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Young Scientists' Circle

In Silico Study of potential drug leads for inhibition of angioensin converting enzyme using Sri Lankan Natural Products

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Almost fifty percent of the active ingredients present in modern medicine is derived from natural products. Natural products were widely used in the past for drug discovery before high-throughput screening and genomic sequencing were practiced. These natural product-based drugs are mostly derived from different organisms, such as plants, microbes and animals as well as a few synthetic or semi-synthetic compounds that are derived from natural products, including tigecycline, everolimus, micafungin and caspofungin.[1] These natural product-based drugs have been used for a range of medicinal indications and they have a wide diversity of chemical structures. Approximately half (49%) of the 877 small-molecule New Chemical Entities (NCEs) introduced between 1981 and 2002 were natural products, semi-synthetic natural product analogues or synthetic compounds based on pharmacophores of natural products.[2]

Studies show that natural products usually have a higher number of chiral centers than either synthetic drugs or compounds derived from combinatorial libraries, and they have increased steric complexity. While a slightly higher number of nitrogen-, sulphur- and halogen-containing groups appear to be present in drugs and combinatorial molecules, natural products on the other hand, carry a higher number of oxygen atoms[3]. Multivariate statistical study of molecular descriptors shows that the ratio of aromatic ring atoms to total heavy atoms (lower in natural products), the number of solved hydrogen bond donors and acceptors (higher in natural products) and higher molecular rigidity of hydrogen bond donors and acceptors (higher in natural

products) differ significantly from synthetic drugs and combinatorial libraries in natural products.[4] Scientific publications on compounds extracted from Sri Lankan medicinal plants during the period of 1970-2014 were analyzed, and about 480 compounds published within this period were selected for virtual screening.

Angiotensin Converting Enzyme (ACE) was chosen as our protein of interest. It plays a major role in the management of blood pressure, cardiovascular function and diabetic kidney disease. In most tissues of the body, ACE is always expressed. However, a high level of expression is found in the lungs, kidneys, testes, duodenum, choroid plexus and placenta. Since ACE is expressed in multiple cell types, it cleaves different substrates, such as endothelial cells, renal tubular epithelial cells, gut epithelial cells and myeloid-derived cells. ACE is best characterized by its function in the cleavage of two carboxy-terminal (C-terminal) amino acids of angiotensin I, thereby generating angiotensin II, a vasoconstrictor. ACE also cleaves bradykinin, a vasodilator, releasing an inactive product of 7-amino acids. The enzyme can cleave substrates as small as 3 amino acids and as large as 42 amino acids, but most ACE substrates are only 15 amino acids in size or less.[5]

The human ACE has two functional domains N and C, each of which has a zinc-ion binding site at its active site. In their substrate specificities, physiological forms, and inhibitors, the N and C domains have several variations. On the one side, the hydrolysis of substrates with similar efficiencies is catalysed by the N and C domains.[6] However, inhibition of the N domain of ACE has been reported to have no effect on blood pressure regulation. It was found that targeting the C domain was adequate for blood pressure regulation, and hence, all inhibitors target this site. An important catalytic component of ACE is zinc. As shown in Fig 1, alpha13 has two histidine (His383 and His387) zinc-binding motifs coordinated with a Zn^{2+} ion. ACE has many substrates and can also be used as a neurotensin. The half-life of bradykinin is extended by ACE inhibition and can lead to accumulation and activity. Angiotensin-converting inhibitory enzyme drugs have for decades been first-class therapeutics.[7] Captopril, lisinopril, enalapril, and ramipril are examples of drugs that are ACE inhibitors. Adverse side effects such as dizziness, coughing, and angioneurotic edema may, however, be caused by prolonged use of these drugs. New alternatives have been widely investigated as substitutes for ACE inhibitors. The main focus of this study is on bioactive compounds obtained from Sri Lankan natural products.

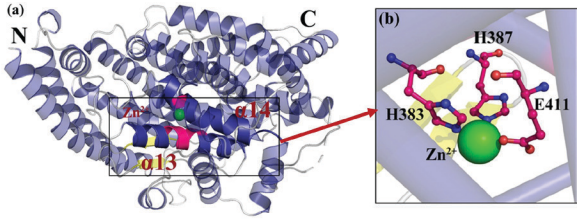

Figure 1: Active sites of angiotensin-converting enzyme; (a) Organization of the ACE protein. The active sites located in α13 and α14 of ACE are indicated by a black rectangle. (b) Zinc-binding motif; The residues that surround Zn^{2+} are represented by pink sticks.

