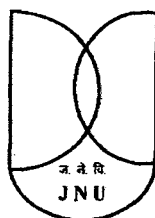# Designing of Novel Inhibitors against Anthrax Toxin (EF & LF)

A thesis submitted in partial fulfillment of the requirements
for the award of the degree of
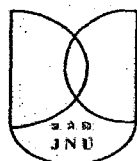
Master of Technology

in

Computational and Systems Biology

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JNU

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May, 2009

CENTRE FOR COMPUTATIONAL BIOLOGY AND BIOINFORMATICS
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NEW DELHI – 110067.

CERTIFICATE

Certified that this M.Tech. dissertation titled "**Designing of novel inhibitors against anthax toxin(LF & EF)**" has been carried out by **Mr. Varun Jaiswal** under my guidance and supervision. This work is original and has not been submitted elsewhere for award of any degree or diploma.

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CONTENTS

1. INTRODUCTION

1.1 ANTHRAX

Anthrax is caused by *Bacillus anthracis,* a gram positive spore forming bacteria. Anthrax is an acute infectious disease. This disease is generally found in agricultural regions of developing countries where it occurs in wild and domestic animals [http://www.bt.cdc.gov/agent/anthrax/faq/] [http://www.bt.cdc.gov/agent/anthrax/needtoknow.asp]. Inhaling anthrax spore is very dangerous. When a person inhales the spores of *B. anthracis,* they(spores) germinate and the bacteria infect the lungs, spreading to the lymph nodes in the chest. As the bacteria grow, they produce anthrax toxin(TA) which results in death of organism[http://www3.niaid.nih.gov/topics/anthrax/overview.htm]. Anthrax affects farm animals more than human. It can cause three forms of disease in human. These are:

1) Cutaneous, it occurs when *Bacillus anthracis* infects the cuts or open sores in the skin of an individual by touching the bacteria.

2) Inhalational, it occurs when an individual inhales the spores of bacteria(highly fatal).

3) Gastrointestinal it occurs by eating undercooked infected meat with *Bacillus anthracis.* [http://www.nlm.nih.gov/medlineplus/anthrax.html)].

The anthrax infection is rare in human and observed in people related to paper, wool and cattle industry. But inhalation of anthrax spore is extremely dangerous and its spore can be stored for a long period and which can be released on place of human gathering. These qualities make anthrax suitable for its use as a biological weapon. Armies of several countries have started its development as a biological weapon and the condition turned more dangerous when terrorists started using it. The accidental release of anthrax spores from a military research laboratory in the former Soviet union in 1979 caused at least 79 cases of respiratory infection and 68 deaths[http://news.bbc.co.uk/2/hi/health/1590859.stm]. The mortality rate for naturally occurring inhalational anthrax has been 75 percent, even with appropriate treatment. In the 2001 anthrax attacks, 11 people were infected with inhalational anthrax and 6 survived. [http://www3.niaid.nih.gov/topics/anthrax/overview.htm]. David L. Craft, a Doctoral student at the MIT Operations Research Center, and Edward H. Kaplan, Professor of management sciences at Yale School of Management analyzed a variety of possible

responses to a scenario in which two pounds of anthrax are dropped in a city of 11 million people and 1.5 million are infected. After an analyzing more than 30 years of data, authors suggested following as a possible scenario: Every person must take antibiotics to survive. However, 123,000 people in the city of 11 million would die by the time all of the drugs are distributed, within four days,[http://news.bio-medicine.org/biology-news-2/Anthrax-threat-needs-aggressive-government-action-plan--say-researchers-5322-2/].

1.1.1 PHYSIOLOGY AND MOLECULAR BIOLOGY OF ANTHRAX DISEASE

Anthrax spore of optimal size are required for being an inhalational type of anthrax. Small spore are exhaled and do not remain in lungs to cause disease. Large spores drop to the earth and do not remain in air because of big size and hence do not cause inhalation on the site of release. Victim of inhalational anthrax do not show very specific symptoms. These includes low grade fever, chills, profound fatigue, nonproductive cough and chest pain. These symptoms are very generic that is why early diagnosis of anthrax is a big challenge.

When a person inhales the spores of *B. anthracis* it goes into the lungs. In lungs spores are engulfed by alveolar macrophages and they are transported to lymph nodes of chest. But spores germinate and proliferate inside the macrophages. After that they come out by bursting the macrophage. How this bacteria encounters this first defense is not well known. There are two ways by which this *Bacillus anthracis* encounter, survive and kill the macrophages. The first is by Lethal Factor (LF) mediated killing. LF by its metalloprotease activity cleave the MAP kinase- kinases.Some of these MAP kinase- kinases also activate antiapoptotic MAPkinase. This leads to the suppression of activation of this antiapoptotic MAPkinase. The real targets of these MAPkinase have not been yet discovered. Researchers found that LF causes oxidative burst and killing of macrophages in a cascade manner. As LF prevent MAPK activity by cleaving MAPkinase kinases,this results in preventing the activation of ribosmal S6 kinase-2 (RSK) because its activation requires MAPK activity. RSK is responsible for phosphorylation of CCAAT/enhancer binding protein-β (C/EBPβ) on threonine 217. The expression of the dominant positive, phosphorylation mimic C/EBPβ-

E217 rescued macrophages from LT-induced apoptosis by blocking the activation of procaspase-8. It shows that C/EBPβ may be playing the critical role in anthrax pathogenesis, at least in macrophages[1].

Researchers are more focused toward anthrax toxin. but some studies have shown that ability of *B. anthracis* to survive inside the phagocyte also depends crucially on their oxidative stress defense system. It is known that Reactive oxygen species are responsible for innate immunity against many microorganisms. These ROS can damage DNA and protein by Fenton Reaction. Studies show that this ROS is tolerate by *Bacillus anthracis* due to presence of NO-synthase (bNOS)-derived NO in protecting germinating *B. anthracis* spores from macrophage oxidative attack[2].

Now from the lymph nodes bacteria spread in different parts of the body with blood and create Septicemic (bloodstream) anthrax. Septicemic anthrax refers to an overwhelming blood infection by anthrax. In this stage bacteria proliferate in blood and secrete anthrax toxin(TA) which consists of three proteins. These are lethal factor(LF), edema factor(EF) and protective antigen(PA). LF with PA called lethal toxin and EF with PA is called edema toxin. PA is common in both cases. PA required for entering the toxin in cytoplasm where toxin act. Anthrax toxin is exotoxin and both LF and EF are intracellular active enzyme. They show their toxic affects inside the cytoplasm. In the initial stages of the intoxication mechanism, full-length PA (PA83) binds opportunistically to one of two cellular receptors (capillary morphogenesis protein (CMG2) or tumor endothelial marker (TEM8)). Then after binding, PA is proteolytically cleaved by a surface protease, furin, to a shorter polypeptide (PA63) that spontaneously heptamerizes to form a so-called pre-pore. Heptamers in turn are able to bind LF and/or EF and to trigger endocytosis of the receptor-bound toxin. Low pH conditions (around pH 5.5) then induce a pre-pore to pore conformational switch that allows the enzymes to enter [3].

LF is a metalloprotease and show its enzymatic activity inside the cell in cytoplasm. In cytoplasm this enzyme specifically cleaves most isoforms of mitogen-activated protein kinase(MAPK)-kinases (MEKs) close to their N termini. LF consists of four domains. Domain iv contains the active site, which is present in bottom part of protein and it accepts the N terminal region of substrate MAP kinase-kinases. The enzyme is selective for peptides that contain the perticular consensus sequence or motif, that include the cleavage site. This

motif is found in the N-terminal sequence of nearly all isoforms of MAP kinase-kinases. MAP kinase and MAP kinase-kinases are involved in several important metabolic pathways some of these are very crucial for survival of cell. Studies have shown that LF can activate caspase-1 by a mechanism involving proteasome activity and potassium efflux[35] and LT-induced apoptosis by blocking the activation of procaspase 8 [1]. Cell death is the result of LF activity which start with cleavage in MAP kinase- kinases. And happened in cascade manner. The sequence motif of MAP kinase- kinases which is identified by LF is also recognised by MAP kinase. Mutation in this motif or loss of motif leads to failure of its recognition by MAP kinase and loss of its activity. LF is very well evolved to act on MAP kinase- kinases as its natural substrate [4].

Inside the cells, the adenylyl cyclase activity of EF is activated by CaM, which leads to the rise of intracellular cyclic (cAMP) to pathological levels (Shen et al,2002) [5]. High level of cAMP disturb the homeostasis of water in the cell leading to abnormalities in the intracellular and stimulationrc of the chloride channel. This causes the edema in mediastinum located between the lobes of the lungs. EF can impair host innate and adaptive immunity by altering the phagocytic activity of macrophages, cytokine production by monocytes and macrophages, and antigen presentation of T cells. Consequently, the disruption of the EF gene results in reduced survival and lethality of anthrax bacteria [5].

In finding the answer to question "why the inhalational infection of anthrax is highly fatal (more than 70%) even with antibiotics treatment and cutaneous form of infection generally remain localized and some time individual survive even without any treatment". Researchers point out that neutrophil are abundant in skin form of anthrax and not in lungs. Neutrophil is known for its antimicrobial action. These antimicrobial properties are oxygen dependent by reactive oxygen species (ROS) and oxygen independent by enzymes and antimicrobial peptides[6]. ROS system is not effective against the B.anthrcis but oxygen independent system kill the bacteria significantly. The antimicrobial peptide alpha-defensin is identified as the main component from neutrophil which kills the bacteria. Sudies have shown that neutrophil efficiently kill the bacteria[7]. Some researcher showed that the alpha-defensin can also inhibit the lethal factor and inhibit the fatal consequence of anthrax disease[8].

1.1.2 GENES FOR TOXIN AND CAPSULE PRODUCTION AND THEIR EXPRESSION

The genes responsible for toxin production and capsule synthesis in *Bacillus anthracis* are encoded by two different plasmid so they are crucial for pathogenic activity. Toxin encoded genes are present in p XO1 (185 kb) plasmid. These genes are cya, lef, and pag, encoding EF, LF, and PA, respectively and present in discontinuous fashion in 30-kb region[9]. The capsule genes (capB, capC, and capA) are present in pXO2 with a dep, gene. which is associated with depolymerization of the capsule these are all located contiguously and in the same direction of transcription[10].

In *in vitro* culture of *B. anthracis* the expression of its toxin and capsule genes depends on culture conditions and states(growth phase). Expression of toxin genes pagA,lef, and cya is highest in late log phase. In increased carbondioxide (more than normal) or in presence of bicarbonate in culture the expression of capsule and toxin genes increases[11]. It is also found that there is more expression of toxin genes and toxin production at 37°C compared to 28°C. One can say that this is the adaptation to sustain in warm blooded host like cattle and human. Production of toxin protein reported is highest during the transition into stationary phase [17-12].

1.2 CURRENT SCENARIO

1.2.1 CURRENT INHIBITORS AGAINST LETHAL FACTOR

Lethal factor toxin protein is directly related with the cell death. Defect in this protein or inactivation of the LF gene results in great loss of virulence of *Bacillus anthracis* strain(about 1000 fold)[23]. LF is main toxin component of anthrax toxin and is required in anthrax treatment in all stages of disease inhibition. The concentration of this toxin is inversely proportional to cell or organism survival in diseased condition. Importance of LF

toxin has been shown by several researchers. It is widely used as a target for inhibitors by several research group working on this area with traditional and computational approach in anthrax drug design. Martino Forino et al [24] by fragment based approach designed several compounds which can inhibit the LF and tested activity of these molecules by enzymatic assays and found that BI-MFM3 is the most potent of them. IC50 value of this compound is1.7μM[24].

Rekha G Panchal et al [25] did high throughput screening of 1990 compounds from NCI diversity set and they selected the compound which showed >70% inhibition of LF activity for HPLC based assay after taking other factor in account (excluding organometallic and charged molecule). They selected two compounds NSC 12155 and NSC 357756. Then according to 3D pharmacophore present in these compound they used 3D database mining approach to identify more LF inhibitors. They got six compound and they performed kinetics studies, docking and cytotoxic assays to study these molecules and thier interaction Three of these compounds had Ki values in the 0.5–5 M range and showed competitive inhibition [25].

The chemical screening to find LF inhibitor was done by Dal- Hee etal and they found that compound DS-998 is able to inhibit LF with IC50 ~2μM [26]. Isabella Dell Aica et al [27] have isolate two polyphenolic compound from tea leaves which inhibited the activity of LF with IC50 μM. These compounds are catechin-gallate (CG) and EGCG[27]. High throughput screening for LF was done by Sherida L. Johnson et al [28] and they identified six compounds which inhibit the activity of LF the IC50 value of these inhibitors ranging from 1.7 to 38.2 μM [28].

Sherida L. Johnsonhave et al [29] have done SAR and QSAR study on some inhibitors of LF. They have identified a set of compounds which inhibit the LF. They study the structure activity relationship and by X-ray structure of representative compound they established the possible alignment rule for superposition of different compound to perform CoMFA (by SYBYL7.0) by taking 17 compound as training set. They did their docking simulation by GOLD, They tested 10 compounds and got good predictivity [29].

Researchers found sulfonylamino]-N-hydroxy-2-(tetrahydro-2H-pyran-4-yl)acetamide, (hydroxamate) is a high affinity inhibitor of LF. Its structure is also solved. The complex LF and hydroxamate is present in protein data bank pdb-id is (1YQY)

deposited by Shoop, W.L. et al [36] and they also found out the IC50 value to be around 60nM[36].

1.2.2 CURRENT INHIBITOR AGAINST EDEMA FACTOR

Edema factor is a important part of anthrax toxin. EF plays crucial role in survival of anthrax spore inside macrophages and establishment of disease. An anthrax strain with defective EF show 100 fold decrease in lethality in mice[30].

It has been found that adefovir a drug which is used against chronic infection of hepatitis B can inhibit EF induced toxicity in cell. The active compound adefovir diphosphate can inhibit the adenylil cyclase activity of EF with Ki=27 nM. This compound shows better interaction with EF than its natural substrate[31].

Very recently researcher have designed some inhibitors for anthrax edema factor. First they did fragment based pharmacophore design then they performed UNITY search using these pharmacophore against NCI-2000 database. Compounds found as hit from this search were docked to EF by using FlexX and compounds with lowest scores were selected for analysis of their interaction with EF. Now initial pharmacophores(fragments) and interaction of selected compounds of NCI database were used to search ZINC database. They found about 10000 compounds from ZINC database. These 10000 compound were finally used for docking to EF by using AutoDock. and low energy compounds were selected. After taking other factors in account they selected 19 compound for enzymatic assay, and finally they showed that 3 of them have IC50 values in the range 1.7-9μM. [32].

2 MATERIAL AND METHODS

2.1 DOCKING

From last some years the databases of biological information are growing rapidly.

These database included the genome sequence, gene sequence, EST, protein sequence, ligand database, Macro molecule structure databases, databases of pathways etc. This time the growth of these databases are in exponential phase. These databases provide the different information. Structural database of biological molecules gives insights about which structural part of a molecule plays important role in their function and interaction. Different techniques of sequencing and structure solving are now well established and used in routine work. These structure solving technique like NMR and X-ray diffraction are responsible for production of structure database. On other hand computational power and efficiency of computer are also increasing as its requirement in different field of science and society. This increase of structural databases of biological molecules, their interactions and function along with computer advancement giving the boast to *in silico* drug designing studies.

Importance and reliability of protein ligand docking also increases with advancement and accuracy of docking software. Increase in accuracy of docking software increases with use of new and refined algorithm.

Docking is finding the correct orientation and conformation of small ligand molecule inside the binding pocket of protein molecule. This conformation and orientation is according to shape and electrostatic complementarity between ligand and protein which provides the maximum favorable interactions.

