to create vaccination. Currently, only four drugs are approved in the USA for influenza A treatment. Oseltamivir and zanamivir are inhibited the viral neuraminidase, while amantadine and its methyl derivative rimantadine is inhibited the viral M2 proton channel (Das, 2012). Emergence of strains with resistance to all approved drugs: oseltamivir (Bright et al., 2005), amantadine (Bright et al., 2006) is a distinct possibility and could have particularly serious repercussions in the event of a new pandemic. M2 is a 97-residue single-pass membrane protein with its N- and C-termini directed toward the outside and inside of the virion (Sugrue & Hay, 1991). The residue 25-46 is a single trans-membrane domain, which is necessary and sufficient for tetramerization, proton conductance and drug binding. Thus, compounds are potential block M2 channel activity able to inhibit influenza A treatment.



Oseltamivir **Zanamivir**

Figure 1. The 2D structure of Oseltamivir and Zanamivir

This paper is to identify potential drugs from Collaborative Drug Discovery in PubChem (see http://pubchem.ncbi.nlm.nih.gov) for inhibition the M2 protein channels of influenza A virus H5N1. Combining Lipinski's rule and docking method were used as a virtual screening tool to find out top hits with the lowest binding energy ΔE_{bind} in the best docking mode with binding energy less than -11.0 kcal.mol^{-1}. Top-leads were selected for further study by the more precise steered molecular dynamics (SMD) method that instead of the binding free energy, the rupture force needed to unbind a ligand from a receptor is used as a measure of binding affinity. The higher is rupture force, and the stronger is binding. Note that, the rupture force is defined as a maximum in the force-time, force-displacement profile.

2. Material and Methods

2.1. Material

2.1.1. Data base of ligands and receptor

Using about 1.4 million compounds from Collaborative Drug Discovery in PubChem, screening of drug candidates has been performed. Concerning the target (receptor), the structural model of proton channel M2 from influenza A in complex with inhibitor rimantadine in the Protein Data Bank with PDB ID: 2RLF (DOI: 10.2210/pdb2RFL/pdb) (Schnell and Chou, 2008), with four 4 chains and residues 18-60. The 3D structure of 2RLF showed Figure 2.

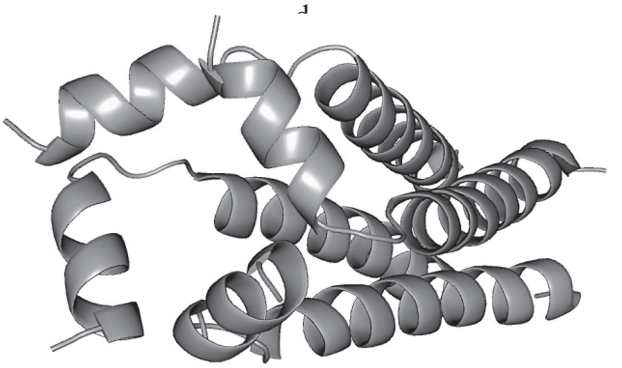


Figure 2. The structure of channel M2 from influenza A (2RLF) virus H5N1

2.1.2. Lipinski's rule

For QSARIS system, the prospective compounds for the potential drugs achieve physicochemical properties of the potential inhibitors, including molecular mass (Da), polarizability (Å3) and volume or size (Å), and dispersion coefficients (logP and logS). However, in this study, potential compounds are set for drug-like properties by Lipinski's rule of five (Lipinski et al., 2012), namely (1) Molecular mass < 500 Da; (2) no more than 5 groups for hydrogen bonds; (3) no more than 10 groups receiving hydrogen bonds; (4) the value of logP is

less than +5 (logP < 5). This applied rule reduced the whole set of about 1.4 million compounds to 5372 compounds.

2.2. Methods

2.2.1. Docking method

Use Autodock Tool 1.5.4 (Sanner, 1999) and prepare PDBQT file for docking ligands to target 2RFL. The Autodock Vina version 1.1 (Trott & Olson, 2010) was performed using the docking simulation. For global search, the exhaustiveness was set to 1000, and the maximum energy difference between the best and worst binding modes was chosen as large as 7.0 kcal.mol^{-1}. Twenty binding modes have been generated starting from random configurations of ligand that had fully flexible torsion degrees of freedom. The box was chosen big enough to cover the entire receptor with minimal distance between ligand and target of 1.4 nm.

