3. MATERIALS AND METHODS

3.1 Materials

The genome sequence of community acquired methicillin *Staphylococcus aureus* (CA-MRSA) also known as *Staphylococcus aureus* subsp. *aureus MW2* is available on the website http://www.genome.jp (Accesion No. NC_003923) which contains 2820462 base pairs and 2624 protein encoding genes. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (http://www.kegg.jp/kegg/pathway.htm) was used for the retrieval of metabolic pathways for the community-acquired methicillin resistant *Staphylococcus aureus* (Entry no. T00086). The genes and their corresponding protein sequences of CA-MRSA were retrieved from the KEGG database in FASTA file format. The databases and softwares/servers used in the present research work are listed in **Table 3.1**.

Table 3.1: Computational softwares/servers and databases utilized

S.No.	Software/Server/ Databases	Description
1.	KEGG Pathway	KEGG pathway mapping is the process to map molecular datasets in genomics, transcriptomics, proteomics, and metabolomics for biological interpretation of higher-level systemic functions
2.	BLASTP	Database searching tool for protein database using the protein query
3.	NRDB	The database which contains non-redundant protein sequences
4.	DEG	Database of Essential Genes required for the survival of organisms
5	PSORTb v3.0.2	Tool for the prediction of subcellular localization of bacterial proteins
6.	STITCH v3.1	Predicts interactions of proteins and chemicals
7.	COBALT	Multiple protein sequence alignment tool. Also used for generating the phylogenetic tree
8.	Pfam	Predicting Pfam families, motifs, repeats and clans
9.	Conserved Domain *Database*	A resource for the annotation of functional units in proteins
10.	Motif search	Motif prediction
11.	ProtParam	Physicochemical properties prediction
12.	GOR IV method	Secondary structures prediction
13.	GlobPlot	Protein order/globularity and disorder analysis
14.	UniprotKB	Primary protein sequence database
15.	PDB	Structural database which contains 3D structure of biomolecules determined by experimental methods
16.	ClustalW	Multiple sequence alignment tool
17.	HHpred	An interactive server for protein homology detection and structure prediction of proteins
18.	MODELLER 9v9	Tool for 3D structure prediction of proteins

Contd.

19.	PROCHECK	Evaluates the stereochemical properties of the protein structure based on Ramachandran plot
20.	VERIFY3D/ ERRAT	Protein structure validation
21.	Whatif	Protein structure validation
22.	ProSA web server	Recognition of errors in the three dimensional structure
23.	GROMACS 4.0.6	Molecular dynamics simulation tool to evaluate the stability of 3D structure of protein
24.	metaPocket 2.0	A meta server for predicting the active site
25.	Autodock vina	Virtual screening of ligands
26.	ZINC	A database of ligand database
27.	Ligplot	Protein-ligand interaction analysis
28.	PreADMET	Server for the prediction of pharmacokinetic (ADMET) properties of ligands

3.2 Methodology

3.2.1 Putative drug target identification in CA-MRSA using metabolic pathways analysis

The entire genome of *Staphylococcus aureus* subsp. *aureus MW2* (CA-MRSA), was sequenced in the year 2002. It is available on the website http://www.genome.jp (Accesion No. NC_003923) and contains 2820462 base pairs and 2624 protein encoding genes. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (http://www.kegg.jp/kegg/pathway.htm) (**Kanehisa *et al.*, 2002**) was used for the retrieval of metabolic pathways for the community-acquired methicillin resistant *Staphylococcus aureus* (Entry no. T00086). The metabolic pathway of CA-MRSA was analyzed, which contains 76 different types of metabolic pathways **(Table 3.2)**.

Table 3.2: List of metabolic pathways present in CA-MRSA

S. No.	KEGG pathway Entry No.	Name of the metabolic pathway	No. of gene products
1.	sam00010	Glycolysis / Gluconeogenesis	37
2.	sam00020	Citrate cycle (TCA cycle)	20
3.	sam00030	Pentose phosphate pathway	20
4.	sam00040	Pentose and glucuronate interconversions	8
5.	sam00051	Fructose and mannose metabolism	16
6.	sam00052	Galactose metabolism	19

Contd.

