JOURNAL OF PURE AND APPLIED MICROBIOLOGY, FEBRUARY 2014. Vol. **8**(1), p. 651-658

In silico Structural Analysis of Ketol-acid Reductoisomerase (KARI) of *Staphylococcus aureus* and it's Docking to Analyze its Drug Targeting Ability

Y. Sandhya Rani and P. Vijaya Lakshmi

Department of Microbiology, Vivekanandha College of Arts and Sciences for Women (Periyar University), Elayampalyam, Tiruchengode, Namakkal, Tamilnadu, India.

(Received: 18 August 2013; accepted: 29 October 2013)

Ketol-acid reductoisomerase (KARI) is a promising target for the design of drugs and herbicides yet there are only few reports on the molecular design of KARI inhibitors. Ketol-acid reductoisomerase(KARI) of *S. aureus* was isolated but its protein is not having any predicted 3-Dimentional structure available in PDB (Protein databank) as elucidated by X-ray crystallography or NMR. Its structure was determined *in silico* by sequence homology. The gene sequence of the KARI of *S. aureus* was known and its protein sequence was subjected to PSI-BLAST at NCBI. There was neither identical sequence available nor the nearest neighbour in the blast analysis. Then an alternative method for finding the homologous protein i.e., fold prediction method was used. The generated model was subjected to several repeated cycles of energy minimization using SPDBV software and the final model was subjected to stereo chemical evaluation. The homology modeled structure of the KARI of *S. aureus* was docked by different inhibitors by Molegro virtual docker and the data were presented.

Key words: Ketol-acid reductoisomerase, inhibitors, SPDBV, Molegro Virtual Docker.

Enzyme Ketol-acid reductoisomerase (KARI; EC 1.1.1.86) is encoded by *ilvC* gene (isoleucine-valine requirement)[1] that catalyzes the first step in the biosynthesis of amino acids such as valine, leucine and isoleucine which are branched-chain[2]. KARI catalyzes the conversion of 2-aceto-2-hydroxyacids to 2-keto-3-hydroxyacids and their subsequent reduction by NADPH TO 2, 3-dihydroxyacids[3]. It is also involved in the biosynthesis of pantothenate and coA[4]. These three amino acids are essential for mammals since they cannot synthesize them. Among the Staphylococci, the major human pathogen is *Staphylococcus aureus*, which causes infections ranging from cutaneous infections and food poisoning to life threatening septicaemia. *S. aureus* produces a large array of exotoxins and exoezymes[5]. The pathogenesis is elicited only with the involvement of these three amino acids. KARI has been considered as a target as a result of comparative pathway analysis between host and parasite[6]. The branched chain amino acid metabolic pathways in microbes are currently the objects of intense study for the development of drugs.

The present study is aimed at homology modeling of KARI of *S. aureus* and docking of various drugs in analyzing it as a suitable drug target.

* To whom all correspondence should be addressed.
E-mail: srb0104@gmail.com

MATERIALS AND METHODS

In silico analysis of Ketol-acid reductoisomerase (KARI) of *Staphylococcus aureus* by Homology modeling

