Venom as a neurotoxin or hemotoxin biology essay

Historically, venoms have been differentiated from poisons by the route of entry into a recipient organism: venoms are injected or introduced into a wound produced by the delivering organism, and poisons are injected (accidentally or intentionally) in the recipient organism. The term venom typically is applied to simple or complex secretions (usually containing multiple toxins) produced in a specialized gland which causes deleterious effects and/or death when injected into a recipient organism (e. g. 4). A toxin, on the other hand, is a biologically produced unique molecular entity, which can damage or kill an organism through its action on specific tissues (e. g. 5). Unfortunately, even in the scientific literature, one still occasionally encounters the description of venom as a “ neurotoxin” or a “ hemotoxin”, particularly in reference to the venoms of front-fanged snakes (families Atractaspididae, Elapidae and Viperidae). The term “ hemotoxin” is really a misnomer, because there are no venom toxins, which specifically target the blood. Though the dominant pharmacological effect of venom may be described superficially as “ neurotoxic” or “ tissue-damaging”, no snake venom described to date contains only a single molecular or pharmacologically-active component. Toxinologists, herpetologists and other should therefore refrain from using such obfuscating language, because these errors become propagated by the lay press and could lead to inappropriate management of human envenomation by a hemotoxic snake. For example, in the United States, the general public considers rattlesnakes to produce “ hemotoxic” venom; however, venom of the Mojave rattlesnake, Crotalusscutulatus (as well as several other species), often contains Mojave toxin, a potent homolog of the presynaptic neurotoxin crotoxin, and bites by this species can rapidly become life-threatening.

Antivenin was first prepared in 1894, and was the result of several investigations carried out simultaneously in different parts of the world. The successfully scientific immunization of an animal by repeated injections of animal venom was first reported by Fornara in 1877. He successfully protected a dog after several inoculations of small amounts of toad skin secretions. Later in Michigan, Sewall described a similar experiment in 1887. He protected pigeons against the equivalent of six lethal doses (LD) of rattlesnake venom after treating them with gradually increasing doses of venom. Subsequently, in France in 1892, Kaufmann reproduced these experiments on a dog inoculated with Viperaaspisvenom. It was demonstrated that animals can be protected against a toxic substance after inoculation of several sub-lethal doses of the same substance. The development of diphtheria and tetanus antitoxins by Behring and Kitasato in early 1890s was based upon a similar principle. But they showed that protection could be transmitted from one animal to another that had never before received the substance. The way to antitoxin or antivenin therapy was opened.

Discovery of antivenin was claimed the same day (February 10, 1984) by the team of Hisalixand that of Calmette (Brygoo in 1985; Calmette in 1884; Phisalix and Bertrand in 1884). Calmette in Paris, Fraser in Edinburgh, São Paulo in Brazil and McFarland in Philadelphia began preparation of antivenin against various species of venomous snakes. The main difficulties in the preparation and use of antivenin appeared soon after the beginning of serotherapy. Some are Calmette (Brygoo in 1985; Calmette in 1884; Phisalix

and Bertrand in 1884). Calmette in Paris, Fraser in Edinburgh, São Paulo in Brazil and McFarland in Philadelphiastill not completely resolved:

Inactivation of venom before inoculation of the animal

Purification of antivenin

Evaluation of antivenin potency

Adverse reactions to antivenin

Today, in addition to the epidemiological, biochemical, and immunological considerations, one must add the commercial and economic points of view. Serotherapy is currently the only specific treatment of snake envenomation; however these accidents often occur in regions where antivenin, if available, is difficult to administer and expensive in relation to the way of life.

Snake bites were considered emergency threats for human life. Perhaps, venomous bites show as double teeth marks than ordinary bites. Snake venom is one of the most amazing and unique adoptions of snakes in animal planet. Venoms are mainly toxic modified saliva consisting of a complex mixture of chemicals called enzymes found in snake poisons throughout the world known to man. Snakes with neurotoxic venom include cobras, mambas, sea snakes, kraits and coral snakes and snakes with hemotoxic venom include rattlesnakes, copper-head and cottonmouths (Blanchard, 2001).