2.2.2. Steered molecular dynamics

The steered molecular dynamics (SMD) method was developed to study mechanical unfolding of biomolecules (Israelewitz et al., 2001; Kumar & Li, 2010) and ligand unbinding from receptor along a given direction (Grubmüller et al., 1996). Since the predictive power of the docking method is limited, the SMD method was employed to refine docking results as a next step in the multi-step screening procedure. Overall, a spring with spring constant k is attached to a dummy atom at one end and to the first heavy atom of ligand in the pulling direction at another end. Moving along the pulling direction with a constant loading rate v, the dummy atom experiences elastic force $F = k(\Delta x - vt)$, where Δx is the displacement of a pulled atom from the starting position. The spring constant k = 600 kJ.(mol.nm^2)$^{-1}$ and v = 5 nm.ns^{-1} (Mai & Li, 2011; Vuong et al., 2015). All Cα-atoms of receptor were restrained to keep the receptor almost at the same place but still maximally maintain its flexibility.

2.2.3. The pulling direction

CAVER 3.0 (Chovancova et al., 2012) and Pymol plugin were used for choosing the easiest path for ligand to exit from receptor as the pulling direction. It showed in Figure 3. After equilibration, to completely pull the ligand out of the binding site, 500 ps SMD runs were carried out in NPT ensemble. To obtain reliable results, five independent trajectories were performed with different random seeds. In the SMD method the maximum force F_{max} in the force-extension/time profile was chosen as a score for binding affinity, the larger is F_{max}, the stronger is the ligand binding.

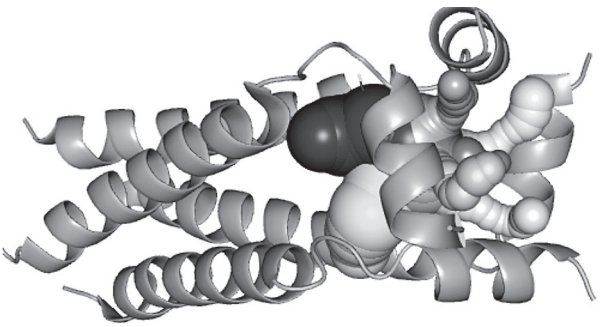


Figure 3. Some pulling directions of CID 5326625 by Caver 3.0

3. Results and Discussion

3.1. Docking results

After the first virtual screening step by Lipinski's rule, the number of compounds is reduced to 5372. The Autodock Vina method was then applied to dock this set to target 2RLF. The binding energies ΔE_{bind}, obtained in the best docking modes for 5327 ligands, vary from -1.2 to -11.9 kcal.mol^{-1}.

Nine compounds are identified with a binding energy lower than -11.0 kcal.mol^{-1}. Locations of these compounds in proton channel M2 from influenza was showed in Figure 4. The compounds are inside proton channel M2.

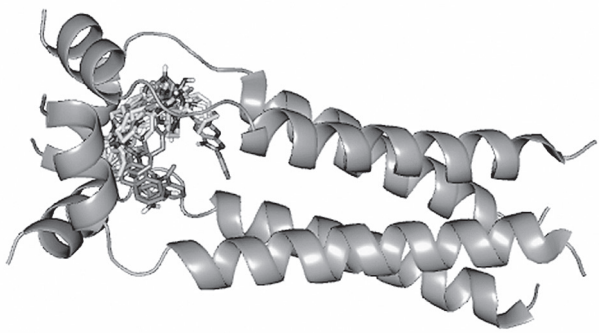
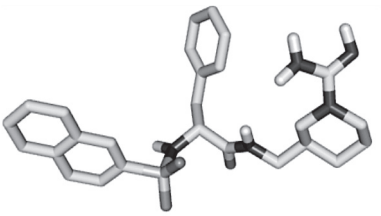
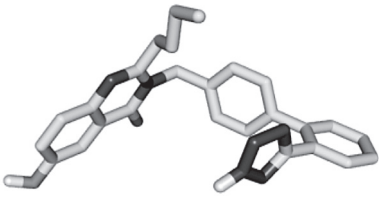
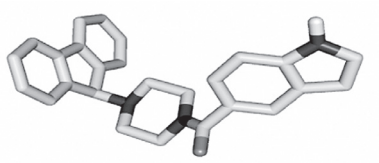
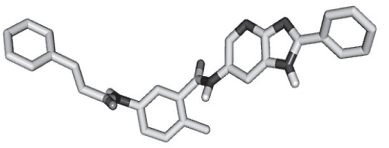
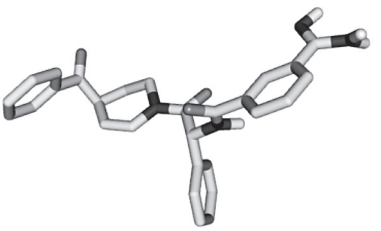
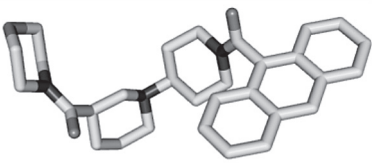
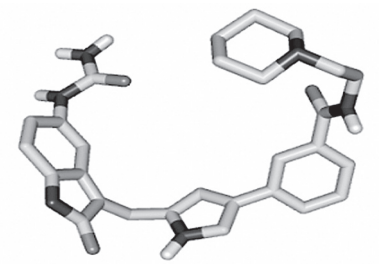
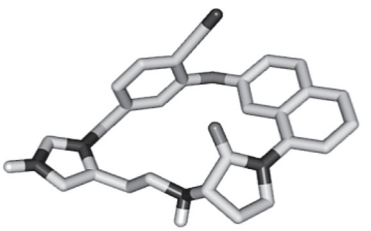
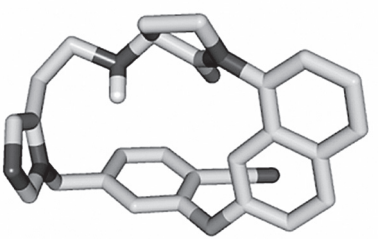