Docking can be divided mainly into two parts, first is search method. This is making of different conformation and orientation (poses) ligand in binding pocket and second is scoring that particular pose means how good this pose is.

Search method can be divide into three main categories these are Systematic method, Random method and Simulation method.

Table 1 : of different algorithm used in different search methods.

Search Method	Algorithm
Systematic method	Incremental construction, Conformational Search Database search
Random method	Monte Carlo, Genetic Algorithm, Tabu Search
Simulation method	Molecular Dynamics, Energy minimization

Scoring is also very important as search method in docking. Scoring evaluate the pose of ligand in the binding pocket. Without accurate scoring function it is hard to find out

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which pose of ligand is best. There are three type of scoring function which are using in docking. These are Force-field based, Empirical and knowledge based [21].

Our chosen software GLIDE uses Systematic and Simulation method for searching the poses and ligand flexibility. In systematic method it uses incremental construction for searching. Scoring function is used by GLIDE is empirical scoring function.

The other software we have used to dock the top molecules is GOLD which uses the Random method for search that is Genetic algorithm. In this way have used all three search algorithm in our docking simulation.

2.2 TARGET SELECTION

From above studies which is going on for Lethal factor and Edema factor we can infer that they are validated known targets for anthrax but there are several other protein of *B. anthracis* which can be targeted in order to cure the disease. But these different proteins have their limitation. In order to find out their importance and which are best to target we have also looked at other different target protein of anthrax. These proteins are

2.2.1 PA AS A TARGET

Protective antigen plays very important role in toxin action. Anthrax toxin shows their toxin action only inside the cell and PA plays main role in translocation of toxin inside the cell. Studies have shown that PA can be a target for anthrax drug. Polyvalent inhibitor against PA were designed to inhibit the interaction of LF and EF. Researchers first screened the phage of 12 amino acid peptide which can inhibit interaction of LF/EF with PA(PA63). To make it more active they attached multiple copies of the peptide on polyacrylamide backbone. For this they synthesized a derivative of polyacrylamide that had multiple covalently linked copies of the peptide. This polyvalent inhibitor shows high inhibition of radio labeled LF binding to PA63, IC50 20nM in terms of molar concentration of linked peptides. Test with animal also gives good result. This experiment clearly proved that inhibitor against PA which prevent the binding of LF/EF can be used as drug against anthrax

toxin. This approach is in experiment phase and lot of study is required [22]

2.2.2 NO-SYNTHASE OF BACTERIA AS TARGET

Macrophages uses reactive oxygen species to encounter the bacterial infection. When they engulf the bacteria they start generating large amount of ROS(Reactive oxygen species). ROS is known for their anti microbial activity. ROS damage protein and DNA by oxidation reaction fentons reaction [33]. Researcher describe that germinating *Bacillus anthracis* inside the macrophage encounter this ROS attack by its own oxidative stress defense system. This is very important part of anthrax pathogenicity. Nitric oxide(NO) produced by bacteria play main role in encountering the ROS. NO is formed by NO-synthase (bNOS) (see fig A). Researchers have shown that *Bacillus anthracis* strain deficient in NO-synthase lose their virulence in model organism. From it became clear that NO-synthase of *B. anthracis* plays key role in establishment of its infection and virulence. So bacterial NO- synthase can be taken as a drug target for anthrax and other infectious disease in which this microbial NO-synthase plays important role to take over ROS immune response [34].

2.2.3 DIHYDROFOLATE REDUCTASE ENZYME OF *Bacillus anthracis* AS TARGET

Dihyrofolate reductase enzyme of *B. anthracis* is important for its survival and pathogenesis of anthrax. DHFR is required for conversion of dihydrofolate to tetrahydrofaolate. DHFR is involved in de novo synthesis of TMP, which is used in synthesis of DNA and RNA. It is very important for survival of bacteria. Dihydrofolate reductase is a validated target for anthrax bacteria [37]. The known inhibitor of DHFR is MTX(methotrixate) which binds with DHFR quite well but it also interacts with human DHFR[38]. Inhibitors directed to Dihydrofolate reductase leads to the killing of bacteria but not does not inhibit bacterial toxin. In later stage of infection, death is due to bacterial

toxin. The Dihyrofolate reductase inhibitor may not be sufficient for treatment of anthrax in later stage as antibiotics treatment.

2.2.4 7,8-DIHYDROPTEROATE SYNTHASE

Dihydropteroate synthase is a known target for antibiotics. It is a key enzyme in folate pathway like DHFR. Dihydropteroate synthase is an enzyme that catalyzes the synthesis of 7,8-dihydropteraoate from p-aminobenzoic acid (pABA) and 6-hydroxystructuresare. 7,8-dihydropteraoate is important intermediate in tetrahydrofolate synthesis. Dihydropteroate synthase complex with ligand (MANIC)was solved by Brad C. Bennett et al in 2007 and is present in PDB (pdb id-1TX2) [42].

2.2.5 AsbF AS TARGET

Sidophore in bacteria play important role in iron uptake. Iron is essential for proper physiological function and survival of bacteria. Two sidephores are reported in *Bacillus anthracis*. Petrobactin is essential sidophore for virulence within host [39]. Jung Yeop Lee et al [40] have shown that asb operon is involved in biosynthesis of petrobactin [40]. In asbABCDEF gene cluster the asbF mutation fails to produce 3,4-DHBA,a key subunit of petrobactin. Studies also found that the asbF mutant strain are completely avirulent at 10 days compare to normal strain which shows 50% mortality in same period. They also shows that AsbF enzymatic activity is responsible for Conversion of 3-Dehydroshikimate (3-DHS) to 3,4-DHBA. On these function of AsbF. they proposed AsbF as a potential new target [41]

TABLE 2 : Different target with their inhibitors

TARGET	VALI DATI ON	FUNCTION	STRUCTU RE	INHIBITO RS	RESULT
LETHAL	VALI	kill the host cell b	PRESENT	915	Give good

FACTOR	DATED	y cleaving the MAP kinase-kinases	IN PDB (pdb id-1J7N)	SD2 NSC1 MFM3 GM6	result
EDEMA FACTOR	VALIDATED	Interfere with signalling pathway and water homeostasis by increasing c-AMP level in cell	Structure present in PDB(pdb id-1K93)	Adefovir	Alone not sufficient
PROTECTIVE ANTIGEN	VALIDATED	Trans locate the EF&EF inside the cell	Structure present in PDB(pdb id-1ACC)	Peptides monoclonal antibodies	Can give good result but hard to target
b NO SYNTHASE	PROPOSED	Encounter the host ROS defense			Alone may not be sufficient
DIHYDROFOLATE REDUCTASE	VALIDATED	Play important role several pathways including nucleic acid synthesis	Structure present in PDB(pdb id-2QK8).	MTX	Alone may not be sufficient
AsbF	PROPOSED	Conversion of 3-Dehydroshikimate (3-DHS) to 3,4-DHBA			Alone may not be sufficient
DIHYDROPTEROATE SYNTHASE	VALIDATED	Play important role in folate pathway	Structure present in PDB(pdb id-1TX2)	MANIC	Alone may not be sufficient

Bacterial NO-synthase is one new proposed target for anthrax. Inhibitor against it can inhibit the bacterial survival in macrophages. But affect of it on circulating bacterial population is not properly known. The condition in later stages of infection is not likely to be controlled by NO-sythase inhibitor because it can not inhibit the bacterial toxin. Further research is required on this NO-sythase inhibitors for using it for anthrax treatment.

Dihyrofolate reductase enzyme of Bacillus anthracis is a new validated target for anthrax. Inhibitor against bacterial DHFR can kill the bacteria very well. But in later stage of infection death is due to circulating anthrax toxin. Like antibiotics and inhibitors against

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NO-synthase the inhibitor may not inhibit the anthrax toxin. Reliability of drug based on DHFR inhibitors in later stage of infection is very low. Further study is required in this issue.

Inhibitors against Edema factor can be a good anthrax drug but alone it can not give good results. It can be use as a combination with Lethal factor inhibitors and antibiotics because lethality of anthrax is mainly due to LF activity. Inhibitor against EF can reduce survival of anthrax spore inside the macrophage and disease development. In model organism only LF with PA can cause death. So EF inhibitor may be a good supporting drug.

Protective antigen is the very important component of anthrax toxin and can be a good target for anthrax drug. Because without PA activity LF and EF both stay out side from the cell and do not show their toxic effect. But all activities of PA is associated with its interaction with another proteins and designing the inhibitor of protein-protein interaction is not easy. In fact inhibition of protein-protein interaction by small molecule is one of the challenges of biology. The polyvalent inhibitor for inhibition of PA has its some practical limitations and it is now in initial stage. Lot of research is required in this section.

At any stage of disease anthrax lethal factor is very important for pathogenic effect, establishment of disease, survival of bacteria and death of host organism. All other target are either not easy to target (eg PA) or their study is in very initial stage (eg bnos,) or they are infective in later stage (antibiotic which kill bacteria only) and they fail to neutralized anthrax toxin. It seems in that LF inhibitor along with antibiotics and EF inhibitor can give the better result. So we have taken Lethal factor and Edema factor as target against anthrax.

2.3 TARGET PROTEINS AND BINDING SITES
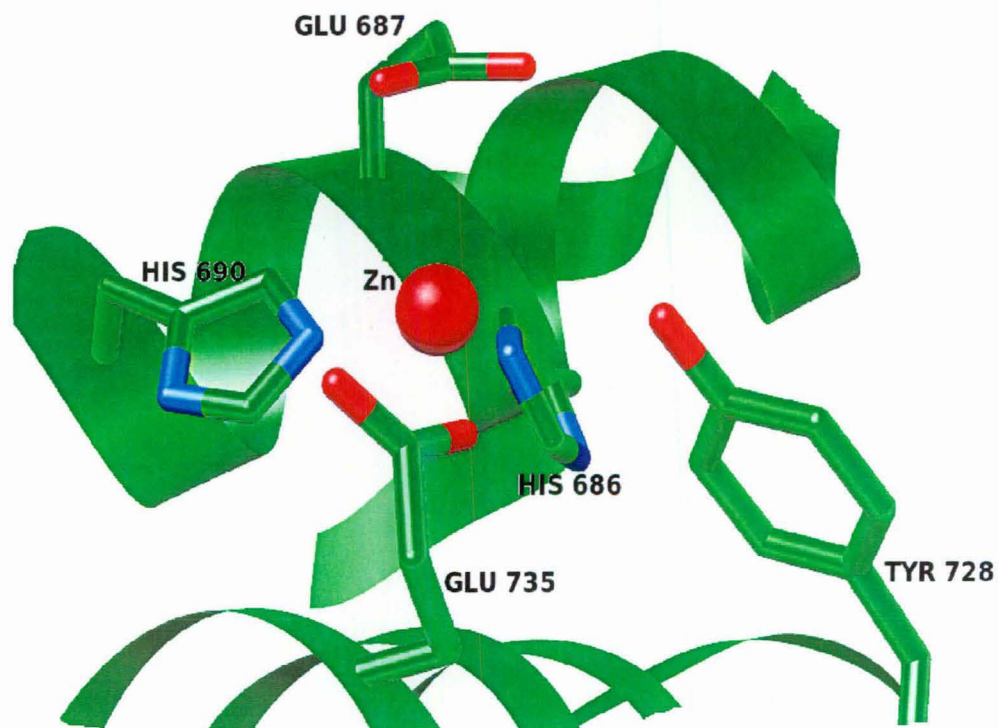
2.3.1 LF LETHAL FACTOR

Structure LF enzyme was solved by Andrew D. Pannifer et al and is present in PDB (pdb id-1J7N)[]. The protein lethal factor is 90KD in weight, contain four domains. Domain I is top part of the protein which attached with domain II. Domain I is only in contact with

domain II and remain faraway from other domains. This domain is responsible for binding with PA and trans location of protein to inside the cell. Domain I also shows 35% sequence similarity with PA binding domain of EF. Domain II, III and IV are together forms a deep grove. The substrate peptide inserts itself in this grove. Domain IV contains the catalytic center. Catalytic HEXXH motif is part of an alpha helix which is conserved in some metalloproteases. In the catalytic site histidine 686 and 690 along with glutanimate 687 and 735 residues holds the Zn.

2.3.1.1 LF SPECIFICITY AND MECHANISM OF ACTION

MAPK-kinase plays major role in signaling pathways because they activate the MAPK which has key importance in many of signaling pathways. LF cleaves specifically the N terminal sequence containing continuous three positive residue followed by some hydrophobic amino acid residues(+++XQXQ). This type of sequence is very well conserved in MAPkinase-kinases. This sequence is near to N terminal region and is found hanging. It has the specificity for MAP kinases. MAP kinases recognize this sequence. Functionally this hanging sequence of MAP kinase kinases is very important it responsible for holding to MAP kinase and after this MAP kinase kinases transfer the phosphate into MAP kinase. Loss of this hanging sequence or mutation in this leads to loss in its enzymatic action. So its seems that LF is very well evolved according to its natural substrate MAP kinase.

The catalytic center which lies near the bottom of the protein in domain IV has trademark HEXXH sequence. This sequence is a part of alpha helix and holds Zn metal ion. The metal ion Zn excites the water molecule which then attacks the amide bond. According to the proposed mechanism the GLU 687 of HEXXH sequence hold the proton of water molecule and transfers it to leaving amino group of protein. It is thought that TYR 728 also plays important role in enzymatic reaction and stabilizes the negative charge of bond carbonyl oxygen in the transition state by its hydroxyl group. Any inhibitor molecule which interacts with these residues shows low IC50 value.

Figure 1. Binding pocket of lethal factor showing catalytic residue and metal ion Zn

2.3.1.2 SELECTION OF LETHAL FACTOR CRYSTAL STRUCTURE

For anthrax lethal factor nine structure are present in PROTEIN DATA BANK and these are following..fin

- LF alone(1J7N) one structure.
- LF with MAPK k2(1JKY) one structure.
- LF complex with optimised peptide substrates(1PWV,1PWW) two structures.
- LF complex with ligands(1YQY,1PWU,1PWP,1PWQ,1ZXV) five structures, ligand names (MFM,SD2,915,NSC1,GM6).

Out of these nine crystal structures five are ligand LF complexes. After analyzing the five LF-ligand complex structures, one crystal structure was selected for docking studies. In which LF is bound with a hydroxamate inhibitor (PDB- 1YQY). The selection is based on the fine resolution of solved crystal structure(2.30 ANGSTROMS) and good enzymatic inhibition (IC50) for the known ligand in the complex.

2.3.2.1 EF EDEMA FACTOR AND ITS MECHANISM OF ACTION

Edema factor does not have significant structure similarity with mammalian adenylase cyclase enzyme and can be used as an example of convergent evolution. It is CaM (calmodulin) depended adenylate cyclase and catalytic rate is 1,000-fold higher than that of mammalian CaM-activated adenylyl cyclase. The catalytic part of EF is formed by three hydrophobic domains. There is considerable difference between structure of EF alone and EF-CaM complex. The carboxy terminal part of EF around 58kD is responsible for its CaM depended adenylate cyclase activity [16].

