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Molecular Docking in Drug Discovery: A Review on Anti-snake Venom Development

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Authors' contributions

This work was carried out in collaboration among all authors. Author ARF wrote the first draft of the manuscript and managed the literature searches. Authors GA and ZH encourage author ARF to investigate (enzymes in snake venom) and supervised the findings. All authors contribute, read and approved the final manuscript.

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ABSTRACT

Snakebite is a frequent accident faced by rural community's dwellers, and it has remained a neglected public health problem in many countries. Snake venom is a complex mixture of proteins, and they participate to envenomation through a diverse array of bioactivities, such as bleeding, inflammation, and pain, cytotoxic, cardiotoxic or neurotoxic effects. The only approved and accepted treatment for snakebite envenoming is the use of antivenoms produced by the purification of IgG immunoglobulins immunized against specific snake venom. However, various technological approaches are being pursued by different research groups, including the use of small-molecule inhibitors, antibody-based bio-therapeutics and peptide-based aptamer against enzymatic toxins and non-enzymatic toxins in snake venom. Modern bioinformatics tools have been recently developed to mine snake venoms, helping focus experimental research on the most potentially interesting toxins. Some computational techniques predict toxin molecular targets, and the binding mode to these targets. This review presents molecular docking studies of potential targeted key enzymes in snake venom.

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1. INTRODUCTION

Snakebite is a frequent accident faced by villagers, and has remained a neglected public health problem in many countries even though it is difficult to be precise about the actual number of cases [1]. In sub-Saharan Africa alone, snakebite is estimated to cause between 435,000 to 580,000 envenomation and 20,000 to 32,000 deaths every year [2]. Amputation and disability, tetanus, gangrene, cortical necrosis of the kidneys etc., are among the medical manifestations of snakebite envenomation [2]. The five families of poisonous snakes are Colubridae, Elapidae, Hydrophidae, Viperidae and Ataspididae. Snake venom is a complex mixture of different enzymes, which are proteins [3]. These enzymes determine the toxicity of the snake venom as to whether it is haemotoxic or neurotoxic but the venoms cannot be classified as being exclusively haemotoxic or neurotoxic. Prompt administration of antivenom is the cornerstone of effective snakebite management, although supportive care is crucial too, including assisted ventilation in case of neurotoxic envenomation [4]. The path to drug discovery is a long, expensive, challenging and arduous tasks, hence, computer aided drug design and discovery (CADDD) is a rapidly growing area that has seen many successes in the last few years [5]. Computational drug discovery can help in identifying potent drug molecules and targets via bioinformatics tools, such as molecular docking. They can also be used to evaluate the target structures for possible binding/active sites, generate active drug molecules, check for their dynamic and kinetic properties [6,7]. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational drug design [8].

2. MOLECULAR DOCKING

Molecular docking is the process that involves placing molecules in appropriate configurations to interact with a receptor [9]. Molecular docking is one of the most frequently used methods in structural based drug design (SBDD) because of its ability to predict, with a substantial degree of accuracy, the conformation of small-molecule ligands within the Molecules appropriate target binding site [10].