Worldwide about 30, 000 to 40, 000 people die annually of snake bites. Of these, about 25, 000 people die in India, mostly in rural areas, about 10, 000

people in United States and rest of in other countries. Under the Wild Life Protection Act, 1972, all snakes are protected (with the venomous once being at the top of the list of the protected species) and there was a ban on the selling of snake skins since 1976. Snake venom is badly needed to produce antivenom required to treat potentially fatal snakebites (Dravidamaniet. al., 2008).

[2. 1] Antivenom Beginnings

In the 1890, Albert Calmette, who was a protégée of Louis Pasteur, found himself in a troubling situation. He was living in what is now Vietnam and his village near Saigonhad just suffered a serious flood. The water wasn't the worst of it. The flooding pushed monocled cobras into village where they bit at least 40 people and killed four. After this experience Calmette began work on a cure that would be similar to the then new science of vaccinations. Calmette eventually caught snakes, " milked" them of their venom and injected it into horses to create antibodies. Drawing the horses' blood for a serum, he was able to create antivenom that worked on humans. Snake venoms are among the best pharmacologically characterized natural toxins, chiefly because of their deleterious effects on humans. While these complex, protein rich mixtures have been extensively separated and fractionated for over half a century, our understanding of the evolution of venomous snakes has relied on comparative morphology (Vidal, 2002; Jackson, 2003) and molecular genetics (Kraus and Brown, 1998; Slowinski and Lawson, 2002). Unfortunately, genetic and morphological analyses alone cannot provide much evidence regarding evolution of venom components, and offer no

insight into the evolutionary and biological utility of snake venoms. This study has two primary functions:

(1) To review and summarize the existing body of toxicological literature regarding the enzyme activities of snake venoms, and

(2) To encourage applied researchers to consider the natural functions and selective forces that have shaped snake venoms over evolutionary time.

These findings should be of particular interest to applied toxicological researchers who deal with these intriguing mixtures exclusively at the pharmacological level. While the primary biological utility of snake venoms is not well understood from an evolutionary perspective, this has not prevented naturalists from speculating about venoms biological utilities for over one hundred years (Mitchell, 1868; Shortt 1870). Most of these hypotheses regarding the function of snake venoms have focused on three adaptive advantages: prey capture, defense, and digestion. Whether a result of difficulties associated with experimental design or the obvious connection between a snake bite and death, very few scientific researchers have attempted to investigate the evolutionary utility of snake venoms (Thomas and Pough, 1979; Daltry et al., 1997; Andrade and Abe, 1999; McCue, 2002).

[2. 2] Evolution of venomous snakes

Venomous snakes are a polyphyletic group of Colubroidea that includes all family members of Elapidae and Viperidae, and some of the members of the families Atractaspidae and Colubridae. Because of the difficulties in definitively identifying which snakes belonging to the families Atractaspidae and Colubridae are venomous (Vidal, 2002), and because venomous

atractaspid and colubrid snakes are so poorly represented in the toxicological literature (Rodriguez-Robles, 1994; Weinstein and Kardong, 1994), these groups are not further addressed here.

This review focuses on the venom characteristics of the three most widely researched venomous subfamilies: the Elapinae (Family Elapidae) and the Viperinae and Crotalinae (Family Viperidae). These lineages are believed to have originated in the Miocene, but remain sparsely represented in the fossil record (Nilson and Andren, 1997; Rage, 1997). Like their fossil record, scientific discussions concerning the evolutionary and selective forces responsible for shaping their venoms are scant. Several studies have shown that the composition of snake venom is genetically controlled, and thus subject to evolution via natural selection like any heritable trait (Jimenez-Porras, 1964; Airdet al., 1989). Therefore, it should be possible to make evolutionary inferences based on the current patterns of venom phenotypes. This paper examines patterns in venom protein content, toxicity, and yield, and compares specific enzyme activities among over one hundred venomous snake species from three subfamilies. The purpose of this investigation is to uncover patterns in the chemical activities and composition of venoms. Such patterns can then be used to address the long-standing hypotheses about the biological function and evolutionary radiation of snake venoms.