EF requires calmodulin protein for its enzymatic action. EF has two functional domains PA binding domain (PABD) and aenylate cyclase domain (ACD). Calmodulin is a protein which plays important role in activation of several protein for diverse biological functions[18]. The structure of ACD domain with CaM and alone was solved by researchers and proposed binding and activation of catalytic domain. [18]. first EF binds to closed conformation of N-CaM domain [19] this facilitate the binding of EF to open conformation of C-CaM domain with high affinity.[20]. It is proposed that EF uses one metal ion and histidine in its catalytic mechanism. One metal ion helps to stabilize the transition state and facilitate the departure of phosphate group. Histidine deprotonates the 3'OH group of ATP[18]. This mechanism differs from mammalian adenylate cyclase action mechanism.
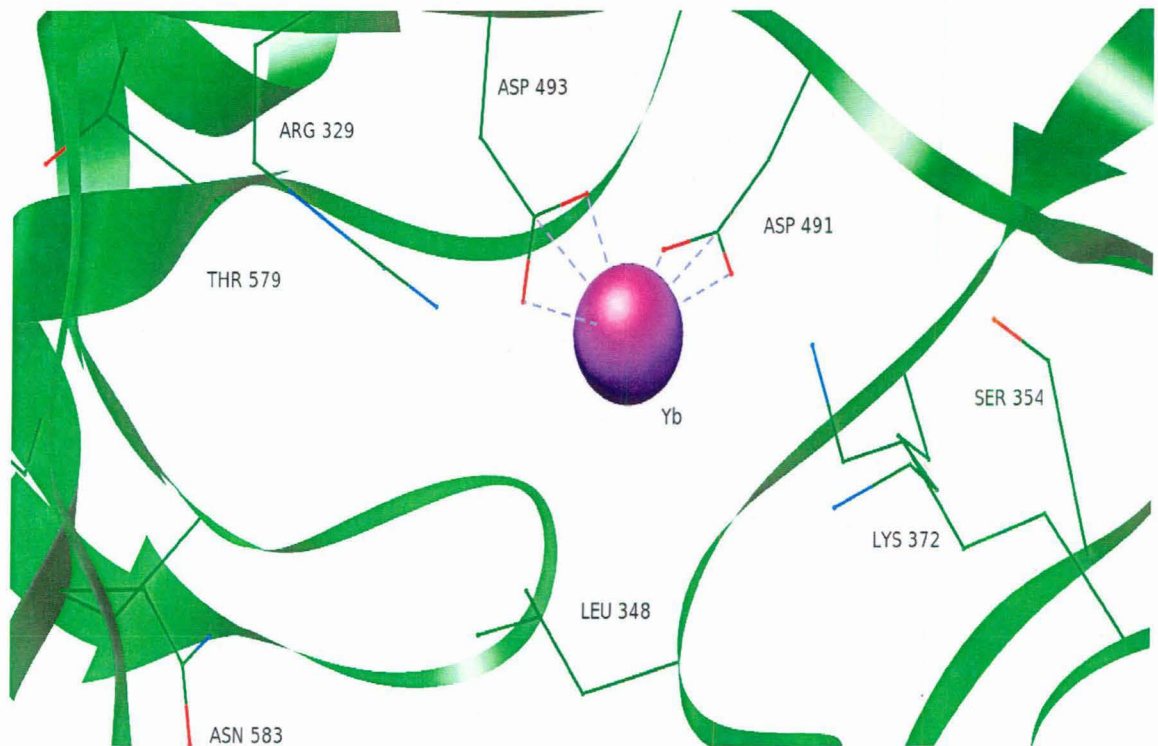
Figure 2. Binding pocket of edema factor showing catalytic residue and metal ion Yb

2.3.2.2 SELECTION OF EDEMA FACTOR STRUCTURE FOR DOCKING

There are fourteen structures for edema factor present in protein data bank. These are the following:-

- two edema factor complex with calmodulin (PDB id- 1K93, 1XFY)
- two edema factor complex with calmodulin in presence of different concentration of calciumchloride (PDB id- 1XFZ, 1XFX)
- two edema factor complex with calmodulin and 3' deoxy-ATP (PDB id- 1K90, 1XFV)
- two edema factor complex with calmodulin and 3' 5' cyclic AMP(PDB id- 1XFW, 1SK6)

one edema factor truncation mutant (EF-delta64) complex with calmodulin(PDB id- 1XFU)

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- one edema factor complex with calmodulin and pyrophosphate (PDB id- 1YOV)

- one edema factor complex with calmodulin and PMEApp (PDB id- 1PK0)

- one edema factor complex with calmodulin and 2' deoxy,3' anthraniloyl ATP (PDB id- 1LVC)

- one crystal structure of adenylate clase domain of anthrax edema factor. (PDB id- 1K8T)

- one edema factor complex with calmodulin-alpha beta methylene adenosine 5' triphosphate. (PDB id- 1S26)

There is no structure which contains ligand (inhibitor) in catalytic site in PDB. After studying all fourteen structures present in PDB one structure is selected for docking studies. The selection is based on resolution of structure. PDB id- 1K90 is selected in this the natural substrate ATP is bounded in catalytic site in protein.

The selected PDB(1K90) structure of edema factor contains three identical chain (these are chain A, B, and C) each containing the catalytic site (binding pocket) in which ATP substrate is bounded. For selection of chain we studied the bounding of ATP and chain A was selected for docking studies. The selection is based on most closely bounded ATP to catalytic site. For this the list of of nearby atoms within 3 Angstrom form bounded ATP is prepared for all three different chains. The chain A ATP is interacting with more atoms of catalytic residues.

Table 3. Interacting residues with ATP in different chains of edema factor.

CHAIN	DISTANCE WITHIN	NUMBER PROTEIN ATOMS
A	3Å	9
B	3Å	6
C	3Å	7

From the interaction list it is clear that in chain A, substrate ATP is well docked were compared to chain B and C, so chain A of (PDB id 1K90) is selected for our docking studies.

2.4 LIGAND LIBRARY

We have taken Zinc database for ligand library. Zinc database is one of the largest database which contains drug like as well as diverse set of molecule. Most of the molecules of Zinc database are commercially available. From Zinc database the database of drug like molecules and natural molecules were downloaded the kinase inhibitor ligand library[http://www.lifechemicals.com/] was also taken out for our ligand library.

In drug like molecule there were around 2.3 million(23,67,377) molecules. These molecules were filtered according to Lipinsky rule of five with Filter V 2.0.1 from Open Eye Software[4600000]. After this filter dataset of drug like molecules reduced to 4,59,403 molecules, in natural molecules library of Zinc database there was around 89 thousand molecules and the kinase inhibitor library about 38 thousand molecules.

2.5 PRE DOCKING PREPARATION

2.5.1 PREPARATION OF LIGAND LIBRARY FOR VIRTUAL SCREENING

We subdivided our ligand library into twelve different sets in which each set contains around 50 thousand molecules. Set number 1 to 9 cover all drug like molecules of Zinc database which we have already filtered by Lipinsky rule of five. The set number 1 to 8 contains 50 thousand molecules each and set no.9 contains 59,403 molecules. Natural molecules(89399) of Zinc database were separated into two sets number 10 and 11. Set ten contains 44399 molecules and set no. 11 contain 45 thousand molecules. Kinase inhibitor dataset was kept in single set of around 38 thousand molecules set no 12.

All molecules of dataset number 1 to 11 of ligand library were converted to maestro format (this is the exclusive format for GLIDE software). Subset number 12 was converted to SDF format. After this the subset was concatenated into single file. Now all different twelve sets are present in twelve different files. These twelve sets were used in our docking study.

Our ligand library contains 12 subsets. Basically these are of three types- drug like

molecules, natural molecules and kinase inhibitor molecules. ZINC drug like molecules and ZINC natural molecules were used for docking in both LF and EF and KINASE inhibitor molecules were used only for EF. Natural substrate of EF is ATP and catalytic site in EF binds and act on ATP, that why the Kinase inhibitor molecules were also chosen for EF.

2.5.2 PROTEIN PREPARATION

Preparation of protein is first and important step in docking study. Protein structures present in PDB do not contain all structural information to perform proper docking studies (For eg. in pdb Hydrogen atoms are not present files,prtonation state and tautomers of Histidine are not accurately present). These information are required for accurate docking. We have done protein preparation by Schrödinger protein preparation wizard

These include the following jobs

● 1 Assigning bond order and adding the hydrogen atoms.

● 2 Identifing heteroatom groups and deleting water molecule.

● 3 Running Protein Assignment. This selects the most likely position of hydroxyl and thiol hydrogens; protonation states and tautomers of His residues; and Chi "flip" assignments for Asn, Gln and His residues.

● 4 Running Impref Minimization. The Protein Preparation Wizard can adjust atom coordinates to optimize the structure. The structure minimization in Schrödinger protein preparation wizard uses OPLS force field.

2.5.3 LIGAND PREPARATION

Before docking, the ligands from our ligand library were prepared by Schrödinger ligprep. In this step, all ligands were prepared. In docking studies ligand should be in 3D format. The following processes were carried out in a step wise manner

● addition of hydrogen was done in very first step because it should be done before the minimization of structure.

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- neutralizing charge group.

- after neutralizing the charge group ionization state was generated.

- generation of tautomers generates different tautomeric state for a structure

- generating alternate chiral structures. This step identifies additional chiral atoms in the structures and generates additional structures with the same molecular formula but different chiral properties.

- generating low energy ring conformation.

- Optimizing the geometries. The structure minimization in Schrödinger ligprep uses OPLS2005 force field.

2.5.4 GRID GENERATION

Final step in protein preparation before docking is grid generation. We have done this with GLIDE by Receptor grid generation panel. Grid is the set up of three dimension space where the different poses of ligand are generated. The shape, structure and charge complementarity of binding pocket are represented on a grid within different grid point and calculated by different sets of fields. This approach gives better and fast scoring of the ligand poses.

In lethal factor, after protein preparation the grid was generated. The grid generation is very important step in docking. For this all five known ligands in the complexes were studied and list of common interacting residues was identified. These common residues and other surrounding residues were used to define the binding site. Metal ion (Zn) was retained in the grid because in all different complexes it shows the interaction with inhibitor. Metal ion Zn is also very important for the catalytic activity of this metallo protease.

In edema factor the chain A is selected for docking studies and grid is formed around catalytic site. Grid is generated by covering all residues which interact with ATP. The metal ion Yb is retained in catalytic site inside the grid because metal ion is very crucial in catalytic mechanism of edema factor and interact with substrate ATP.

2.6 DOCKING USING GLIDE

After protein and ligand preparation and defining the grid in protein binding pocket the docking was carried out with Lethal factor and Edema factor.

For lethal factor ligand of Set no1 to 11 from ligand library were docked by high throughput screening. From here top 10 percent ligand poses from set no. 1 to 9 and top 20 percent ligand poses from set no. 10 and 11 were taken for further docking studies. Top ligand poses from high throughput screening were subjected to standard precision docking in GLIDE. From here top 1000 ligand poses form all data set were subjected to final extra precision docking in GLIDE.

For Edema factor ligand of Set no1 to 12 from ligand library were docked by high throughput screening. From here top 10 percent from set no. 1 to 9 and top 20 percent ligand poses from set no. 10, 11 and 12 were taken for further docking studies. These top ligand poses from high throughput screening were then subjected to standard precision docking. From here top 1000 ligand poses form all data set were subjected to final extra precision docking in GLIDE as in the case of lethal factor.

After GLIDE standard precision docking we got ranking of ligand according to GLIDE score.

GLIDE score is the modified chem score. The chem score was given by Eldridge et al in 1997 [14]. GLIDE score is given as:

$$\Delta G_{bind} = C_{lipo\text{-}lipo}\ \Sigma f(r_{lr}) + C_{hbond\text{-}neut\text{-}nuet}\ \Sigma g(\Delta r)\ h(\Delta \alpha)$$

$$+ C_{hbond\text{-}neut\text{-}charged}\ \Sigma g(\Delta r)\ h(\Delta \alpha)$$

$$+ C_{hbond\text{-}charged\text{-}charged}\ \Sigma g(\Delta r)\ h(\Delta \alpha)$$

$$+ C_{max\text{-}metal\text{-}ion}\ \Sigma f(r_{lm}) + C_{rotb}H_{rotb}$$

$$+ C_{polar\text{-}phob}V_{polar\text{-}phob} + C_{coul}E_{coul}$$

$$+ C_{vdW} + \textbf{solvation terms}$$

Figure 3. GLIDE scoring function.

In figure 3 of GLIDE scoring function, the first term is to score lipophilic interaction,

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2nd, 3rd and 4th term is for scoring hydrogen bond, 5th term is for metal interaction, 7th term to score polar -hydrophilic interaction, next two terms for electrostatic and van der waals, and in last is the solvation term [44-45].

2.7 VALIDATION OF DOCKING RESULTS

2.7.1 CALCULATION OF PROTEIN LIGAND INTERACTIONS USING PERL PROGRAM

Interaction between protein and ligand like hydrogen bond and hydrophobic interaction are the key for inhibition of protein enzymatic action. So we were interested in finding these interaction precisely. We have developed our own code for finding hydrogen bond and hydrophobic interaction between ligand and protein. For finding the interaction in protein ligand complex which comes as the GLIDE result, we have used this own code. Our code finds interactions in following steps.

1) Scanning of ligand for forty functional groups.

2) Assigning the hydrogen bond and hydrophobic properties to functional group atoms.

3) Assigning the hydrogen bond and hydrophobic properties to amino acid atoms.

4) Calculation the distance between the ligand and protein atoms within certain cutoff(we have taken 3.6Å).

5) Checking the hydrogen bond and hydrophobic properties of ligand protein atom pairs whose distance is smaller than cutoff distance.

6) If both ligand and protein atoms are hydrophobic then there is hydrophobic interaction.

7) If there is hydrogen bond complementarity between protein and ligand atom then the angle between these atom with hydrogen is calculated.

8) If this angle is more than cutoff angle(we have taken 120) then there is hydrogen bond between protein and ligand atom.
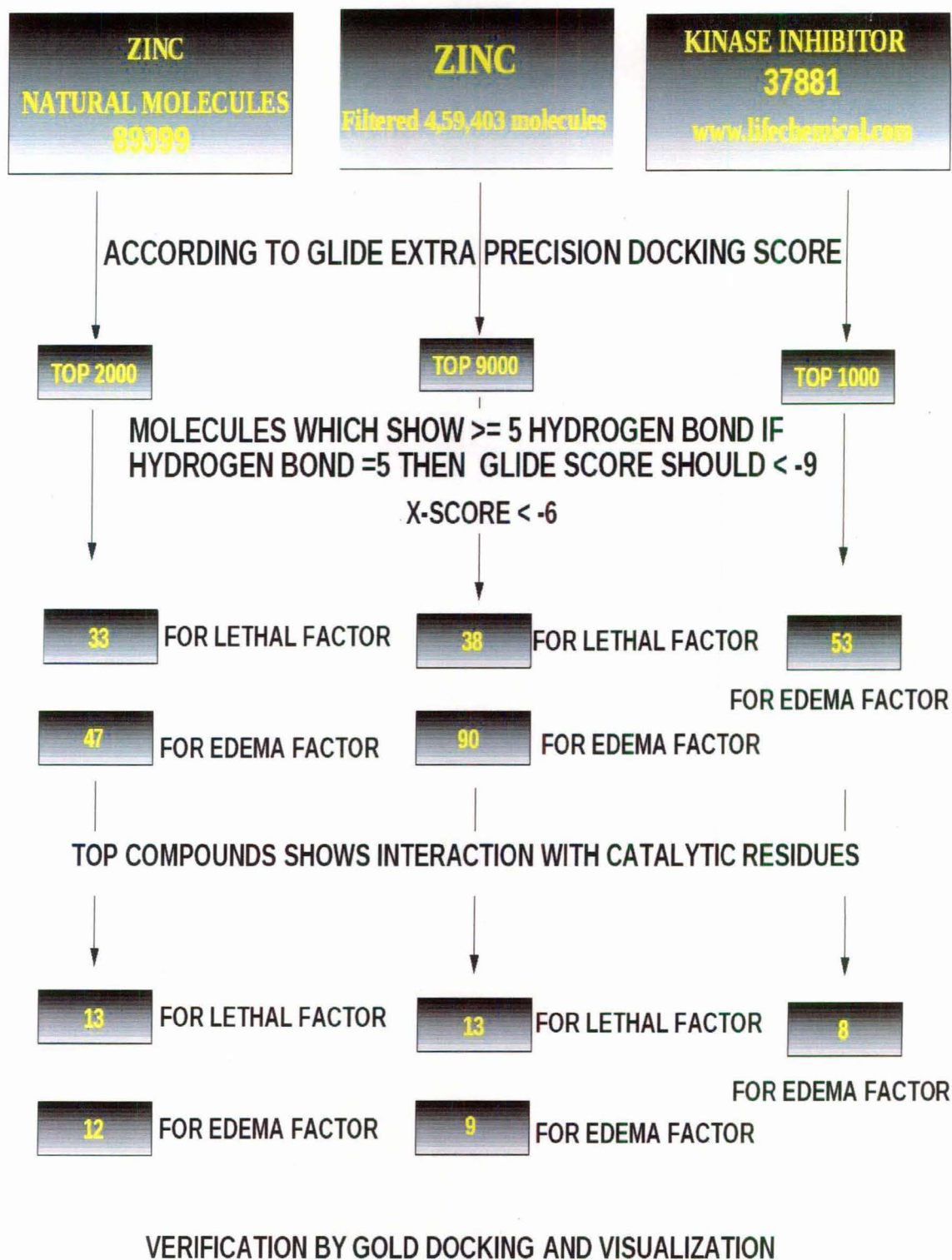
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2.7.2 X-score v2.1

For comparison and cross checking of GLIDE docking result we have used x-score program. The GLIDE output, docked complex structures were used for calculation of X-score(predict binding energy).