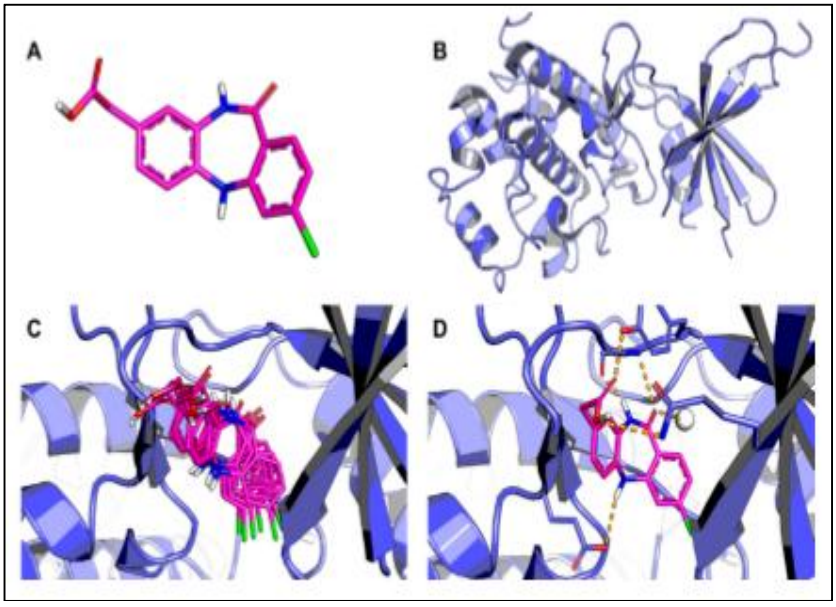

Fig. 1. Outline of the molecular docking process. (A) Three-dimensional structure of the ligand; (B) Three-dimensional structure of the receptor; (C) The ligand is docked into the binding cavity of the receptor and the putative conformations are explored; (D) The most likely binding confirmation and the corresponding intermolecular interactions are identified [11]

2.1 Molecular Docking Models

Over the years, biochemists have developed numerous models to capture the key elements of the molecular recognition process. The models are:

I. **The Lock and Key Theory:** As far back as 1890, Emil Fischer proposed a model called the "lock-and-key model, where a substrate fits into the active site of a macromolecule, just like a key fits into a lock. Biological 'locks' have unique stereo-chemical features that are necessary to their function [12].
II. **The Induced-Fit Theory:** In 1958, Daniel Koshland introduced the "induced-fit theory". The basic idea is that in the recognition process, both ligand and target mutually adapt to each other through small conformational changes until an optimal fit is achieved [13].

2.2 Application of Molecular Docking in Drug Development

Drug discovery can be described as the process of identifying chemical entities that have the potential to become therapeutic agents [14]. The process of drug discovery is time consuming, tedious and expensive. Therefore, Molecular docking has become one of the most important modeling tools in modern drug discovery because is a very convenient and cheap means to study protein-ligand interactions, which can be used to develop more potent, selective and efficient drug candidates [15]. The application of molecular docking in drug discovery process include the following:

i. **Hit identifications:** Docking in combination with scoring function can be used to evaluate large databases for finding out potent drug candidate, which can target the molecule of interest.
ii. **Side effect prediction:** Docking-based tools have predicted the efficacy of potential therapeutic compounds and have also helped in predicting the range of unintended and undesired interactions between the specific compound and the human proteome. Italso plays a prominent role in the initial prediction of drug's binding properties to nucleic acid. This information establishes the correlation between drug's molecular structure and its cytotoxicity.
iii. Molecular docking can be used to establish mechanisms of action a potent drug candidate against a molecular target.

3. VENOM MOLECULAR TARGETS

Numerous snake venom proteins have been identified as druggable or potentially druggable targets, including phospholipases A_2 (PLA_2s), metalloproteinases (SVMP), serine proteases (SVSP), kallikrein-like serine proteases, 5'nucleotidase, ATPase, alkaline phosphomonoesterase and acetylcholine esterase, and many of these have been characterized crystallographically. These protein crystal structures serve as structural models for in-silico screening using molecular docking techniques. Often, there are different structures, usually with different co-crystallized ligands, that can provide slightly different and complementary binding sites for docking studies [6].

