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MM-PBSA and per-residue decomposition energy studies on 7-Phenyl-imidazoquinolin-4(5H)-one derivatives: Identification of crucial site points at microsomal prostaglandin E synthase-1 (mPGES-1) active site

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ABSTRACT

The huge therapeutic potential and the market share of painkillers are well-known. Due to the side effects associated with traditional NSAIDs and selective cyclooxygenase (COX-2) inhibitors, a new generation of painkillers is the need of the hour. In this regard, microsomal prostaglandin E synthase-1 (mPGES-1) offers great potential as an alternative drug target against inflammatory disorders. The present study is aimed at identifying the amino acids crucial in effective inhibitor binding at the mPGES-1 active site by performing molecular dynamics based studies on a series of 7-Phenyl-imidazoquinolin-4(5H)-one derivatives. Molecular dynamics (MD) simulations, MM-PBSA, per-residue energy decomposition and Dimensionality Reduction through Covariance matrix Transformation for Identification of Differences in dynamics (DIRECT-ID) analysis were performed to get insights into the structural details that can aid in novel drug design against mPGES-1. The high correlations of electrostatic and polar energy terms with biological activity highlight their importance and applicability in *in silico* screening studies. Further, per-residue energy decomposition studies revealed that Lys42, Arg52, Arg122, Pro124, Ser127, Val128 and Thr131 were contributing more towards inhibitor binding energy. The results clearly show that MM-PBSA can act as a filter in virtual screening experiments and can play major role in facilitating various mPGES-1 drug discovery studies.

1. Introduction

mPGES-1 is the abbreviation for Microsomal Prostaglandin E Synthase-1, an enzyme belonging to the MAPEG superfamily of proteins [1]. It is a glutathione dependent enzyme which is at the terminal step of arachidonic acid pathway converting prostaglandin H2 (PGH2) to prostaglandin E2 (PGE2), a major mediator of inflammation and pain [2]. Arachidonic acid is metabolized with the help of three different pathways; cyclooxygenase, lipoxygenase and cytochrome P-450 producing prostaglandins, leukotrienes and epoxygenases respectively [3]. Cyclooxygenase-2 (COX-2) and lipoxygenases are already identified as important drug targets against inflammation and there are a variety of inhibitors both in market and in developmental phase targeting these two [4–8]. However, the prolonged use of these compounds is associated with wide range of cardiac and gastro-intestinal side effects [9].

mPGES-1 is an induced enzyme activated by a pro-inflammatory stimuli and is believed to be functionally coupled with COX-2 [10]. As mentioned earlier, mPGES-1 is involved in the conversion of PGH2 to PGE2, whose elevated concentrations are found in patients suffering from inflammatory disorders like arthritis, osteoarthritis, sclerosis and a variety of cancers and neurological disorders [11–21]. It is assumed that designing mPGES-1 specific drug candidates can significantly improve the safety profile of painkillers and other anti-inflammatory drugs [22–27].

The number of reported studies on mPGES-1 showed dramatic increase over the last decade, however, there are very few *in silico* studies conducted on mPGES-1. Earlier we reported a rescore to improve the predictions of various docking programs against mPGES-1 and also identified the key residues involved in effective inhibitor binding at the mPGES-1 active site using structure based drug design (SBDD) techniques [28,29]. *In silico* techniques are crucial as they tend to limit the time and cost involved in the development of novel drug like molecules. In this regard, molecular dynamics simulations are considered important both in terms of accuracy and robustness as they offer real time environment for the protein-ligand complexes [30].

In the recent times there have been many success stories of molecular dynamics simulation studies where they assisted in the development of many drug candidates against various drug targets [7,31–36]. The

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strength of any bimolecular interaction is usually measured in terms of the binding energies and MD studies offer a variety of approaches to compute the binding free energies. The most commonly used approaches include free energy perturbations (FEP). Linear interaction energies (LIE), thermodynamic integration (TI), molecular mechanics Poisson–Boltzmann surface area (MM-PBSA), and molecular mechanics Generalized Born surface area (MM-GBSA) [37]. FEP and TI methods are most accurate but require higher computational expenses. On the other hand, LIE and MM-PBSA/GBSA approaches are more computationally cheaper. These techniques rely on the ensemble of conformations at the preliminary and final stages to estimate the binding energies. This path independent nature of these approaches is the main reason behind their computational efficiency [38]. MM-PBSA methods coupled with per residue energy decomposition studies are also crucial in finding the role of individual amino acids towards effective inhibitor binding [39]. MM-PBSA approach is the most widely used technique and it has been successfully applied to a number of protein-ligand systems [31–34].

Despite all these advantages, there are only a few MD simulation based studies on mPGES-1. The prime reason for this might be that mPGES-1 is a membrane protein and performing MD studies on a membrane system is complicated. Hamza et al. [40], performed molecular modeling and simulations studies on mPGES-1 for the first time. They identified the key residues participating in effective binding of substrate and inhibitors at the mPGES-1 active site by performing MM-PBSA studies and validated the computational results by comparing them with experimental data. The results of these studies were further utilized by the same research group to identify novel mPGES-1 inhibitors by performing structure based virtual screening [25]. In one of the studies, Chang et al. [41] have performed docking, QSAR, pharmacophore mapping and MD based studies to identify novel drug candidates against mPGES-1 using the traditional Chinese medicine database.