7.	sam00053	Ascorbate and aldarate metabolism	6
8.	sam00061	Fatty acid biosynthesis	13
9.	sam00071	Fatty acid metabolism	11
10.	sam00072	Synthesis and degradation of ketone bodies	3
11.	sam00121	Secondary bile acid biosynthesis	1
12.	sam00130	Ubiquinone and other terpenoid-quinone biosynthesis	8
13.	sam00190	Oxidative phosphorylation	23
14.	sam00230	Purine metabolism	56
15.	sam00240	Pyrimidine metabolism	44
16.	sam00250	Alanine, aspartate and glutamate metabolism	18
17.	sam00260	Glycine, serine and threonine metabolism	30
18.	sam00270	Cysteine and methionine metabolism	21
19.	sam00280	Valine, leucine and isoleucine degradation	13
20.	sam00290	Valine, leucine and isoleucine biosynthesis	12
21.	sam00300	Lysine biosynthesis	13
22.	sam00310	Lysine degradation	10
23.	sam00312	Beta-Lactam resistance	3
24.	sam00330	Arginine and proline metabolism	27
25.	sam00340	Histidine metabolism	16
26.	sam00350	Tyrosine metabolism	5
27.	sam00360	Phenylalanine metabolism	3
28.	sam00362	Benzoate degradation	6
29.	sam00380	Tryptophan metabolism	9
30.	sam00400	Phenylalanine, tyrosine and tryptophan biosynthesis	19
31.	sam00401	Novobiocin biosynthesis	3
32.	sam00410	Beta-Alanine metabolism	6
33.	sam00430	Taurine and hypotaurine metabolism	6
34.	sam00450	Selenocompound metabolism	7
35.	sam00460	Cyanoamino acid metabolism	3
36.	sam00471	D-Glutamine and D-glutamate metabolism	3
37.	sam00472	D-Arginine and D-ornithine metabolism	1
38.	sam00473	D-Alanine metabolism	6
39.	sam00480	Glutathione metabolism	7
40.	sam00500	Starch and sucrose metabolism	13
41.	sam00520	Amino sugar and nucleotide sugar metabolism	29
42.	sam00521	Streptomycin biosynthesis	3
43.	sam00550	Peptidoglycan biosynthesis	20

Contd.

44.	sam00561	Glycerolipid metabolism	15
45.	sam00562	Inositol phosphate metabolism	4
46.	sam00564	Glycerophospholipid metabolism	17
47.	sam00590	Arachidonic acid metabolism	3
48.	sam00592	Alpha-Linolenic acid metabolism	1
49.	sam00620	Pyruvate metabolism	35
50.	sam00621	Dioxin degradation	2
51.	sam00622	Xylene degradation	2
52.	sam00625	Chloroalkane and chloroalkene degradation	5
53.	sam00626	Naphthalene degradation	2
54.	sam00627	Aminobenzoate degradation	3
55.	sam00630	Glyoxylate and dicarboxylate metabolism	13
56.	sam00640	Propanoate metabolism	21
57.	sam00642	Ethylbenzene degradation	2
58.	sam00650	Butanoate metabolism	17
59.	sam00660	C5-Branched dibasic acid metabolism	9
60.	sam00670	One carbon pool by folate	9
61.	sam00680	Methane metabolism	20
62.	sam00730	Thiamine metabolism	8
63.	sam00740	Riboflavin metabolism	6
64.	sam00750	Vitamin B6 metabolism	4
65.	sam00760	Nicotinate and nicotinamide metabolism	7
66.	sam00770	Pantothenate and CoA biosynthesis	16
67.	sam00780	Biotin metabolism	12
68.	sam00785	Lipoic acid metabolism	4
69.	sam00790	Folate biosynthesis	18
70.	sam00860	Porphyrin and chlorophyll metabolism	16
71.	sam00900	Terpenoid backbone biosynthesis	14
72.	sam00903	Limonene and pinene degradation	4
73.	sam00906	Carotenoid biosynthesis	5
74.	sam00910	Nitrogen metabolism	13
75.	sam00920	Sulfur metabolism	8
76.	sam01040	Biosynthesis of unsaturated fatty acids	2

