IDENTIFICATION OF A SUITABLE INHIBITOR FOR SNAKE VENOM PLA2 BY MOLECULAR DOCKING

A PROJECT REPORT

Submitted by

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BONAFIDE CERTIFICATE

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INTERNAL EXAMINER

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DECLARATION

We hereby declare that the project report entitled "**IDENTIFICATION OF A SUITABLE INHIBITOR FOR SNAKE VENOM PLA2 BY MOLECULAR DOCKING**" submitted to the Department of Biotechnology, Prathyusha Engineering College, affiliated to the Anna University, Chennai, in partial fulfillment of the award of the degree Bachelor of Technology in Biotechnology, is the record of the original work carried by us under the guidance of **Dr.M.THENMOZHI M.Sc., Ph.D.**, Assistant professor, Department of Biotechnology, Prathyusha Engineering College, during the period of Dec 2019 to April 2020. We further declare that the results of the work have not been previously submitted for the award of any degree or diploma.

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ABSTRACT

Snakebite is a significant health concern, especially in rural populations of tropical and subtropical countries. High mortality is due to poor health services in rural areas and delay in getting the victim to a health care facility, where anti-venom can be administered. The important bioactive molecules in the venom are serine proteases, acetylcholinesterase, phospholipase A2, neurotoxins, and cardiotoxins. Among this, PLA2s are most abundant and fatal toxin in Snake Venom. PLA2 hydrolyses the sn-2 bond of fatty acids by nucleophilic attack and disrupts the host cell membrane. By inhibiting the activity of secreted PLA2, the toxic effects of snakebite can be cured. Protein from *Naja naja* venom was retrieved from Protein Data Bank and its binding sites were predicted using SEESAR. The Compounds DB00398-Sorafenib (-9.9 kcal/mol), DB09280-Lumacaftor (-10.5 kcal/mol), and DB13879-Glecaprevir (-10.1 kcal/mol) had the highest binding energy against the protein PLA2. Visualization was done using PyMOL to find the binding interactions. The ligand interactions noticed that TYR27, TYR63 are playing a vital role in bond formation. These three small molecules have the potential to inhibit the activity of secreted PLA2 enzyme from snake venom.

Key words: Snake bite, *Naja naja*, Phospholipase A2, SEESAR, Sorafenib, Lumacaftor, Glecaprevir, PyMOL.

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LIST OF SYMBOLS

Å	Armstrong
Da	Dalton
Ft	Feet
Inch	inch
Ka	kilocalories
M	metre
µg/ml	microgram
ml	millilitre
mol	mole
ΔS/ΔH	Change in entropy/change in enthalpy

LIST OF ABBREVIATIONS

AA	Amino acids
Ach	Acetylcholine
AchE	Acetylcholinesterase
ADP	Adenosine diphosphate
Ala	Alanine
AtxA	Ammodytoxin A
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
Bp	Bothrops pauloensis
Bmoo	Bothrops moojeni
ca	Calcium
CADD	Computer-aided drug design
CASTp	Computed atlas of surface topology of proteins
Cys	Cysteine
DB	Drug bank
DNTx-III	Daboia neurotoxin III
FTX	Finger toxin
Glu	Glutamic acid
Gly	Glycine
His	Histidine
HIV/AIDS	Human immunodeficiency virus/Acquired immuno deficiency syndrome

Ile	Isoleucine
Leu	Leucine
MD	Molecular dynamics
nAchR	Nicotinic acetylcholine receptors
NMJ	Neuromuscular junction
OHV	*Ophiophagus hannah* venom
p-BPB	Para-bromophenacyl bromide
PAF	Platelet aggregation factor
PDB	Protein data bank
Phe	Phenylalanine
PhTx-I	Porthidium hyoproratoxin I
PLA2	Phospholipase A2
SNARE	Soluble N-ethylmaleimide-sensitive-factor attachment receptor
Trp	Tryptophan
Tyr	Tyrosine
VipTx-II	Viperatoxin II
VS	Virtual screening

AIM AND OBJECTIVE

AIM:

The aim of project is to identify suitable inhibitor for *N. naja* snake venom PLA2 by molecular docking.

OBJECTIVES:

- To identify a suitable protein (PLA2 from *N. naja* snake venom).
- To undergo the prediction of active site.
- To perform virtual screening and docking.
- To find a suitable drug candidate to inhibit the activity of secreted PLA2 from snake venom.

CHAPTER 1
INTRODUCTION

1.1 SNAKE BITE:

Snake bite is an injury especially caused by the bite of venomous snake. Snakebite envenomation is a critical public health problem and fieldwork hazard, particularly in tropical and subtropical regions, causing high mortality and morbidity. As most of the ophidian incidents occur in rural areas of developing countries, accurate statistical data concerning the number of victims is crucial to obtain (Warrell, 2010). Snake bite may result in redness, swelling, and severe pain at the area, which may take up to an hour to appear and vomiting, blurred vision, tingling of the limbs, and sweating may also result (Gold, Barry *et al*., 2002). Unfortunately, snakebite was neglected by international health agencies and governments for a long time, even though the snake bite mortality rate is equivalent to half of the deaths from HIV/AIDS in India and one-fifth of the deaths from malaria worldwide. An estimated 2.5 million people are bitten each year by snakes and these are estimated to result in up to as high as 95000 deaths worldwide (Chippaux *et al*., 1998; Kasturiratne, *et al*., 2008).

1.2 TYPES OF VENOMOUS SNAKES IN INDIA:

* **Indian krait:** The Indian krait or blue krait, *Bungarus caeruleus*, is one of the highly venomous snake that contributes to the snakebite envenoming problem in

South Asia. The average length is 0.9 m (3.0 ft), but adult can grow to 1.75 m (5 ft 9 in).

Fig 1.1 Indian krait (*Bungarus caeruleus*) (Source: Walk through India.com)

SCIENTIFIC CLASSIFICATION

Kingdom	:	Animalia
Phylum	:	Chordata
Class	:	Reptilia
Order	:	Squamata
Suborder	:	Serpentes
Family	:	Elapidae
Genus	:	*Bungarus*
Species	:	***B. caeruleus***

* **Russell's viper:** The Russell's viper, (*Daboia russelii*), also known as daboia, ortic polonga, abundant, highly venomous terrestrial snake of the family Viperidae. It is found from India to Taiwan and Java, most usually in open country. It is a major cause of snakebite deaths within its range because it generally exists in farmlands where human contact and rodent prey are abundant. The viper grows to a maximum of about 1.5 m (5 feet) and is marked with three rows of reddish brown spots outlined in black and once more in white. *Daboia* is a live-bearer, and females frequently produce litters of more than 25 neonates.

Fig 1.2 Russell's viper (*Daboia russelii*) (Source: www.pinterest.com)

SCIENTIFIC CLASSIFICATION

Kingdom	:	Animalia
Phylum	:	Chordata
Class	:	Reptilia
Order	:	Squamata
Suborder	:	Serpentes
Family	:	Viperidae
Genus	:	*Daboia*
Species	:	***D. russelii***

* **Saw scaled viper:** Saw-scaled viper, (genus *Echis*), any of eight species of miniature venomous snakes (family Viperidae) that inhabit arid regions and dry savannas north of the Equator across Africa, Arabia, and southwestern Asia to India and Sri Lanka. They are characterized by a stout body with a pear-shaped head that is distinct from the neck, vertically elliptical pupils, rough and strongly keeled scales, and a precise thin tail. On two sides of the body are several rows of obliquely arranged serrated scales. Length of adults range from 0.3 to 0.9 metre (1 to 3 feet). *Echis* coloration includes different shades of brown, gray, or orange with darker dorsal blotches and lateral spots.

Fig 1.3 Saw-scaled viper (*Echis*) (Source: timesofindia.indiatimes.com)

SCIENTIFIC CLASSIFICATION

Kingdom	:	Animalia
Phylum	:	Chordata
Class	:	Reptilia
Order	:	Squamata
Suborder	:	Serpentes
Family	:	Viperidae
Subfamily	:	Viperinae
Genus	:	*Echis*

* **King cobra:** The Great King Cobra (*Ophiophagus hannah)* is the biggest snake in India, with an average length of 13-15 ft. The unique adult King cobras can reach upto 18 feet (5.5 meters) in length, making them the world's longest venomous snake. The fierce and agile King cobra habitat includes rainforest, humid jungle, thick undergrowth, cool swamps and bamboo clusters forested areas.

Fig 1.4 King Cobra (*Ophiophagus hannah)* (Source: Walk through India.com)

SCIENTIFIC CLASSIFICATION

Kingdom	:	Animalia
Phylum	:	Chordata
Class	:	Reptilia

Order	:	Squamata
Suborder	:	Serpentes
Family	:	Elapidae
Subfamily	:	Elapinae
Genus	:	*Ophiophagus*
Species	:	***O. hannah***

* **Malabar pit viper:** Malabar pit viper (*Trimeresurus malabaricus)*, also called as rock viper, is a master of camouflage. It is venomous and also deadly in its precision when striking its prey. These nocturnal reptiles are commonly found on ground rocks and trees near streams in South-western India. Many various colour morphs are known to exist, including colours such as yellow, green, and brown.

Fig 1.5 Malabar pit viper (*Trimeresurus malabaricus*) (Source: Commons.wikimedia.org; Author: Amitayu)

SCIENTIFIC CLASSIFICATION

Kingdom	:	Animalia
Phylum	:	Chordata
Class	:	Reptilia
Order	:	Squamata
Suborder	:	Serpentes
Family	:	Viperidae
Genus	:	*Trimeresurus*
Species	:	***T. malabaricus***

* **Indian cobra or spectacled cobra:**

Among these venomous snakes, Indian cobra are most commonly found in India and cause high death rate.

1.3 INDIAN COBRA:

The Indian cobra (*Naja naja*), also known as spectacled cobra, Asian cobra, or binocellate cobra, is a species of the genus Naja found in INDIA, PAKISTAN, BANGALADESH, SRI LANKA, NEPAL and BHUTAN, and a member of big four species that cause the most snakebites on humans in INDIA (Whitaker *et al.*, 2004; Mukherjee *et al*., 2012). It is a smooth-scaled snake with a wide neck and head, black eyes, and a medium-sized body. Its colour varies from black, to dark brown, to a creamy white. The body is usually covered with a spectacled white or yellow pattern, which rarely forms ragged bands. It may grow from 1.8 m to 2.2 m (Breen, 1974).

Fig 1.6 Indian cobra (*Naja naja*) (Source: Walk through India.com)

SCIENTIFIC CLASSIFICATION

Kingdom	:	Animalia
Phylum	:	Chordata
Class	:	Reptilia
Order	:	Squamata
Suborder	:	Serpentes
Family	:	Elapidae
Genus	:	*Naja*
Species	:	***N. naja***

1.4 SIGNS AND SYMPTOMS OF *N.naja:*

A. **Neurological and Neuromuscular:** These signs and symptoms will usually declare earliest. Not all of these symptoms will necessarily develop, even with severe envenomation.

