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Research Article

SOLVATION FREE ENERGY OF THIENO [3,2-b] PYRIMIDINE ANALOGS COMPRISING INTERMOLECULAR SOLVATION AND INTRAMOLECULAR SELF-SOLVATION

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ABSTRACT

Objective: The aim was to estimate the various physiochemical properties of a molecule and the de-solvation cost for its binding to macromolecular receptors, the solvation free energy is a fundamental thermodynamics that has to be used. Here, a new solvation free energy carried out through the improvement of the existing solute-solvent interaction model and test its applicability in estimating the solvation free energies of vascular endothelial growth factor receptor-2 (VEGFR-2) inhibitors are discussed.

Methods: The molecular dynamics program GROMACS, which is designed for free energy calculations and bond simulations, has been used to understand the solvation free energies.

Results: The estimates of the solvation free energies of VEGFR-2 inhibitor molecules showed a reasonable accuracy by combining the effects from the solvent exposed and self-solvation regions. This significant contribution of free energies is thus consistent with the stability of the inhibitors in the solvent.

Conclusion: The estimated solvation free energies from the new model illustrated a good association with the solute-solvent interaction. The current solvation model is thus expected to be more useful in supporting the stability of the inhibitors within a solvent.

Keywords: GROMACS, Solvation free energy, VEGFR-2.

INTRODUCTION

The molecular solubility in aqueous solution is very crucial characteristic feature in various chemical and biological process including the structural change, inter and intramolecular interactions [1]. The solubility is also important because the entire drug reactions are involved in an aqueous medium and bioactivity of the binding site decides the stability of the protein-ligand complex. The calculation of free energies using molecular simulation has been in the research field for so long. It also helps to study the probability of a system adopting a given state and the underlying process at atomic level [2]. The estimates can be calculated using the numerous simulations, which will reduce the experiment measurement of solubility, which is a very time-consuming procedure, which prevents its use for the purpose of screening a large number of compounds. Considering the above statements, it has been very much attracted by the material science and the rational drug design [3]. The computation of solvation free energy has been a challenge for structure based drug design because the de-solvation effect plays a significant role in determining the binding mode and the binding affinity of the protein-ligand complex. The prediction of solvation free energies has been more reliable computational method in recent years as it more important for the development of combinatorial chemistry [4]. However, solvation free energy has been considered as one of the most calculation-difficult energy terms due to the complexity of solvent-solute interactions. There are many methods available for the solubility prediction. In this paper, GROMACS package has been used due to the accuracy of prediction [5]. To be more precise, there are numerous statistical modeling methods that have been investigated using the molecular concept such as artificial neural network, fragmental substructures, surface area model, topological parameters, general solubility equation, free energy perturbation and multiple linear regressions [6]. For smaller and simple systems, the absolute free energy can be calculated directly using analytical expressions. On the contrary, analytical expression systems are not suitable to calculate the absolute free energy for larger systems [7]. The absolute free energy calculations can be derived when the free energy of the reference state is known, like as for an ideal gas, for a gaseous system, or a perfect crystal, for a solid phase system. The appropriate reference is very difficult to build in liquid phase system; however, it is necessary to embed that model in this work to study more about the atomic changes [8].

In this work, the continuum electrostatic models of solvation have also been proposed to deal with molecular solvation free energy. The simplest way to develop the distant dependent to model electrostatic screening by solvent is by adjusting the dielectric constant [9]. The Poisson–Boltzmann equation has been used to calculate the electrostatic potential, which will be easier to derive the precise model for predicting the molecular solvation free energies. In the continuum models, however, the structural change of a solute upon solvation could not be taken into account, which has limited their usefulness to the solutes of simple ions and small molecules [10]. Earlier, Stouten et al. 1990, suggested a solvation model for a protein molecule by extending the solvent contact model. The maximum atomic occupancy, the atomic solvation parameters and the atomic fragmental volume are the key parameters involved in representing the solvation free energy per unit volume. In most of the cases, the aim would be calculate the relative energies of the two different compounds binding to the same receptor. The "absolute" free energy is defined as the free energy between the unbound and bound state of a single molecule [11]. In general, the estimate of the free energy differences is done between two States A and B (or possibly a series of pairs of States A and B). This simple solvation model proved to be very successful in estimating the structural properties of a protein as well as in saving computation time in molecular dynamics simulations when compared to the explicit solvent model [12]. All the atomic parameters in the solvation free energy function are optimized by the operation of a standard genetic

