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Research paper

Predicted PAR1 inhibitors from multiple computational methods

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ABSTRACT

Multiple computational approaches are employed in order to find potentially strong binders of PAR1 from the two molecular databases: the Specs database containing more than 200,000 commercially available molecules and the traditional Chinese medicine (TCM) database. By combining the use of popular docking scoring functions together with detailed molecular dynamics simulation and protein-ligand free energy calculations, a total of fourteen molecules are found to be potentially strong binders of PAR1. The atomic details in protein-ligand interactions of these molecules with PAR1 are analyzed to help understand the binding mechanism which should be very useful in design of new drugs.

1. Introduction

Atherothrombosis is triggered by the rupture of an atherosclerotic plaque in the coronary artery and it is the major cause of cardiovascular death [1]. Plaque rupture results in a battery of clinical conditions, collectively known as acute coronary syndrome (ACS), which ranges from unstable angina to acute myocardial infarction [2,3]. Platelets play a central role in the progression of atherothrombosis and have become a key target for therapeutic intervention [4,5]. The currently available antiplatelet agents include: (1) aspirin, participates in the pathway of cyclo-oxygenase (COX)-1-mediated thromboxane A2 (TXA2) synthesis and activation via the TXA2 receptor. (2) clopidogrel, aimes at adenosine diphosphate (ADP) via the P2Y12 receptor. They all have modest potency, however, they are associated with an increased risk of bleeding. Another pathway involved in platelet activation is triggered by thrombin via the protease-activated receptor (PAR)-1, with thrombin being the most potent agonist [6–8]. The PARs are composed of four members belonging to the seven transmembrane G protein-coupled receptor family [9]. In humans, PAR1 is the main thrombin receptor on platelets and is widely distributed among cells and tissues such as endothelial cells, smooth muscle cells, monocytes and fibroblasts [10], but not in fibrin generation [11]. It is recognized as a promising antithrombosis target with potentially less severe bleeding side effects [11,12]. The coagulation protease thrombin activates the prototypical PAR, PAR1, by specific cleavage of the N-terminal exodomain of the receptor to generate a new N-terminus. This new N-terminus functions as a tethered peptide agonist that binds intramolecularly to the seven-transmembrane helix bundle of the receptor to affect G-protein activation [13–16].

Several inhibitors of PAR1 have been developed. The most advanced one in clinical trials is vorapaxar [17,18] (brand name Zontivity, formerly known as SCH530348), which was developed from a lead identified by a radioligand binding approach using a high affinity Thrombin Receptor Agonist Peptide [6,19]. The vorapaxar, recently approved by FDA, is a potent inhibitor of PAR1 but is associated with an increased risk of intracranial bleeding when used in combination with standard therapy in a phase III trial (TRA-CER) [19,20]. There are other PAR1 inhibitors under study, such as atopaxar [21–23] (formerly known as E5555), which has completed Phase II clinical investigation, BMS-200261 [24], RWJ56110 [25,26], RWJ58259 [27], FR17113 [28], F16618 [29,30] and F16357 [31], etc. [32]. They are analogues of the tethered peptide agonist of PAR1 or reformed from natural products. Thus, novel PAR1 inhibitors that may have new core structures are desired. The recent determinations of crystal structure of Vorapaxar-bound human PAR1 (PDB id: 3VW7) provides an excellent opportunity in structure-based drug discovery for PAR1 [13].

In this study, the structure-based virtual screening is carried out in hope to find novel PAR1 inhibitors. Although there are various molecule databases available, the Specs database has been chosen since the molecules in this database are all commercially available. In addition, the traditional Chinese medicine (TCM) database was

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also chosen for virtual screening. Subsequently, molecular dynamic simulation and binding free energy calculation are carried out to further validate the potential potency of the possible inhibitors for PAR1 target. The molecules obtained from this study can be useful lead compounds as novel potential inhibitors of PAR1.

2. Computational approaches

2.1. Molecular docking

The crystal structure of PAR1-vorapaxar complex is retrieved from Protein Data Bank (see Fig. 1). The docking study with the native ligand was first performed using both SYBYL [33] and Glide [34]. When using the SYBYL software, the protein was prepared using the BIOPOLYMER module. All the crystallographic water molecules were removed. Hydrogen atoms were added based on the Amber ff99SB force field [40]. Then the docking protocol file was generated using the Surflex-Dock module [35]. The active site was confirmed with the reference of the native ligand. Vorapaxar was prepared using Ligprep option. The protein was prepared using Protein Preparation Wizard option when utilizing Glide for docking. The grid was generated using the Receptor Grid Generation module in Glide. The native ligand was docked to the protein using SYBYL and Glide as a reference for the structure-based virtual screening.