- Drowsiness (>90%)
- Eyelid drooping (Ptosis) (75-85%)
- Respiratory paralysis or Dyspnea (70-80%)
- Ophthalmoplegia (35-45%)
- Palatal paralysis (30-40%)
- Glossopharyngeal paralysis (30-40%)
- Limb paralysis (20-30%)
- Convulsions (10-20%)
- Head drooping (Cervical muscle paresis or paralysis)
- Headache
- Sudden loss of consciousness
- Stumbling gait (Ataxia)

B. **General:** These symptoms typically display within one to four hours following the bite if envenomation occurred.

- Nausea and Vomiting
- Hypotension
- Flushing of the face
- Warm skin
- Pain around bite site
- Abdominal Pain

C. **Cardiotoxicity:** Increased Blood Pressure and Cardiac Output followed by Myocardial Depression and Asystole. If cardiotoxic complications occur, mortality approaches 100%.

D. **Local Symptoms:** Local tissue destruction and necrosis can dominate the clinical presentation in some Cobra bites. Gangrene requiring amputation can take place. Local tissue damage appears to be less frequent and less severe in most cases of *N. naja* envenomation, but may include:

- Localized discoloration of skin
- Vesiculation (usually small and localized)
- Necrosis (can be extensive, but is characteristically localized to the bite site)
- Local edema (usually minimal)

E. **Fang Marks:** Fang marks may be present as one or more well defined punctures, as a series of small lacerations or scratches, or there may not be any noticeable or obvious markings where the bite appeared. The absence of fang marks does not prevent the possibility of a bite (especially if a juvenile snake is involved). In general, the fang marks from an *N. naja* tend to be small, but deep. The snake in providing the bite may hold on and chew savagely, and may inject up to 60% of its venom. Multiple bites inflicted by a one snake or by more than one snake are also possible, and should be noted if present. The presence of fang marks does not always mean that the injection or deposition of venom into the bite wound (envenomation) actually occurred (Haffkine, 1978; Tiger *et al*., 1975; Stueven *et al*., 1983; Reid, 1964).

1.5 TYPES OF VENOMS:

The most important bioactive molecules in the venom are serine proteases, acetylcholinesterase, phospholipase A2.

1.5.1 SERINE PROTEASES:

Serine proteases are major components and have been identified mainly in the venoms of snakes belonging to the 13odelled13 family with a few occurring in members of the elapidae, colubridae and hydrophidae families (Serrano *et al*., 2005). Many snake venom serine proteases exert their effects through the capacity to disrupt the normal haemostasis of envenomed prey and victims (Meier *et al*., 1991). Some of the serine proteases have both fibrinogenolytic and fibrinolytic activities, but a number of them have only fibrinogenolytic activity and are also known as `thrombin-like' proteases if they show 'fibrinogen clotting' activity (Markland, 1991 and 1998; Pirkle, 1990, 1991 and 1998). However, their actions toward fibrinogen and the other substrates of thrombin are not exactly identical to those of thrombin. Instead of fibrin(ogen)olytic activity, several venom serine proteases have the activity for releasing bradykinin from kininogen like mammalian kallikrein (or kininogenase) (Iwanaga *et al*., 1976; Bjarnason *et al*., 1983) and are also known as `kallikrein-like' proteases (Bjarnason *et al*., 1983). In addition, there have been some reports on the venom serine proteases with a unique activity, such as the activation of factor V (Tokunaga *et al*., 1988), protein C (Kisiel *et al*., 1987), plasminogen (Zhang *et al*., 1995 and 1997) or platelets (Serrano *et al*., 1995).

1.5.2 ACETYLCHOLINESTERASE:

The presence of acetylcholinesterase (EC 3.1.1.7; AchE) in cobra venom was first reported in 1938 (Lyenger *et al*., 1938). Acetylcholine (Ach) is the first chemical agent known to create a communication link between two distinct mammalian cells, and acts by propagating an electrical stimulus across the synaptic junction. AchE is a member of the cholinesterase family (Frobert *et al*., 1997) and plays a important role in Ach transmission in the nervous system by ensuring the hydrolysis of Ach to choline and an acetate group, thereby terminating the chemical impulse. The transmission of a chemical impulse takes place within 1 ms and demands accurate integration of the structural and functional components at the synapse (Aldunate *et al*., 2004). Incidentally, AchE may also be one of the fastest enzymes known, hydrolyzing Ach at a rate that is quite close to the diffusion-controlled rate (Bazelyansky *et al*., 1986). The estimated turnover values of the enzyme range are relatively 7.4×10^5 to 3×10^7 Ach molecules per minute per molecule of enzyme (Rothenberg *et al*., 1947; Wilson *et al*., 1961). The rapid hydrolysis of Ach forms the basis of fast, repetitive responses at the synapse.

1.5.3 PHOSPHOLIPASE A2 (PLA2):

PLA2 (EC 3.1.1.4) are enzymes which release fatty acids from the second carbon group of glycerol. This particular PLA2 specifically recognizes the sn-2 acyl bond of phospholipids and catalytically hydrolyzes the bond releasing arachidonic acid and lysophospholipids. PLA2 are most commonly found in mammalian tissues as well as insect and snake venom (Nicolas *et al*., 1997). Due to the increased presence and activity of PLA2 resulting from a snake bite, arachidonic acid is released from the phospholipid membrane disproportionately. As a result, inflammation and pain occurs at the site of bite (Argiolas *et al*., 1983).

1.5.3.1 CHARACTERISTICS OF SNAKE VENOM PLA2:

Snake venom PLA2 hydrolyze glycerophospholipids at the sn-2 position of glycerol backbone, freeing lysophospholipids, and fatty acids. It shares 44-99% amino acid identify in their primarily structure, which results to high similarity in their tertiary structure. Based on their size, location, function, substrate specificity, and calcium requirement, PLA2s are classified into 6 families. Snake venom PLA2 belongs to the secretory PLA2 family (groups IA, IIA, and IIB) (14-16). Cobras, kraits and Gaboon vipers have snake venom PLA2s in groups IA, IIA, and IIB, respectively (Scott *et al*., 1997). In group I there are about 115–120 residues, 7 disulfide bonds (the unique disulfide linking residues 11 and 77), and group IA has a characteristic surface loop between residues 63 to 67 called elapidic loop (Six *et al*., 2000). While group IB has a five amino acids residues (residues 62–67) extension termed pancreatic loop, some group IB snake venom PLA2 even has an eight-residue propeptide segment in their mature state (Pearson *et al*., 1993; Mackessy *et al*., 2010). Group II has a C-terminal extension, the unique disulfide linking residues 50 and 137. Group IIA have a 7-residue C-terminal extension and 7 conserved disulfide bonds, while in group IIB, the C-terminal extension is 6 residues, and only 6 disulfides remained in which a universally conserved 61–95 disulfide is lacking (Six *et al*., 2000). Replacement of the 49th residue (asparagine) with lysine results in inactive or weakly toxic PLA2. This lysine residue can interact with other amino acids in the "calcium-binding loop" which ultimately results in the loss of calcium-dependent catalytic activity (Petan *et al*., 2007; Ward *et al*., 2002). Most snake venom PLA2s exist as monomers, but some exist in complexes, which mainly exhibit presynaptic neurotoxicity through combination of isoenzymes or other proteins (Bon, 1997).

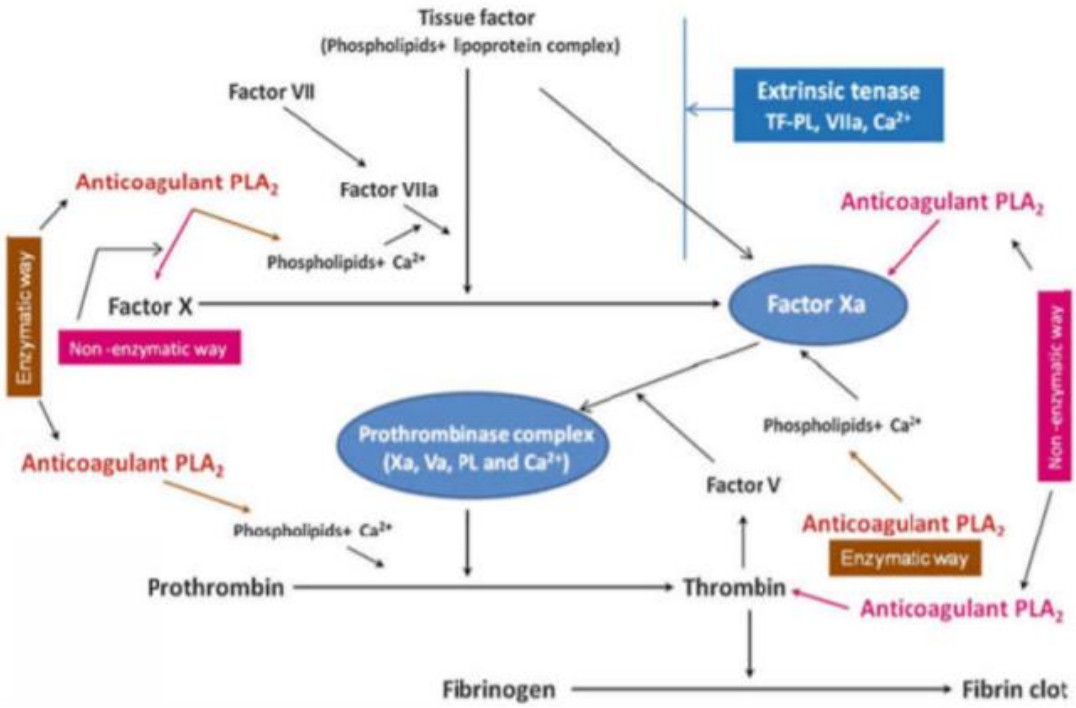

Fig 1.7 Effect of snake venom anticoagulant PLA2 enzymes in different stages of the extrinsic pathway of blood coagulation. Enzymatic way: Enzymatic way of anticoagulant mechanism of snake venom PLA2s; non-enzymatic way: Non-enzymatic way of anticoagulant mechanism of snake venom PLA2s; Anticoagulant PLA2: Anticoagulant PLA2s of snake venom. (Source: Saikia and Mukherjee, In: P. Gopalakrishnakone *et al*., (eds.), snake venoms, toxinology, 2017).