All enzymes involved in the different metabolic pathways were listed in a table. The most important criteria for selecting any enzyme or protein as a potential drug target in a pathogen is that it should be non-homologous to the host i.e. *Homo sapiens*. All 914 gene products involved in different metabolic pathways of CA-MRSA genome were subjected to a database search against the proteome of the *Homo sapiens* using the BLASTp program (**Altschul *et al.*, 1997**). The threshold of E-value (expect value) was set to 0.001. The E-value is a parameter with the

intention of describing the number of hits one can expect to see just by chance when searching a database of particular size. Essentially, the E-value describes the random background error that exists for matches between sequences which ranges from 0 to 10. It decreases exponentially as the alignment score (S) of the match increases. The lower the E-value, or the closer it to zero reveals the more significant match between sequences (http://www.ncbi.nlm.nih.gov/BLAST/tutorial/Altschul-1.html#head2). In order to find the significant non-homologous gene products of metabolic pathways CA-MRSA with *Homo sapiens* proteome, the threshold E-value was set to 0.001 in BLASTp program (reduced from its default value 10). The similar protein sequences which were having less than 25% identity and/or less than 80% query coverage to the *Homo sapiens* proteome were considered as non-homologous to humans. These enzymes can be considered as unique putative drug targets for the drug designing.

After performing the database searching of all metabolic enzymes (gene products) of CA-MRSA against the human proteome, 220 targets were identified as non-homologous to *Homo sapiens*. These enzymes were involved in 50 different metabolic pathways. Further analysis for all 220 targets was carried out and it was found that some duplicate targets were involved in more than one metabolic pathway. The list of all putative targets was further refined and duplicates were removed. Finally 152 targets were identified as unique putative drug targets. After identifying the novel putative drug targets from metabolic pathways of CA-MRSA, the genes coding for the important enzymes were further searched in the DEG 6.8 database **(Zhang and Lin, 2009)** to identify the essentiality or non-essentiality of the genes for the survival of the pathogen. DEG provides the database of essential genes which are indispensable for the survival of an organism (http://www.essentialgene.org/). DEG database has been classified into two categories prokaryotes and eukaryotes. In the pathogens, essential gene products provide unique potential drug targets for antimicrobial targets. Among all 152 putative drug targets, 39 genes which encode for potential drug targets were identified as essential for the survival of the CA-MRSA.

3.2.1.1 Sub-cellular localization of putative targets in CA-MRSA

The sub-cellular localization (SCL) of all 39 essential gene products in CA-MRSA was predicted using the PSORTb v3.0.2 program (http://www.psort.org/psortb/). It is the first sub-cellular localization predictor exclusively devised for all prokaryotes, including archaea and bacteria with atypical membrane/cell wall topologies. It handles

archaeal sequences as well as Gram-positive and Gram-negative bacterial protein sequences. This program consists of various analytical modules, each of which analyzes one biological feature known to influence or be characteristic of subcellular localization. The modules may act as a binary predictor, classifying a protein as either belonging or not belonging to a particular localization site, or they may be multi-category, able to assign a protein to one of several localization sites (**Yu *et al,* 2010**).

3.2.1.2 Protein interaction network analysis of FBA in CA-MRSA

In order to analyze the molecular interaction networks of candidate drug target fructose bisphosphate aldolase (FBA), the protein-protein interaction study was carried out from the architecture of CA-MRSA (strain MW2) interactome using the STITCH v3.1web server (http://stitch.embl.de/). This server explores known and predicted interactions of proteins and chemicals. It contains interactions for between 300,000 small molecules and 2.6 million proteins from 1133 organisms (**Kuhn *et al.,* 2012**). For predicting interactions of proteins and chemicals, the server uses 7 active prediction methods i.e., Neighborhood, Gene fusion, Co-occurance, Co-expression, Experiments, Databases and Textmining. Based on these prediction methods, a combined association score is calculated which is called as confidence score. The confidence score is the approximate probability that a predicted link exists between two enzymes in the same metabolic map in the KEGG database. Using the parameter of highest confidence score (0.9) and 20 interactors, the chemical-protein interactions of FBA were predicted in CA-MRSA interactome.

3.2.2 Phylogenetic analysis of candidate target Fructose-bisphosphate aldolase

In order to establish the evolutionary relationship of fructose biphosphate aldolase (FBA) of CA-MRSA with other bacteria, the similar sequences were identified by database searching against non-redundant databases (NRDB) using the BLASTp program (**Altschul *et al.,* 1997**). This program searches protein databases using the protein query, which returned 100 similar sequences on first page. Since the FBA sequences of almost all species of *Staphylococcus aureus* were 100% identical to the CA-MRSA, they were excluded for the phylogenetic analysis. Only non-aureus staphylococcal species were selected apart from the other bacterial species for the multiple sequence alignment (MSA) and phylogenetic analysis. The similar sequences