It is a consensus scoring function derived from three empirical scoring functions. These scoring functions include terms accounting for van der Waals interaction, hydrogen bonding, deformation penalty, and hydrophobic effect. X-score is the arithmetic mean of these functions. A special feature of these scoring function is that each scoring function calculate hydrophobic interaction with different algorithms. These empirical scoring function were calibrated on two hundred known protein ligand complex structures which are present in PDB. The accuracy of predicted binding energy by X-score is Renxiao et al[43].

2.7.3 DOCKING USING GOLD

GOLD has been used for docking the top ranking molecules for comparison with GLIDE and X-score values. We have used GOLD version 4 in our docking studies. As we mentioned in methodologies that GOLD uses genetic algorithm for searching the poses of molecules in side the binding pocket. There is option in GOLD to chose scoring function. We have chosen GOLD fitness function as scoring function. It is composed of van der waal energy, ligand torsion strain energy, hydrogen bond energy, ligand internal van der waals energy [15].

FLOW CHART OF STEPS IN PROCEDURE

| ZINC NATURAL MOLECULES 89399 | ZINC Filtered 4,59,403 molecules | KINASE INHIBITOR 37881 www.lifechemical.com |

ACCORDING TO GLIDE EXTRA PRECISION DOCKING SCORE

| TOP 2000 | TOP 9000 | TOP 1000 |

MOLECULES WHICH SHOW >= 5 HYDROGEN BOND IF
HYDROGEN BOND =5 THEN GLIDE SCORE SHOULD < -9

X-SCORE < -6

33 FOR LETHAL FACTOR	38 FOR LETHAL FACTOR	53
		FOR EDEMA FACTOR
47 FOR EDEMA FACTOR	90 FOR EDEMA FACTOR	

TOP COMPOUNDS SHOWS INTERACTION WITH CATALYTIC RESIDUES

13 FOR LETHAL FACTOR	13 FOR LETHAL FACTOR	8
		FOR EDEMA FACTOR
12 FOR EDEMA FACTOR	9 FOR EDEMA FACTOR	

VERIFICATION BY GOLD DOCKING AND VISUALIZATION

figure 4. flow chart of procedure.

3 RESULT AND DISCUSSION

In this section we presents the results docking scoring and interactions of LF and EF separately.

3.1 *IN SILICO* SCREENING RESULTS FROM DATABASE MOLECULES FOR LF

We have docked all Drug like molecule(filtered) and natural molecules from ZINC database to lethal factor protein with High throughput virtual screening wizard in GLIDE. From HTVS results we have taken top ten percent ligand poses for GLIDE standard precision docking. From standard precision results, top 9000 from drug like molecules and top 2000 from natural molecules were subjected to detailed docking from GLIDE extra precision wizard as explained in the methodology. After GLIDE docking binding energies were also caculated for all top ligand poses (9000 +2000) using X-score. Graphical representation of GLIDE scores of top ranking 1000 molecules form druglike like molecules.

GLIDE RESULTS FOR ONE THOUSAND LIGAND POSES
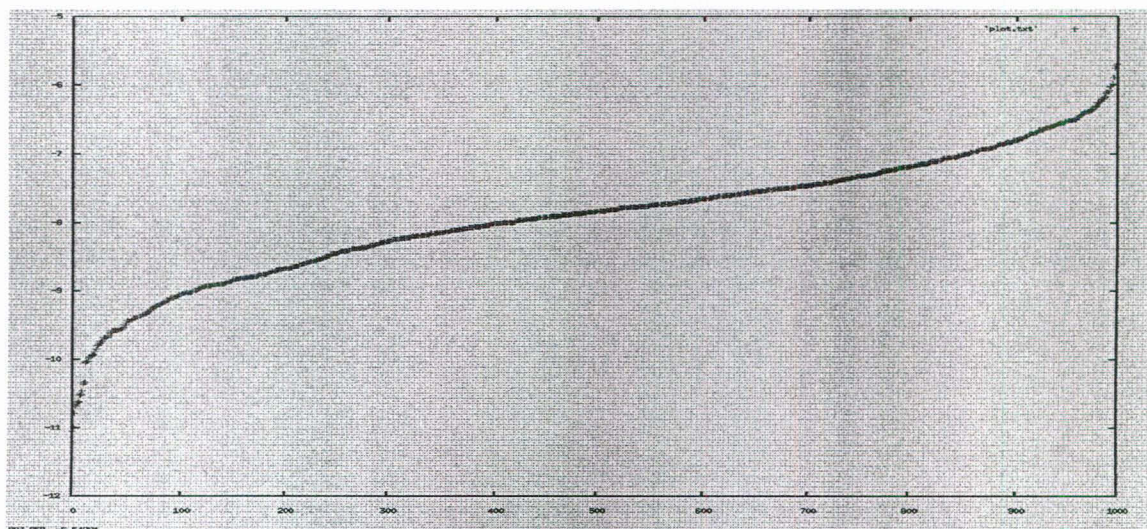
Figure 5. Graphical representation of GLIDE score for top one thousand poses of ligand from ligand library sub set one with lethal factor

3.1.1 PROGRAM FOR CALCULATING PROTEIN LIGAND INTERACTION USING PERL

We have developed a program to calculate protein ligand interaction using perl. The number of hydrogen bonds and other interactions listed in the tables were calculated by our program. Details of the program is given in the methodology.

3.1.2 RESULTS OF TOP RANKING LIGANDS FOR LETHAL FACTOR

After post docking analysis of eleven thousand ligands we have selected those ligands which have at least five hydrogen bond and minimum GLIDE score of -9. We also have selected those ligand which shows more than five hydrogen bonds irrespective of the GLIDE score. Thus we obtained 38 ligand poses in case of drug like molecules and 33 from natural molecules. We have shown the top 30 molecules from each set.

Table 4. Top ranking molecules form drug like molecules against lethal factor

ZINC-id	GLIDE-SCORE	X-SCORE	H – BOND
ZINC00129143	-11.31	-7.72	5
ZINC00522450	-11.15	-8.67	5
ZINC4317277	-10.81	-8.67	6
ZINC05154623	-10.57	-8.27	6
ZINC00281476	-10.46	-7.55	6
ZINC05154644	-10.12	-8.29	7
ZINC05154424	-9.75	-9.23	5
ZINC00984902	-9.72	-8.11	6
ZINC00150649	-9.71	-7.85	6
ZINC02718714	-9.68	-8.87	6
ZINC05154628	-9.67	-8.18	6
ZINC04225064	-9.65	-8.23	5
ZINC05154431	-9.65	-8.92	5
ZINC01830227	-9.6	-8.47	5
ZINC04610792	-9.59	-9.2	5
ZINC00543655	-9.58	-8.91	5
ZINC03408011	-9.53	-7.9	5
ZINC04029809	-9.43	-8.94	5
ZINC04905100	-9.4	-8.04	5
ZINC00270525	-9.37	-7.98	6
ZINC04771922	-9.36	-6.9	5
ZINC02169924	-9.35	-7.75	6
ZINC04225076	-9.24	-8.39	5
ZINC02422699	-9.2	-7.89	5
ZINC05154622	-9.11	-8.02	5
ZINC04992575	-9.07	-7.56	6
ZINC00143077	-9.04	-8.26	5
ZINC04606934	-8.92	-8.32	6
ZINC03870194	-8.85	-7.09	6
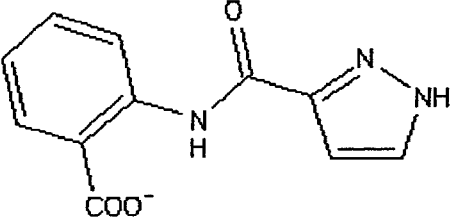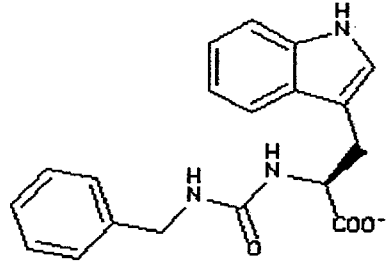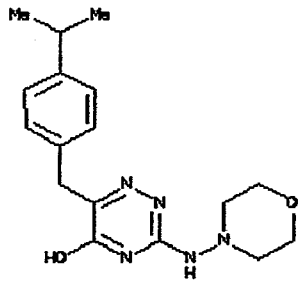
Table 5. Top ranking molecules form natural molecules against lethal factor

ZINC-id	GLIDE SCORE	X-SCORE	H BOND
ZINC02087229	-11.65	-8.03	5
ZINC04995790	-11.41	-8.98	5
ZINC12882480	-11.29	-9.64	5
ZINC00281475	-11.14	-7.62	5
ZINC08856538	-10.89	-7.96	5
ZINC04026906	-10.84	-9.17	6
ZINC00281476	-10.46	-7.55	6
ZINC06624611	-10.34	-6.55	5
ZINC12661647	-10.26	-9	5
ZINC04027430	-10.16	-8.93	5
ZINC04701676	-10.01	-7.5	5
ZINC04026907	-9.87	-9.37	5
ZINC02087228	-9.77	-7.34	6
ZINC02099979	-9.73	-7.88	5
ZINC00517217	-9.48	-7.37	5
ZINC04029809	-9.43	-8.94	5
ZINC00034161	-9.42	-7.07	5
ZINC04060815	-9.36	-7.47	5
ZINC04089172	-9.35	-6.65	6
ZINC03843466	-9.35	-7.12	5
ZINC00119344	-9.33	-7.4	5
ZINC02139623	-9.33	-8.8	5
ZINC02100717	-9.24	-8.56	5
ZINC01569529	-9.19	-7.47	5
ZINC01280452	-9.18	-6.83	5
ZINC04060819	-9.11	-7.45	5
ZINC05224470	-9.06	-8.65	6
ZINC08583964	-9.05	-6.77	5
ZINC09033911	-9.04	-9.09	5
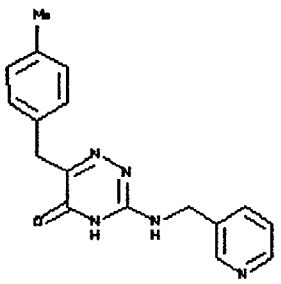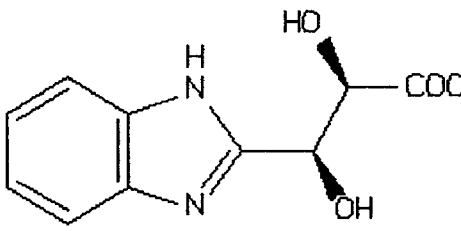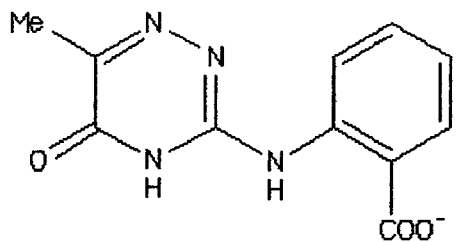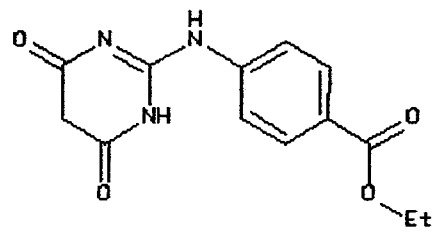
In table 4 the molecular docking results for drug like molecules against lethal factor are displayed. The GLIDE scores for molecules range from -8.85 to -11.31, X-score values (predicted binding energies) ranges from -6.9 to -9.23, and there are 5 to 7

hydrogen bonds between protein and ligands. In table 5 the result of natural molecules against edema factor is displayed. The GLIDE scores for molecules range from -9.04 to -11.65, X-score values ranges from -6.55 to -9.64, and there are 5 to 7 hydrogen bonds between protein and ligands. Molecules which are shown in the tables (4-5) were then manually visualized and examined by us and from there we have taken top molecules for lethal factor. These molecules are showing good interaction visually. These molecules were then subjected to GOLD docking. For Lethal factor we are showing the 13 top compounds from drug like molecule and 13 molecules from natural molecules.
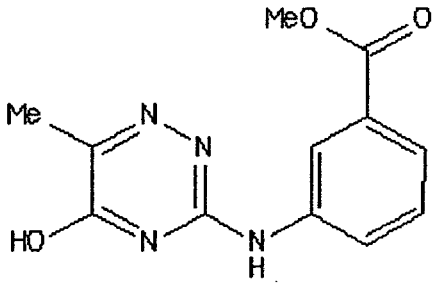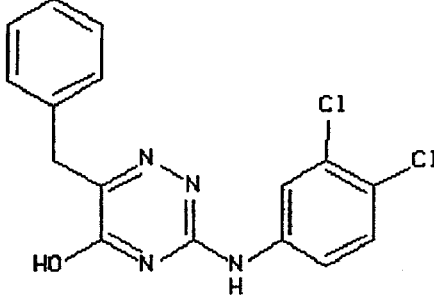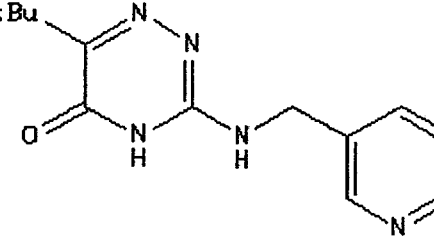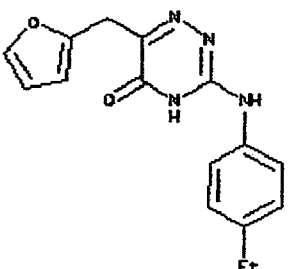
Table 6. Top ranking molecules from drug like database as potential inhibitors for LF