1.5.3.2 BIOLOGICAL ACTIVITIES OF SNAKE VENOM PLA2 ENZYMES:

In spite of having sequence similarity and conserved structure, the PLA2 enzymes exhibit a plethora of pharmacological activities on prey or victim due

to the presence of different pharmacological sites apart from the common catalytic site. The most profound biological activities reported so far from snake venom PLA2 enzymes include neurotoxicity (pre-synaptic and post-synaptic), myotoxicity (local and systemic), cardiotoxicity, platelet aggregation initiation or inhibition, anticoagulant, haemolytic, antibacterial, edema inducing, internal hemorrhage, hypotension, tissue and organ damaging activities.

Neurotoxicity: Snake venom PLA2 enzymes are reported to reveal both pre-synaptic (inhibit neurotransmitter release from the nerve terminal) and post-synaptic (inhibit neurotransmitter action on the muscles) neurotoxic effect. Several hypothesis were proposed to describe the neurotoxic effect of PLA2 enzymes like their hydropathy profile or hydrophobicity in the region from 80-110th residues. However, the most latest and convincing mechanism was proposed by Curin-Serbec and co-workers where the antibodies raised against the C-terminal loop of ammodytoxin A (AtxA) (*Vipera ammodytes ammodytes*) inhibited the neurotoxic effect of the enzymes. Further site directed mutagenesis studies conducted by Pungercar and co-workers on AtxA showed that the substitution of Phe124, an aromatic amino acid residue at the exposed region of the C-terminal loop by aliphatic amino acid residue like Ile124 lowered the neurotoxic effect by several folds. This proposed the significance of the Phe124 residue in execution of the neurotoxic effect by PLA2 enzymes. In particular, chemical modification of Trp residues of *Daboia* neurotoxin III (DNTx-III) (*D. russelii*) lowered the twitches in *Rana hexadactyla* sciatic nerve gastrocnemius muscle preparations. This suggests the significance of aromatic residues in AtxA and DNTx-III for neurotoxic activity.

Myotoxicity: Snake venom PLA2 enzymes are frequently reported with both local myonecrosis and systemic myotoxic effects, the severity of which can be estimated by quantifying the release of creatine phosphokinase into the plasma of the victim. The catalytic activity of these enzymes are noticed to be insignificant for the execution of myotoxic effects. Kini and Iwanaga suggested the importance of cationic sites (+00+++00+) at the amino terminal end of the hydrophobic helix E and hydrophobic regions for executing the myotoxic effect. Lomonte and co-workers presented the crucial role of C-terminal cationic and the hydrophobic segment with Lys36 and Lys38 for the activity. In particular, chemical modification of His, Lys and Tyr residues in PhTX-I, a basic PLA2 enzyme isolated from *Porthidium hyoprora* venom altered its myotoxic effect. This observation manifested the crucial role of both catalytic and pharmacological sites for displaying myotoxic effect in this enzyme. In another study, Asp49 and Lys49 PLA2 enzymes from *Bothrops asper* venom are reported to act synergistically to activate myotoxicity and cell death in C2C12 myotubes along with increase in intracellular Ca2+ ions levels. The catalytically active Asp49 PLA2 enzymes are reported to create anionic patches with the hydrolysed products to collaborate with Lys49 myotoxins electrostatically and exert cumulative myotoxic effects.

Cardiotoxicity: Although the exact mechanism of cardiotoxic effect by PLA2 enzymes is not yet clear, but it is suggested to be independent of the enzymatic activity. Huang and co-workers reported an acidic PLA2 enzyme, OHV A-PLA2 from the venom of *Ophiophagus hannah* which was proposed to reveal the cardiotoxic effect in rat heart by raising the influx of intracellular Ca2+ ions. Two basic PLA2 enzymes (Lys49 and Asp-49) isolated from the venom of *Agkistrodon piscivorus piscivorus* were reported to influence the functioning of a preparation of phrenic nerve-diaphragm and isolated ventricle strip of heart. The cardiotoxic effects of these enzymes were established to be comparable

with that of catalytically active Asp-49 PLA2 enzymes from *Naja nigricollis*, *Hemachatus haemachatus* and *Naja naja atra*. This indicates that the toxic effects are independent of the catalytic activity of the enzymes.

Antimicrobial activity: A number of snake venom PLA2 enzymes are reported with the bactericidal activity which is found to be either dependent or independent of the catalytic activity. PLA2 enzymes with Lys49 from *Bothrops asper* are reported to exhibit antibacterial activity suggesting the role of non-enzymatic mechanism for antimicrobial activity. Weiss and co-workers proposed overall basicity and cluster of basic residues on the surface of N-terminal helix of PLA2 enzymes to be significant for antibacterial activity. For the bactericidal activity on gram-positive bacteria, recognition of the anionic site and phospholipid hydrolysis were crucial while for gram-negative bacteria along with phospholipid hydrolysis cumulative effect of the bactericidal/permeability increasing protein were reported to be necessary. VRV-PL-V from the venom of *Daboia russelii pulchella*, NN-XIb-PLA2 and NNXIa-PLA2, from the venom of *Naja naja* are reported to reveal potent antibacterial activity against both gram-positive and gram-negative bacteria. In the case of VRV-PL-V the antimicrobial activity persisted even after its modification with para-Bromophenacyl bromide (p-BPB) suggesting the existence of properties alike to bactericidal/membrane permeability-increasing protein. Treatment of NN-XIb-PLA2 and NN-XIa-PLA2 with p-BPB lowered their antibacterial activity suggesting their dependence on the catalytic activity. In addition to this, Viperatoxin (VipTx-II) isolated from the venom of *Daboia russelii* (Indian Russell's viper) is reported to reveal antimicrobial effect (*Staphylococcus aureus* and *Burkholderia pseudomallei* (KHW and TES), *Proteus vulgaris* and *P. mirabilis*) by pore formation and membrane damage.

Edema inducing: The edema-inducing action of PLA2 enzymes were first reported by Vishwanath and co-workers in *Trimeresurus flavoviridis* (Habu snake) venom. The manifestation of the edema formation is because of the enzymatic action of these enzymes where the arachidonic acid released during the phospholipid hydrolysis participates in inflammatory pathways generating leukotrienes, prostaglandins and thromboxane A2. These eicosanoids then expand vascular permeability and cause the formation of edema at the site of envenomation. PLA-A and PLA-B, (Asp49 PLA2 enzymes) isolated from *Trimeresurus flavoviridis* venom showed edema formation with PLA-B exhibiting stronger activity compared to PLA-A. Both sequences were reported to be similar to each other except at position 79 with PLA-A having an aspartate residue while PLA-B with an asparagine residue. The beta-turn segment at this position was proposed to be critical for edema-inducing activity for both the enzymes. Thus, apart from the enzymatic activity, structural conformations also seem to be critical for explicating the inflammatory responses.

Platelet aggregation initiation or inhibition: Some PLA2 enzymes are also described to cause platelet aggregation initiation or inhibition induced by various agonists like collagen or ADP causing delay in clot formation. Most of the acidic PLA2 enzymes described so far are known to inhibit platelet aggregation induced by ADP or collagen. The platelet deaggregation and platelet binding properties of NnPLA2-I, an acidic PLA2 enzyme isolated from the venom of *Naja naja* is reported to be lowered upon alkylation of the active site histidine residue suggesting the significance of catalytic action for exhibiting antiplatelet activity. Apart from this, several other acidic PLA2 enzymes like Bp-PLA(2) from *Bothrops pauloensis* and BmooTX-I from

Bothrops moojeni have also described reduction in platelet aggregation inhibition induced by collagen or ADP upon treatment with p-BPB. Superbins II purified from the venom of *Austrelaps superbus* also exhibited anticoagulant effect on the extrinsic tenase complex and inhibited platelet aggregation caused by collagen. Thus, catalytic activity of these enzymes seems to play a critical role in platelet aggregation inhibition; however, the exact mechanism of action is not clear. In particular, the ability of the PLA2 enzymes to activate platelet aggregation is mostly attributed to the release of the lytic products like arachidonic acid and/or platelet aggregation factor (PAF) which in turn facilitate blood coagulation at the site of injury.

Anticoagulation: The ability of the snake venom PLA2 enzymes to inhibit blood coagulation was first reported by Boffa & Boffa in *Vipera berus* venom. Since then a number of PLA2 enzymes have been isolated from the different venomous snake with potent anticoagulant activity. Most of these anticoagulant PLA2 enzymes are described to act either enzymatically by hydrolysing the procoagulant phospholipids required for the formation of the coagulation complexes or non-enzymatically by targeting FXa in prothrombinase complex inhibiting the conversion of prothrombin to thrombin or thrombin directly affecting the fibrin clot formation. Verheij and co-workers classified the anticoagulant PLA2 enzymes into strong, weak and non-anticoagulant based on the concentration required to delay the plasma clot formation. The strong anticoagulant PLA2 enzymes exhibit anticoagulant effect at a concentration lower than approximately 2 µg/ml while the weak anticoagulant PLA2 enzymes display at 3-10 µg/ml while the non-anticoagulant PLA2 enzymes show above 15 µg/ml. In particular, Kini & Evans have classified PLA2 enzymes into these three groups based on the amino acid composition at the anticoagulant region (54th to 77th residue based on Renetseder numbering system). They proposed

the significance of positively charged amino acid residues at the anticoagulant region for determining the anticoagulant potency of these enzymes. The strong anticoagulant PLA2 enzymes are suggested to have positive amino acid residues in this region while the weak or non-anticoagulant enzymes contain neutral or negatively charged residues. Most of the anticoagulant PLA2 enzymes described so far are basic in nature while only a few of them are reported to be neutral or acidic in nature. Three basic PLA2 enzymes, CM-I, II and CM-IV with anticoagulant activity were secluded from the venom of *Naja nigricollis*. CM-I and CM-II exhibited anticoagulant effect on the extrinsic tenase complex by phospholipid hydrolysis. In particular, CM-IV exhibited anticoagulant effect on both extrinsic tenase complex and prothrombinase complex both enzymatically and non-enzymatically. It inhibited the prothrombinase complex enzymatically by phospholipid hydrolysis and non-enzymatically by competing with FVa for binding to FXa thus, interfering the development of the prothrombinase complex. AtxA, a PLA2 enzyme isolated from the venom of *Vipera ammodytes ammodytes* inhibits the prothrombinase complex by binding to FXa and by phospholipid hydrolysis. Vipoxin, a heterodimeric PLA2 complex isolated from the venom of *Vipera ammodytes meridionalis* is announced to inhibit the intrinsic tenase complex by phospholipid hydrolysis. TI-Nh isolated from the venom of *Naja haje* is described to be a strong anticoagulant PLA2 enzyme delaying the thrombin time. It exhibits its anticoagulant activity by inhibiting the fibrinogenolytic, amidolytic and platelet aggregating properties of alpha-thrombin, but does not show any result on prothrombin time or activated partial thromboplastin time.