which were having more than 75% identity were selected for the MSA. Total 39 sequences were chosen for the MSA and phylogeny consequently, to establish the evolutionary relationship between FBA of CA-MRSA and other bacterial species. Using the COBALT program multiple sequence alignment of all 40 sequences was performed. This is a multiple protein sequence alignment tool which finds a collection of pairwise constraint derived from conserved domain databases, protein motif database, and sequence similarity, using the RPS-BLAST, BLASTp and PHI-BLAST. Subsequently, pairwise constrains are incorporated into a progressive multiple alignment (**Papadopoulos and Agarwala, 2007**). Multiple aligned sequences of FBA from different species were carefully examined.

Based on COBALT multiple alignment, phylogenetic tree was generated which was incorporated into the same program itself. The phylogenetic guide tree was computed by the Fast Minimum Evolution (FME) method (**Desper and Gascuel, 2004**) which is a distance-based method for phylogeny reconstruction applied on large sets of taxa. The evolutionary distance between two sequences was calculated using the Grishin (protein) algorithm (**Grishin, 1999**). Four methods i.e., Rectangle, Slanted, Radial and Force were used for rendering the phylogenetic tree and all of them show the same guide tree compute with a method selected in 'Tree method' option. Rectangle view is the rectangular shaped rooted tree, where root is placed in the longest edge. The Slanted view is similar to rectangle, but with triangular shape. The radial view is un-rooted tree. The Force view is similar to radial, where nodes are pushed away from one another for the improved appearance of the tree. The phylogenetic tree computed for all 40 FBA sequences were viewed in different forms and their evolutionary relationship was judiciously analyzed.

3.2.3. Proteomic analysis of Fructose biphosphate aldolase in CA-MRSA

3.2.3.1 Pfam prediction

The protein sequence of fructose biphosphate aldolase (FBA) in CA-MRSA was searched in the pfam database (http://pfam.sanger.ac.uk/) to identify the protein family of the protein. The Pfam database is a huge collection of protein families, each represented by *multiple sequence alignments* and *hidden Markov models (HMMs) (***Finn *et al.,* 2010***)*. This database has been classified into two classes Pfam-A and Pfam-B. ***Pfam-A*** entries are high quality, manually curated families. ***Pfam-B***

contains supplement of families which are commonly of lower quality. Using the pfam database searching, domain of the FBA has been identified which provides insight into their function.

3.2.3.2 Conserved domain prediction

In order to identify the conserved domain in fructose biphosphate aldolase (FBA) in CA-MRSA, the protein sequence was searched against the Conserved Domain Database (CDD) which has been provided by the NCBI. CDD is the collection of sequence alignments and profiles representing protein domains conserved during molecular evolution (http://www.ncbi.nlm.nih.gov/cdd) (**Marchler-Bauer *et al.*, 2011**). It encompasses alignments of the domains to known 3-dimensional protein structures in the MMDB database as well. The amino acid sequence of FBA in FASTA file format was submitted to the CDD v3.10 database using the default parameters such as E-value (Expect value) of 0.01 and maximum number of hits 500, and the result was obtained in concise mode.

3.2.3.3 Motif prediction

The motif(s) in fructose biphosphate aldolase (FBA) of CA-MRSA was identified by the Motif Search tool provided by GenomeNet (http://www.genome.jp/tools/motif/). It searches with a protein query sequence against Motif Libraries (**Bucher and Bairoch, 1994**). The amino acid sequence of FBA was submitted in FASTA file format, subsequently searched against the PROSITE pattern database, which is the secondary protein database derived from the Swiss-Prot database (primary database).

3.2.3.4 Prediction of physico-chemical properties

Various physico-chemical properties of the fructose biphosphate aldolase (FBA) in CA-MRSA were computed using the ProtParam tool, which is provided by ExPASy server (http://web.expasy.org/protparam/). This tool allows the computation of different physical and chemical parameters for a given protein that can be deduced from a protein sequence. Number of parameters were computed such as molecular weight, theoretical pI (Isoelectric point), amino acid composition, atomic composition, extinction coefficient, estimated half life, instability index, aliphatic index and grand average of hydropathicity (GRAVY) (**Gasteiger *et al.*, 2005**).