[2. 3] Comparing chemical activities of snake venoms

Snake venoms are complex mixtures composed chiefly of varied enzymatic and non-enzymatic toxins. Although a single snake venom sample may contain dozens of enzymatic toxins, these enzymes are generally grouped into a few classes by Toxinologists. The most commonly quantified classes of

snake venom enzymes include phospholipase A2 (PLA2), phosphodiesterase, phosphomonoesterase, L-amino acid oxidase, specific endopeptidases, and nonspecific endopeptidases (Iwanaga and Suzuki, 1979). The specific activities of each of these can be measured using different substrates. Most comparative studies of snake venoms do not quantify enzyme concentration directly, but rather, measure venoms' specific activities on various molecular substrates. Because the enzyme composition of particular venom fractions can vary widely (Boumrahet. al., 1993; Komori et. al., 1995), measurements of specific activity of whole venoms are employed in these analyses. Furthermore, many venom components are believed to work synergistically with each other, or with components of prey tissue (Teng et al., 1984; Tan and Armugam, 1990), and thus fractionated venoms offer a less complete collection of potential synergies. As a result, this study references only toxicological studies that use either fresh venoms or freshly reconstituted whole venoms. Reconstituted venoms are most commonly used in laboratory research and are well known to be pharmacologically equivalent to fresh venoms (Minton and Weinstein, 1986; Hayes et. al., 1995). Several studies have demonstrated that venom from conspecific snakes can vary ontogenetically (Bonilla et. al., 1973; Meier and Freyvogel, 1980; Meier, 1986; Andrade and Abe, 1999), seasonally (Gregory-Dwyer et al., 1986), interdemically (Aird, 1985; Minton and Weinstein, 1986; Wilkinson et. al., 1991; Rodrigues et. al., 1998), and with physical condition (Klauber, 1997). Because of the numerous sources of qualitative and quantitative variation among venoms, this study draws from a broad range of primary sources to explore patterns in toxicological pharmacological properties of a diverse collection of snake venoms.

[2. 4] Mechanism of Action on Human

Cobra snake venom cardiotoxins and bee venom melittin share a number of pharmacological properties in intact tissues including hemolysis, cytolysis, and contractures of muscle, membrane depolarization and activation of tissue phospholipase C and, to a far lesser extent, an arachidonic acid-associated phospholipase A2. The toxins have also been demonstrated to open the Ca2+ release channel (ryanodine receptor) and alter the activity of the Ca(2+)+Mg(2+)-ATPase in isolated sarcoplasmic reticulum preparations derived from cardiac or skeletal muscle. However, a relationship of these actions in isolated organelles to contracture induction has not yet been established. The toxins also bind to and, in some cases, alter the function of a number of other proteins in disrupted tissues. The most difficult tasks in understanding the mechanism of action of these toxins have been dissociating the primary from secondary effects and distinguishing between effects that only occur in disrupted tissues and those that occur in intact tissue.

[2. 5] Symptoms of Venom on Humans

Intense Pain.

Swelling [dysphagia]

Necrosis (Muscles damaged)

Hemorrhaging

Internal Organ Breakdown

Blood Cells (i. e. WBC and RBC) destroyed [Hemolysis]

Weakness

Rapid pulse

Numbness

Tingling Sensation

Bruising

Bleeding disorder [Disrupt blood clotting]

Vomiting

Drooping of eyelids [Ptosis]

Double Vision [Diplopia]

[2. 6]

[2. 7] Treatment with Antivenom

Antivenom acts to neutralize the poisonous venom of the cobra and causes the venom to be released from the receptor site. Thus, the receptor sites that were previously blocked by venom are now free to interact with the acetylcholine molecule and normal respiration resumes. The spent antivenom and the neutralized venom are then excreted from the body.