1.5.3.3 MECHANISM OF PLA2:

PLA2s catalyze the hydrolysis of 2-acyl ester bonds of 3-sn-phospholipids producing fatty acids and lysophospholipids. The Ca2+ ion, an essential cofactor, and an Asp residue at position 49 are required for catalysis on artificial substrates.

The disarrangement of phospholipids components can result in severe alterations of the structural and functional membrane integrity, with subsequent influx of Ca2+ ions, causing 23odelled23 contraction, activation of Ca2+ ion-dependent proteases and endogenous PLA2s, besides the envenoming of mitochondria. Cell death is a possible result of the sum of all these alterations. Snake venoms constitute a rich source of PLA2 enzymes, which show remarkable functional diversity.

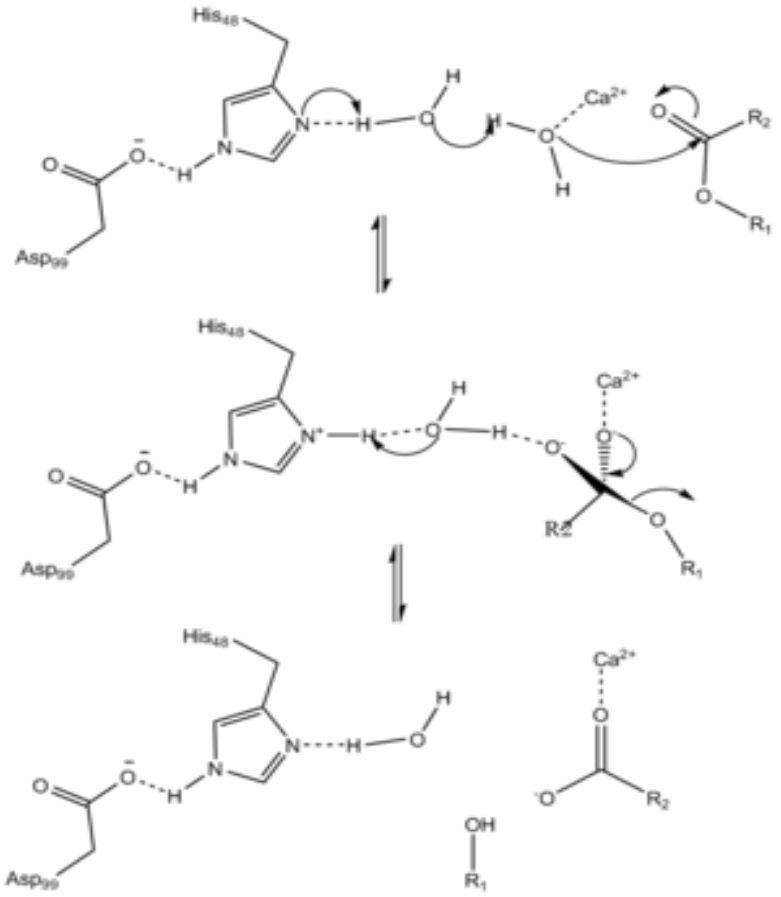

Fig 1.8(a) Mechanism of hydrolysis catalysed by PLA2

(source: Berg *et al*., 2001)

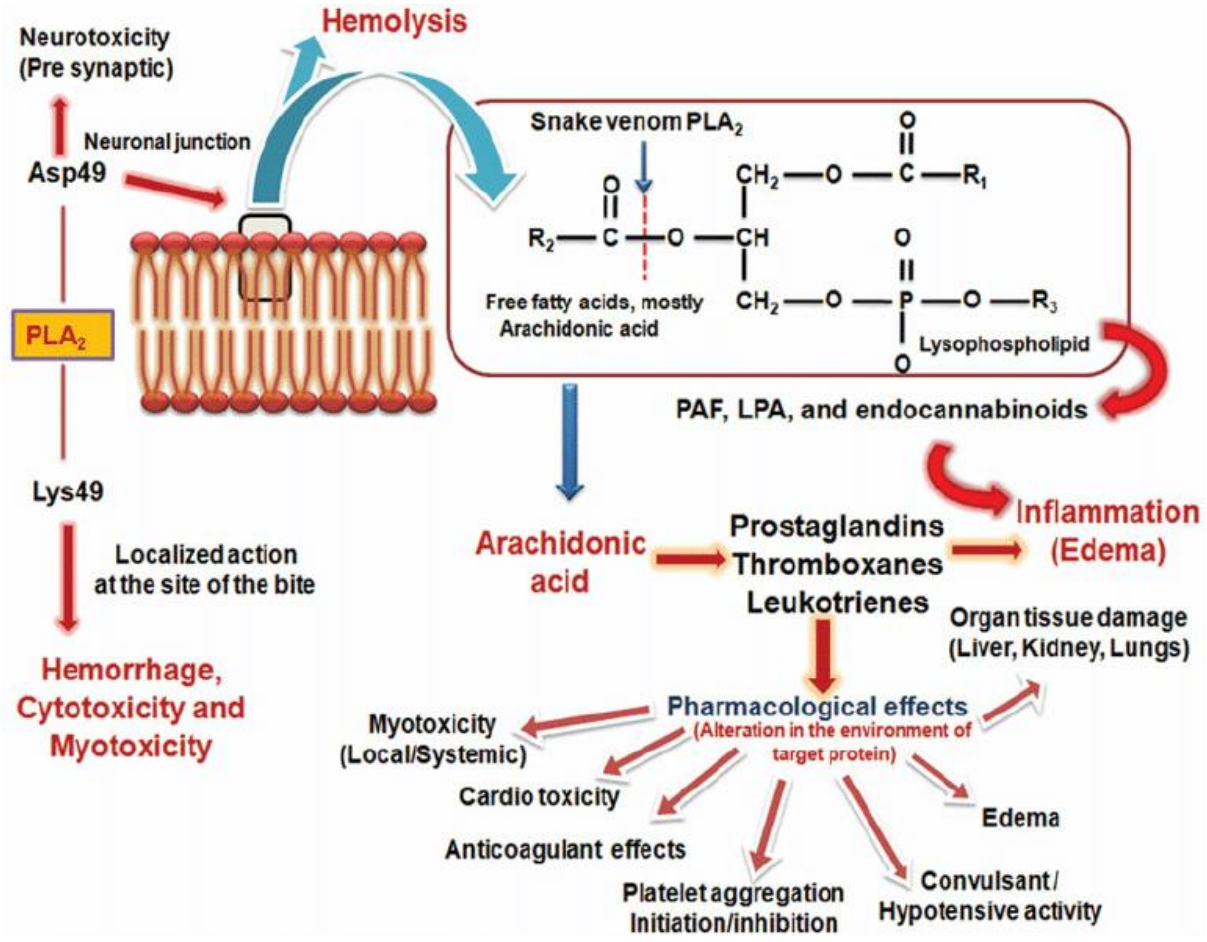

Fig 1.8(b) Mechanism of action of snake venom PLA2. PLA 2: phospholipase A 2; Asp49: Aspartate49; Lys49: Lysine49; PAF: platelet. (Source: Kini and Evans, 1990).

1.6 CASTp (COMPUTED ATLAS OF SURFACE TOPOLOGY OF PROTEINS):

The CASTp web server aims to provide a comprehensive and detailed quantitative characterization of interior voids and surface pockets of proteins,

which are the important concave regions of proteins that are frequently associated with binding events (Lakowski *et al*., 1996; Liang *et al*., 1998).

CASTp depends on the alpha shape and the pocket algorithm developed in computational geometry (Liang *et al*., 1998; Edeslbrunner *et al*., 1998).

In CASTp, voids are defined as buried unfilled empty space inside proteins after removing all hetero atoms that are unattainable to water molecules (25odelled as a spherical probe of 1.4 A°) from outside (Lee *et al*., 1971). Pockets are determined as concave caverns with constrictions at the opening on the surface regions of proteins. Unlike voids, pockets permit easy access of water probes from the outside. CASTp identifies all pockets and voids on the protein structure and provides detailed delineation of all atoms participating in their formation. It also measures the volume and area of each pocket and void analytically, using both the solvent accessible surface model (Richards' surface) and molecular surface model (Connolly's surface). It measures the size of mouth openings of individual pockets, which supports to assess the accessibility of binding sites to various ligands and substrates.

CASTp computation has been useful in a number of biological studies (Paetzel *et al*., 1999; Kim *et al*., 1997; Licata *et al*., 1998; Ory *et al*., 1999; Li *et al*., 1999; Thompson *et al*., 1999). The CASTp server, in its most current release, was updated in January 2003 and its previous version was launched in 1998 at the University of Minnesota. It is listed in the Research Consortium of Structural Bioinformatics Protein Data Bank (PDB) website at the San Diego Supercomputing Center (http://www.rcsb.org/pdb/), a central portal of structural bioinformatics worldwide. CASTp permits access to information of computed pockets and voids for structures in the PDB. The server currently contains characterization of 1,322,538 pockets and voids that had been computed from

19,161 protein structures from the PDB. The CASTp server is updated with new PDB entries.

1.7 PyMOL:

PyMOL (version 2.3.5) is a free cross-platform molecular graphics system created by Warren Lyford Dano. It provides most of the capabilities and performance of traditional molecular graphics packages written in C or Fortan. PyMOL has been released under a completely unrestrictive open-source software license so that all scientists and software developers can freely use PyMOL and distribute derivative works based on it without cost or limitation.

Molecular graphics representation:

PyMOL supports most of the common representations for macromolecular structures such as cylinders, wire bands, spheres, solid surfaces, ball-and-stick, wire mesh surfaces, backone ribbons and cartoon ribbons which are comparable to those generated by molscript.

Labels can be displayed for atoms, and dashed bonds can be used for hydrogen bonding interactions and distances. Surfaces can be transparent, and molecules can be loaded from Protein Data Bank (PDB) files and several other common file formats.

1.8 SEESAR:

SeeSAR (version 9.0) is a software tool for interactive, visual compound prioritization and compound evolution. Structure-based design work supports a multi-parameter optimization to maximize the likelihood of success, rather than affinity alone. Hang the relevant parameters in combination with real-time

visual computer assistance in 3D is one of the strengths of SeeSAR. Stimulating exploration with SeeSAR have embarked on persuing a new cheminformatics compute paradigm of “Propose & Validate” with these first four themes accomplished so far:

1. **Affinities:**
   A sophisticated graphics are implemented to visualize atom-based affinity contributions; that allow for a rough estimate of the ΔS/ΔH -split of the Free Energy. (This is an ongoing co-development between BAYER, the University of Hamburg and BioSolveIT.)
2. **Physical and chemical properties:**
   Relevant parameters are computed on-the-fly or carried to be taken into consideration throughout the design process.
3. **Torsional heat:**
   Torsional statistics analysis (developed between Hoffmann-LaRoche and the University of Hamburg); is readily available through intuitive color-coding.
4. **Explorable space:**
   A tight fit is the prerequisite for affinity and specificity. Therefore, as guidance for the user, a potent computation combined with refined graphics provides on-the-fly visualization of gaps in the binding interface and positions where a tighter fit is likely to be gained.