CHEMICAL STRUCTURE   ID   SCORE   PROPERTIES

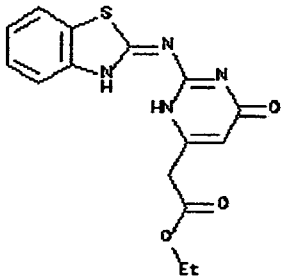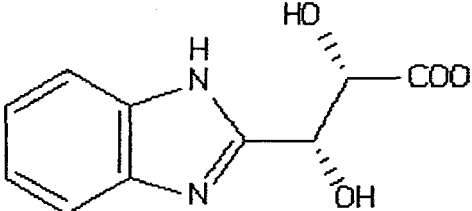| CHEMICAL STRUCTURE	ID	SCORE	PROPERTIES
	ZINC 00129143 2-(1H-pyrazole-3-carbonylamino)benzoic acid	Glide score -11.31 Gold fitness 45.67 X-score -7.72	Molecular weight 230.203 hbond donor 2 hbond accepter 6 log p 1.49
	ZINC 00522450 2-(benzylcarbamoyl amino)-3-(1H-indol-3-yl)propanoic acid	Glide score -11.15 Gold fitness 71.93 X-score -8.67	Molecular weight 336.371 hbond donor 3 hbond accepter 6 log p 0.88
	ZINC 4317277 6-[(4-isopropylphenyl)m ethyl]-3-(morpholinoamino)-1,2,4-triazin-5-ol	Glide score -10.81 Gold fitness 68.79 X-score -8.67	Molecular weight 329.404 hbond donor 2 hbond accepter 7 log p 3.34

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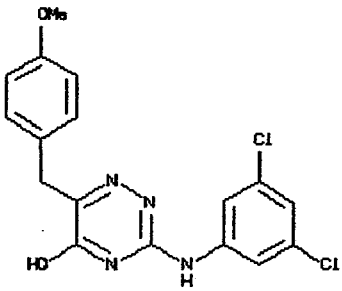
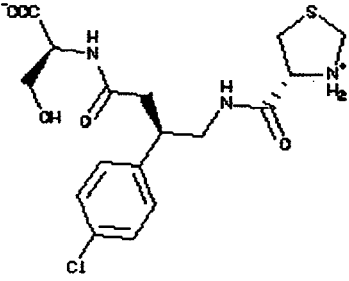| CHEMICAL STRUCTURE	ID	SCORE	PROPERTIES
	ZINC 05154623 6-(p-tolylmethyl) -3-(3-pyridylmeth ylamino)-4H -1,2,4-triazin-5-one	Glide score -10.57 Gold fitness 67.28 X-score -8.27	Molecular weight 307.357 hbond donor 2 hbond accepter 6 log p 2.13
	ZINC 00281476 (2R,3S)-3-(1H-benzimidazol -2-yl)-2,3-dihydroxy-propanoic acid	Glide score -10.46 Gold fitness 57.53 X-score -7.55	Molecular weight 221.192 hbond donor 3 hbond accepter 6 log p -0.68
	ZINC 05154644 2-[(6-methyl-5-oxo-4H-1,2, 4-triazin-3-yl)amino]be nzoic acid	Glide score -10.12 Gold fitness 22.25 X-score -8.29	Molecular weight 245.218 hbond donor 2 hbond accepter 7 log p 1.72
	ZINC 00984902 ethyl 4-[(4,6-dioxo-1H-pyrimidin-2-yl)amino]ben zoate	Glide score -9.72 Gold fitness 54.88 X-score -8.11	Molecular weight 275.264 hbond donor 2 hbond accepter 7 log p 0.81
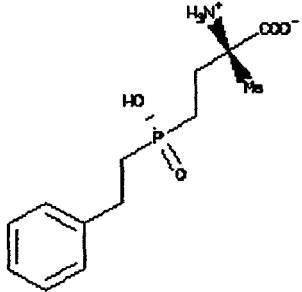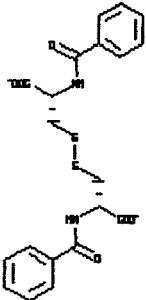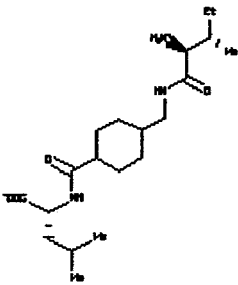
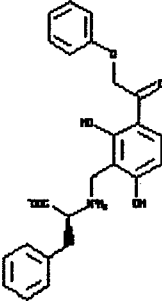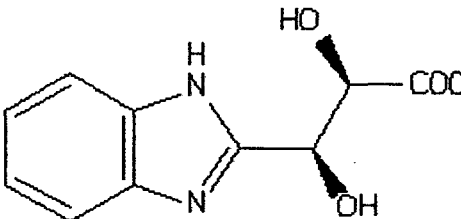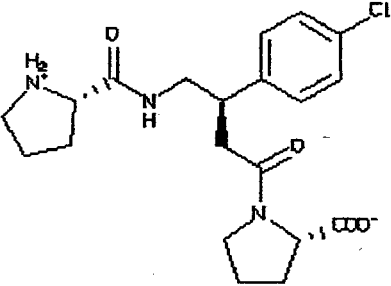| CHEMICAL STRUCTURE	ID	SCORE	PROPERTIES
	ZINC 00150649 methyl 3-[(5-hydroxy-6-methyl-1,2,4-triazin-3-yl)amino]benzoate	Glide score -9.71 Gold fitness -9.52 X-score -7.85	Molecular weight 260.253 hbond donor 2 hbond accepter 7 log p2.28
	ZINC 02718714 6-benzyl-3-[(3,4-dichlorophenyl)amino]-1,2,4-triazin-5-ol	Glide score -9.68 Gold fitness 35.49 X-score -8.87	Molecular weight 347.205 hbond donor 2 hbond accepter 5 log p 4.94
	ZINC 05154628 3-(3-pyridylmethylamino)-6-tert-butyl-4H-1,2,4-triazin-5-one	Glide score -9.67 Gold fitness 53.37 X-score -8.18	Molecular weight 259.313 hbond donor 2 hbond accepter 6 log p 1.43
	ZINC 04225064 3-(4-ethylphenyl)amino-6-(2-furylmethyl)-4H-1,2,4-triazin-5-one	Glide score -9.65 Gold fitness 54.88 X-score -8.23	Molecular weight 296.33 hbond donor 2 hbond accepter 6 log p 3.83
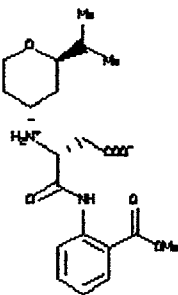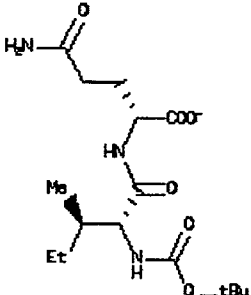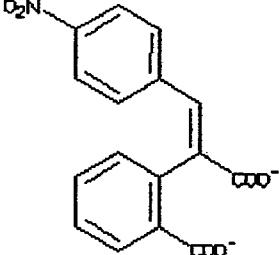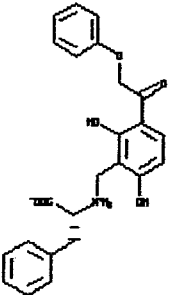
33

CHEMICAL STRUCTURE	ID	SCORE	PROPERTIES
	ZINC 05154431 3-[(3,5-dichlorophenyl)amino]-6-[(4-methoxyphenyl)methyl]-1,2,4-triazin-5-ol	Glide score -9.65 Gold fitness 24.87 X-score -8.92	Molecular weight 377.231 hbond donor 2 hbond accepter 6 log p5.00
	ZINC 01830227 ethyl 2-[2-(3H-1,3-benzothiazol-2-ylideneamino)-6-oxo-3H-pyrimidin-4-yl]acetate	Glide score -9.6 Gold fitness 56.31 X-score -8.47	Molecular weight 330.369 hbond donor 2 hbond accepter 7 log p3.20

34

Table 7. Top ranking molecules from natural molecules as potential inhibitors for LF
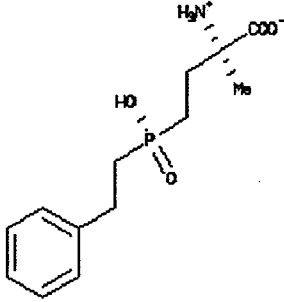
CHEMICAL STRUCTURE	ID	SCORE	PROPERTIES
	ZINC02087229 2-amino-4-(hydroxy-phenethyl-phosphoryl)-2-methyl-butanoic acid	Glide score -11.65 Gold fitness 62.52 X-score -8.03	Molecular weight 285.28 hbond donor 4 hbond accepter 5 log p -1.44
	ZINC04995790 2-benzoylamino-3-(2-benzoylamino-2-carboxy-ethyl)disulfanyl-propanoic acid	Glide score -11.41 Gold fitness 67.15 X-score -8.98	Molecular weight 446.506 hbond donor 2 hbond accepter 8 log p-1.47
	ZINC12882480 (2R)-2-[[4-[[[(2S,3S)-2-amino-3-methyl-pentanoyl]amino]methyl]cyclohexanecarbonyl]amino]-4-methyl-pentanoic acid	Glide score -11.29 Gold fitness 52.33 X-score -9.64	Molecular weight 383.533 hbond donor 5 hbond accepter 7 log p 0.13
	ZINC 00281475 (2S,3R)-3-(1H-benzimidazol-2-yl)-2,3-dihydroxy-propanoic acid	Glide score -11.14 Gold fitness 54.94 X-score -7.62	Molecular weight 221.192 hbond donor 6 hbond accepter 3 log p-0.68

CHEMICAL STRUCTURE	ID	SCORE	PROPERTIES
	ZINC08856538 2-[3-(4-chlorophenyl)-4-thiazolidin-4-ylcarbonylamino-butanoyl]amino-3-hydroxy-propanoic acid	Glide score -10.89 Gold fitness 69.97 X-score -7.96	Molecular weight 415.899 hbond donor 5 hbond accepter 8 log p-1.17
	ZINC4026906 2-[[2,6-dihydroxy-3-(2-phenoxyacetyl)-phenyl]methylamino]-3-phenyl-propanoic acid	Glide score -10.84 Gold fitness 62.86 X-score -9.17	Molecular weight 421.449 hbond donor 4 hbond accepter 7 log p 1.91
	ZINC00281476 (2R,3S)-3-(1H-benzimidazol-2-yl)-2,3-dihydroxy-propanoic acid	Glide score -10.46 Gold fitness 57.32 X-score -7.55	Molecular weight 221.192 hbond donor 3 hbond accepter 6 log p -0.68
	ZINC06624611 1-[3-(4-chlorophenyl)-4-pyrrolidin-2-ylcarbonylamino-butanoyl]pyrrolidine-2-carboxylic acid	Glide score -10.34 Gold fitness 63.51 X-score -6.55	Molecular weight 407.898 hbond donor 3 hbond accepter 7 log p 0.14

CHEMICAL STRUCTURE	ID	SCORE	PROPERTIES
	ZINC12661647 (3R)-3-[[(2R,4R)-2-isopropyltetrahydro pyran-4-yl]amino]-4-[(2-methoxycarbonylph enyl)amino]-4-oxo-butanoic acid	Glide score -10.16 Gold fitness 58.04 X-score -9	Molecular weight 392.452 hbond donor 3 hbond accepter 8 log p 2.19
	ZINC04027430 5-amino-2-(3-methyl-2-tert-butoxycarbonylam ino-pentanoyl)amino-5 -oxo-pentanoic acid	Glide score -10.16 Gold fitness 64.74 X-score -8.93	Molecular weight 358.415 hbond donor 4 hbond accepter 9 log p 0.70
	ZINC 04701676 2-[1-carboxy-2-(4-nitrophenyl)-vinyl]benzoic acid	Glide score -10.01 Gold fitness 57.32 X-score -7.5	Molecular weight 311.249 hbond donor 0 hbond accepter 7 log p 2.73
	ZINC04026907 2-[[2,6-dihydroxy-3-(2-phenoxyacetyl)-phenyl]methylami no]-3-phenyl-propanoic acid	Glide score -9.87 Gold fitness 61.83 X-score -9.37	Molecular weight 421.449 hbond donor 4 hbond accepter 7 log p 1.91

CHEMICAL STRUCTURE	ID	SCORE	PROPERTIES
	ZINC02087228 2-amino-4- (hydroxy- phenethyl- phosphoryl)-2- methyl- butanoic acid	Glide score -9.77 Gold fitness 57.51 X-score -7.34	Molecular weight 285.28 hbond donor 4 hbond accepter 5 log p -1.44

3.1.1 *IN SILICO* RESULTS FOR KNOWN INHIBITORS OF LF

We have five known inhibitors of lethal factor. The docking, scoring and interaction calculation were done with known inhibitors for comparison with our designed molecules. From literature we got the KI dissociation constant of inhibitors. These data are shown in the table.

Table 8. Results of known inhibitors.

LIGAND NAME	INIBITION IN TERMS OF KI	GLIDE SCORE	GOLD SCORE	X-SCORE	H-BOND
915	24 nM	-8.86	63.73	-8.82	6
NSC1	0.5 µM	-6.57	50.37	-8.05	2
MFM	0.8 µM	-8.28	64.89	-7.65	5
GM6	2.1 µM	-9.08	62.38	-9.04	8
SD2	11 µM	-8.59	71.95	-8.31	6

3.1.2 IDENTIFICATION OF POTENTIAL INHIBITORS FOR LF

In case of LF we have five reference molecules (known inhibitors) so we have compared two top designed molecules with two top known inhibitors(according to Ki) of Lethal Factor.

Table 9. Comparison table between known and designed ligand.

LIGAND NAME	INIBITION IN TERMS OF KI	GLIDE SCORE	GOLD SCORE	X-SCORE	H-BOND
915	24 nM	-8.86	63.73	-8.82	6
NSC1	0.5 µM	-6.57	50.37	-8.05	2
ZINC05154623	Designed ligand	-10.57	67.28	-8.27	6
ZINC00281476	Designed ligand	-10.46	57.32	-7.55	6

From comparison table (Table 9) it was found that designed ligands are much better in GLIDE and GOLD score. Hydrogen bonds which are important in specificity are more in the designed ligands than in known inhibitors. The known inhibitors are having slightly better X-score but we know the accuracy of X-score is ±2 Kcal and the difference between known and designed is not more than 0.5. As we got several molecules form drug like and natural molecules of Zinc database as potential inhibitors which show better scores and interactions. For showing the binding of designed molecules to LF visually and detailed interactions we have selected one molecule from Drug like data set (table 6) and one molecule from natural molecules (table 7). Interaction with catalytic residues are essential to inhibit the action of target enzyme and so we have considered involvement of catalytic residues. This study was done by our program to calculate the interactions and manually visualizing the complex structure which are shown below