Venom composition (and its corresponding toxicity) can vary among cobras from the same species and even from the same litter it can also vary for an individual cobra during its lifetimeand all of this makes each cobra bite truly

unique. In order to insure correct treatment, antibodies specific to each form of cobra venom must be developed. The correct antibodies may be synthesized by injecting horses with a small amount of cobra venom, and then collecting the antibodies produced by the horses' immune systems. Of course, large samples of cobra venom must be collected for this process, and many snake farms around the world make significant amounts of money by harvesting the deadly snake toxin.

[2. 8] Phospholipase A2 (PLA2)

PLA2 enzymes are esterolytic enzymes which hydrolyseglycerophospholipids at the snâˆ'2 position of the glycerol backbone releasing lysophospholipids and fatty acids. Snake venoms are rich sources of PLA2 enzymes. Several hundred snake venom contains PLA2 enzymes which have been collected, purified and characterized. Amino acid sequences of over 280determined as PLA2 enzymes (Adatabaseis available at http://sdmc. lit. org. sg/Templar/DB/snaketoxin_PLA2/index. html). They are made of approx. 13kDa proteins and contain 116-124 amino acid residues and six or seven di-sulphide bonds. They are rarely glycosylated. So far, three-dimensional structures of more than 30 PLA2 enzymes have been determined (for a comprehensive list). The structural data indicate that snake venom PLA2 enzymes share strong structural similarity to mammalian pancreatic as well as secretory PLA2 enzymes. They have a core of three Î±-helices, a distinctive backbone loop that binds catalytically important calcium ions, and a Î²-wing that consists of a single loop of antiparallel Î²-sheet. The C-terminal segment forms a semicircular ' banister', particularly in viperid and crotalid PLA2 enzymes, around the Ca2+-binding loop. In addition, they have a

similar catalytic function in hydrolyzing phospholipids at the snâˆ’2 position. However, in contrast with mammalian PLA2 enzymes, many snake venom PLA2 enzymes are toxic and induce a wide spectrum of pharmacological effects. These include neurotoxic, cardiotoxic, myotoxic, hemolytic, convulsive, anticoagulant, antiplatelet, oedema-inducing and tissue-damaging effects. Thus PLA2 enzymes also form a family of snake venom toxins, which share a common structural fold but exhibit multiple functions. These factors make the structure-function relationships and the mechanisms of action intriguing, and pose exciting challenges to scientists.

Some snake venom PLA2 enzymes inhibit blood coagulation. Boffa and colleagues studied the anticoagulant properties of a number of PLA2 enzymes and classified them into strongly, weakly and non-anticoagulant enzymes. Strongly anticoagulant PLA2 enzymes inhibit blood coagulation at concentrations below 2Î¼g/ml. weakly anticoagulant PLA2 enzymes show effects between 3 to 10Î¼g/ml. A number of venom PLA2 enzymes do not prolong the clotting times significantly even at 15Î¼g/ml. Thus the anticoagulant activity of different PLA2 enzymes varies significantly. Evans et. al., purified three anticoagulant proteins (CM-I, CM-II and CM-IV) from Najanigricollis (black-necked spitting cobra) venom and showed their identity with PLA2 enzymes. CM-IV shows at least 100-fold more potent anticoagulant activity than CM-I and CM-II. On the basis of their anticoagulant properties, they were classified as strongly (CM-IV) and weakly (CM-I, CMII) anticoagulant PLA2 enzymes respectively. Since phospholipids play a crucial role in the formation of several coagulation complexes, intuitively one might anticipate that the destruction of phospholipid surface would be the primary

mechanism to account for anticoagulant effects of PLA2 enzymes. However, strongly anticoagulant PLA2 enzymes also affect blood coagulation by mechanisms that are independent of phospholipid hydrolysis.