1.9 PyRx:

PyRx (version 0.7) is a Virtual Screening software for Computational Drug Discovery that can be used to screen libraries of compounds against potential drug targets. PyRx enables Medicinal Chemists to run Virtual Screening from any platform and helps users in every step of this process-from data preparation to job submission and analysis of the outcomes. While it is true that there is no

magic button in the drug discovery process, PyRx includes docking wizard with easy-to-use user interface which makes it a beneficial tool for Computer-Aided Drug Design. PyRx includes chemical spreadsheet-like functionality and powerful visualization engine that are essential for Rational Drug Design.

Features:

- 3D Viewer
- Virtual Screening
- Computer-Aided Drug Design
- AutoDock 4
- AutoDock Vina

1.10 Motivation:

Anti-venoms are prevalently used for treating a snake bite. However, the geographical and species variation restrict the use of anti-snake venom. It is a tedious and time-consuming process to find the type of snake to which the venom belongs. In addition to this, anti-venoms are given by assuming the type of snake based on the demography. Hence, there is a certain amount of ambiguity to the treatment which makes reduces its efficiency. In order to overcome this issue, a ligand with the potential to inhibit the activity of venom has to be identified.

CHAPTER 2
REVIEW OF LITERATURE

2.1 SNAKE VENOM:

Kularatne et al., (2009) studied that despite the fact that cobra venoms are primarily neurotoxic, transient coagulopathies leading to increase in whole blood clotting time have been observed in envenomed patients. A few anticoagulant proteins have been purified from the venom of different cobras, which exhibit anticoagulant activity via different modes of action. Most of the cobra venom anticoagulant toxins belong to enzymatic snake venom protein families of phospholipase A2 as well as metallo-proteinase. However, to date, only one non-enzymatic anticoagulant three-finger toxin has been purified and characterized from cobra (*Naja nigricollis crawshawii*) venom. The anticoagulant activity of the cobra venom enzymes may or may not be directly correlated with their enzymatic activity. Further, some of these anticoagulant proteins have shown significant promise for the development into potent drug prototypes for treatment of thrombosis associated cardiovascular disorders.

Lu et al., (2017) reported that snake venom proteins/toxins exhibit a wide variety of pharmacological effects, including modulation of the hemostatic system. To date, several snake venom proteins affecting hemostasis have been purified and characterized.

2.2 SNAKE VENOM TOXINS AND NEUROMUSCULAR BLOCK:

Harris JB (1999); Goonetilleke A (2004) ; Prasarnpun et al., (2005) reported that the pre-synaptically active neurotoxins (beta-neurotoxins-mostly neurotoxic phospholipase A2 toxins, PLA2s) bind to the motor nerve terminals, leading to

depletion of synaptic ACh vesicles, impaired release of ACh, and later, degeneration of the motor nerve terminal. They produce neuromuscular block that occurs in three phases: an immediate depression of ACh release, followed by a period of enhanced ACh release, and then complete inhibition of NMJ transmission.

Neuromuscular Transmission and Neuromuscular Block:

Hirsch (2007); Ruff (2003; Fagerlund, Eriksson (2009); Martyn et al., (2009); Shear, Martyn (2009) studied that at the pre-synaptic level, the motor nerve axon terminal is responsible for the synthesis, packaging, transport, and release of the neurotransmitter acetylcholine (ACh). Release of ACh in response to an incoming nerve action potential is triggered by the opening of voltage-gated calcium channels and the influx of calcium ions. Increased intracellular calcium concentration triggers a cascade of events that leads to the formation of a fusion complex made up of SNARE (Soluble N-ethylmaleimide-sensitive-factor Attachment REceptor) proteins, which enables fusion of ACh vesicles to the nerve terminal membrane and ACh release. Nicotinic acetylcholine receptors (nAChRs) at the nerve terminal (pre-synaptic neuronal autoreceptors -α3β2) facilitate release of increasing quantities of ACh, by mobilising ACh vesicles from a reserve pool to a releasable pool, in response to high frequency stimulation via positive feedback systems. Interference with neuromuscular transmission at a pre-synaptic level can occur at voltage-gated calcium channels (e.g., Lambert Eaton myasthenic syndrome), SNARE proteins (e.g., botulism), potassium channels (e.g., neuromyotonia), or at the neuronal nAChRs.

2.3 ANTIVENOM:

Lomonte., et al (2007) reported that immunotherapy is the only specific treatment for snake bite envenoming. Antivenoms are produced by fractionation of plasma obtained from immunized animals, usually horses.

Joseph., et al (2007) and Ariaratnam., et al (2008) studied that they can be either monovalent or polyvalent, depending on the number of species (single or multiple, respectively) whose venoms are used for immunization. Although monovalent antivenom has often been considered more efficacious, the production of polyvalent antivenom is preferred in many countries as snake species identification is generally not possible for the attending physician. Antivenoms have been available in South Asia for the past 60 years, and all existing products are manufactured by Indian companies. Traditionally, the production has focused on four species believed to be responsible for most deaths: *N. naja, B. caeruleus, D. russelii, and E. carinatus.* However, a number of other species that contribute to morbidity and mortality in the region have not been considered, and envenoming by these species usually does not respond adequately to existing antivenoms.

2.4 *N. naja:*

Wuster and Thorpe (1991); Shashidharamurthy et al., (2002); Mukherjee and Maity (2002); Shashidharamurthy et al., (2010); Venkatesan (2014); Tan et al., (2017) reported that India is inhabited with four species of cobras, *N. naja*, *N. kaouthia*,*N. sagittifera*, and *N. oxiana,* and out of these, *N. naja* is identified to be the most distributed species of *Naja* in India. Therefore, India has already given their much concern to address this issue of venom variability due to regional difference of *N. naja.* Several studies already have revealed that

composition and clinicopathological effects on vital organs by venom of *N. naja* have a significant variation in accordance with their originated location.

Somaweera (2006); Dissanayake et al., (2017) studied that similar to *N. naja* in India, the spectacled cobra in Sri Lanka inhabits a wide range of terrestrial habitat, up to an elevation of 1500m. Though the distribution of Sri Lankan *N. naja* is highly variable, Sri Lanka still lacks these systematics studies on Sri Lankan venomous snakes to verify each venom composition and their effect on body according to their geographic diversity and other associated factors.

2.5 ANTICOAGULANT PHOSPHOLIPASE A2 ENZYMES:

Saikia, et al., (2007) evaluated that most of the anticoagulant proteins isolated from cobra venom belong to the PLA2 superfamily of snake venom proteins. It has been postulated that after binding of snake venom PLA2s to the target site, they can induce their pharmacological effects through mechanisms that are either dependent on or independent of their enzymatic activity.

2.6 CARDIOTOXINS:

Kumar et al., (1997) assessed that the secondary structure of cardiotoxins consists of antiparallel β-pleated sheets while the tertiary structure consists of unique asymmetric distribution of non-polar and polar amino acid residues.

Lachumanan et al., (1998) and Chang et al., (2000) studied that similar to other 3FTXs, cardiotoxin encoding genes have three exons interrupted by two introns and preferential mutations are observed in specific segments in exon 2 and 3 which contribute to diverse functions of cardiotoxins. Kini (2002) studied that

cardiotoxins are the second largest group of 3FTXs and only present in cobra venoms.

2.7 VIRTUAL SCREENING AND DOCKING:

Friesner, et al., 2004; Lee and Sun, 2007; Hou, et al., 2013 studied that careful evaluation shows that accuracy is a major problem with docking studies, because if the docking is not approached with precision then these papers will be of little value. Questionable docking results can be found, even in high-profile journals. There are frequent problems such as an inaccurate binding site of the target protein, screening using an unsuitable small-molecule database, the choice of docking pose, high dock score (binding affinity) but failed in MD simulation, lack of clarity over whether the compound is an inhibitor or agonist, or the docking results are inconsistent with bioassays. Although some papers declare docking results with a high accuracy by comparing the ligand pose before and after docking, here I present some evidence that the docking might be still questionable. In some cases, the accuracy of docking can even change from 0% to 92.66%.

Merz, et al., 2010; Warren, et al., 2012; Zheng, et al., 2013; Pei, et al., 2014 reported that since its beginnings in the 1960s, docking, along with the tremendous developments in physics, chemistry, informational technology, biochemistry, and computers, has become a powerful tool and an essential technique, not only in drug screening but also in protein–protein interactions and the behavior of nanomaterials. The current field of computer-aided drug design (CADD) is dominated by technologies used to dock small molecules into macromolecules, particularly protein targets, and its use is increasing year by

year. In modern CADD, structure-based drug design is essential and most big pharmaceutical companies have this department. Protein-ligand or protein-protein docking is a computational technology to predict the orientation of a ligand when it is bound to a protein receptor or enzyme. In most cases, one can choose the best 'binding affinity' to be the potent ligand for further biochemistry experiments and development. Because docking is simple and the equipment requirement is low (it even works well on a personal computer), docking related papers have sharply increased over the past decade. It can provide a critical survey of the field, pointing out the strengths and weaknesses of the current family of docking protocols.

CHAPTER 3
EXPERIMENTAL WORK/METHODOLOGY

3.1 IDENTIFICATION OF PROTEIN:

Phospholipases include phospholipase A1, phospholipase A2, phospholipase B, phospholipase C, and phospholipase D PLA2 is present invariably in all snake venom. For the Virtual Screening and Docking studies, Monomer structure of PLA2 from Cobra (*Naja naja* venom) with 1.8Å resolution was retrieved from Protein Data Bank. The PDB ID is 1A3D.