The Ketol-acid reductoisomerase (KARI) of *Staphylococcus aureus* has no 3-Dimentional structure available in PDB (Protein databank), as the 3-Dimentional structure was not elucidated either by using X- ray crystallographic or NMR studies. The protein sequence was subjected to PSI-BLAST at NCBI from the DNA sequence obtained by sequencing. The DNA sequence was

```
ATGACAACAGTTTATTATGATCAAGATGTAAAAACGGACGCTTTACAAGGCAAAAAAATTGCAGTAGTAGGTTAT
GGATCACAAGGTCACGCGCATGCACAAAACTTAAAAGACAATGGATATGATGTAGTCATCGGCATTCGCCCAGGT
CGTTCTTTTGACAAAGCTAAAGAAGATGGATTTGATGTGTTCCCTGTTGCAGAAGCAGTTAAGCAAGCTGATGTA
ATTATGGTGCTATTACCTGATGAAATTCAAGGTGATGTATACAAAAACGAAATTGAACCAAATTTAGAAAAACAT
AATGCGCTTGCATTTGCTCATGGCTTTAACATTCATTTTGGTGTTATTCAACCACCAGCTGATGTTGATGTATTT
TTAGTAGCTCCTAAAGGACCGGGTCATTTAGTTAGACGTACATTTGTTGAAGGTTCTGCTGTACCATCACTATTT
GGTATTCAACAAGACGCTTCAGGTCAAGCACGTAATATTGCTTTAAGTTATGCAAAAGGTATTGGTGCAACTCGT
GCAGGTGTTATTGAAACAACATTTAAAGAAGAAACTGAGACAGATTTATTTGGTGAACAAGCAGTACTTTGCGGT
GGTGTATCGAAATTAATTCAAAGTGGCTTTGAAACATTAGTAGAAGCGGGTTATCAACCAGAATTAGCTTATTTT
GAAGTATTACATGAAATGAAATTAATCGTTGATTTGATGTATGAAGGCGGTATGGAAAATGTACGTTACTCAATT
TCAAATACTGCTGAATTTGGTGACTATGTTTCAGGACCACGTGTTATCACACCAGATGTTAAAGAAAATATGAAA
GCTGTATTAACTGATATCCAAAATGGTAACTTCAGTAATCGCTTTATCGAAGACAATAAAAATGGATTCAAAGAA
TTTTATAAATTACGCGAAGAACAACATGGTCATCAAATTGAAAAAGTTGGTCGTGAATTACGCGAAATGATGCCT
TTTATTAAATCTAAAAGCATTGAAAAATAA
```

The protein sequence was subjected to PSI-BLAST at NCBI. Protein parameters were analysed by using the tool Prosite[7]. All the protein parameters with respect to amino acid composition, secondary structure prediction, hydrophobicity, isoelectric point etc were analyzed. The generated model was subjected to several repeated cycles of energy minimization using SPDBV software[8] and the final model was subjected to docking.

Ligand Drawing

Inhibitors used in the study were amides which are having the ability to act as herbicides extracted from literature sources published elsewhere[9]. The ligands were searched against pubchem and chemspider database for the 2D structures and then with the help of open babel [http://openbabel.org/wiki/Main_Page] these 2D structures are converted to 3D structures. ISIS/ Draw is a user friendly drawing package that enables the drawing of chemical structures with the same specific signs and symbols employed in paper sketches. ISIS/Draw is primarily a 2D drawing program though it is equipped with some 3D rotation features and can interface with Rasmol for 3D visualization and rendering. Structures drawn were imported to TSAR software to convert them into 3D structures and energy of all molecules were also minimized[10]. The energy minimized ligand structures were employed for docking. The 2-dimensional inhibitor structures of the ligands were given (Fig.1)

1. 2-(4-Methoxy-benzoylamino)-benzoic acid; 2. N-(5-Methyl-thiazol-2-yl)-2-morpholin-4-yl-acetamide; 3. 2-(2-Piperidin-1-yl-ethyl)-isoindole-1,3-dione; 4. 5-(2-Morpholin-4-yl-acetyl)-5,10-dihydro-dibenzo[1,4]diazepin-11-one; 5. 5-(2-Piperidin-1-yl-acetyl)-5,10-dihydro-dibenzo[b,e][1,4]diazepin-11-one; 6. 2-(4-Benzyl-piperazin-1-yl)-N-(3,4-dichloro-phenyl)-acetamide; 7. 2-(4-Benzyl-piperazin-1-yl)-N-pyrimidin-2-yl-acetamide; 8. 2-(4-Benzyl-piperazin-1-yl)-N-(4-methyl-pyrimidin-2-yl)-acetamide

Docking of Ketol-acid reductoisomerase (KARI) of *Staphylococcus aureus* with certain Inhibitors (Amides)