In another report, Shan et al., have used MD (molecular dynamics) simulations, mutation analysis, hybrid experiments and co-immunoprecipitation studies to understand the conformational changes in mPGES-1 during catalysis [42]. They identified Arg73 as crucial for interactions with the co-factor GSH and Arg126 as crucial for binding of PGH2 (the substrate).

In one of our previous reports our group reported a number of amino acids as crucial for effective inhibitor binding by performing molecular docking and in detailed interaction analysis [29]. In continuation to that, in the present study we attempted to perform SAR on a series of compounds by performing MD simulation studies followed by MM-PBSA. The SAR of the inhibitors was quantified using per residue energy decomposition. The aim of the study was not only to identify the amino acids crucial for inhibitor binding but also to single out amino acids that can distinguish binding modes of analogs in terms of better prediction of experimental activities.

2. Materials and methods

2.1. Dataset preparation

For the present study, ten 7-Phenyl-imidazoquinolin-4(5H)-one derivatives (Fig. 1) were selected from existing literature [43]. The compounds were prepared initially in Accelrys Draw 4.2 [44] and later geometrical optimization was performed in R.E.D. server [45-49] using Hartree-Fock method. After structural optimization these molecules were used for generation of protein ligand complexes. Ligand topology and parameters were obtained using ParamChem [50-53].

2.2. Preparation of the system

Being a membrane protein, a lipid bilayer was to be generated for mPGES-1 to complete the system before any MD simulation studies could be carried out. To achieve this CHARMM-GUI, a web based graphical interface was used. CHARMM-GUI generates input files for MD simulations for a variety of platforms like CHARMM, GROMACS, NAMD, AMBER and Desmond *etc.* [54]. The membrane builder [55-57] option was utilized for generation of the lipid bilayer and a POPC lipid bilayer was generated for the protein-ligand complexes. We prepared the input files for GROMACS [58,59]. For the present study, the trimer structure of mPGES-1 (PDB id 4yl3) [60] was obtained from the Orientations of Proteins in Membranes (OPM) database [61]. The OPM database provides the spatial arrangement of membrane proteins and peptides in the lipid bilayer aiding in systematic positioning of the lipid bilayer around the target protein. The optimized compounds were then overlaid with the co-crystalized inhibitor in the protein structure to generate the protein-ligand complexes. The protein structure was fed as input and the bilayer components were constructed around the protein and later assembled together to generate the complete system. CHARMM-GUI uses the CHARMM36 Additive Force Field for the generation of input files for MD simulation studies [59,62]. For solvating the system TIP3P [63] water model was used and system was neutralized by adding counter-ions in CHARMM-GUI itself. The same protocol was followed for preparation of all protein-ligand complexes.

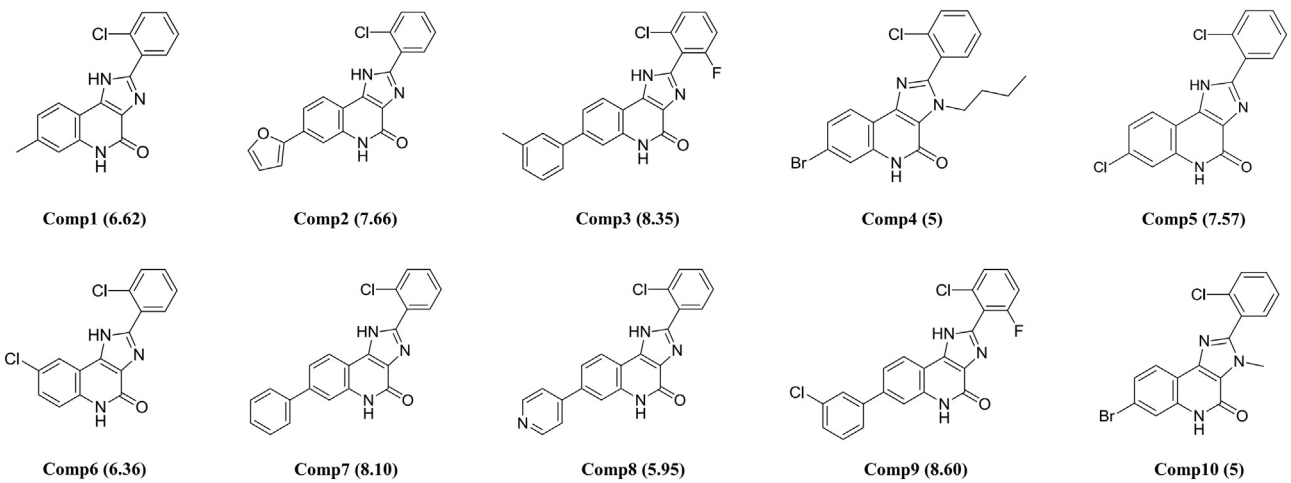

Fig. 1. Chemical structures of the investigational compounds with their experimental biological activity, pIC_{50} (in parenthesis).