To explain the functional specificity and mechanism of induction of various pharmacological effects, the target model was proposed. Accordingly, the susceptibility of a tissue to a particular PLA2 enzyme is due to the presence of specific ‘ target sites’ on the surface of target cells or tissues. These target sites are recognized by specific ‘ pharmacological sites’ on the PLA2 molecule that are complementary to ‘ target sites’ in terms of charges, hydrophobicity and van der Waals contact surfaces. Proteins (or glycoproteins) could act as specific target sites for PLA2 enzymes. The affinity between PLA2 and its target protein is in the low nanomolar range, whereas the binding between PLA2 and phospholipids is in the high micromolar range. Such a four to six orders of magnitude difference in affinity between the protein-protein interaction and the protein-phospholipid interaction explains why the interaction of PLA2 and its target protein governs the pharmacological specificity.

The target proteins such as membrane-bound receptors/acceptors are identified through studies using radiolabelled PLA2 enzymes and specific binding studies, as well as photo affinity labeling techniques. Anticoagulant PLA2 enzymes, on the other hand, target one or more soluble proteins or their complexes in the coagulation cascade. Furthermore, the enzymes may interact with the active, but not the zymogen, form of the coagulation factor. Therefore different strategies have been used to identify the soluble target

protein in order to understand the mechanism of anticoagulant effects of PLA2 enzymes.

A2 cleavage site

[2. 9] PLA2 as Target

PLA2 disrupts biological membranes and can lead to permanent damage or even lysis (splitting or breaking of cells). The body secretes its own versions of PLA2 (pancreatic [I] or non-pancreatic [II]) that have totally different functions. Human PLA2 aid in: digestive enzymes, cell contraction, cell proliferation, destruction of pathogens (Disease producing organisms) Venom PLA2 is classified as group III and has a similar structure to I & II only when bound to a receptor. The various physiological effects of PLA2 are determined by the type of receptor to which it binds. Receptors include N-receptors (neurological- III) and M-receptors (muscular- bind only I & II). It may act pre- or post-synaptically at the neuromuscular junction by binding to acetylcholine receptors (N-receptor). The binding of PLA2 to acetylcholine receptors block the binding of acetylcholine, which causes flaccid (limp) paralysis. The binding of the receptor affects in a variety of ways in different muscles. This suggests that there are differences in affinity of the binding in different muscle types. Respiratory failure often accompanies the paralysis because there is likely a high affinity for PLA2 in phrenic nerve-diaphragm endplate receptors.

A large number of different types of plasma membrane receptors, including many that act via heterotrimeric GTP-binding proteins or tyrosine kinases, have been demonstrated to induce activation of PLA2. This enzyme cleaves

the sn-2 fatty acyl bond of phospholipids, producing a free fatty acid and a lysophospholipid. AA is the precursor of a large family of compounds known as the eicosanoids (based on their derivation from the precursor), which includes cyclooxygenase-derived prostaglandins and lipoxygenasederived leukotrienes. The eicosanoids possess a wide spectrum of biological PHOSPHOLIPASE A2 REGULATION 177

[2. 10] Ursolic Acid as Inhibitor

Ursolic acid is a pentacyclictriterpenoid, present in many fruit plants such as apples, bilberries, cranberries, elder flower, peppermint, lavender, oregano, thyme, hawthorn, prunes and i. e. why it is used in cosmetics. Ithas medicinally action, both topically and internally. Ursolic acid can serve as a starting material for synthesis of more potent bioactive derivatives, such as anti-tumor agents. It is capable of inhibiting various types of cancer cells by inhibiting the STAT3 activation pathway and human fibrosarcoma cells by reducing the expression of matrix metalloproteinase-9 by acting through the glucocorticoid receptor. It may also decrease proliferation of cancer cells and induce apoptosis. Ursolic acid and its native compositions are used in pharmacology (one can find more than 1, 500 sources in scientific literature) predominantly as a component of preventive medicine for various diseases including lymphocytic leukemia, neoplastic tumors, and as a modifier of protein synthesis.