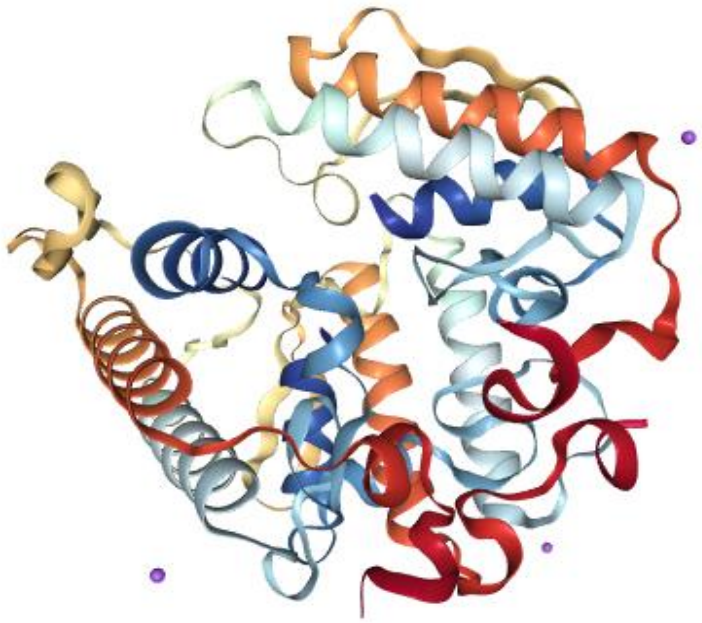

Fig. 3.1 Phospholipase A2 structure from *Naja naja* Venom

- PDB ID: 1A3D
- Resolution: 1.8Å
- Chains: A
- Sequence length: 119 a.a
- Mass: 13346 Da
- UNIPROT ID: P15445

3.2 PREDICTION OF ACTIVE SITE:

The active site is the region of an enzyme where substrate molecules bind and undergo a chemical reaction. The active site consists of residues that form temporary bonds with the substrate (binding site). Although the active site is small relative to the whole volume of the enzyme, it is the most important part of the enzyme as it directly catalyzes the chemical reaction. Each active site is evolved to be optimised to bind a particular substrate and catalyse a particular reaction, resulting in high specificity. An active site can catalyse a reaction repeatedly as residues are not altered at the end of the reaction. The active site residues of the protein were identified using SEESAR.

Table 3.1 List of active amino acid residues with its position

POSITION	AMINO ACID RESIDUES
2	LEU
5	PHE
9	ILE
18	TRP
21	PHE
22	ALA
27	TYR
28	CYS
29	GLY
30	ARG
31	GLY

44	CYS
47	HIS
48	ASP
51	TYR
52	ASN
55	GLU
63	TYR
100	PHE

PyMol:

File ➡ open ➡ PDB file was choosen where it was actually located or the file can be loaded through command line using the command load <file path>

E.g., “load C: Downloads1A3D.pdb”.

By default, the loaded PDB file structure was shown with line representation in PyMol. In display option, the sequence was selected and the specific amino acids obtained from SEESAR were selected. A, S, H, L and C labels in the two rows as different columns that implies, Action, Show, Hide, Label and Color respectively were used to get performed actions. Among the selected amino acid sequence, hide-everything and show-surface was selected to obtain the active site. The image file was saved by using the file menu option, file ➡ save image.

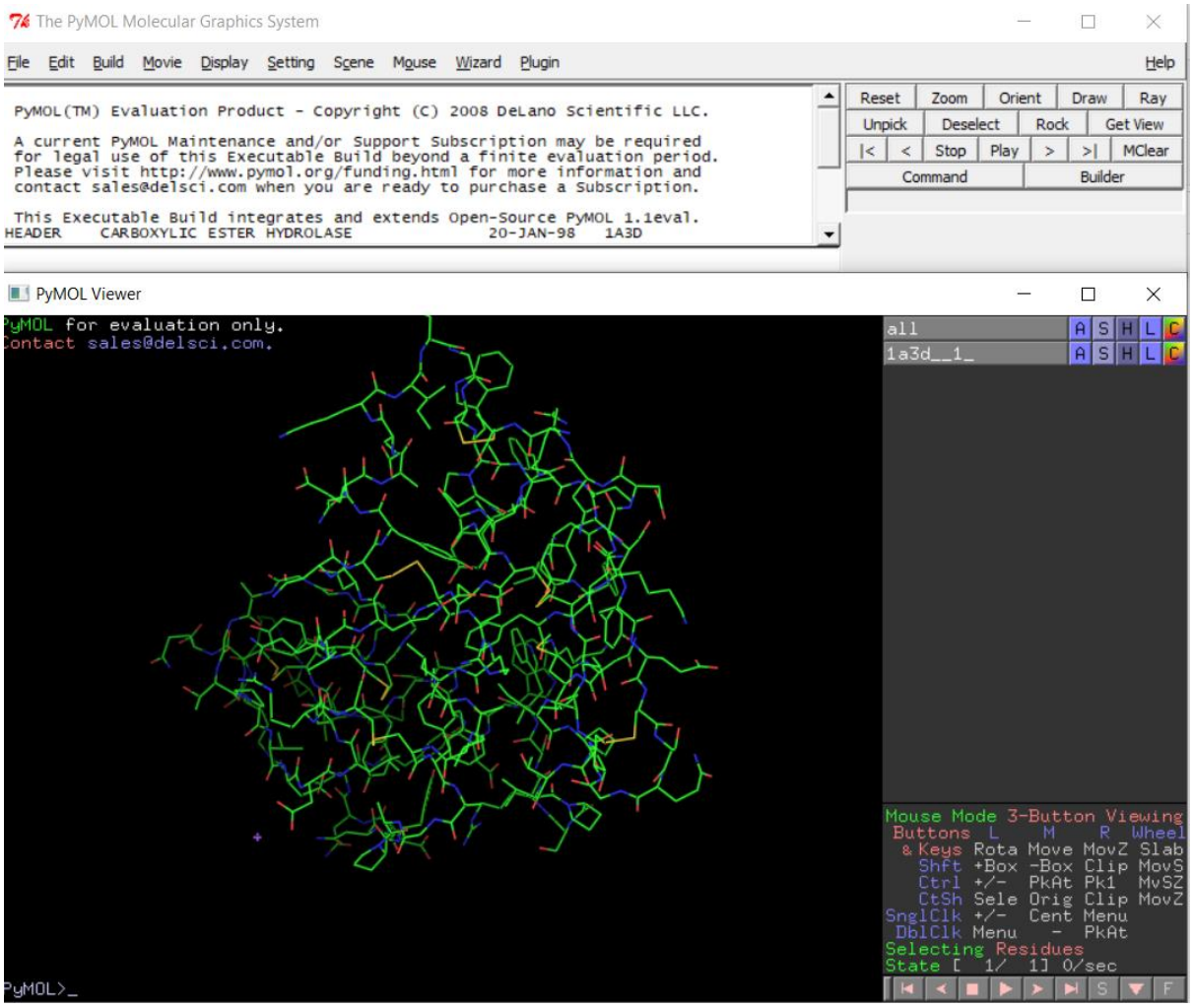

Fig 3.2 PyMol with a loaded molecule

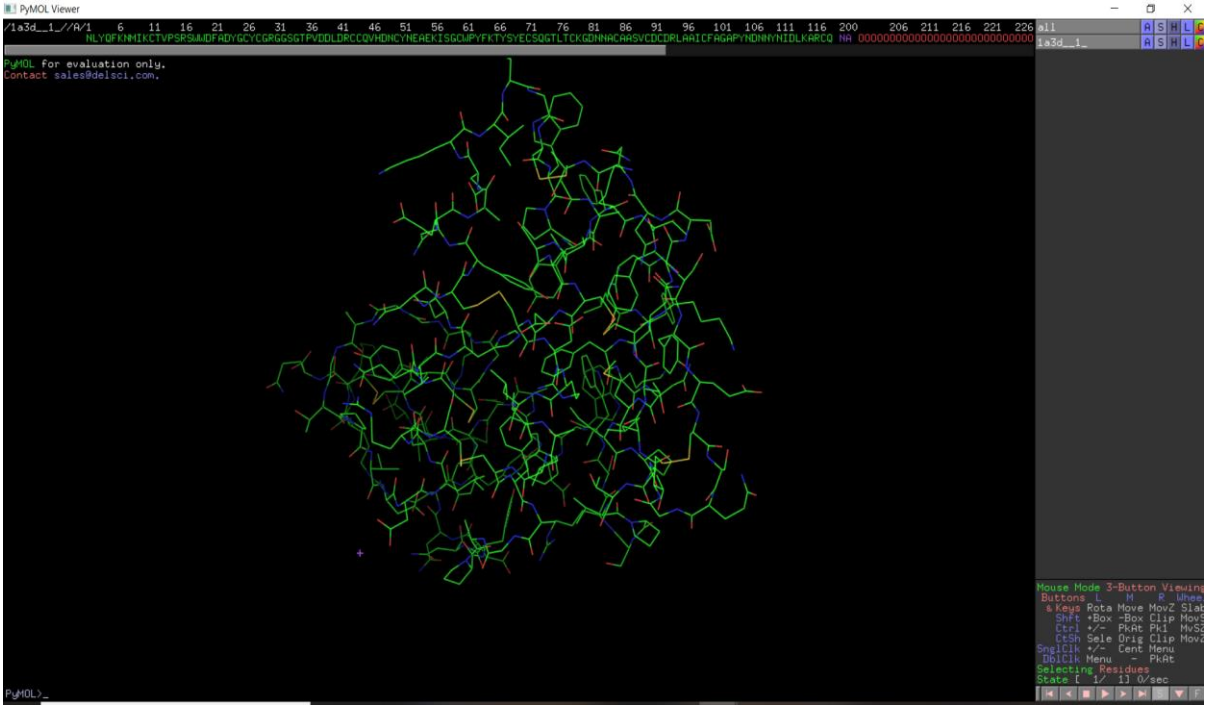

Fig 3.3 Visualization of amino acid sequence for a loaded molecules

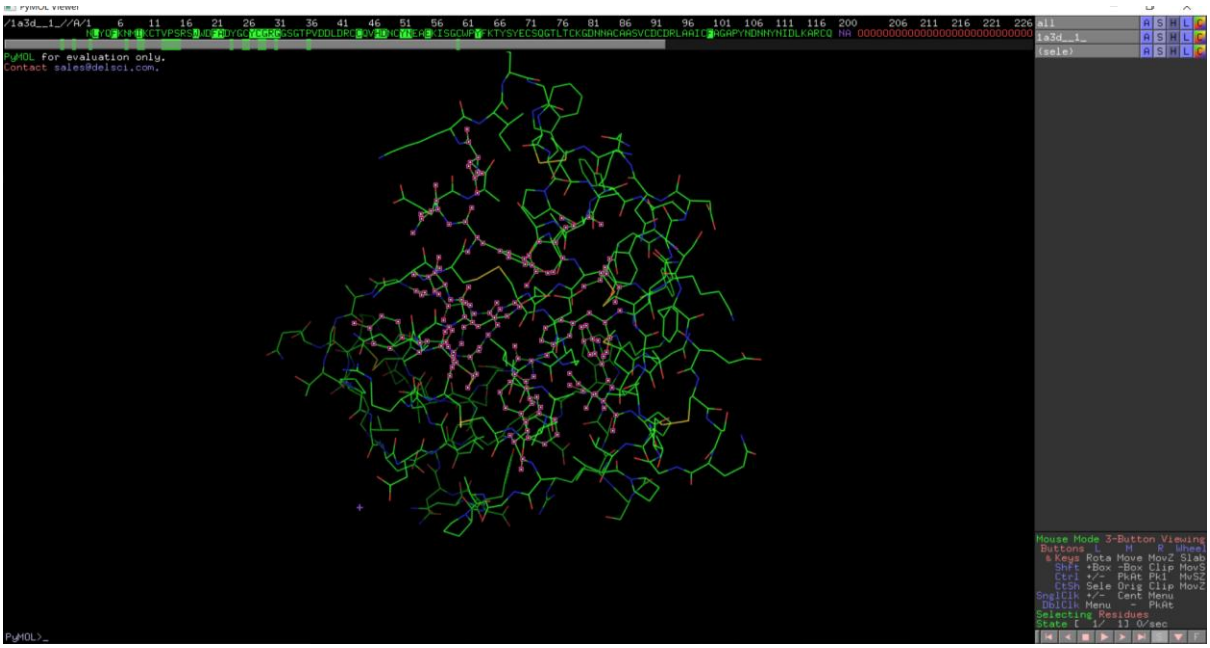

Fig 3.4 Display of selected amino acid sequence

3.3 VIRTUAL SCREENING AND DOCKING:

Virtual screening (VS) is a computational technique used in drug discovery to search libraries of small molecules in order to identify those structures which are most likely to bind to a drug target, typically a protein receptor or enzyme. Virtual screening of compound libraries has become a standard technology in modern drug discovery pipelines. If a suitable structure of the target is available molecular docking can be used to discriminate between putative binders and non-binders in large databases of chemicals and to reduce the number of compounds to be subjected to experimental testing substantially.