One of the selected compound for LF From Drug like library ZINC05154623
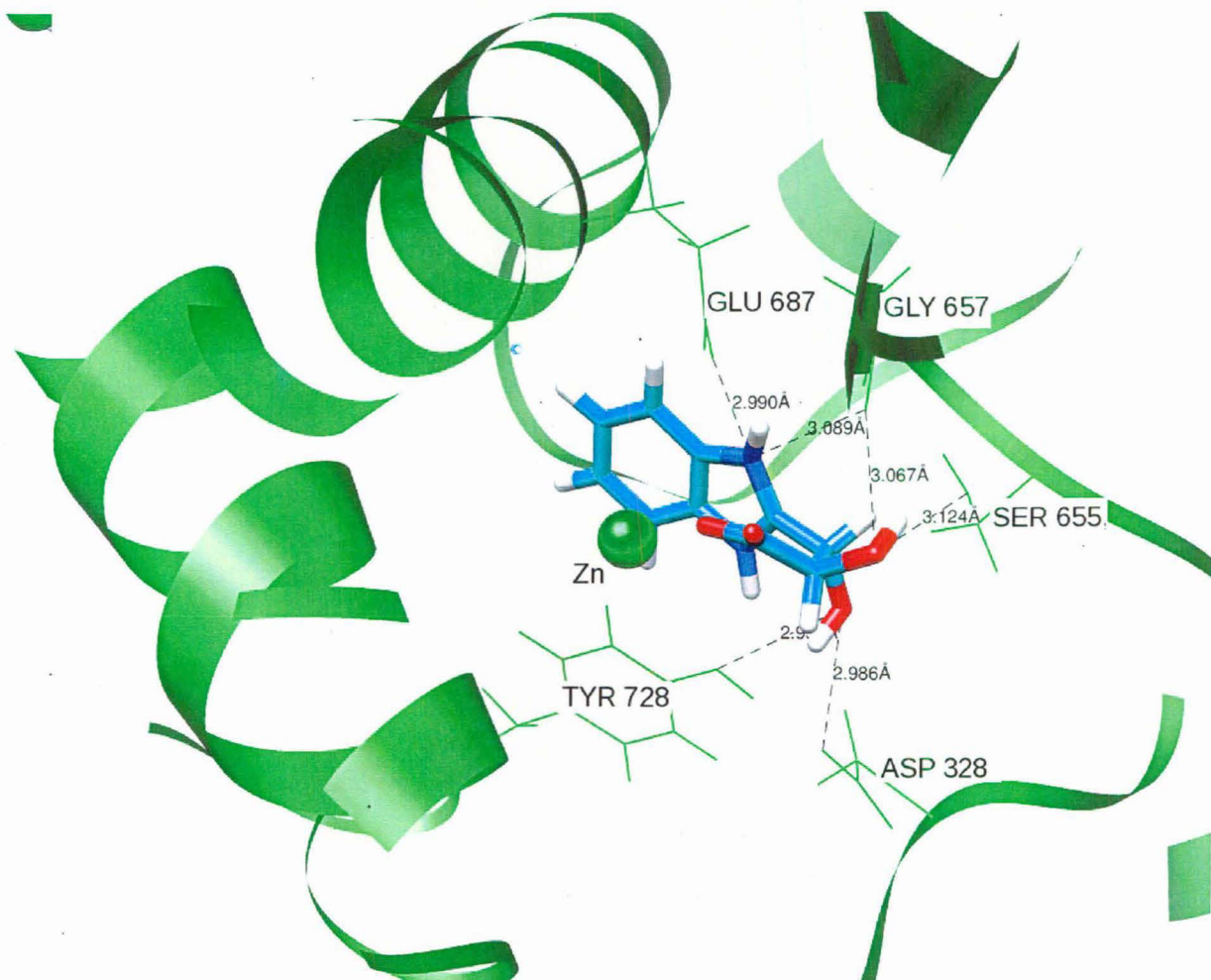
Figure 6. Ligand bound to active site of LF and hydrogen bond between them.
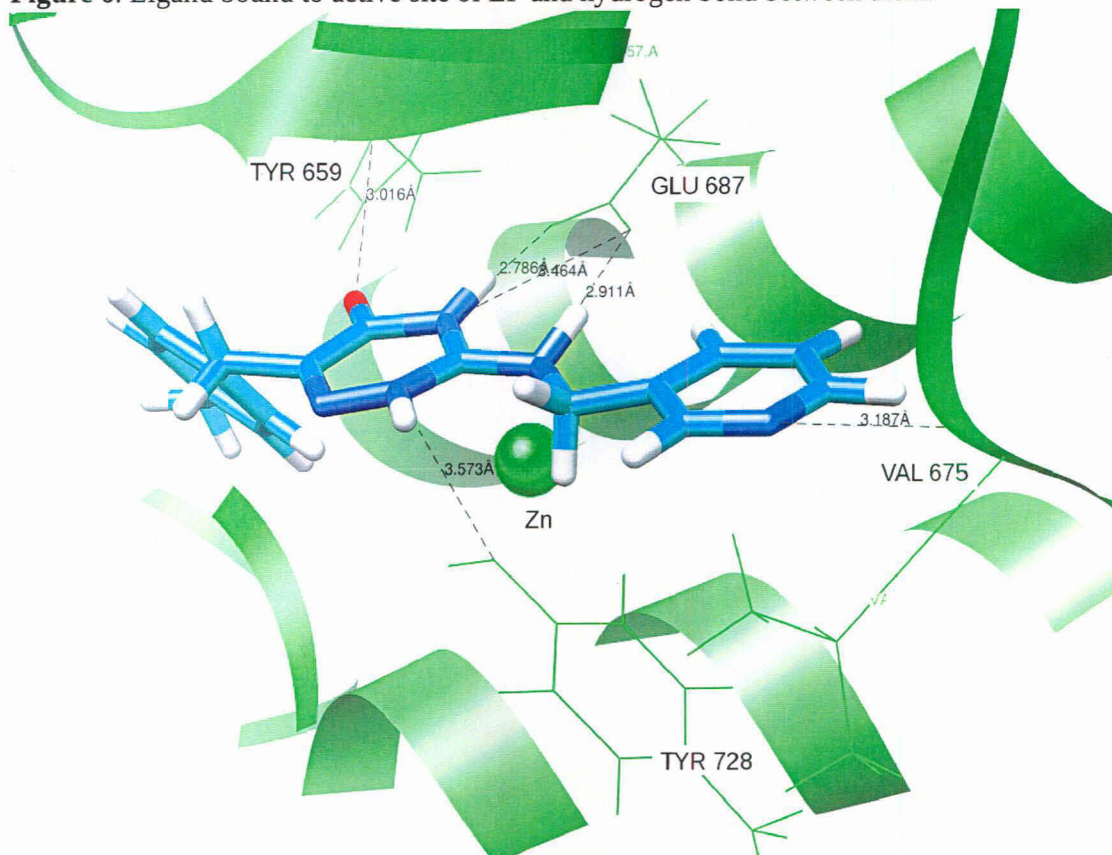
TABLE 10. List of strong interaction within 3.6 å range

LIGAND ATOM	PROTEIN ATOM	DISTANCE	INTERACTION
11 O	N TYR A 659	3.016 Å	HYDROGEN BOND
12 N	OE1 GLU A 687	2.785 Å	HYDROGEN BOND
15 N	OH TYR A 728	3.573 Å	HYDROGEN BOND
17 N	OE2 GLU A 687	2.91 Å	HYDROGEN BOND
12 N	OE2 GLU A 687	3.464 Å	HYDROGEN BOND
23 N	N VAL A 675	3.187 Å	HYDROGEN BOND
1 C	CG PRO A 661	3.113 Å	HYDROPHOBIC INTERACTION
1 C	CD1 LEU A 707	3.106 Å	HYDROPHOBIC INTERACTION
3 C	CD PRO A 661	3.398 Å	HYDROPHOBIC INTERACTION

One of the selected compound for LF From Natural molecules ZINC 00281476


Figure 7. Ligand bound to active site of LF and hydrogen bond between them.

TABLE 11. LIST OF STRONG INTERACTION WITHIN 3.6 Å RANGE

LIGAND ATOM	PROTEIN ATOM	DISTANCE	INTERACTION
7 N	O GLY A 657	3.088 Å	HYDROGEN BOND
16 O	OG SER A 655	3.123 Å	HYDROGEN BOND
17 O	ASP A 328	2.985 Å	HYDROGEN BOND
7 N	OE2 GLU A 687	2.989 Å	HYDROGEN BOND
16 O	O GLY A 657	3.067 Å	HYDROGEN BOND
17 O	OH TYR A 728	2.955 Å	HYDROGEN BOND
2 C	CD2 LEU A 677	3.493 Å	HYDROPHOBIC INTERACTION
3 C	CE2 TYR A 728	3.472 Å	HYDROPHOBIC INTERACTION

3.2 *IN SILICO* RESULTS FROM DATABASE MOLECULES FOR EF

We have docked all molecules from our ligand library to edema factor protein with High throughput virtual screening wizard in GLIDE. From HTVS result we have taken top ten percent ligand poses for GLIDE standard precision docking. From standard precision result top 9000 from drug like molecules, top 2000 from natural molecules and top 1000 from kinase inhibitors molecules were subjected to detailed docking from GLIDE extra precision wizard. After GLIDE docking all top ligand poses (9000 +2000 + 1000) were also scored with X-score. Graphical representation GLIDE score of kinase inhibitor molecules are shown in given graph
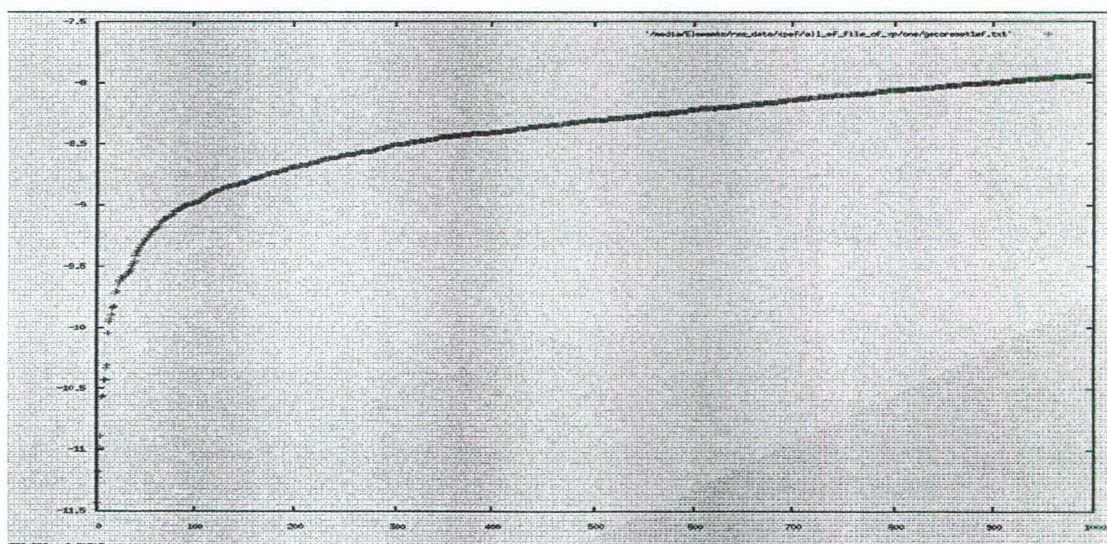
Figure 8. Graphical representation of GLIDE score of 1000 kinase inhibitor molecules of edema factor

3.2.1 RESULTS OF TOP RANKING LIGANDS FOR LETHAL FACTOR

After post docking analysis of twelve thousand ligands, we have selected those ligands which have at least five hydrogen bond and minimum GLIDE score of -9. We also have selected those ligand which shows more than five hydrogen bonds

irrespective of GLIDE score. Thus we obtained 90 ligand poses in case of drug like molecules, 53 from natural molecules and 47 from kinase inhibitor molecules. We have shown the top 30 molecules from each set.

Table 12. Top ranking molecules form drug like molecules against EF

ZINC-ID	GSCORE	XSCORE	H – BOND
ZINC03881816	-12.64	-7.03	6
ZINC00125011	-12.05	-6.55	9
ZINC04501578	-11.92	-7.75	6
ZINC03871853	-11.64	-7.24	5
ZINC04671354	-11.43	-7.89	10
ZINC05009081	-11.42	-7.29	5
ZINC04993329	-11.31	-7.28	5
ZINC04993328	-11.25	-7.65	5
ZINC04625033	-10.98	-7.14	6
ZINC00136594	-10.91	-6.7	5
ZINC05050492	-10.89	-7.09	5
ZINC02932488	-10.89	-7.85	5
ZINC045015277	-10.87	-7.82	6
ZINC02381763	-10.62	-7	5
ZINC02214851	-10.57	-8.07	5
ZINC03096639	-10.5	-6.61	6
ZINC05413181	-10.47	-7.52	5
ZINC01806872	-10.4	-7.9	5
ZINC05214746	-10.4	-7.89	5
ZINC02634436	-10.25	-6.68	5
ZINC03846664	-10.13	-6.43	7
ZINC05252763	-10.12	-7.42	6
ZINC04090458	-9.97	-7.23	5
ZINC04819999	-9.91	-7	7
ZINC04976542	-9.87	-8.33	5
ZINC04759984	-9.84	-6.89	5
ZINC04769867	-9.82	-8.14	6
ZINC04945256	-9.82	-7.27	6
ZINC01233325	-9.8	-7.28	6

Table 13. Top ranking molecules form natural molecules against EF

ZINC-ID	GSCORE	XSCORE	H – BOND
ZINC04090445	-12.35	-7.55	8
ZINC12652334	-11.81	-6.63	6
ZINC04089174	-11.58	-6.31	7
ZINC02038400	-11.56	-6.67	6
ZINC12652337	-11.39	-6.37	7
ZINC00308060	-11.16	-8.14	6
ZINC04501392	-11.14	-6.28	6
ZINC12659657	-10.91	-7.4	7
ZINC02132705	-10.75	-7.77	7
ZINC04089172	-10.67	-6.59	9
ZINC04235568	-10.56	-8.35	6
ZINC02093008	-10.35	-6.27	6
ZINC04044291	-10.24	-7.39	7
ZINC01530283	-10.21	6.18	6
ZINC04501392	-10.11	-6.21	5
ZINC04265699	-10.09	-8.38	6
ZINC12658893	-9.88	-7.77	5
ZINC12659848	-9.82	-8.38	7
ZINC03844926	-9.79	-7.77	6
ZINC12896941	-9.71	-7.03	5
ZINC13410592	-9.63	-7.26	5
ZINC08877541	-9.59	8.57	5
ZINC12659850	-9.58	-8.61	5
ZINC04023371	-9.56	-7.36	6
ZINC00711801	-9.49	-8.4	9
ZINC12661132	-9.43	-9	6
ZINC12659848	-9.3	-8.39	5
ZINC04064606	-9.27	-9.1	5
ZINC04028704	-9.24	-6.12	6