The three dimensional structure of target Ketol-acid reductoisomerase (KARI) of *Staphylococcus aureus* was generated by homology modelling using SPDBV (Swiss Protein Data Bank Viewer) at 2.6 ú RMSD resolution. The 3D structures of ligands which are obtained were minimized using Hyperchem's MM+ force field. Molegro Virtual Docker V4.2 was used to detect the active sites and docking was performed by moldock function, which is an implementation of evolutionary algorithms (EAs), focused on molecular docking simulations. Docking was performed with all the potential active sites detected on Ketol-acid reductoisomerase (KARI) of *Staphylococcus aureus* enzyme. During

Docking at first the molecules were prepared and bonds, bond orders, explicit hydrogens, charges, flexible torsions, were assigned if they were missing by the MVD program to both the protein and ligands. From the docking wizard ligands were selected and the scoring function used is Moldock score. The model was calibrated using a data set from the PDB bind database with known binding affinities (expressed in kJ/mol)[11].

Docking analysis was performed using default parameters of Molegro. Before docking, the protein structure was searched for possible cavities using cavity detection algorithm of Molegro. A big sub surface cavity of 2266 ú was resulted as first cavity and the remaining possible four cavities are found to be very small. Hence, NADPH bound to 1NP3 was extracted and utilized as ligand for active site identification on model protein. NADPH is allowed to rotate, vibrate and translate in all possible degrees of freedom and the best pose was evaluated based on dock score or binding energies reported as kcal/mol. The experiment was performed thrice (Table.1).

RESULTS AND DISCUSSION

In silico analysis of Ketol-acid reductoisomerase (KARI) of *Staphylococcus aureus* by Homology modeling

The translated protein sequence was

MTTVYYDQDVKTDALQGKKIAVVGYGSQGHAHAQNLKDNGYDVVIGIRPGRSFDKAKEDGFDVFPVAEAVKQADVIMVLLP
DEIQGDVYKNEIEPNLEKHNALAFAHGFNIHFGVIQPPADVDVFLVAPKGPGHLVRRTFVEGSAVPSLFGIQQDASGQARNIAL
SYAKGIGATRAGVIETTFKEETETDLFGEQAVLCGGVSKLIQSGFETLVEAGYQPELAYFEVLHEMKLIVDLMYEGGMENVRYSIS
NTAEFGDYVSGPRVITPDVKENMKAVLTDIQNGNFSNRFIEDNKNGFKEFYKLREEQHGHQIEKVGRELREMMPFIKSKSIEK

ProtParam

The parameters computed by ProtParam include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY). Molecular weight and theoretical pI are calculated as to compute pI/Mw[12].

The number of amino acids present in the Organophosphorus hydrolase of *Kocuria* sp. was 325 with a molecular weight: 35597.9 whose theoretical pI was 9.82.

Amino acid composition

Ala (A)	25	7.5%
Arg (R)	12	3.6%
Asn (N)	15	4.5%
Asp (D)	20	6.0%
Cys (C)	1	0.3%
Gln (Q)	16	4.8%
Glu (E)	29	8.7%
Gly (G)	32	9.6%
His (H)	9	2.7%
Ile (I)	21	6.3%
Leu (L)	21	6.3%
Lys (K)	23	6.9%
Met (M)	8	2.4%
Phe (F)	19	5.7%
Pro (P)	13	3.9%
Ser (S)	14	4.2%
Thr (T)	13	3.9%
Trp (W)	0	0.0%
Tyr (Y)	12	3.6%
Val (V)	31	9.3%

Total number of negatively charged residues (Asp + Glu): 49

Total number of positively charged residues (Arg + Lys): 35

Atomic composition

Carbon	C	1656
Hydrogen	H	2576
Nitrogen	N	442
Oxygen	O	503
Sulfur	S	9

Formula: $C_{1656}H_{2576}N_{442}O_{503}S_9$

Total number of atoms: 5186

Extinction coefficients

This protein does not contain any Trp residues. Experience shows that this could result in more than 10% error in the computed extinction coefficient. Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm measured in water.