PyRx is a Virtual Screening software for Computational Drug Discovery that can be used to screen libraries of compounds against potential drug targets. Features of PyRx software are Autodock, Autodock Vina, and Computer-Aided Drug Design. The DrugBank database is a unique bioinformatics and cheminformatics resource that combines detailed drug data with comprehensive drug target information.

A library containing 3003 small molecules from DrugBank were screened using Autodock Vina. All molecules were loaded into PyRx workspace. After loading molecules, it was converted into AutoDock input files (pdbqt files) as:

Right click on ligand > AutoDock > Make ligand

Right click on protein > AutoDock > Make Macromolecule

After converting pdb files into AutoDock input files (.pdbqt files), now protein and ligands were ready for docking. For docking, the dimension of the grid was set as X: 25.8442 Y: 29.9598 Z: 19.5071. When 3003 small molecules were

docked against the protein, 2117 small molecules were found to be interacting with the protein.

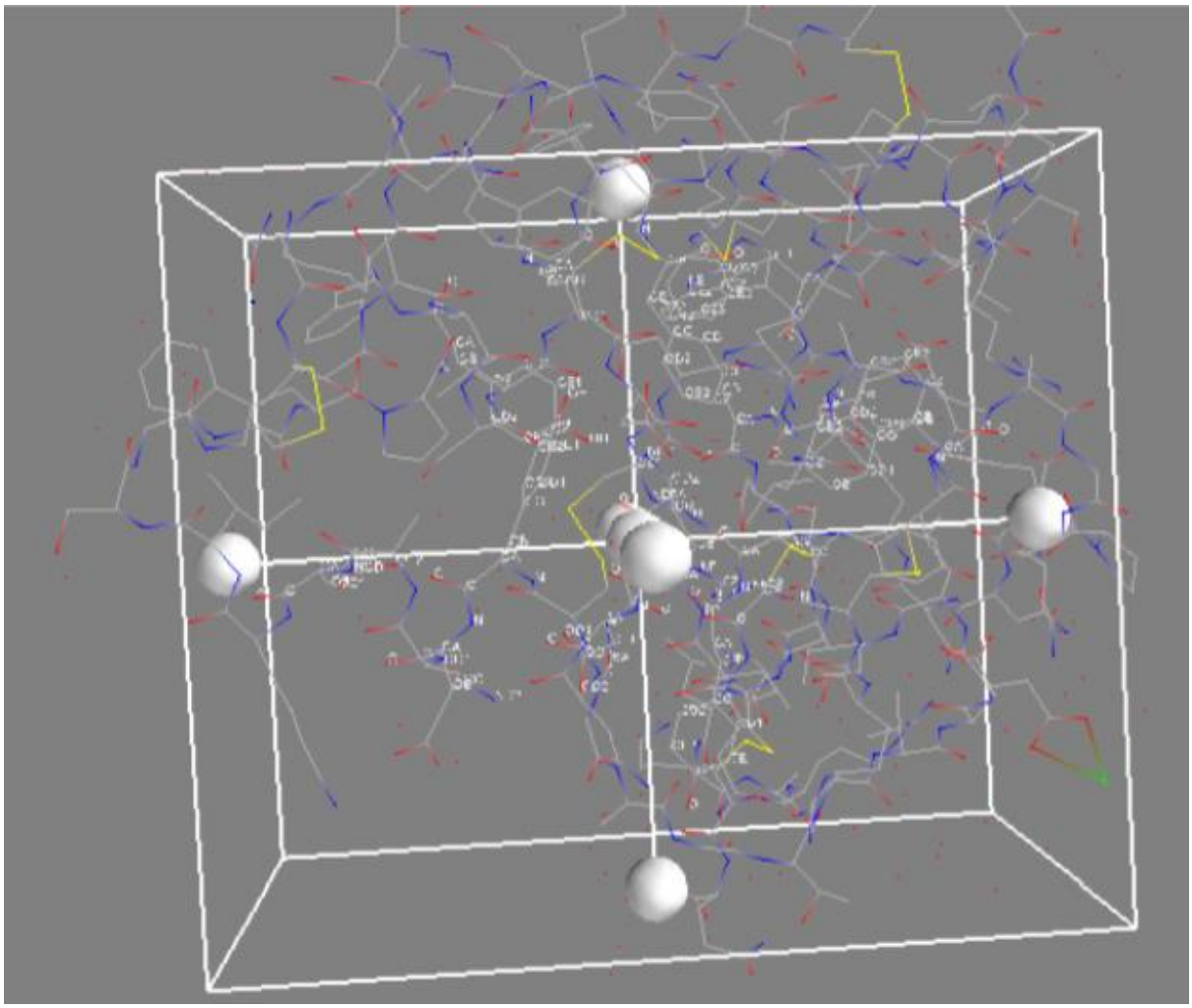

Fig 3.5 Display of grid box with selected dimensions

CHAPTER 4

RESULTS AND DISCUSSION

4.1 PYMOL RESULT:

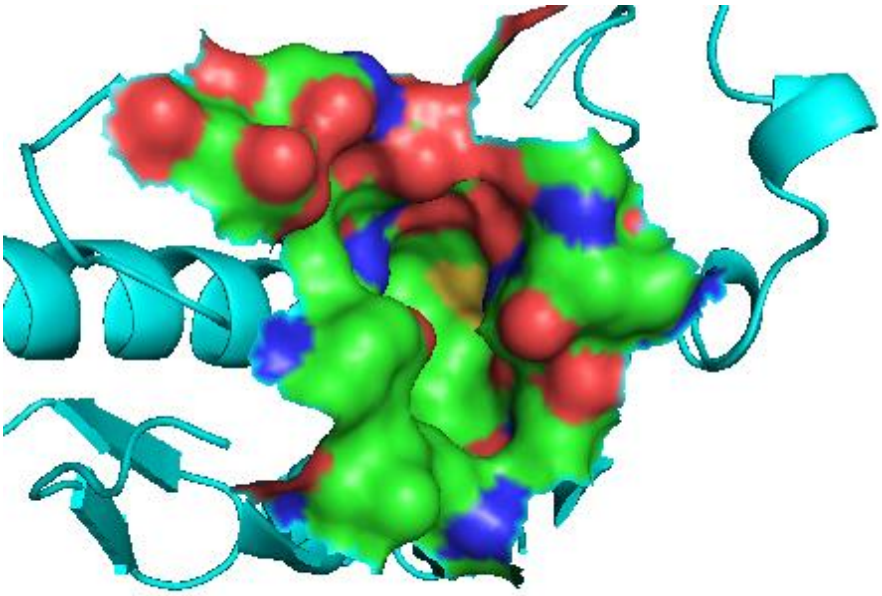

Fig. 4.1 Active Site of PLA2 (Green-carbon, White-hydrogen, Blue-nitrogen, Red-oxygen, Orange-sulfur).

The active site or binding pocket of PLA2 was obtained by using PyMol. Among 1a3d, hide-everything, show-cartoon was selected and among selected amino acid sequence, hide-everything and show-surface was selected to obtain the active site.

Green colour indicates carbon, white colour indicates hydrogen, blue colour indicates nitrogen, red colour indicates oxygen and orange indicates sulphur.

4.2 VIRTUAL SCREENING AND DOCKING RESULT:

Many conformations for one protein-ligand complex were generated. Out of all the conformations, the one with the best binding energy for each ligand was selected. From 2117 docked compounds, 10 were selected on the basis of binding energy.

Table 4.1 Table representation of inhibitors with its specific interacting residues and binding energy

LIGAND	INHIBITOR	SITE OF INTERACTION	INTERACTING RESIDUES	BINDING ENERGY (kcal/mol)
DB00398	Sorafenib	27,47,63	TYR, HIS, TYR	-9.9
DB00549	Zafirlukast	29,55,63	GLY, GLU, TYR	-9.9
DB00696	Ergotamine	18,55	TRP, GLU	-9.7
DB01232	Saquinavir	22,63	ALA, TYR	-9.7
DB04868	Nilotinib	55,63	GLU, TYR	-9.8
DB06817	Raltegavir	29,55,63	GLY, GLU, TYR	-9.7
DB09280	Lumacaftor	27,29,44,47	TYR, GLY, CYS, HIS	-10.5
DB09534	Ecamsule	27,29,63	TYR, GLY, TYR	-9.7
DB11691	Naldemidine	48,63	ASP, TYR	-9.6
DB13879	Glecapivir	29,63	GLY, TYR	-10.1

DB00398-Sorafenib, DB09280-Lumacaftor, DB13879-Glecaprevir were found to be having the best binding energy among the 10 complexes. Visual examination of predicted binding geometries (docking poses) thereby contributes crucially to the further development of a lead compound either towards enhanced binding affinity, towards reduced side effects or towards reduced susceptibility to drug resistance related mutations. The binding interaction of DB00398-Sorafenib, DB09280-Lumacaftor, and DB13879-Glecaprevir were visualized using PyMOL.