Ursolic acid was found to be a weak aromataseinhibitor (IC50 = 32 Î¼m).

Other names for ursolic acid include 3-beta-3-hydroxy-urs-12-ene-28-oic-acid, 3-Î²-hydroxy-urs-12-en-28-oic acid, urson, prunol, and malol.

Figure-2. 1: Structure of Ursolic Acid

[2. 11] Molecular Docking

Drug discovery is often evolved from serendipitous and fortuitous findings. For example penicillin discovery by alexander Fleming in 1928 brought revolution in drug discovery which contributed tremendously for longevity of human beings. Such discovery may be achieved through random systematic experimentation where combinatorial libraries are synthesized and screened potent activities. Such an approach is time consuming, labor intensive and high cost effective. A more lucrative solution to overcome this problem is to rationally drugs design using computer aided tools such as molecular modeling, molecular docking simulation and virtual screening for the purpose of identifying promising candidates prior to synthesis.

Docking and are design are the measure computational approach towards understanding and affecting receptor-ligand interaction. Molecular docking is a key tool in structural molecular biology and computer assisted drug design. Now a day, the goal of the molecular docking in modern drug design and discovery to help in understanding the drug -receptor interaction. It has been show in literature that these computational techniques can strongly support and help in the design of novel, more potent inhibitors by revealing the mechanism of drug receptor interaction. The computational concepts and offered the following strategy for flexible docking and design (a) Monte Carlo/molecular dynamics docking (b) in-site combinatorial search (c) ligand build-up and (d) site mapping and fragment assembly(Rosenfeld et al., 1994). Significant advances in computer based ligand-receptor docking

techniques and related rational drug design tools helped significantly to generate lead compound for target proteins (Lybrand, 1995).

Autodock predicts the conformations of a small and flexible ligand to a macromolecular target of known structure with the help of C program. It combines simulated annealing for conformation searching with a rapid grid-based method of energy evaluation (Goodsellet al., 1996). In general, there are two key component of molecular docking(Leach and Gillet, 2003): (a)Accurate pose prediction or binding confirmation of the ligand inside the binding site of the target protein and (b) Accurate binding free energy prediction, which later is use to rank order the docking poses. The docking algorithm usually carries out the first part of the docking (predicting binding confirmation) and the scoring function associated with the docking program carries out the second part i. e. binding free energy calculations.

Docking algorithm usually perform pose prediction. Identifying molecular features which are responsible for molecular recognition or pose prediction are very complex and often difficult to understand and even more so, when simulated on a computer (Kitchen et. al., November 2004).

After the pose prediction by the docking algorithm, the immediate step in the docking process is activity prediction, which is also termed as scoring. Docking score is achieved by the scoring functions associated with the particular docking software. Scoring functions are design to calculate the biological activity by estimating the interaction between the compound and protein target.

[2. 11. 1] Docking Algorithm

Depending on the flexibility of protein of ligand, docking algorithms can be divided in 3 types:

Rigid docking: Protein and ligand are considered to be rigid.

Semi-flexible docking protein is fixed and ligand is flexible.

Flexible docking: Both protein and ligand are flexible.

Based on the principle of confirmation generation, the search methods are categorized into Stochastic, Systematic and Deterministic method.

The two most popular stochastic methods are genetic algorithm (GA) and Monte Carlo algorithm (MC) (Clark and Ajay 1995; Jones et. al., 1995; Oshiroet. al., 1995). The Monte Carlo method is capable of generating ensembles of confirmations statistically consistent at room temperature. While generating the pool of random conformations, with each iteration of the process, either the internal confirmation of the ligand (by rotating around a bond) is changed or the entire ligand is subjected to the rotation or translation within the active site of the protein. An energy function evaluates the newly formed confirmation and except the confirmation only if the energy is lower than the one derived from the previous step or if, it is higher, is within the ranged defined by Boltzmann factor (Miteva, 2008). Ligand fit Monte Carlo algorithm. GA Starts with population of random ligand confirmations with random orientation and at random translations. In genetic algorithm (GA), each chromosome in a population encodes for one ligand confirmation along with its orientation in its binding sites of the proteins.