Ext. coefficient 17880

Abs 0.1% (=1 g/l) 0.483, assuming all pairs of Cys residues form cystines.

Ext. coefficient 17880

Abs 0.1% (=1 g/l) 0.483, assuming all Cys residues are reduced.

Estimated half-life

The N-terminal of the sequence considered is M (Met).

The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro).

Instability index

The instability index (II) is computed to be 38.66

This classifies the protein as stable.

Aliphatic index: 83.44

Grand average of hydropathicity (GRAVY): -0.304

SOPMA

The Sequence length was 334 whose Alpha helix (Hh) accounts 170 amino acids of about 50.90%. The extended strand (Ee) had 52 amino acids accounting 15.57%, Beta turn (Tt) made up of 28 amino acids making up 8.38% and random coil (Cc) made up of 84 amino acids accounting 25.15%. There was no 3_{10} helix (Gg), Pi helix(Ii), Beta bridge (Bb), Bend region (Ss), Ambigous states and other states. The parameters were window width of 17 with a similarity threshold 8 and the number of states is 4[13] (Fig.2).

PepWheel

PepWheel draws a helical wheel diagram for a protein sequence. This displays the sequence in a helical representation as if looking down the axis of the helix. It is useful for highlighting amphipathicity and other properties of residues around a helix. By default, aliphatic residues are marked with squares; hydrophilic residues are marked with diamonds, and positively charged residues with octagons, although this can be changed (Fig.3).

Dimensional Structure determination of Organophosphorus hydrolase of *Kocuria* sp