Table 14. Top ranking molecules form kinase inhibitor molecules against EF
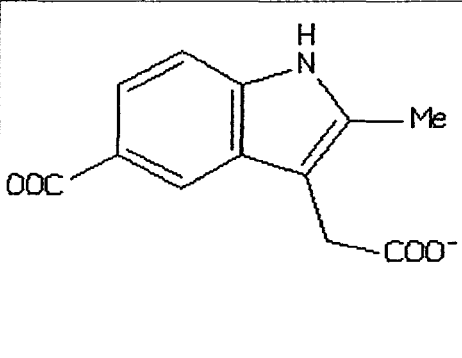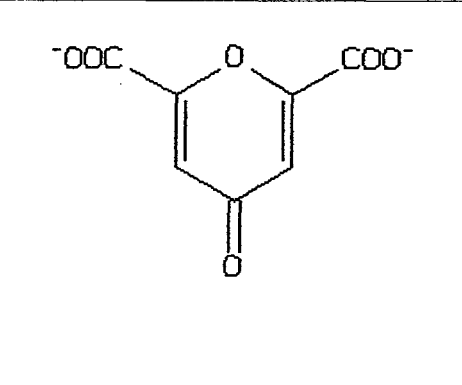
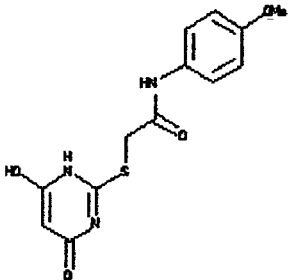| Ligand no.	GSCORE	XSCORE	H – BOND
10797	-13.17	-7.84	8
459	-12.58	-8.25	7
10797	-11.65	-7.84	9
10797	-11.65	-7.84	8
388	-10.28	-8.82	6
20887	-9.65	-8.22	6
10797	-9.56	-7.92	6
16800	-9.54	-9.27	7
32731	-9.28	-7.31	5
33227	-9.26	-8.05	5
14476	-9.24	-8.26	5
18031	-9.17	-8.19	8
32753	-9.15	-7.76	7
32730	-9.14	-7.2	6
9574	-9.11	-8.66	5
3288	-9.1	-7.76	5
32750	-8.76	-7.44	8
32751	-8.74	-7.71	7
32745	-8.71	-6.72	7
32768	-8.69	-7.62	7
18033	-8.62	-8.05	6
32953	-8.6	-7.78	8
370	-8.54	-5.96	8
32972	-8.46	-7.53	7
18036	-8.45	-8	6
18004	-8.38	-7.48	7
28595	-8.37	-8.29	6
3345	-8.3	-7.17	7
32970	-8.25	-7.15	9
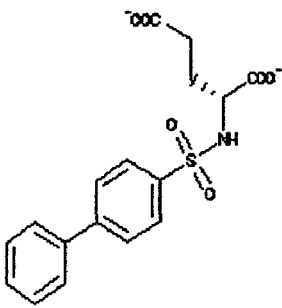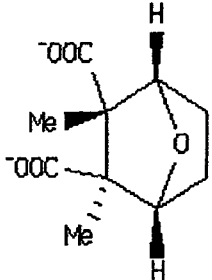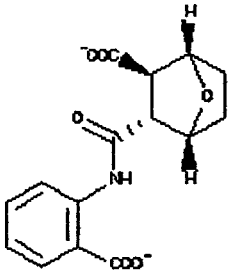
In table 12 the molecular docking results for drug like molecules against EF
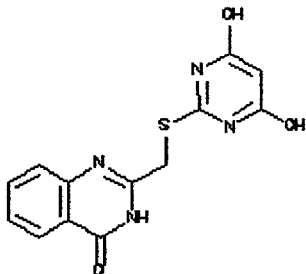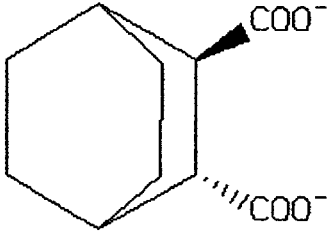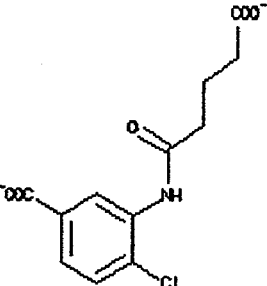
are displayed. The GLIDE scores for molecules range from -9.8 to -12.64, X-score values (predicted binding energies) ranges from -6.43 to -8.33, and there are 5 to 10 hydrogen bonds between protein and ligands. In table 13 the result of natural molecules against edema factor is displayed. The GLIDE scores for molecules range from -9.24 to -12.35, X-score values ranges from -6.12 to -9.1, and there are 5 to 9 hydrogen bonds between protein and ligands. In table 14 the result of kinase inhibitors molecules against edema factor is displayed. The GLIDE scores for molecules range from -8.25 to -13.17, X-score values ranges from -5.96 to -9.27, and there are 5 to 9 hydrogen bonds between protein and ligands. Molecules which are shown in the tables (12,13 and 14) are then manually visualized and examined by us and from there we have taken top molecules for edema factor. These molecules are showing good interaction visually. These molecules were then subjected to GOLD docking. For Edema factor we are showing the 9 top compound from drug like molecule, 12 molecules from natural molecules and 8 molecules from kinase inhibitor molecules.
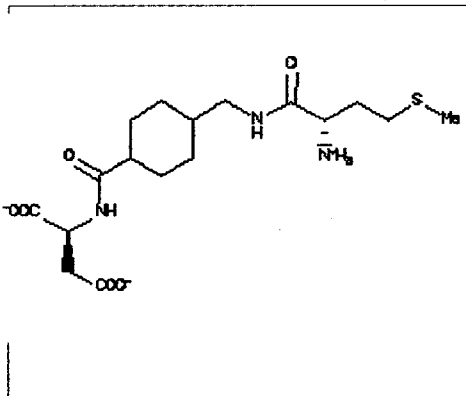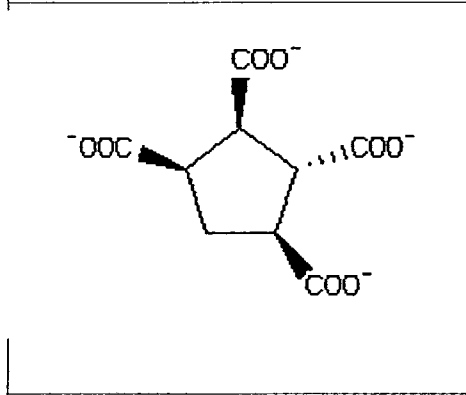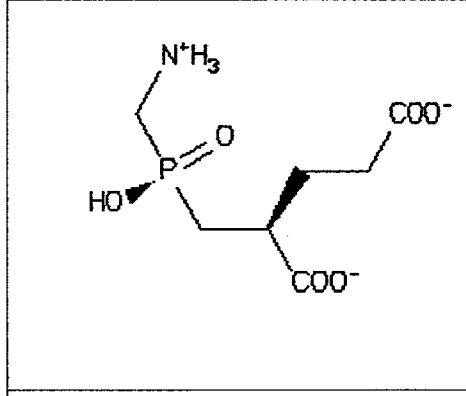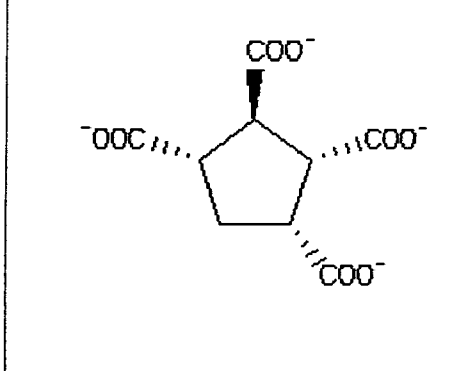
Table 15. Top ranking molecules from drug like molecules as potential inhibitors for EF
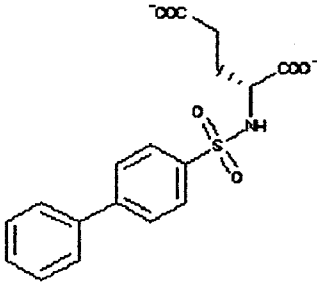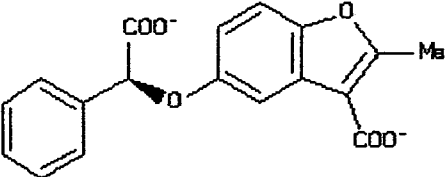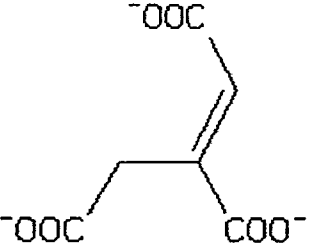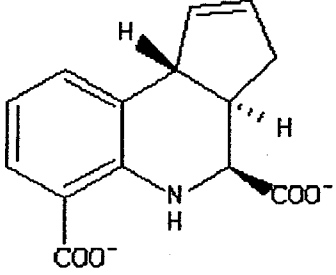
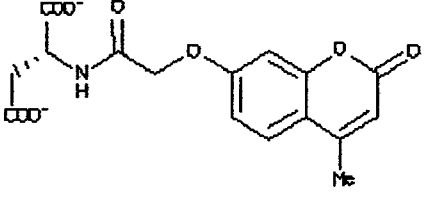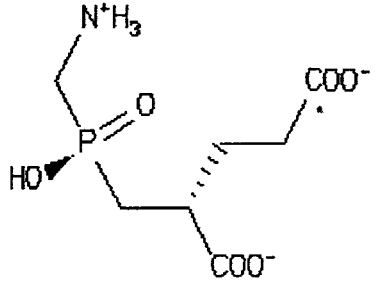| CHEMICAL STRUCTURE	ID	SCORE	PROPERTIES
	ZINC03881816 3-(carboxymethyl)-2-methyl-1H-indole-5-carboxylic acid	Glide score -12.64 Gold fitness 56.54 X-score -7.03	Molecular weight 231.207 hbond donor 1 hbond accepter 5 log p 1.62
	ZINC00125011 4-oxopyran-2,6-dicarboxylic acid	Glide score -12.05 Gold fitness 41.89 X-score -6.55 -6.55	Molecular weight 182.087 hbond donor 0 hbond accepter 6 log p 0.13
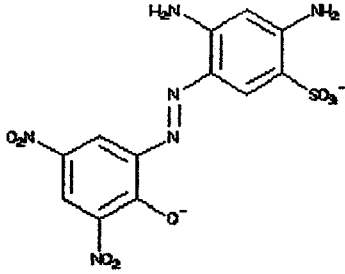
CHEMICAL STRUCTURE	ID	SCORE	PROPERTIES
	ZINC04501578 2-(4-phenylphenyl)sulfonylaminopentanedioic acid	Glide score -11.92 Gold fitness 67.68 X-score -7.75	Molecular weight 361.375 hbond donor 1 hbond accepter 7 log p 0.36
	ZINC03871853 (1R,4S,5S, 6S)-5,6-dimethyl-7-oxabicyclo[2.2.1]heptane-5,6-dicarboxylic acid	Glide score -11.64 Gold fitness 28.97 X-score -7.24	Molecular weight 212.201 hbond donor 5 hbond accepter 0 log p 0.26
	ZINC 4671354 6-[(2-carboxyphenyl)carbamoyl]-7-oxabicyclo[2.2.1]heptane-5-carboxylic acid	Glide score -11.43 Gold fitness 51.52 X-score -7.89	Molecular weight 303.27 hbond donor 1 hbond accepter 7 log p:1.37
	ZINC05009081 6-[(2-carboxyphenyl)carbamoyl]-7-oxabicyclo[2.2.1]heptane-5-carboxylic acid	Glide score -11.42 Gold fitness 62.89 X-score -7.29	Molecular weight 306.323 hbond donor 2 hbond accepter 7 log p 1.92

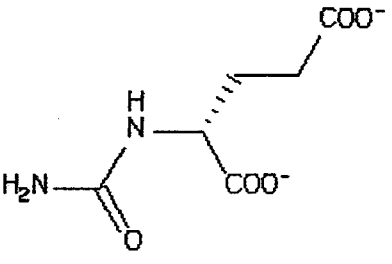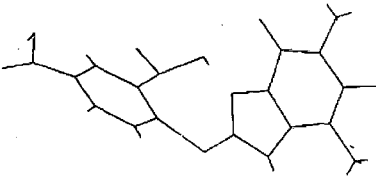| CHEMICAL STRUCTURE	ID	SCORE	PROPERTIES
	ZINC04625033 2-[(4,6-dihydroxypyrimidin-2-yl)sulfanylmethyl]-3H-quinazolin-4-one	Glide score -10.98 Gold fitness 63.75 X-score -7.14	Molecular weight 302.315 hbond donor 3 hbond accepter 7 log p 2.26
	ZINC00136594 2-[(4,6-dihydroxypyrimidin-2-yl)sulfanylmethyl]-3H-quinazolin-4-one	Glide score -10.91 Gold fitness 41.23 X-score -6.75	Molecular weight 196.202 hbond donor 1 hbond accepter 4 log p 1.09
	ZINC05050492 3-(4-carboxybutanoylamino)-4-chloro-benzoic acid	Glide score -10.89 Gold fitness 53.13 X-score -7.09	Molecular weight 283.667 hbond donor 1 hbond accepter 6 log p 1.93
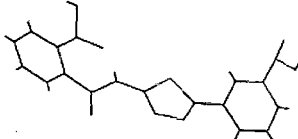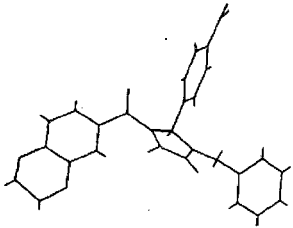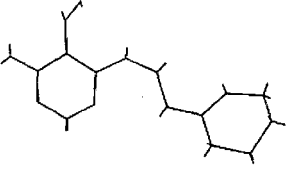
Table 16. Top ranking molecules from natural molecules as potential inhibitors for EF

CHEMICAL STRUCTURE	ID	SCORE	PROPERTIES
	ZINC04090445 2-[4-[(2-amino-4-methylsulfanyl-butanoyl)aminomethyl]cyclohexyl]carbonylaminobutanedioic acid	Glide score -12.35 Gold fitness 74.02 X-score -7.55	Molecular weight 402.493 hbond donor 5 hbond accepter 9 log p -2.84
	ZINC12652334 (1S,2R,3R,4R)-cyclopentane-1,2,3,4-tetracarboxylic acid	Glide score -11.81 Gold fitness 46.76 X-score -6.63	Molecular weight 242.139 hbond donor 0 hbond accepter 8 log p -1.09
	ZINC04089174 4-[(aminomethyl-hydroxy-phosphoryl)methyl]pentanedioic acid	Glide score -11.58 Gold fitness 69.36 X-score -6.31	Molecular weight 238.156 hbond donor 4 hbond accepter 7 log p -1.83
	ZINC02038400 cyclopentane-1,2,3,4-tetracarboxylic acid	Glide score -11.56 Gold fitness 47.25 X-score -6.67	Molecular weight 242.139 hbond donor 0 hbond accepter 8 log p -1.09

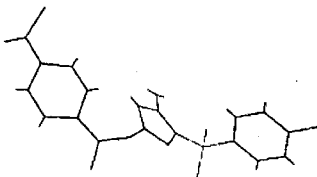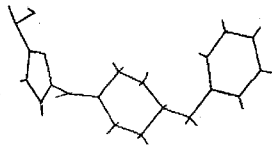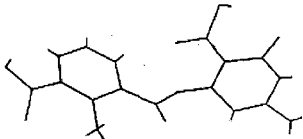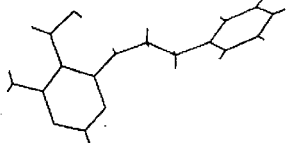| CHEMICAL STRUCTURE	ID	SCORE	PROPERTIES
	ZINC12652337 (1S,2R,3R,4S)-cyclopentane-1,2,3,4-tetracarboxylic acid	Glide score -11.39 Gold fitness 46.13 X-score -6.37	Molecular weight 242.139 hbond donor 0 hbond accepter 8 log p -1.09
	ZINC00308060 5-(carboxy-phenyl-methoxy)-2-methyl-benzofuran-3-carboxylic acid	Glide score -11.16 Gold fitness 61.55 X-score -8.14	Molecular weight hbond donor hbond accepter log p
	ZINC04501392 (E)-prop-1-ene-1,2,3-tricarboxylic acid	Glide score -11.14 Gold fitness 46.17 X-score -6.28	Molecular weight 324.288 hbond donor 0 hbond accepter 6 log p 2.85
	ZINC12659657 (3aR,4S,9bR)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-4,6-dicarboxylic acid	Glide score -10.91 Gold fitness 46.50 X-score -7.4	Molecular weight 257.245 hbond donor 1 hbond accepter 5 log p 0.15

CHEMICAL STRUCTURE	ID	SCORE	PROPERTIES
	ZINC02132705 2-[2-(4-methyl-2-oxo-chromen-7-yl)oxyacetyl]aminobutanedioic acid	Glide score -10.75 Gold fitness 72.85 X-score -7.77	Molecular weight 347.279 hbond donor 1 hbond accepter 9 log p -1.18
	ZINC04089172 4-[(aminomethyl-hydroxy-phosphoryl)methyl]pentanedioic acid	Glide score -10.67 Gold fitness 62.25 X-score -6.59	Molecular weight 238.156 hbond donor 4 hbond accepter 7 log p -1.83
	ZINC04235568 2,4-diamino-5-(2-hydroxy-3,5-dinitro-phenyl)azo-benzenesulfonic acid	Glide score -10.56 Gold fitness 56.54 X-score -8.35	Molecular weight 396.297 hbond donor 4 hbond accepter 14 log p -0.50
	ZINC02093008 2-ureidopentanedioic acid	Glide score -10.35 Gold fitness 48.09 X-score -6.27	Molecular weight 188.139 hbond donor 3 hbond accepter 7 log p -3.06

Table 17. Top ranking molecules from kinase inhibitor molecules as potential inhibitors for EF

CHEMICAL STRUCTURE	ID	SCORE	PROPERTIES
	10797	Glide score -13.17 Gold fitness 70.89 X-score -7.84	Molecular weight 380 hbond donor 3 hbond accepter 9 log p 0.927
	388	Glide score -10.28 Gold fitness 69.64 X-score -8.82	Molecular weight 373 hbond donor 3 hbond accepter 7 log p 2.557
	16800	Glide score -9.54 Gold fitness 74.08 X-score -9.27	Molecular weight 473 hbond donor 2 hbond accepter 7 log p 2.934
	32731	Glide score -9.28 Gold fitness 40.51 X-score -7.31	Molecular weight 264 hbond donor 3 hbond accepter 4 log p 2.092

CHEMICAL STRUCTURE	ID	SCORE	PROPERTIES
	33227	Glide score -9.26 Gold fitness 71.29 X-score -8.05	Molecular weight 407 hbond donor 3 hbond accepter 7 log p 3.140
	14476	Glide score -9.24 Gold fitness 66.65 X-score -8.26	Molecular weight 317 hbond donor 2 hbond accepter 5 log p 2.049
	18031	Glide score -9.17 Gold fitness 49.96 X-score -8.19	Molecular weight 335 hbond donor 3 hbond accepter 7 log p 1.220
	32730	Glide score -9.14 Gold fitness 37.69 X-score -7.2	Molecular weight 260 hbond donor 4 hbond accepter 3 log p 1.444

• We are retrieving chemical id information for kinase inhibitors from lifechemicals [http://www.lifechemicals.com/]

# 3.2.1 IDENTIFICATION OF POTENTIAL INHIBITORS FOR EF

We have docked two known EF inhibitors which are downloadable from ZINC database. So we have compared two designed molecules with two known inhibitors(according to Ki) of Edema Factor.