4. PHOSPHOLIPASE A_2

PLA_2 are a group of esterolytic enzymes present in snake venoms that typically catalyze the breakdown of glycerophospholipids, the main component of biological membranes, into lysophospholipids and a fatty acid [16]. PLA_2s are found in the venoms of Viperidae, Elapidae, and Colubridae snakes. The lengths of PLA_2s vary from 119–134 amino acids, and they share a common scaffold of four main helices with seven intrachain disulfide bonds [17]. The snake venom PLA_2s are split into two groups, group I PLA_2s are found predominantly in elapid and some colubrid snakes, while group II are found only within Viperidae [18,15]. Group I are generally β-neurotoxins which act pre-synaptically, sometimes binding to voltage gated potassium channels [18]. After binding, neurotoxic PLA_2s can sometimes hydrolyze nerve terminal phospholipids causing permanent neurotoxicity [16]. This has the effect of causing paralysis, while group II PLA_2s tend to act cytotoxically, predominantly as myotoxins, causing myonecrosis via the disruption of the plasma membrane [17]. Indeed, both natural and synthetic PLA_2 inhibitors are able to attenuate the morbidity and mortalityof snake bite envenomation [19].

5. COMPUTATIONAL STUDIES ON PHOSPHOLIPASE A_2INHIBITORS

There are many reports on computational molecular modeling methods used for the development of PLA_2 inhibitors that contribute to the attenuation of snake venom toxicity [20].These applications use the x-ray crystallographic, 3D structural information

generated in the last few decades, and methods such as molecular dynamics (MD) simulations and docking. Structurally, snake venom PLA_2s is divided into classes I and II, this is based on their amino acid sequence and disulfide bonding pattern [21]. However, they have a conserved structure which contains an N-terminal α-helix (H1), a Ca^{2+}-binding loop, two antiparallel α-helices (H2 and H3), a two-stranded antiparallel sheet (β-wing), and a long C-terminal loop. In general, folding is stabilized by seven disulfide bonds (with different pattern in classes I and II). Nargotra and his co-researcher [22] evaluated alibrary of natural products and synthetic molecules through docking studies on *D. russelii* PLA_2 to identify possible inhibitors. Their study lead to in silico identification of several molecules as PLA_2 Inhibitors, with most of them belonging to phenolic and substituted benzaldehydic compounds [20]. The same authors proposed the docking poses inside PLA_2 of *D. russelii* for synthetic phenolic compounds effective against snake venom. They found that phenolic compounds having hydroxyl and methoxyl groups in their benzene ring showed maximum inhibitory potency.The majority of molecular modeling applications in literature for studying PLA_2s are oriented to rational design of novel inhibitors for the treatment of different Viperidae snakebites. In another work, Zhang and his collaborators [23] docked structural elements of the persimmon tannin PT40 (a highly galloylated condensed tannin with an unusual flavonol terminal unit) inside Chinese cobra (Naja atra) PLA_2 binding site, to understand the inhibitory mechanism of this natural product. They found that the residues Trp18, Try27, Gly29, His47, and Tyr63 are involved in the interactions. Pereañez and his colleagues [24] studied the mode of action of morelloflavone with PLA_2 of *Crotalus durissus*, using docking. Authors found that morelloflavone occupies part of the substrate binding cleft of *C.durissus* PLA_2, forming hydrogen bonds with the residues Gly33, Asp49, Gly53, and Thr68 of the enzyme, and π–π stacking with the residue Tyr52. The same authors used docking to investigate the interactions between *C. durissus* PLA2 and bile acids, such as cholic acid and ursodeoxycholic acid. Authors found that bile acids interact with the binding active site of PLA_2 through different interactions, cholic acid showed hydrogen bonds with His48, whereas, ursodeoxycholic acid showed hydrogen bonds with Asp49andTyr28.Also, Chavan and Deobagkar [25] applied molecular docking simulation techniques to propose the putative interactions of LT10 peptide (small synthetic peptide derived from N-terminal of the lethal toxin neutralizing factor) with *N. naja* PLA_2. Molecular docking was performed to analyze the stability of the complex obtained by docking method. Villar and his collaborators [26] demonstrated that synthetic inhibitor derivatives from nitrostyrene that contain typical nitro groups at the ortho-, meta-, and para- positions on the aromatic ring were more efficient against the enzymatic, edematogenic, and myotoxic activities of PLA_2s from *B.jararacussu* venom. In a related research, Da-silva and co-researchers [27] performed molecular modeling studies between Asp49-PLA_2 from *C. adamanteus* venom and synthetic derivatives polyhydroxy phenolic compounds, they concluded that some conformations of these groups might positively influence enzymatic activity inhibition.