Figure 4. Locations of these compounds in proton channel M2 from influenza A

Table 1. Nine compounds with a binding energy lower than -11.0 kcal.mol^{-1}

CID	ΔE_{bind} (kcal.mol^{-1})	CID	ΔE_{bind} (kcal.mol^{-1})	CID	ΔE_{bind} (kcal.mol^{-1})
10323441	-11.3	16062971	-11.4	16129585	-11.1
3846	-11.0	445296	-11.2	446906	-11.1
447767	-11.2	449097	-11.2	5326625	-11.1

Table 2. The 3D structure of compounds top leads

CID	3D structure	CID	3D structure
10323441		3846	
447767		16062971	
445296		449097	
16129585		446906	
5326625			

In general, the compounds top leads have aromatic rings (the role of aromatic rings do not present this report). These results can assess important role of aromatic rings by MM-PBSA method.

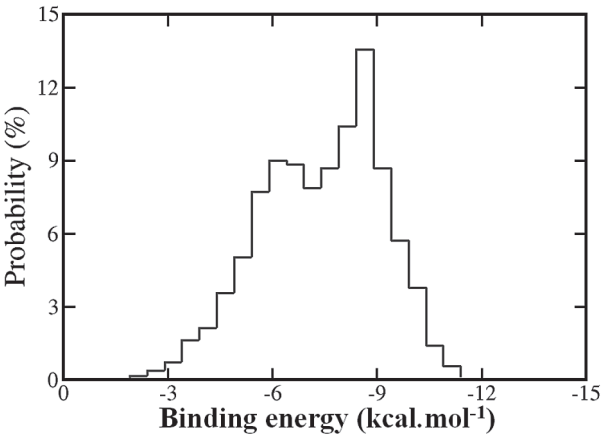


Figure 5. Distributions of binding energies of 5732 ligands to receptor

Figure 5 showed that the distributions of binding energies of 5732 ligands to receptor 2RFL are focused mainly with a level of binding energy -8.4 kcal.mol^{-1} about 13.6%, while -11.0 kcal.mol^{-1} about 0.15%.

3.2. SMD results

Using the Caver 3.0, one can obtain several possible pulling directions but the easiest pathway with the lowest rupture force F_{max} was chosen. For each ligand, five independent SMD runs were performed, and the results were averaged over all trajectories. Typical force-time curves are presented in Figure 8 showing the sensibility of rupture force on SMD runs. The SMD method was applied to study the binding affinity of 09 top leads. The SMD and docking results are shown in Table 3. The ranking of binding affinities based on docking energies is different from that predicted by SMD (Mai & Li, 2011, Vuong et al., 2015).

The compound CID 16062971 is champion in docking, but it is seventh in SMD, while SMD predicts that among 09 top hits compound, CID 3846 is the strongest, but it is the lowest in docking. Correlation coefficient between rupture force (F_{max}) by SMD method and binding energy by docking method is R = 0.48 (Figure 7). This result suggests that the SMD method may be used the binding affinity exactly than docking method (Mai et al., 2011) because the dynamics of receptor atoms were neglected. In general, within the error, the rupture (F_{max}) of compounds is similar, average about 846 pN ± 30 pN.