To classify the possible targets for different ligands, computational and bioinformatics tools have become very important resources. Modern drug research and development (R&D) relies on the discovery of low-molecular weight compounds that interact with disease-related biological macromolecules, known as receptors or molecular targets, in a selective way. The use of structural information of molecular targets to improve aspects related to ligand binding is a core approach in the pharmaceutical industry, and it is commonly known as structure-based drug design (SBDD).[8] Methodologies for SBDD play a growing role in the development of drugs, and are important for the cost-effective identification of promising drug candidates. These computational methods of analysis are important in restricting the use of animal models in pharmacological study, they support the rational design of novel and potent drug candidates and in the repositioning of marketed medicines, and provide invaluable support for medicinal chemists and pharmacologists throughout the process of drug development. Lately, increasing attention has been paid to the concept of using direct or indirect structural knowledge on specific antitargets to enhance ligand selectivity and minimize off-target interactions, leading to increased protection and even improved pharmacokinetic profiles. *In silico* screening, which is generally combined with drug design, typically relies on exploration of the established chemical universe for the identification of new active motifs.[8] The objective of virtual screening is to uncover an unexplained or concealed interaction between

known chemicals and a given biological activity, and not in the chemistry of the emerging hits as this information is most likely available. Advances in SBDD over the past two decades have been achieved by the integration of spectroscopic data and other methods. These include X-ray crystallography, and in silico techniques such as molecular dynamics, homology modeling and molecular docking. The combined use of these methodologies has made it possible to determine the 3D structures of many biological macromolecules along with the precise characterization of their binding site features, such as steric and electrostatic properties. The understanding of ligand-receptor molecular recognition phenomena has been extremely useful. SBDD has successfully assisted the development of pioneering treatments for highly complex and prevalent disorders by combining these data with current technologies in pharmaceutical R&D.

The use of SBDD strategies enables the conception of ligands with specific steric and electrostatic properties that will effectively interact with a target pharmacological receptor. SBDD consists of a cyclic process that begins with the resolution of the 3D structure of the molecular target, in this case angiotensin converting enzyme.[9] The x-ray crystallographic 3D structure can be found on the RCSB protein databank. Each PDB entry contains information on experimental details, related literature, the biological relevance of the macromolecule, and statistical indicators on the quality of the 3D model. Next, molecular modeling investigations are performed to find putative ligands. Molecular docking is prominently used for structure-based virtual screening. The binding event is simulated, and a scoring function is used to predict, for the most probable binding poses, the free energy difference due to the binding of the screened compounds to the target starting from the experimental structure of the target. A homolog from other organisms or another protein belonging to the same family may be used if the structural information of the desired protein is not available, and this is referred to as comparative or homology modeling. Both rigid and flexible approximations are possible. Though the rigid approach is computationally less demanding, the more accurate flexible approximation is computationally challenging. Molecular docking, compared to ligand-based approaches, can be considered a computationally demanding virtual screening approach. To produce a variety of ligand-binding orientations which are rigid body approximations, a search/sampling algorithm is used.

There are currently various software programs and instruments available for use at each point of the molecular docking process. Of all the molecular docking software currently available, AutoDock Vina[10] is one of the most cited software. An effective stochastic conformational search algorithm, and precise and well-rated force-field and empirical scoring functions are combined in AutoDock Vina[10]. Moreover, AutoDock Vina[10] has the capability to combine its productivity and competency in order to produce parallel computational results with a low computational cost. This makes AutoDock Vina[10] an ideal tool for structure based virtual screening against large compound libraries. Free-energy simulations, which employ molecular dynamics or Monte Carlo simulations, provide a more rigorous solution to binding free-energy estimation. Molecular dynamics (MD) simulations can be used to explore the dynamics of the target protein. This method has become popular for studying protein structures and in extracting conformational ensembles of these targets. These simulations can be carried out in the context of the solvent, ions, and various physiological parameters. In this way, one will not only able to identify and understand the flexibility of a given binding site but will be also able to explore its interaction with water molecules and ions.[11] Moreover, by analyzing the MD trajectory, one can also measure the persistence of the binding site, duration of the hydrogen bonds, and varying depth and width of the binding site.

In this study, promising compounds that are identified by in silico studies are thereafter commercially purchased or synthesized, and evaluated for potency, affinity, and selectivity against the receptor, ACE. Once active molecules are identified by wet studies, the 3D structure of the ligand–receptor complex is determined, enabling the identification of the intermolecular interactions that drive the molecular recognition process. Additionally, determining the structure of the ligand–receptor complex enables the construction of relationships between biological activity and structural features. Finally, taking these results into account, molecular optimization efforts may be conducted to improve the ligand properties, mainly those related to

affinity, selectivity, and efficiency.

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