We chose a commercially available compound dataset, Specs database, (downloaded from the ZINC website [36]) for virtual screening. The Specs database contains more than 200,000 small molecules. We first performed molecular docking to PAR1 using SYBYL. About 400 compounds which have relatively high total scores (>11) were selected for next round screening using Glide. We docked those small molecules to PAR1 utilizing both Glide SP and XP scores. Finally, top 5 molecules from each of three docking scores (namely, SYBYL total score, Glide SP and XP) were selected. The selected molecules were labeled by the database, the docking method, and the ranking number under the method which the molecule was selected by. For example, ZINC08424651 was selected using SYBYL software and ranked No. 1 in Specs database, hence, the assigned label for this molecule is Specs_sybyl1. Among the top 15 molecules selected from Specs database, Specs_sp2 and Specs_xp2 are the same molecules. Hence, we just chose Specs_xp2 out of these 2 molecules. The detailed work flow is presented in Fig. 2.

The molecules from traditional Chinese medicine (TCM) database were also chosen for virtual screening of the target protein. The database containing more than 30,000 molecules was also downloaded from the ZINC website. Since the number of molecules in TCM is small, the database was screened using each docking score, respectively. The top five hits of each ranking score (namely, SYBYL total score, Glide SP and XP) were also selected for further study. Furthermore, there are no duplicated molecules among these 15 molecules. Therefore, 29 molecules were finally selected from Specs and TCM databases for subsequent molecular dynamics simulations and more rigorous binding affinity calculation using MM/PBSA.

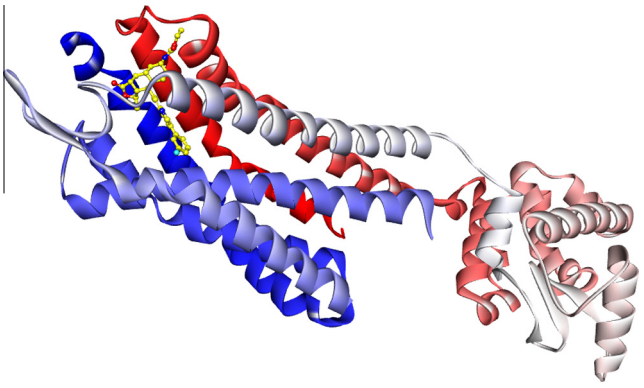

Fig. 1. The 3D structure of PAR1-vorapaxar binding complex. Vorapaxar is shown in ball-and-stick mode.

2.2. Molecular dynamics simulation

The initial structure of PAR1-Vorapaxar complex was taken from the Protein Data Bank. Hydrogen atoms of protein were added by the Tleap module based on the Amber ff99SB [37] force field in Amber12 [38]. The geometry of ligand was optimized at the HF/6-31G* level. Then, force field parameters of the ligand were obtained using ANTECHAMBER [39] module based on the generalized Amber force field (GAFF [40]) at the HF/6-31G* level. This complex was then soaked in a TIP3P water box with 10 Å buffer. Eleven chloride ions were added to neutralize the whole system.

A 10,000 step minimization was carried out using a quadratic constraint on all residues of PAR1 with force constant k = 500 kcal/(mol·Å^2), followed by a 30,000 step minimization on all atoms without any restraints. Then the whole system was heated up to 300 K in 50 ps using a weak restraint force constant k of 10 kcal/(mol·Å^2) on PAR1 with a time step of 1 fs. Finally, 2 ns simulation with a time step of 2 fs was carried out at 300 K with a quadratic constraint on the backbone atoms of the seven transmembrane helical domains of the PAR1 residues beyond 15 Å from the ligand, with the force constant k = 100 kcal/(mol·Å^2). Most region of the binding pocket located in the extra-membrane domain of the protein, and other parts of the protein away from the extra-membrane region have less impact on the binding. The restraint was used to simulate the membrane environment around the protein.

All minimization and MD simulation were performed using Amber 12. The SHAKE algorithm [41] was used to constrain all chemical bonds containing hydrogen atoms. For long-range electrostatic interactions, the particle mesh Ewald (PME) [42] method is used, and a typical 10 Å cutoff is used for van der Waals interactions. Langevin dynamics [43] is applied to control the temperature with a collision frequency of 1.0 ps^{-1}. The configurations were collected every 2 ps for all the complexes.

2.3. Protein-ligand binding free energies from MM/PBSA calculation

The binding free energy (ΔG) of the protein–ligand complex is calculated using molecular mechanics/Poisson–Boltzmann surface area (MM/PBSA) approach. The calculation only includes the unrestrained residues during MD simulation. The binding free energy is calculated as follows,

$$\Delta G = \Delta E_{ele} + \Delta E_{vdw} + \Delta G_{PB} + \Delta G_{nonpolar} - T\Delta S \quad (1)$$

where ΔE_{ele} and ΔE_{vdw} are the electrostatic and van der Waals interaction energies between the protein and ligand; ΔG_{PB} and $\Delta G_{nonpolar}$ are the polar and nonpolar components of the desolvation energy based on the PB model, respectively; and TΔS is the change of conformational entropy upon ligand binding. 50 snapshots from the equilibrated 0.5 ns simulation time with 10 ps interval were used to compute the ensemble-averaged binding free energies using the MM/PBSA method. In MM/PBSA calculation, the value of the exterior dielectric constant was set to 80, and the solute dielectric constant was set to 1. The nonpolar solvation term is calculated from the solvent-accessible surface area (SASA [44]): $\Delta G_{nonpolar} = \gamma \times \Delta SASA$ [where γ = 0.0072 kcal/(mol·Å^2), and the