Table 3. The ranking of binding affinities based on docking energies (ΔE_{bind}) and rupture force (F_{max})

No.	CID	F_{max}(pN)	ΔE_{bind} (kcal.mol^{-1})
1	3846	1048.4 ± 39.9	-11.0
2	445296	991.5 ± 30.7	-11.2
3	5326625	900.9 ± 29.4	-11.1
4	447767	833.8 ± 29.5	-11.2
5	449097	820.7 ± 18.2	-11.2
6	446906	792.6 ± 14.8	-11.1
7	16062971	755.8 ± 40.8	-11.4
8	16129585	743.2 ± 16.5	-11.1
9	10323441	727.4 ± 51.8	-11.3

Typical force-time profiles are obtained for five systems at v = 0.005 nm.ps^{-1}. Figure 8 and Figure 9 show the position and time dependence of force, obtained from one MD run for 09 top leads (Mai & Li, 2011; Vuong et al., 2015).

Unbinding pathways might be divided into two parts. Before the maximum, the system behaves like a spring as *f* grows with Δx linearly. After the peak the behavior becomes more complicated because of occurrence of a weak peak at large time scales, when a ligand is about to move out from the binding pocket (Mai & Li, 2011, Vuong et al., 2015).

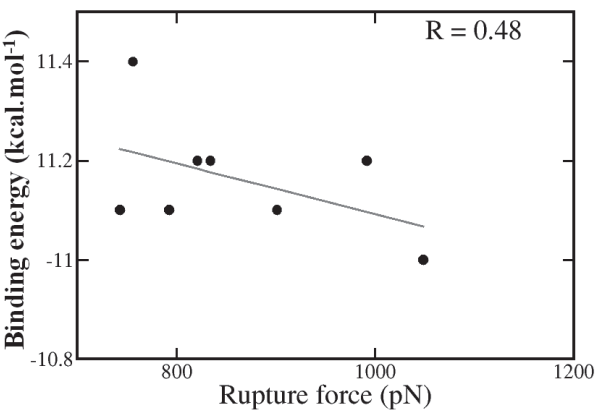


Figure 7. The Correlation coefficient between rupture force and binding energy

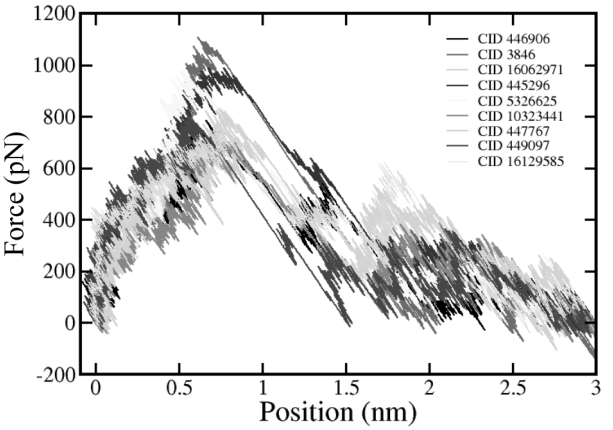


Figure 8. Force-position profiles obtained by the SMD method

If one uses the position of the cantilever from its original position, Δz, as a reaction coordinate, then peaks occur at Δz ≈ 0.5 - 0.7 nm (Figure 8) and Δt ≈ 280-380 ps (Figure 9). After passing the peak, the force decreased rapidly.

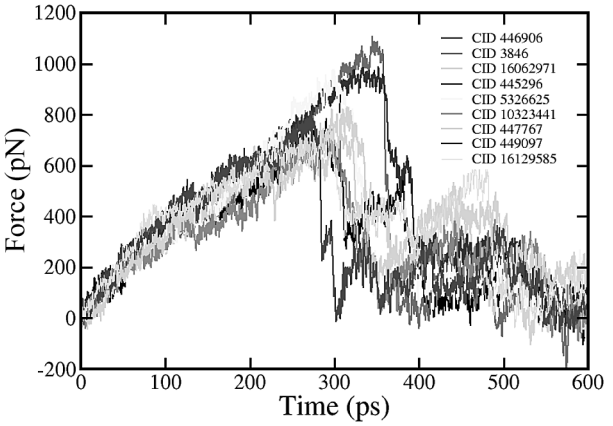


Figure 9. Force-time profiles obtained by the SMD method

4. Conclusions

We suggest that the SMD can serve as a very promising method for drug design because the SMD is shown to be more accurate than the docking approach, which exhibited rupture force. The correlation level R=0.48 showed that the correlation coefficient between rupture force (F_{max}) by SMD method and binding energy by docking method is appropriated. Motivated by this observation, we applied it to study binding of 09 ligands to target 2RLF. The ranking of binding affinities based on docking energies is different from that predicted by SMD. The compound CID 3846 has rupture force strongest in 09 top leads. Therefore, we recommend it for further *in vitro* and *in vivo* studies. The reliability of SMD approach has been also checked by computation of binding free energies for seven systems using the MM-PBSA method, which was not shown in this paper./.

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