PROSITE method (with tools and information) covered by this documentation for

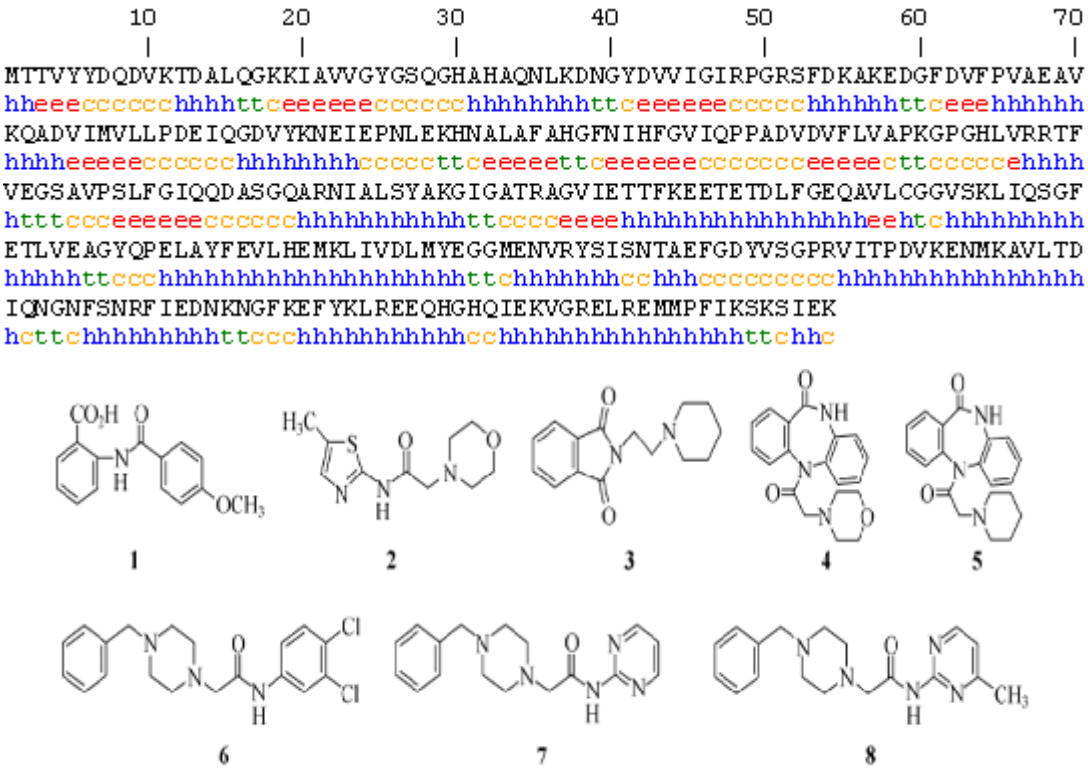

Fig. 1. Ligands : 1. 2-(4-Methoxy-benzoylamino)-benzoic acid; 2. N-(5-Methyl-thiazol-2-yl)-2-morpholin-4-yl-acetamide; 3. 2-(2-Piperidin-1-yl-ethyl)-isoindole-1,3-dione; 4. 5-(2-Morpholin-4-yl-acetyl)-5,10-dihydro-dibenzo[1,4]diazepin-11-one; 5. 5-(2-Piperidin-1-yl-acetyl)-5,10-dihydro-dibenzo[b,e][1,4]diazepin-11-one; 6. 2-(4-Benzyl-piperazin-1-yl)-N-(3,4-dichloro-phenyl)-acetamide; 7. 2-(4-Benzyl-piperazin-1-yl)-N-pyrimidin-2-yl-acetamide; 8. 2-(4-Benzyl-piperazin-1-yl)-N-(4-methyl-pyrimidin-2-yl)-acetamide

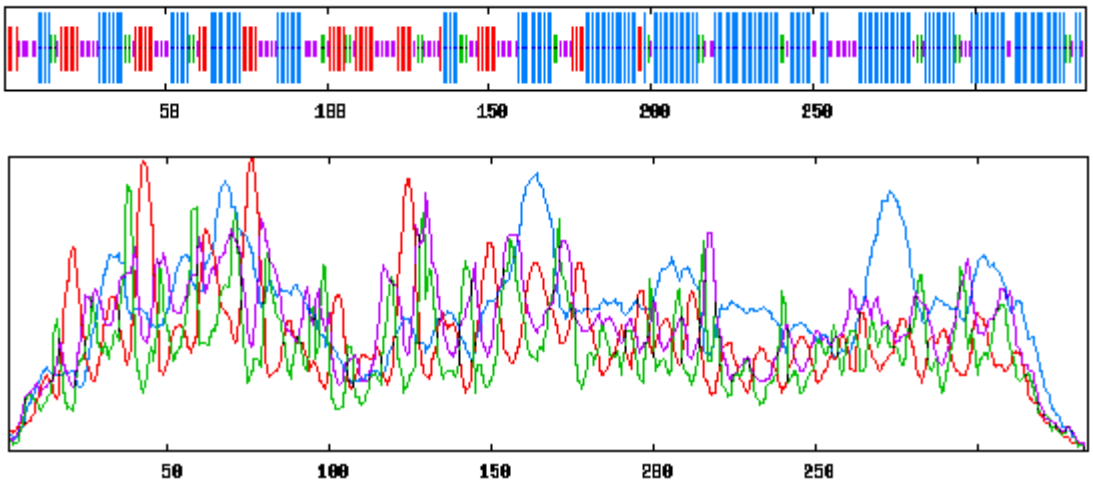

Fig.2. Significant improvement in protein secondary structure prediction by consensus prediction from multiple alignments (SOPMA)