Finally, Mohanapriya and his colleagues [28] investigated phospholipase A_2 inhibitory activity of some medicinal plants against *Naja. naja* venom using auto Dock. They observed that the molecular docking analysis of plant compounds against PLA_2 molecule shows the effective binding site in the C-terminal end (Ile104, Ala101), α- helix 3 (Val 47, Phe 46) and α- helix 4 (Asp122, Pro121). Therefore, they concluded that *Aerva lanata* could be an effective treatment in treating snake bite.

6. METALLOPROTEINASES (SVMPs):

Snake Venom Metalloproteinases (SVMPs) are zinc-dependent proteinases ranging from 20 to 110 kDa in size and are categorized into P-I, P-II, and P-III classes according to their structural domains [29]. Studies have found that the SVMP is most abundant components in snake venom particularly viper species. Previous research has shown that SVMP induces hemorrhage by directly affecting the capillary blood vessels by clearing key bonds of the basement membrane components in a highly selective fashion, and thus affecting the interaction between the basement membrane and the endothelium [30]. Previous research as revealed that metalloproteinase is a mediator for edema, local tissue damage, inflammation, and hemorrhage [31].

7. COMPUTATIONAL STUDIES ON SVMPs INHIBITORS

Sathish kumar and co [32] provides in depth analysis on model the SVMP protein and also the

identification of potent lead compounds (BD17344, BD905, BD 904, BD 25279, BD16837, BD13364, BD5228, BD17338, BD7951, BD26458, BD20708, BD16010, BD4991, BD5001, and BD15993) against SVMP. They model 3D structure of the SVMP using different software and the best model was selected based on the stereochemical properties. The best lead compounds for SVMP were screened using different databases viz. Binding, Maybridge, Hitfinder, and TOSLab databases. They identified fifteen potent hits through the Glide score and Glide energy. It was conclusively shown that all the compounds are quite stable in the active site of SVMP and thus, the isolated compounds might show promising activity against SVMP when compare to screening compounds.

Senkatachalaiah and his colleagues [33] reported the inhibitory effect of compound 5d, an apigenin based molecule against SVMPs both *in silico* and *in vivo*. The researchers found that the molecular docking of compound 5d and bothropasin demonstrated the direct interaction of hydroxyl group of compound with Glu146 present in hydrophobic pocket of active site and does not chelate Zn^{2+}. Hence, it is concluded that compound 5d could be a potent agent in viper bite management.

Pithayaukul and his collaborators [34] studied the ethanolic extract from seed kernels of Thai mango (Anacardiaceae) and its major phenolic principle (pentagalloylglucopyranose) inhibitory effects on the caseinolytic and fibrinogenolytic activities of Malayan pit viper and Thai cobra venoms in in vitro tests. Molecular docking studies revealed that the binding orientations of the phenolic principles were in the binding pockets of snake venom metalloproteinases (SVMPs). The phenolic principles could form hydrogen bonds with the three histidine residues in the conserved zinc-binding motif and could chelate the Zn^{2+} atom of the SVMPs, which could potentially result in inhibition of the venom enzymatic activities and thereby inhibit tissue necrosis.

In another research, Lina and co-researchers [35] investigated the interactions between triterpenes (Ursolic acid, Oleanolic acid, Madecassic acid, β-boswellic acid, Betulin and Betulinic acid) and snake venom metalloproteinase using molecular docking simulation. The simulations revealed the atomic interactions that underlie binding between the triterpenic acids, most notably the electrostatic interaction between carboxylate groups of the compounds. The researcher's findings suggested that the occlusion of the S10 sub-site is essential for inhibition of proteolytic activity of metalloproteinases. They also found out that pentacyclic triterpenes having a carboxylate group at their C-17 position (betulinic, madecassic, oleanolic and ursolic acids) inhibit metalloproteinase proteolytic activity in experiment and exhibit favorable binding free energies and occlusion of the S10 subsite in simulation.