Table 18. Comparison table between known and designed ligand.

LIGAND NAME	INIBITION IN TERMS OF KI	GLIDE SCORE	GOLD SCORE	X-SCORE	H-BOND
ZINC00075209	1.7-5 µM	-9.15	52.31	-9.00	3
ZINC00132711	9 µM	-4.25	54.19	-8.82	10
ZINC0012501 1	Designed ligand	-12.05	41.89	-6.55	9
ZINC040904 45	Designed ligand	-12.35	74.02	-7.55	8

From comparison table (Table 18) it was found that designed ligands are much better in GLIDE score, comparable in GOLD score. Hydrogen bonds which are important in specificity are more in the designed ligands than in known inhibitors. The known inhibitors are having slightly better X-score but we know the accuracy of X-score is ±2 Kcal. We got several molecules from kinase inhibitors database as well as drug like and natural molecules of Zinc database as potential inhibitors which show better scores and interactions. For showing figure and interactions with protein we have selected one molecule from Drug like molecules (table 15), one molecule from natural molecules (table 16) and one molecule from kinase inhibitors (table 17). The selection is based on interactions. Interaction with catalytic residue can inhibit the action of target enzyme so we have considered involvement of catalytic residues in interaction. This study was done by our interaction finding code and manually visualizing the complex structure. The figure and interaction are :

One of the selected compound for EF From Drug like molecules ZINC 00125011
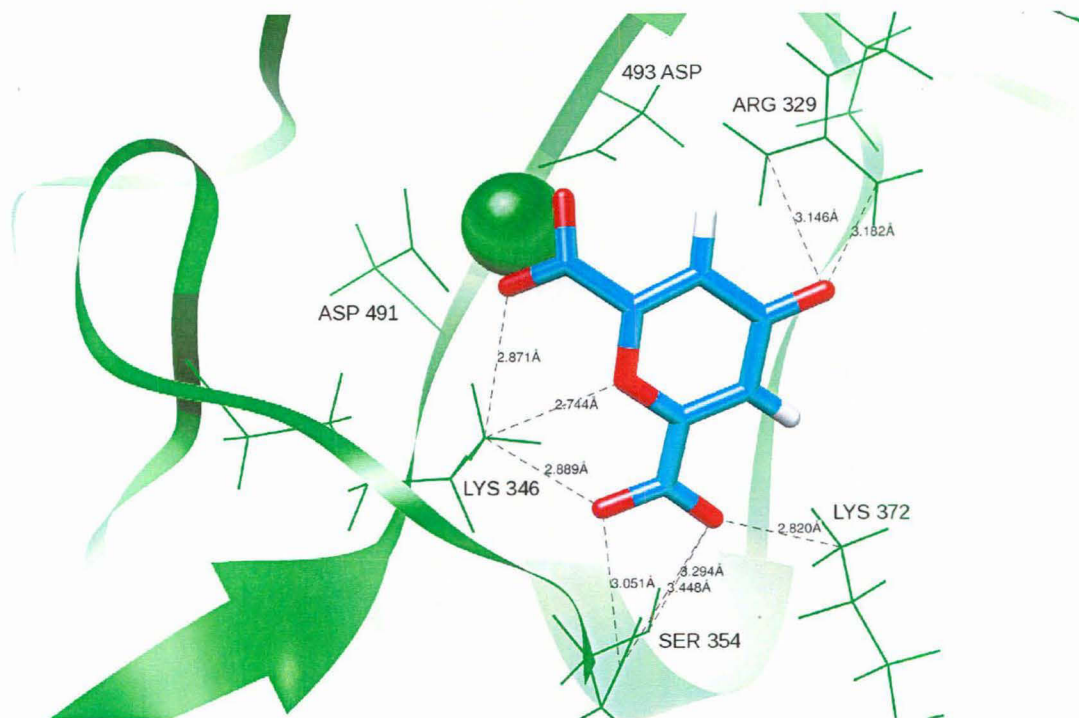
Figure 9. Ligand bound to active site of EF and hydrogen bond between them.

TABLE 19. LIST OF STRONG INTERACTION WITHIN 3.6 Å RANGE

LIGAND ATOM	PROTEIN ATOM	DISTANCE
3 O	NH1 ARG A 329	3.182 Å
3 O	NH2 ARG A 329	3.145 Å
6 O	NZ LYS A 346	2.743 Å
9 O	NZ LYS A 346	2.871 Å
11 O	NZ LYS A 346	2.889 Å
11 O	N SER A 354	3.050 Å
15 O	N SER A 354	3.448 Å
15 O	OG SER A 35	3.294 Å
15 O	NZ LYS A 372	2.819 Å

One of the selected compound for EF From Drug like molecules ZINC 04090445
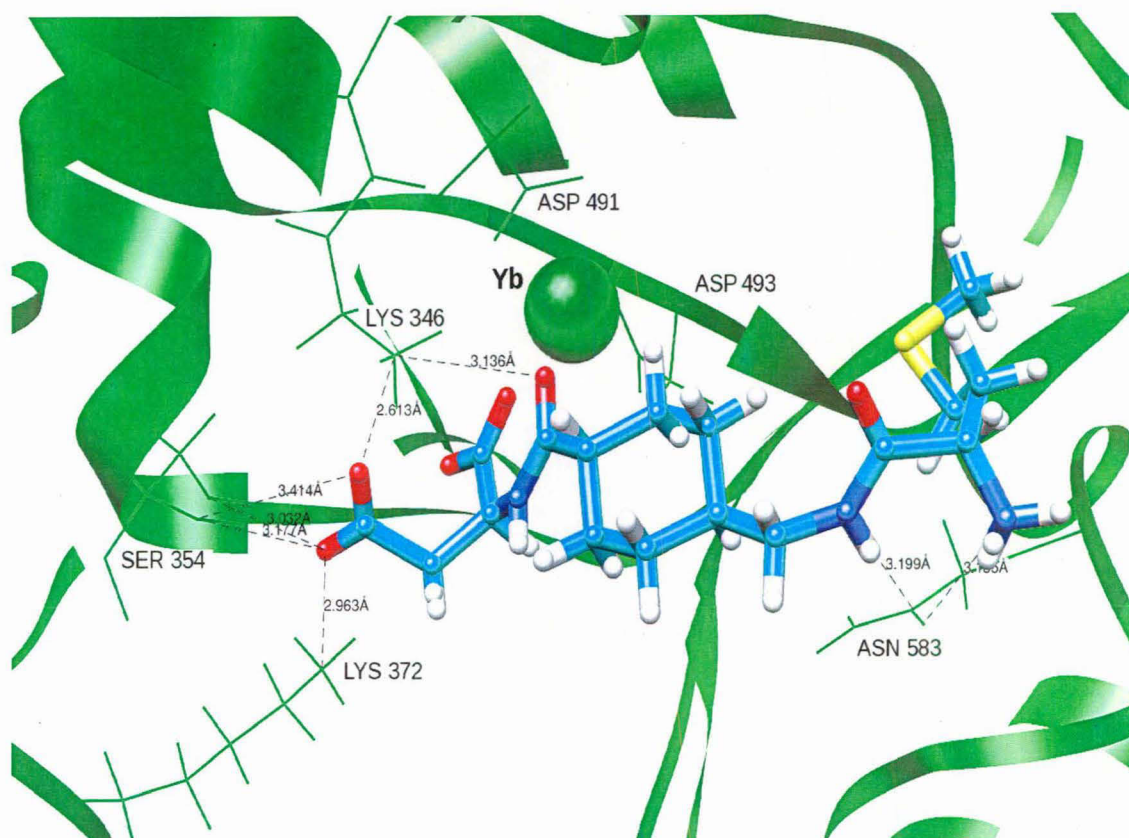
Figure 10. Ligand bound to active site of EF and hydrogen bond between them.

TABLE 20. List of strong interaction within 3.6 Å range

LIGAND ATOM	PROTEIN ATOM	DISTANCE
9 N1	OD1 ASN A 583	3.199 Å
20 O2	NZ LYS A 346	3.136 Å
26 O3	NZ LYS A 346	2.612 Å
26 O3	N SER A 354	3.414 Å
52 O5	N SER A 354	3.172 Å
52 O5	OG SER A 354	3.032 Å
52 O5	NZ LYS A 372	2.961 Å
54 N3	OD1 ASN A 583	3.135 Å

One of the selected compound for EF From Drug like molecules 10970
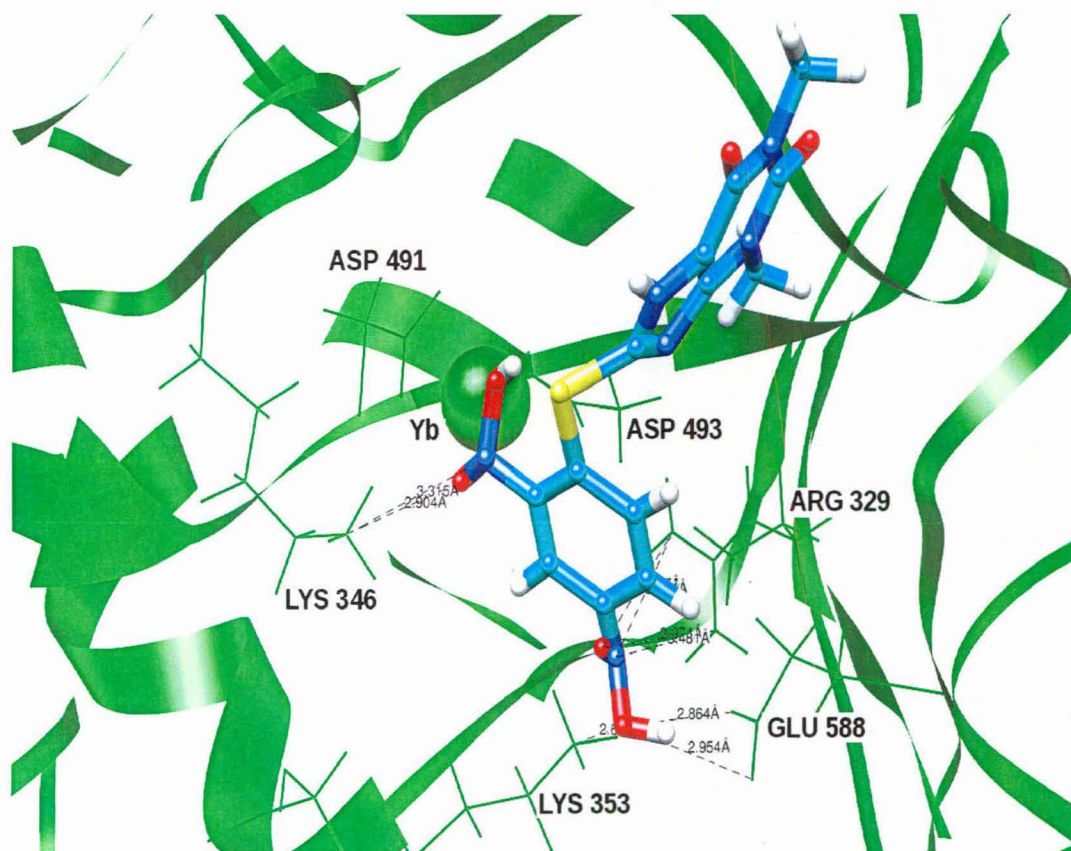
Figure 11. Ligand bound to active site of LF and hydrogen bond between them.

TABLE 21. LIST OF STRONG INTERACTION WITHIN 3.6 Å RANGE

LIGAND ATOM	PROTEIN ATOM	DISTANCE
11 N11	NZ LYS A 346	3.31 Å
12 N12	NH1 ARG A 329	3.48 Å
12 N12	NH2 ARG A 329	3.14 Å
19 O19	NZ LYS A 353	2.60 Å
19 O19	OE1 GLU A 588	2.95 Å
19 O19	OE2 GLU A 588	2.86 Å
21 O21	NZ LYS A 346	2.90 Å
22 O22	NH1 ARG A 329	2.97 Å
22 O22	NH2 ARG A 329	2.74 Å

We have done *in silico* docking of large number of molecules and screened out some top scoring molecules according to GLIDE score after post docking analysist. Predicted binding energy using X-score and GOLD docking for top ranking molecules were performed to validate the results. From our results we find many top ranking molecules have better binding affinity and specificity when compared with known inhibitors. They also show interactions with catalytic residues of active sites. Virtual docking is the computational part which enables potential molecules that can be tested in wet lab. Prof. Rakesh Bhatnagar and his group at JNU have been working on *Bacillus anthracis* for many years to develop vaccine and novel inhibitors. Prof. Bhatnagar's group has shown interest to test the designed molecules.

4 CONCLUSION

As anthrax is an important disease which can be used as a warfare, it is highly required to establish the very accurate post exposer treatment of anthrax. We have done *in silico* docking for large number of small molecules from three different databases against lethal factor and edema factor to design novel inhibitors. For reliability of docking results, X-score and interactions in docked structure were also calculated. GOLD docking was also done for top molecules to validate the results. In this work we have identified few potential inhibitor molecules for LF and EF. The *in silico* designed molecules have better GLIDE score when compared to currently known inhibitors. These molecules have more number of interactions, including interaction with catalytic residues. These molecules also follow lipinsky rule of five thus they can be good inhibitors. From our result we will take up 10-15 molecules for each edema factor and lethal factor which are available commercially for invivo and invitro testing to identify few lead molecules.

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