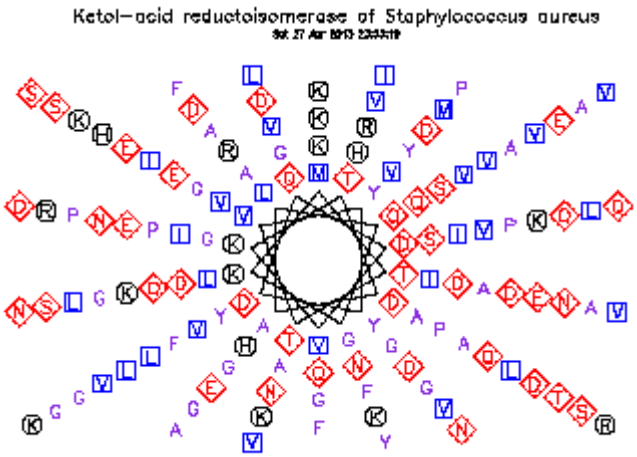

Fig.3. PepWheel for Ketol-acid reductoisomerase (KARI) of *Staphylococcus aureus*

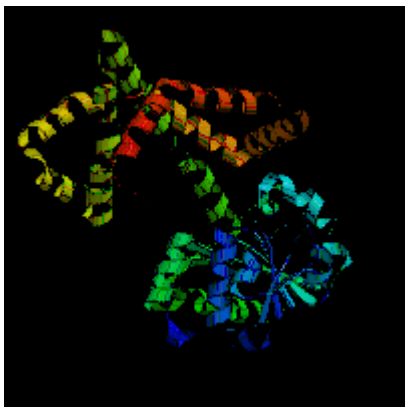

Fig.4. *In silico* 3-Dimensional Structure of Ketol acid Reductoisomerase of *Staphylococcus aureus*

the active site residues Gln28, Leu79, Leu80, Asp82, Ala106, His107, Pro129, Lys130, Gly131, Pro132, Glu186, Asp190, Glu194, Cys199. This was confirmed by performing Pfam - Protein Family Analysis (http://pfam.sanger.ac.uk/family?acc=PF00704)[14]. Alignment of Protein Sequence Ketol-acid reductoisomerase (KARI) of *Staphylococcus aureus* and template >1NP3A with chain length of 327 is as follows.

```
Score =  504 bits (1299), Expect = e-144,   Method: Composition-based stats.
Identities = 185/327 (56%), Positives = 236/327 (72%), Gaps = 1/327 (0%)

Query: 2   TTVYYDQDVKTDALQGKKIAVVGYGSQGHAHAQNLKDNGYDVVIGIRPGRS-FDKAKEDG 60
             V+YD+D     +QGKK+A++GYGSQGHAHA NLKD+G DV +G+R G +   KA+  G
Sbjct: 1   MRVFYDKDCDLSIIQGKKVAIIGYGSQGHAHACNLKDSGVDVTVGLRSGSATVAKAEAHG 60

Query: 61  FDVFPVAEAVKQADVIMVLLPDEIQGDVYKNEIEPNLEKHNALAFAHGFNIHFGVIQPPA 120
             V  V  AV  ADV+M+L PDE QG +YK EIEPNL+K   LAFAHGF+IH+  + P A
Sbjct: 61  LKVADVKTAVAAADVVMILTPDEFQGRLYKEEIEPNLKKGATLAFAHGFSIHYNQVVPRA 120

Query: 121 DVDVFLVAPKGPGHLVRRTFVEGSAVPSLFGIQQDASGQARNIALSYAKGIGATRAGVIE 180
           D+DV ++APK PGH VR  FV+G  +P L  I QDASG A+N+ALSYA G+G  R G+IE
Sbjct: 121 DLDVIMIAPKAPGHTVRSEFVKGGGIPDLIAIYQDASGNAKNVALSYACGVGGGRTGIIE 180

Query: 181 TTFKEETETDLFGEQAVLCGGVSKLIQSGFETLVEAGYQPELAYFEVLHEMKLIVDLMYE 240
           TTFK+ETETDLFGEQAVLCGG  +L+++GFETLVEAGY PE+AYFE LHE+KLIVDLMYE
Sbjct: 181 TTFKDETETDLFGEQAVLCGGCVELVKAGFETLVEAGYAPEMAYFECLHELKLIVDLMYE 240

Query: 241 GGMENVRYSISNTAEFGDYVSGPRVITPDVKENMKAVLTDIQNGNFSNRFIEDNKNGFKE 300
           GG+ N+ YSISN AE+G+YV+GP VI  + +  M+  L  IQ+G ++  FI +    +
Sbjct: 241 GGIANMNYSISNNAEYGEYVTGPEVINAESRAAMRNALKRIQDGEYAKMFITEGAANYPS 300

Query: 301 FYKLREEQHGHQIEKVGRELREMMPFI 327
               R     H IE++G +LR MMP+I
Sbjct: 301 MTAYRRNNAAHPIEQIGEKLRAMMPWI 327
```