algorithm using the experimental solvation free energy data. It will be shown that the improved solvent contact model with the newly developed atomic parameters can be an appropriate tool for predicting solvation free energies of organic molecules in aqueous solution [13]. 3-D box whose length, width, and height correspond to the maximum distances along the three axes defining the co-ordinate system of the van der Waals volume of the molecule. Monte Carlo simulations involving random selections of a point in the predefined 3-D box were then carried out to calculate the total volume of the molecule embedded in the box. In this simulation, it could be obtained by the volume of the box multiplied by the ratio of the number of trials to select a point in the molecular van der Waals volume to the total number of trials [14]. In the present study, the ligands in aqueous solution are simulated to know the solvation free energy of the best vascular endothelial growth factor receptor-2 (VEGFR-2) inhibitors, which can be used for future clinical research.

METHODS

System preparation

The molecular dynamics simulations and free energy calculations of top six thieno [3,2-b] pyrimidine analogs (ZINC01056202, ZINC06091460, ZINC06091450, ZINC04107510, ZINC04623218, and ZINC81582433) is essential to identify the stability. The initial simulation system is prepared with the GROMACS 4.6.5 utility PDB2GMX with default protonation states, with GROMACS AMBER 9642 force field [15]. There are no difficulties using the protonation status because the binding cavity is mostly occupied with hydrophobic groups. Later, the protein is placed in a dodecahedral simulation box roughly filled with 6000 water molecules which are pre-equilibrated for 1 ns. In it, the protein is fixed prior to the equilibration [16].

Topology orientation

The ligand orientation is prepared using the PRODRG2 server with AMBER force field [17]. The charges that were generated in the server are retained and tried to differentiate the parameter difference and methodology difference [18]. Finally, the server produced the topology and coordinated files that are used for further analysis. Prior to the simulation, the ligand topology and the coordinate files are merged for the pre-solvated system. A separate 1 ns molecular dynamics simulation is performed to understand the total number of ligand orientation in the solvent. Finally, only one orientation is used for free energy calculation [19]. In general, the restrained orientation is also considered to have relative binding site to the ligand that will subsequently be easy for calculating accurate free energy of solvation. The positions are randomly picked considering the degree of freedom as determined during the simulation process, although in principle this choice is arbitrary [20].

Solvation free energy calculations

Independent binding free calculation is performed for each kinetic distinct orientation. Using the orientation decomposition procedure, the final binding free energy is derived from the effective binding free energy of each orientation ($\Delta G°_{multiple}$) [21]. The binding free energy of a single potential bound orientation is also calculated with symmetric corrections, as done in docking ($\Delta G°_{single}$). The entire experiment is performed using GROMACS 4.6.5 using the Bennett acceptance ration method to the difference in free energy [22]. The thermodynamic cycle is developed to calculate the absolute binding free energies. In this method, the ligand is allowed to restrain harmonically with the solvent [23]. Later the system is annihilated using the ligand's partial changes, and then decoupled the rest of the system with Lennard-Jones (LJ) interaction. The final state of the ligand is equivalent to a non-interacting ligand with no electrostatics, restrained, in vacuum or water [24]. By removing the restraints, the free energy is calculated and derives the free energy of restoring first the LJ and then the electrostatic interactions in water. In the complete process forms a thermodynamics cycle which transfer the ligand to a standard system of bulk water from the binding site [25]. The measurement of the absolute binding site energy is easy to get when the whole system is in converged, $\Delta G°$, for the selected force field and the solvent model. The independent free energy calculations are taken at sequential alchemical states (denoted by the parameter λ) as part of each of the step [26].

Simulation process

At each λ value, the simulations are performed using the following parameters. The velocities are taken from Maxwell–Boltzmann distribution at 300 K and the isothermal molecular dynamics is carried out at 10 ps [27]. This is followed by isothermal-isobaric dynamics at 100 ps using the Berendsen weak-coupling method. The final production simulation is run using the Langevin integrator for temperature control with a fixed cell size simulation. The particle-mesh-Ewald parameters are also modified to tune the accuracy of the process [28]. In addition, van der Waal correction is used to correct for the effect of truncating the long range dispersive interactions at a finite cut-off. These interactions have a significant role since the compounds have a higher density of attractive sites than water. The long and short cut-offs in LJ interactions are compared in order to estimate the decoupling free energy. A relative selection has increased the binding affinity and also increases when the ligand size increases. Only one symmetric orientation of benzene is considered to reduce the complications in convergence [29].