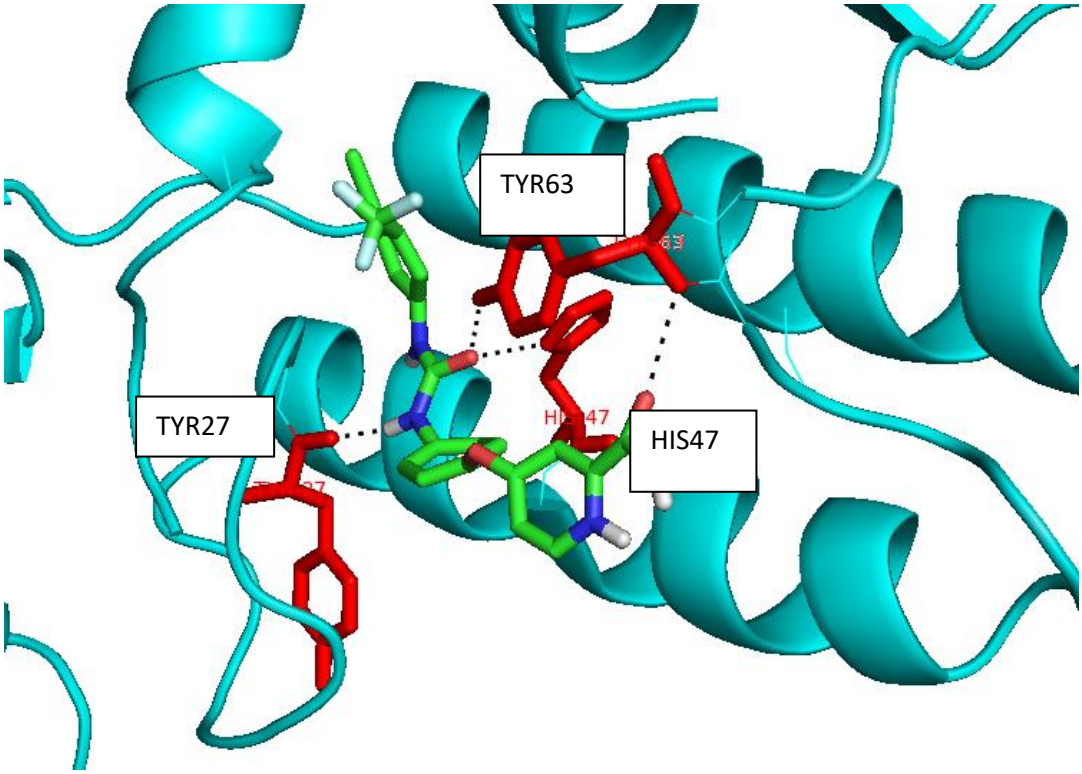

Fig. 4.2 Binding Interaction of Sorafenib

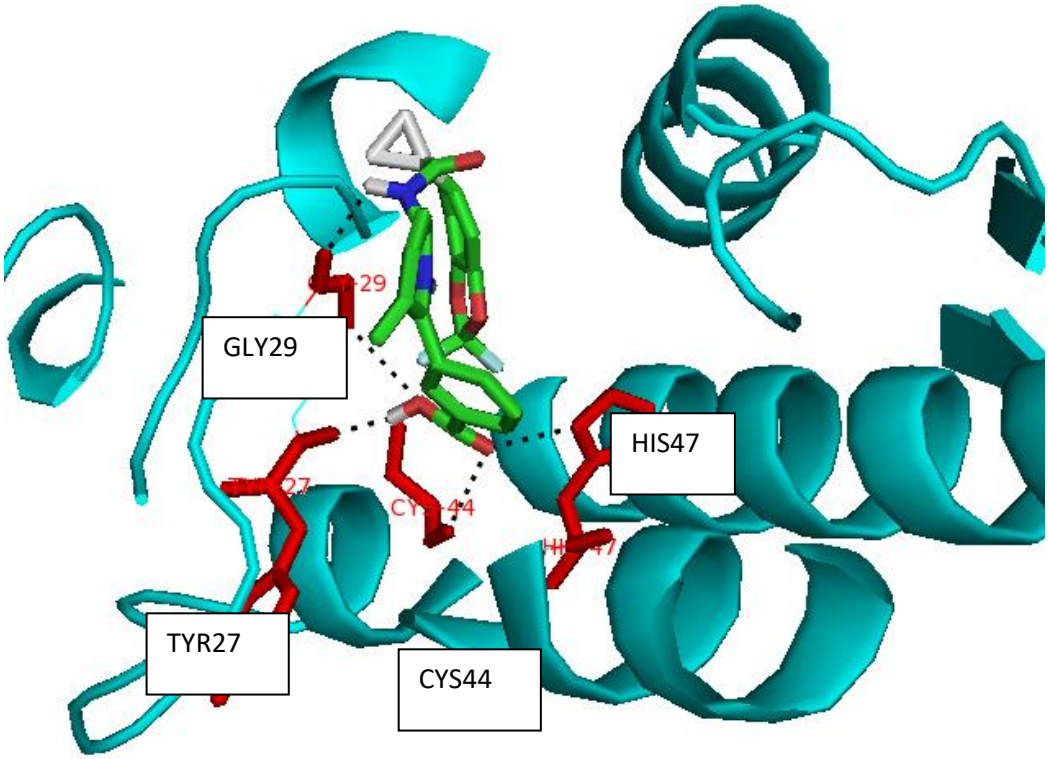

Fig. 4.3 Binding Interaction of Lumacaftor

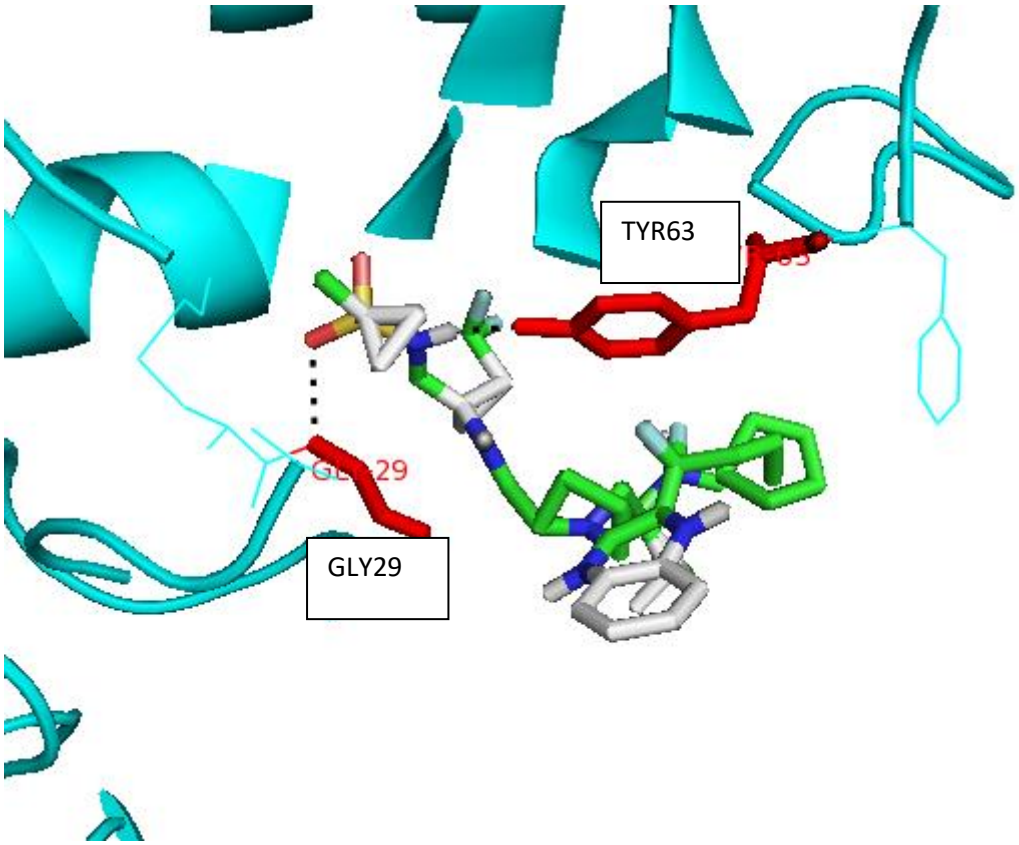

Fig. 4.4 Binding Interaction of Glecaprevir

- Red colour- The amino acid present in the protein which are interacting with the ligand. They are in stick representation.
- The black colour dots represent the polar interaction between protein and ligand
- The ligand is also represented in stick format.
- Blue colour- rest of the protein in cartoon format other than the amino acids interacting with the ligand.

Glecaprevir is a direct acting antiviral agent and Hepatitis C virus NS3/4A protease inhibitor that targets the viral RNA replication. In combination with pibrentasvir, glecaprevir is a useful therapy for patients who experienced therapeutic failure from other NS3/4A protease inhibitors. It is marketed under trade name Maviret and Mavyret and administered through oral route. Glecaprevir disrupts the intracellular processes of the viral life cycle through inhibiting the NS3/4A protease activity of cleaving downstream junctions of hepatitis C virus polypeptide and proteolytic processing of mature structural proteins.

Lumacaftor is a drug used in combination with Ivacaftor as the fixed dose combination product Orkambi for the management of Cystic Fibrosis in patients aged 6 years and older. Lumacaftor improves cystic fibrosis symptoms and underlying disease pathology by aiding the conformational stability of F508del-mutated cystic fibrosis transport regulator proteins, preventing misfolding and resulting in increased processing and trafficking of mature protein to the cell surface. It is administered through oral route. More specifically, lumacaftor acts as a protein-folding chaperone, preventing misfolding of cystic fibrosis transport regulator ion channels and consequent destruction during processing in the endoplasmic reticulum. The half-life of lumacaftor is approximately 26 hours. Lumacaftor is extensively protein bound in the plasma (99%), and binds primarily to albumin

Sorafenib, marketed as Nexavar by Bayer, is a drug approved for the treatment of advanced renal cell carcinoma (primary kidney cancer). Sorafenib is a small molecular inhibitor of Raf kinase, platelet-derived growth factor, vascular endothelial growth factor receptor 2 & 3 kinases and c Kit the receptor for Stem

cell factor. The originality of Sorafenib lays in its simultaneous targeting of the mitogen activated protein kinase/extracellular signal related kinase pathway. It is administered through oral route and it has a half life of about 25-48 hours.

Among these 10 drugs, Glecaprevir, Lumacaftor, Sorafenib may have the potential for inhibiting the snake venom PLA2 as they possess higher negative binding energy. By changing the structure of these drugs and altering its pathways through lead optimization, these 3 drugs can be used to inhibit the snake venom PLA2.

CHAPTER 5

CONCLUSION

Anti-venoms remain the only specific treatment that can potentially prevent or reverse most of the effects of snakebite when administered early in an adequate therapeutic dose. In addition to anti-venom, additional medical measures, including administration of other drugs are needed to effectively treat snakebite patients. From the above result DB00398-Sorafenib, DB09280-Lumacaftor, DB13879-Glecaprevir were found to be having the best binding energy among the complexes that had the lowest binding energy. These three small molecules have the potential to inhibit the activity of secreted PLA2 enzyme from snake venom.

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