3.2.3.5 Secondary structure prediction of FBA

The secondary structures for fructose biphosphate aldolase (FBA) were predicted using the GOR V server (http://gor.bb.iastate.edu/) (**Kloczkowski *et al.*, 2002**). The alpha-helices, beta-sheets, coils, and turns comprise the fundamental elements of the

secondary structure of proteins. The GOR (Garnier-Osguthorpe-Robson) method uses both information theory and Bayesian statistics for predicting the secondary structure of proteins (**Garnier *et al.*, 1978**). The knowledge of the secondary structure provides an input for prediction of three-dimensional structure of protein. By secondary structure prediction complex three-dimensional problem can be reduced to a much simpler one-dimensional problem, which helps to find the location of fundamental elements not in the three-dimensional space but along the protein amino acid sequence as well (**Kloczkowski *et al.*, 2002**).

3.2.3.6 Protein globularity and disorder prediction

The tendency of protein disorder and globularity of the fructose biphophate aldolase (FBA) in CA-MRSA was predicted by using the GlobPlot v2.3 server. It is a CGI (Common Gateway Interface) based server accessible at http://globplot.embl.de, that allows the user to plot the tendency within the query protein for order/globularity and disorder (**Linding *et al.*, 2003**). The amino acid sequence (in single letter IUPAC code) of FBA was submitted to the GlobePlot server using the following parameters:

JOB-ID:	Protein globularity and disorder prediction
Paramters:	propensities=Russell/Linding smooth=10 dy/dx_smooth=10
Disorder frames:	peak-frame=5 join-frame=4
Globularity frames:	peak-frame=74 join-frame=15
Name:	fructose bisphosphate aldolase
Plot title/ID:	none
Sequence length:	286

3.2.4 Homology modelling of candidate target Fructose biphosphate aldolase

The amino acid sequence of target protein fructose 1, 6-biphosphate aldolase (FBA) was retrieved from the UniProtKB database (Uniprot accession no: P67478) (www.uniprot.org). The length of target protein was 286 amino acid residues. In order to find the most similar template(s) for the target protein, database search was performed against the PDB database using the BLASTp program (**Altschul *et al.*, 1997**). Based on high sequence identity (76%) a template (pdb id: 3Q94) was selected, subsequently multiple sequence alignment was carried out between the target and template sequences using the clustalW2 program (http://www.ebi.ac.uk/Tools/msa/clustalw2/). The target-template aligned sequences were submitted in PIR/NBRF file format to the HHpred server (http://toolkit.tuebingen.mpg.de/hhpred). HHpred is an interactive server for

protein homology detection and structure prediction (**Soding *et al.,* 2005**). The 3D structural models of FBA were predicted by the MODELLER 9v9 software from those target-template alignments (http://www.salilab.org/modeller). To evaluate and identify any anomalies in the predicted model of FBA, it was submitted to the Structural Analysis and Verification Server (http://nihserver.mbi.ucla.edu/SAVES/). SAVES is a metaserver for analyzing and validating protein structures which integrates five modules i.e. Procheck, What_check, Errat, Verify_3D and Prove. To improve the quality of predicted model of FBA, energy minimization was performed with the GROMOS 96 forcefield (**van Gunsteren *et al.,* 1996**) implementation of DeepView v4.04 (spdb viewer) tool. This force field permits to evaluate the energy of the modeled structure as well as overhaul distorted geometries through energy minimization. All computations during energy minimization were done in vacuo, without reaction field. The minimized 3D structure of fructose biphosphate aldolase (FBA) has been revalidated by analysing the protein structure using the ProSA server. The ProSA (Protein Structure Analysis) is a tool which calculates an overall quality score (z-score) for a specific input structure. If this score is beyond a range characteristic for native proteins the structure might contain errors. A plot of local quality scores points to erroneous parts of the model (**Wiederstein and Sippl, 2007**). The predicted 3D structure of FBA was visualized by PyMOL v1.3 Viewer (http://www.pymol.org/).