Table 1. H-bond interactions and dock score (kcal/mol) of NADPH against Ketol-acid reductoisomerase (KARI) of *Staphylococcus aureus*

Ligand	Dock score (kcal/mol)	H-bond interactions
NADPH	-155.482	Gln28, Leu79, Leu80, Asp82, Ala106, His107, Pro129, Lys130, Gly131, Pro132, Glu186, Asp190, Glu194, Cys199
Ligand 1	-72.979	Gln28, Lys130
Ligand 2	-57.076	Nil
Ligand 3	-57.636	Nil
Ligand 4	-64.524	Nil
Ligand 5	-70.126	Gln28
Ligand 6	-74.799	Asp82
Ligand 7	-62.147	Asp82
Ligand 8	-85.161	His107, Asp190, Glu194

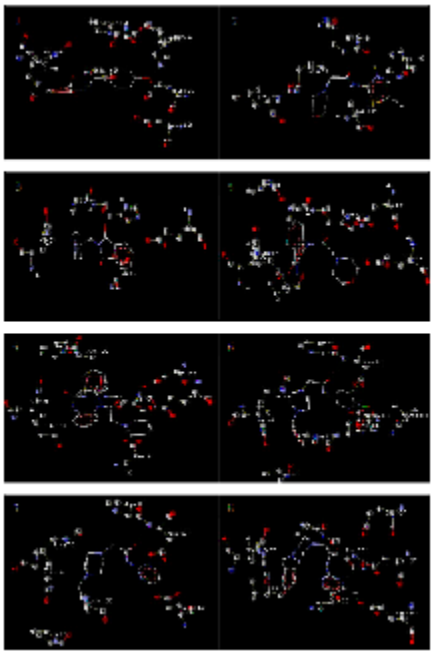

Fig.5. Docking of Drugs onto Ketol-acid reductoisomerase (KARI) of *Staphylococcus aureus*.

Then an alternative method for finding the homologous protein i.e., fold prediction method was used. There is an automated server for protein modeling which searches the homologous protein by fold prediction and sequences are modeled with high degree of accuracy. The generated model was subjected to several repeated cycles of energy minimization using SPDBV software and the final model was subjected to stereo chemical evaluation. The fold prediction method found out Template 1NP3 from protein data bank of Crystal structure of class I acetohydroxy acid isomeroreductase from *Pseudomonas aeruginosa* was found to be the best homologue and modeling was carried out. The generated model was subjected to several repeated cycles of energy minimization using modeler software that is performed by satisfaction of spatial restraints and the final model was subjected to stereo chemical evaluation[15]. After Energy minimization, the energy of the protein model is found to be -1.10 KJ/mol that fits Ramachandran Plot (Fig.4).

Docking of ligands onto Ketol-acid reductoisomerase (KARI) of *Staphylococcus aureus* with certain Inhibitors (Amides)

Docking studies Ketol-acid reductoisomerase (KARI) of *Staphylococcus aureus* were initiated using inhibitors reported in literature, designated as ligand 1 to 8 respectively. Due to the presence of big cavity within the protein, template docking was performed by considering NADPH as template (Table.1; Fig.5).