Conclusively, Muthusamy and his colleagues [36] analyzed the role of Zn^{2+} and Ca^{2+} ions in the protein metalloproteinase activity using a compound clerodane diterpenoid by molecular docking. They performed molecular dynamics simulations up to 50ns using Desmond to understand the role of ligand in the active site of SVMP protein by various combinations. It was discovered that in the absence of both Zn^{2+} and Ca^{2+} ions from the protein, molecular docking simulation were fluctuating, particularly absence of Zn^{2+} ion with the protein. Based on the results, they concluded that the $Zn^{2+}$and Ca^{2+} ions have a vital role in the active site of SVMP protein.

8. SERINE PROTEINASES (SVSPs)

SVSPs are found in venoms of the snake families Viperidae, Elapidae, and Colubridae [37]. Venom Serine Proteinases (SVSPs) belong to the S1 family of serine proteinases and display molecular masses ranging from 26 to 67 kDa with two distinct structural domains [38]. It catalyzes the cleavage of covalent peptide bonds in proteins and play key roles in diverse biological processes ranging from digestion to the control and regulation of blood coagulation, the immune system and inflammation [39]. The anticoagulant SVSPs activate protein C via a thrombomodulin-independent mechanism. The most studied SVSP enzyme is from *Agkistrodon contortrix* venom, commercially referred to as Protac, which specifically converts protein C to activated protein C by hydrolyzing the Arg169-Leu170 bond, functioning independently of plasmatic factors [40].

Subhamay and Iman [41] studied molecular interaction between serine protease and Hesperetin (one of the major flavonoid glycosides naturally present in several citrus fruits such as lemons and oranges) to investigations the likelihood of inhibition. The researchers obtained the amino acid sequence of thrombin-like serine protease from sharpnosed

pit viper snake venom from the online database system of National Centre for Biotechnology Information. Employing AutoDock as molecular docking analysis software, they discovered the ideal ligand binding pose of hesperetin which is in close proximity to catalytic residues of snake venom serine protease, i.e., serine, histidine, and aspartic acid. Therefore, the generated in silico results by the researchers suggests that the novel structure hesperetin - flavanone might act as a potent inhibitor of thrombin-like snake venom proteases, and unlocks the possibilities for designing drugs of the inhibitors of snake venom serine proteases.

In a similar research, Roney and his colleagues [42] investigated the inhibitory activity of citrus bioflavonoid, hesperetin on two thrombin-like snake venom serine proteases isolated from *Crotalus simus.* They performed the computational molecular docking studies to assess the possibility of serine protease thrombin-like being inhibited by flavonoids. Considering seven possible binding sites, their results suggest that hesperetin could form a hydrogen bond with Arg60 and His57 with the hydroxyl group on the carbon-5 from its A ring and oxygen atom 1 in the ring C could form hydrogen bonds with the Gly193 and Lys192. In addition, the methoxy group of the B ring could form hydrogen bond with the Gly216 and Asp217. They concluded that flavonome is an interesting inhibitor for snake venom serine proteases, and could open up a possibility for drug design of snake venom serine protease inhibitors.

9. CONCLUSION

Snake venoms are amongst the most fascinating animal venoms regarding their complexity and evolution. The generation and visualization of venom enzyme-inhibitor binding data from molecular docking simulation provide opportunity to explain structural features of enzyme inhibition mechanism. In this regard, molecular docking could be used as a pre-screen to identify compounds that are more likely to have different activity against a potential venom molecular target, which could lead to effective treatment of snake bite envenomation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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