Then, in the next step, scoring functions evaluate the fitness of each individual in a population and less fit individuals are being killed (or not passed on onto the next generation). Pairs or survived individuals are mated leading to children with the new chromosomes derived from the parents by mutations and recombination. (Chromosome in this text refers to position, orientation, and confirmation of the ligand). GA differs from the Monte Carlo methods by performing a numbers of runs and selecting the structure with highest scores. GOLD (Verdonket. al., 2003), Autodock (Morris et. al., 1999) and DARVIN (Taylor et. al., November 2000) are the some of the few docking programs with rely on genetic algorithms.

With the availability of more and more information on protein and nucleic acid molecular docking is considered as a lead method for drug design and discovery. The computer added Drug Design (CADD) has facilitated the discovery of new lead compounds and three dimensional structural optimization. The main direction in CADD are based on the availability of the experimentally determined 3Dimentiona Structure of protein molecules. The methods of structure based drug design are used wherever the 3D structure of protein molecule is known. In other indirect methods of CADD based on ligand based drug design system is used. The structural information does obtain can be invaluable in the generation of novel molecules or in the redesign of existing molecules which do not have optimal activity.

Therefore computational approaches like ‘ Dock’ small molecules into the binding cavity of macromolecular target and ‘ Score’ there potential complementarity to binding sites are widely used in potent hit identification lead optimization.

[2. 12] Quantitative Structure Activity Relationships

QSAR make possible to predict the activities of a given compound as a function of its molecular substituent. QSAR has great potential for modeling and designing novel compounds with robust properties. QSAR has its origin in the field of toxicology whereby Cross in 1863 proposed a relationship which existed between the toxicity of primary aliphatic alcohol with their water solubility (Cross, 1963), shortly after, Richet (Richet, 1893), Meyer (Molecular Networks GmbH Computer chemie., 2008), and Overton (Overton, 1901) separately discovered a linear correlation between lipophilicity (e. g. oil-water partition coefficient) and biological effects (e. g. Narcotic effects an toxicity). In 1956, Taft proposed an approach for separating polar, steric and resonance effect of substituents in aliphatic compounds (Taft, 1956) these contributions by Hammet and Taft formed the mechanist basis for the development of QSAR by other investigators like Hensch and Fujita (Hensch and Fujita, 1964). An excellent account on the development of QSAR is presented by Hensch and Leo (Hensch and Leo, 1995).

Classical QSAR often correlate biological activities of drug with physiochemical properties which encode certain structural features (Hensch and Leo, 1995; Ramsden, 1994; Kudinyi, 1993; Kubinyi, 1995; Ven de waterbeemt, 1996). In addition to lipophilicity, polarizability, electronic properties and steric parameters are also frequently used to describe the different size of substituents. Cramer and Milne were the first to attempt to compare molecules by aligning them in space and by mapping their molecular field to a dimensional grid (kim, 2007). In order to correlate the field values with the biological activities, svantewold in 1986 developed the

used of partial least squares analysis instead of principal component analysis. So many different approaches to QSAR have been developed over the years. The rapid increase in three dimensional structure information (3D) of bioorganic molecules, coupled with the development of fast method for 3D structure alignment (e. g. active analogue approach), has led to the development of 3D structural descriptors and associate 3D QSAR methods. The most popular 3D QSAR method is comparative molecular field analysis (CoMFA) (Cramer et. al., 1988) and comparative molecular similarity analysis (CoMSIA) (Klebeet. al., 1994). The CoMFA method involves generation of a common three-dimensional lattice around a set of molecules and calculation of the steric and electrostatic interaction energies at the lattice points. The interaction energies are numerically very when a lactic point is very close to an atom and special care needs to be taken in order to avoid problems arising because of this. The CoMSIA method avoids these problems by using simi