3.2.5 Molecular dynamics simulation of predicted 3D structure of FBA

The molecular dynamics simulation of modelled 3D structure of the Fructose 1,6 biphosphate aldolase was performed using the Gromacs ver 4.53 program (**Berendsen *et al.,* 1995**) to track the motion of individual atoms. Two methods-energy minimization and molecular dynamics were employed to optimize structure and simulate the natural motion of atoms respectively. Before starting simulation, Gromacs environment was set up and input files necessary for the simulation were prepared, and the structure was solvated in water, minimized and equilibrated. In order to prepare the topology from the pdb file, 'pdb2gmx' tool was used. The solvent water was added around the protein to generate a simulation box using the 'genbox' program. The dimension of the box was set to as 0.9 nm from the protein molecules periphery. The energy minimization was performed in 1000 steps using the steepest descent minimization algorithm. GROMOS 96 force field (**van Gunsteren *et al.,***

1996) was chosen for the calculation of potential energy of the structure. A standard cut-off of 1.0 nm, both for the neighbourlist generation and the Coulomb & Lennard-Jones interactions was employed. The system was neutralized by adding a Cl^- ion in the model. To avoid the unnecessary distortion of the protein, an equilibrium run of water around the protein was performed using the 100 pico second (ps) time scale and 50000 steps (iterations). Finally molecular dynamics simulation was performed using the 2000 ps time scale and 1000000 steps at 300° K temperature and 1 atm. pressure. The simulation results were analyzed using the 'grace' program.

3.2.6 Virtual screening of fructose biphosphate aldolase as a drug target

In order to perform the virtual screening for fructose 1, 6-biphosphate aldolase (FBA) in CA-MRSA, the active site was predicted in the modelled structure using the metaPocket 2.0 server (http://projects.biotec.tu-dresden.de/pocket/). The metaPocket server (**Huang, 2009; Zhang *et al.*, 2011**) is a meta server to identify ligand binding sites on protein surface based on a consensus method, in which the predicted binding sites from eight methods: LIGSITEcs, PASS, QSiteFinder, SURFNET, Fpocket, GHECOM, ConCavity and POCASA are combined together to improve the prediction success rate. In the 3D structure of 1, 6-biphosphate aldolase, 3 potential ligand binding sites were predicted and ranked according to their z-score. Out of all 3 predicted active sites, second active site was chosen for the screening of a set of ligand databases. This active site was predicted by LIGSITEcs (LCS) and SURFNET (SFN) methods and average was taken from these two methods.

Using the protein-ligand docking method, virtual screening was performed for the fructose 1, 6-biphosphate aldolase against the NCI diversity subset-II molecules retrieved from the ZINC databases. ZINC is a free database of commercially-available compounds for virtual screening (**Irwin and Shoichet, 2005**). ZINC contains over 21 million purchasable compounds in ready-to-dock, 3D formats. The ZINC database is provided by the Shoichet Laboratory at the University of California, San Francisco (UCSF) (http://zinc.docking.org/). The virtual screening was carried out using the Autodock-vina package (http://vina.scripps.edu/). Before performing the screening process, a set of 1,364 compounds (NCI Diversity subset-II) available in mol2 file format were converted into pdbqt file format using a small python script prepare_ligand4.py. The receptor molecule (target) was also converted into pdbqt format using prepare_receptor4.py script available in Autodock Tools package. After

performing the virtual screening, a python script in the MGL tools package was used to analyze the best docked ligands for the fructose 1, 6-biphosphate aldolase (FBA) based on their energy score. Top ranked ligand-receptor complexes were further analyzed to study the protein-ligand interactions using the LigPlot^{+} v.1.4.3 program (**Laskowski and Swindells, 2011**). LigPlot^{+} is a successor to the original LIGPLOT program for automatic generation of 2D ligand-protein interaction diagrams (http://www.ebi.ac.uk/thornton-srv/software/LigPlus/). Using this program the hydrogen and hydrophobic interactions between the ligand and amino acid residues within the active site of the FBA were analyzed.

3.2.7 *In silico* prediction of Pharmacokinetic properties

The pharmacokinetic properties i.e. absorption, distribution, metabolism, excretion and toxicity (ADMET) of the top screened ligands were evaluated by using the PreADMET server (http://preadmet.bmdrc.org) (**Lee *et al.*, 2003**). Since large number of drugs fail to clear the various phases of clinical trials due to unacceptable pharmacokinetic properties, therefore scientists now consider absorption, distribution, metabolism, excretion and toxicity (ADMET) properties of drugs as prerequisite conditions to consider any ligand as lead molecule. The PreADMET server can predict the human intestinal absoption (HIA), plasma protein binding, permeability for different cells such as skin permeability, blood brain barrier, Caco-2 cell etc. It also predicts mutagenecity and carcinogenicity of ligands, helping researchers to avoid the toxicity in the advance stage of drug development.