The solvation free energy is most important quantity in thermodynamic analysis because it demonstrates the level of molecular solubility in the solvent. Here is an illustration of top six ligands, which are selected from the docking studies. Based on the interaction level, those molecules

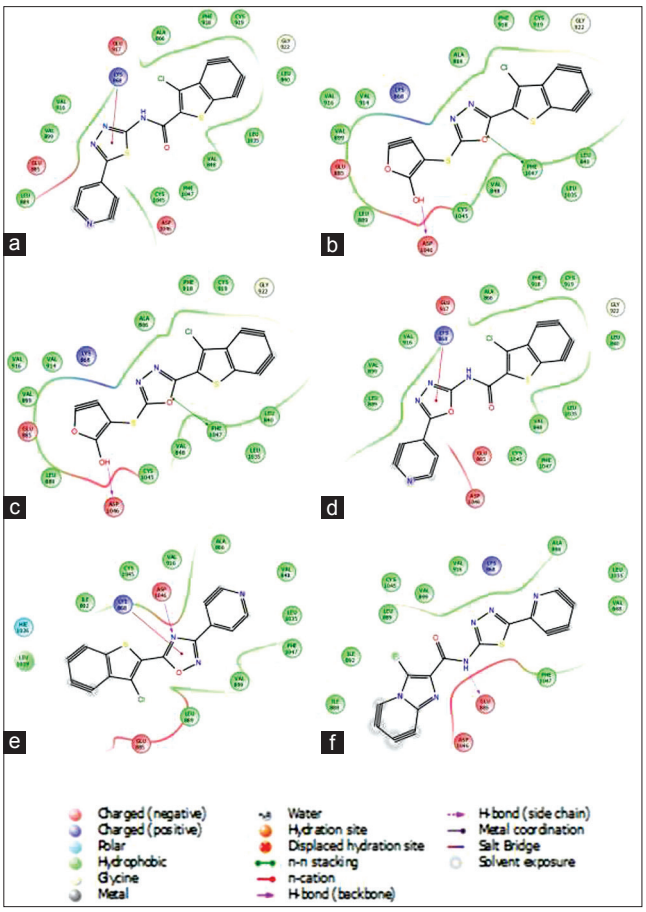

Fig. 1: Binding mode illustration of protein ligand complex. (a) ZINC01056202 - vascular endothelial growth factor receptor-2 (VEGFR-2) complex, (b) ZINC06091460 - VEGFR-2 complex, (c) ZINC06091450-VEGFR-2 complex, (d) ZINC04107510 - VEGFR-2 complex, (e) ZINC04623218-VEGFR-2 complex, (f) ZINC81582433-VEGFR-2 complex

are put into molecular dynamics studies to study the solvation free energy [30]. To avoid the system crash during the simulation process, the charge interactions and LJ terms are turned off, which is of minimal impact on the final estimation. The observation of Hamiltonian energy perturbation is done at various points from the State A (λ=0) to State B (λ=1), which has allowed us to collect the adequate data for analysis and to produce the reliable $\partial H/\partial \lambda$ curve [31]. The decoupling of Coulombic and van der Waals interactions are done at equidistant λ spacing from 0 to 1, to produce the transformations correctly. The following steps steepest descents minimization, L-Broyden–Fletcher–Goldfarb–Shanno minimization, NVT equilibration, NPT equilibration and data collection under an NPT ensemble are performed sequentially to get the data values at converged and stable state.