From Table 1, it was evidenced that ligands 2,3,4 and 7 displayed weak to average activity with model protein whereas ligands 1, 5, 6 and 8 represented moderate activity when compared to NADPH (dock score: -155.482 kcal/mol). It was observed that ligands 2, 3 and 4 are devoid of any H-bond interactions and remaining all showed one h-bond except ligand 8. The high score (-85.161 kcal/mol) of ligand 8 might be attributed to the mode of h-bond interaction with His107, Asp190 and Glu194 residues, respectively.

CONCLUSION

The 3- dimensional structure Ketol-acid reductoisomerase (KARI) of *Staphylococcus aureus* was predicted by using SPDBV. Later by using this model it was docked by different ligands which are amides acting as amides. The *in silico* model proved that this enzyme is an effective drug target and thereby many diseases caused by different bacteria can be treated and cured.

REFERENCES

1. Riley BM, Mansilla MA, Raffensperger L, Maher B, Marazita ML, Mohammadi M, Murray JC. Mutations in FGF signaling pathway genes contribute to cleft lip and palate. Plenary Presentation, 2006 ASHG Annual Meeting, New Orleans, LA.
2. Satyanarayana T, Radhakrishnan AN. Biosynthesis of valine and isoleucine in plants. 3. Reductoisomerase of Phaseolus radiatus. *Biochim. Biophys. Acta.,* 1965: **110** (2): 380-388.
3. Hill RK, Sawada S and Arfin SM. Stereochemistry of valine and isoleucine biosynthesis. IV Synthesis, configuration, and enzymatic specificity of alpha-acetolactate and alpha-aceto-alpha-hydroxybutyrate. *Bioorg. Chem.,* 1979: **8** (2): 175–189.
4. Primerano B, Tassone F, Hagerman RJ, Hagerman P, Amaldi F, Bagni, C. Redced *FMR1* mRNA translation efficiency in fragile X patients with premutations. *RNA.*, 2002: **8**:1482–1488.
5. Zhang YQ, Ren SX, Li HL, Wang YX, Fu G, Yang J, Qin ZQ, Miao YG, Wang WY, Chen RS, Shen Y, Chen Z, Yuan ZH, Zhao GP, Qu D, Danchin A, Wen YM. Genome-based analysis of virulence genes in a non-biofilm-forming *Staphylococcus epidermidis* strain (ATCC 12228). *Molecular microbiology*., 2003: **49**(6):1577-1593.
6. Morya VK, Kumari S, Kim Eun-Ki . Imperative pathway analysis to identify the potential drug target for Aspergillus infection. *International Journal of Latest Trends in Computing*.,2011: **2**(1):178-182.
7. www.expasy.org/prosite
8. Guex N and Peitsch MC. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. *Electrophoresis*,1997: **18**(15):2714-23.
9. Baolei W, Yi MA, Yonghong LI, Suhua W, Zhengming LI. The design, synthesis of amide KARI inhibitors and their biological activities. *Front. Chem. China.*, 2009: **4**(2): 186–190.
10. Shoichet BK, Kuntz ID, Bodian DL. Molecular docking using shape descriptors. *J. Comput. Chem.*, 2004; **13** (3): 380–397.
11. Thomsen R, Christensen MH. MolDock: a new

technique for highaccuracy molecular docking. *J Med Chem*, 2006: **49**: 3315-3321.

12. Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD, Bairoch A. *Protein Identification and Analysis Tools on the ExPASy Server;* (In) John M. Walker (ed): The Proteomics Protocols Handbook, Humana Press.2005: 571-607
13. Geourjon C and Deleage G. SOPMA: significant improvements in protein secondary structure prediction by consensus prediction from multiple alignments. *Comput Appl Biosci*,1995: **11**(6):681-4.
14. Sigrist CJA, Cerutti L, Hulo N, Gattiker A, Falquet L, Pagni M, Bairoch A, Bucher P. *PROSITE: a documented database using patterns and profiles as motif descriptors. Brief Bioinform.*, 2002: **3**:265-274.
15. Sali A, Blundell TL. Comparative protein modelling by satisfaction of spatial restraints. *J Mol Biol.*, 1993: **234**(3):779-815.