RESULTS AND DISCUSSION

The single-reference (SR) thermodynamic integration (TI) approach is computed for the series of thieno [3,2-b] pyrimidine analogs, for a total of top six best-fit ligands with VEGFR-2. The computational data for docking is summarized in Fig. 1, along with the different interaction regions with various amino acids of VEGFR-2 protein. In general, the current SR-TI is more accurate than the previous TI methodologies. The accuracy calculations are performed using the NVT simulations whereas the entire molecule is annihilated. On the other hand, NPT ensemble is used in SR-TI simulations and particular molecules are annihilated beyond the common reference sub-structure. The Hamiltonian Replica Exchange (HREX) SR-TI is validated by comparing the hydration free energies with and without HREX option. In this study, mostly benzene core is used as a reference. The water phase contributions create the observed disparity. The mean force profile λ=0, wherein the molecule is totally absent in the water phase along with GROMOS soft core potential properties. This soft core effect of hydrogens is observed without the LJ interactions. To see a trend in more atoms, the molecule A is switched off and turned on to the reference State B on sequential phase. After calculating the differences in free energy the regular molecular dynamics, suggested the sufficient stability of the molecule in the water phase. To assess the solvation free energy calculated by HRES SR-TI approach, more complex set of molecules were turned on to provide additional torsional degrees of freedom. The error bars are always within the estimated values, indicating a reduction in a large variation of molecule inside the solvent phase. The focus is also given to the predominant N-C amide bond for understanding the rotation of those bonds. The trans isomer had slight favorable result compared with cis isomer. Using the regular calculation of SR-TI simulations, the cis/trans ratios are compared in the water phase. Increasing the number of atoms is directly proportional to the results of HREX SR-TI simulations and their derived values. Is also depends on the surrounding water molecules and increasing it will reduce the standard deviation of the computed solvation free energy. The simulation is performed at every 500 steps (every 1 ps) as opposed to every 1000 steps. There is a significant increase in value near to the experimental value when the simulations are performed for a longer period. Before changing the Hamiltonian states, the entire system is equilibrated in the same way to avoid the difference in energy calculations. Fig. 2 shows the calculated the Hamiltonian energy at each state from λ 0 to 1, corresponding to the cis and tans conformations. Fig. 3 illustrated the integral of the individual free energy on top the individual free energy at various points. The introduction of equilibration time has increased the quality of simulations. This work has demonstrated the solvation free energy calculation to get high-quality results for ligands with multiple configurations that have unique solvation properties. The solvation free energy of this approach is independent of cis or trans isomer initiated simulation. All these results provided confidence in the approach, necessary for the calculation of free energy of protein-ligand complex.

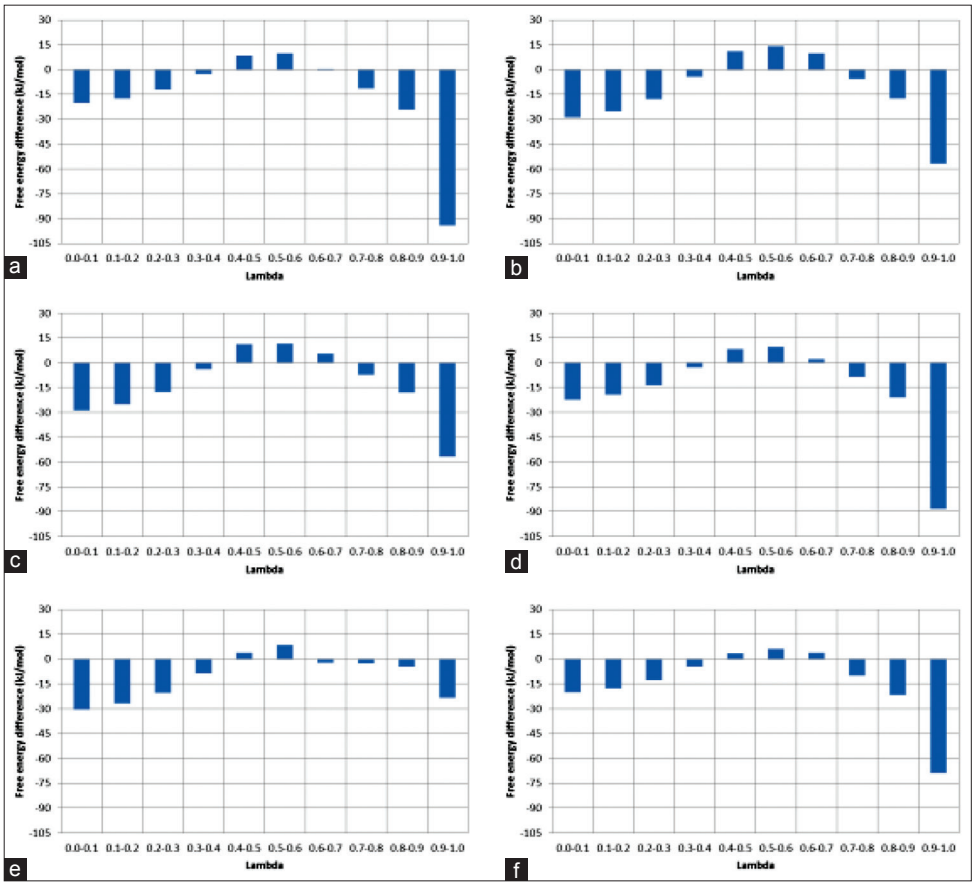

Fig. 2: Free energy difference of the ligands. (a) ZINC01056202, (b) ZINC06091460, (c) ZINC06091450, (d) ZINC04107510, (e) ZINC04623218, (f) ZINC81582433

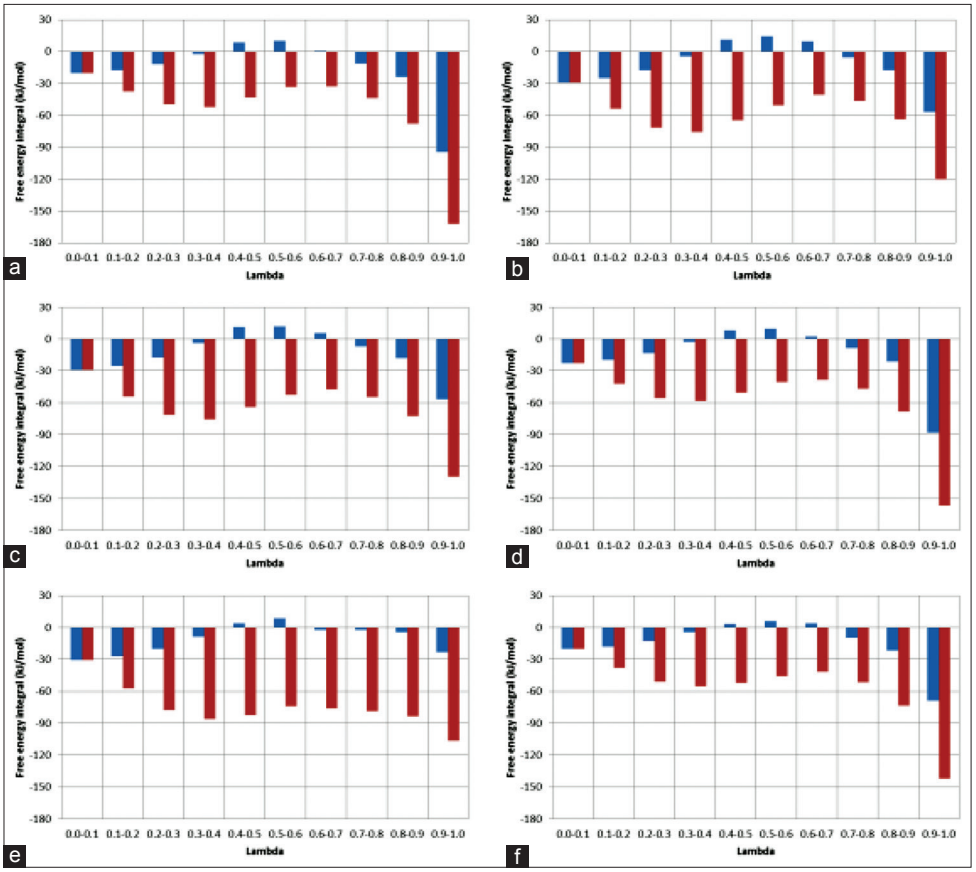

Fig. 3: Free energy integral of the ligands. (a) ZINC01056202, (b) ZINC06091460, (c) ZINC06091450, (d) ZINC04107510, (e) ZINC04623218, (f) ZINC81582433

CONCLUSION

The performance of the modified solvent contact model has been demonstrated involving the ligand in aqueous solution with predicted molecular solvation free energies. The current model contains 3D molecular coordinates with no additional molecular structures being required to calculate solvation of free energy. The solvation model was developed with the top six VEGFR-2 inhibitors (ZINC01056202, ZINC06091460, ZINC06091450, ZINC04107510, ZINC04623218, and ZINC81582433), which illustrated the stability of it in aqueous solution. The accuracy of the calculations is dependent on the presence of multiple ligand orientations and its conformational changes. It also relies on the duration of the molecular dynamics simulation done for each molecule. The observations in the model will also be found in biological relevant binding sites. The alchemical free energy methods hold good benefits in understanding the stability of the approximate protein-ligand complexes.

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