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Original Article

RECEPTOR IDENTIFICATION AND LEAD MOLECULAR DISCOVERY OF PHAGE ENCODED PROTEIN IN *TCH8431/19A* STRAIN OF *STREPTOCOCCUS PNEUMONIAE*: A COMPUTATIONAL APPROACH

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ABSTRACT

Objective: Lead receptor identification and drug Discovery of Phage encoded protein in *TCH8431/19A* Strain of *Streptococcus pneumoniae* using computation analysis.

Methods: In this article we focus on the computational modeling of Phage encoded protein (D6ZPG9) and validating the nature of the proteins as a future drug target of TCH8431/19A in *Streptococcus pneumoniae*. We have also focused on identifying specific ligands for the above mentioned protein by using the statistical method of high throughput screening based on structure activity relationship along with the validation of ADMET descriptors.

Results: Based on docking studies, we conclude that 5-O-ethyl 3-O-methyl4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate with Phage encoded protein (D6ZPG9) in strain TCH8431/19A has a Dock score of 40.7 is the best ligand molecule.

Conclusion: Based on our Insilco analysis we conclude that Phage encoded protein can be considered as the best drug target and the above mentioned ligand having highest dock score may be considered as the drug candidate.

Keywords: Pneumonia, *Streptococcus pneumoniae,* Phage encoded protein, TCH8431/19A, Homology, Docking

INTRODUCTION

Pneumonia is an inflammation in lung which is caused by the infection with viruses, bacteria and other pathogens. *Streptococcus pneumoniae* is the most significant microbe which is responsible for causing inflammatory disorders in humans [1,2]. *S. pneumoniae* is mainly responsible for causing bacterial infections in humans. The equilibrium shift of *S. pneumoniae* from a commensal bacterium to an opportunistic pathogen occurs in the respiratory tract of humans.

Host tissues are invaded and colonizd by pathogens with various factors for causing virulence. These virulence factors are displayed on the cell surface with adhesins that mediates its attachment to host cells. Pneumolysin is an important toxin and it is produced by all strains of *S. pneumoniae* [3]. As part of its life cycle, pneumococcus exists as a commensal bacterium that inhabits and colonizes the nasopharynx [4,5].

It has been estimated that more than a million people die every year from pneumococcal infections in a global spectrum [6,7]. General vaccination with the 7-valent pneumococcal conjugate vaccine was recommended in Germany during July 2006 for children greater than 2 years [8,9]. In United States and elsewhere, resistance to a range of antibiotics is increasing among the clinical isolates of *S. Pneumoniae* [10-13].

In genome of *S. pneumoniae* TCH8431*/19A* is virulent strain of single chromosome with 2088772 base pairs having 39.8% of GC content. In this present study the Phage encoded protein protein (D6ZPG9) is been studied. TCH8431/19A strain of *Streptococcus pneumoniae* is extremely virulent in pneumococcal pathogenesis which contains coding gene (HMPREF0837_10280) for the synthesis of Phage encoded protein. In this article we performed the homology modeling of above mentioned protein the potential of the receptor as a future drug target for TCH8431/19A strain of *Streptococcus pneumoniae*. We have also focused on identifying specific ligands for the above mentioned proteins by using the statistical method of high throughput screening based on structure activity relationship along with the validation of ADMET descriptors.

MATERIAL AND METHODS

Template for the target protein (Phage encoded protein (D6ZPG9) was identified on the basis of sequence similarity using PDBsum and cross validated with BLAST search [14,15]. Multiple sequence alignment was performed between the template and target protein using CLUSTAL W. Homology modeling of the target proteins were executed by MODELLER 9v7 [16]. .The structural confirmation of the modeled proteins were validated using Structural Analysis and verification Server on the basis of ψ and φ angles of amino acids in maximum favored regions [17].

In order to obtain stable confirmation, amino acids in partially allowed regions were subjected to energy minimization and certain residues of partial helical nature were subjected to loop refinement using Swiss PDB viewer [18]. Ligands of target proteins were obtained using DrugPort and their corresponding analogs were obtained using PubChem as shown in **Fig 1**. Binding pockets of the target protein were obtained using CASTp server and the binding energy of protein-ligand complex were obtained using ARGUS lab [19]. Various structural confirmations of the protein-ligand complex were subjected to pose based dock score in Ligand fit module of Discovery Studio under CHARMm force field and the energy function is based on pairwise structural analysis between the nonbonding interactions of protein-ligand complex [20,21]. Finally, ADMET properties of ligands were studied through DISCOVERY STUDIO [22,23].

RESULTS AND DISCUSSION

Homology modeling

Homology modeling was performed for Phage encoded protein (D6ZPG9) and was modeled using the template structure (PDB Id: 3PWQ: C). The modeled protein was validated through SAVS and the validation results are shown in **Table 1**. From the result it is found that 91.4% residues in target proteins are present in the allowed region of Ramachandran Plot. The final modeled protein structure and its corresponding Ramachandran plots are shown in **Fig 2.**

Ligand Search

Ligands for Phage encoded protein (Sequence ID: D6ZPG9) were retrieved from DrugPort sharing more identity with related protein sequence for which already a drug exists. The best analogs for each ligands were obtained from PubChem were chosen from the hit. The docking was performed with those analogs using Discovery Studio software. Dock score was calculated for all the analogs.

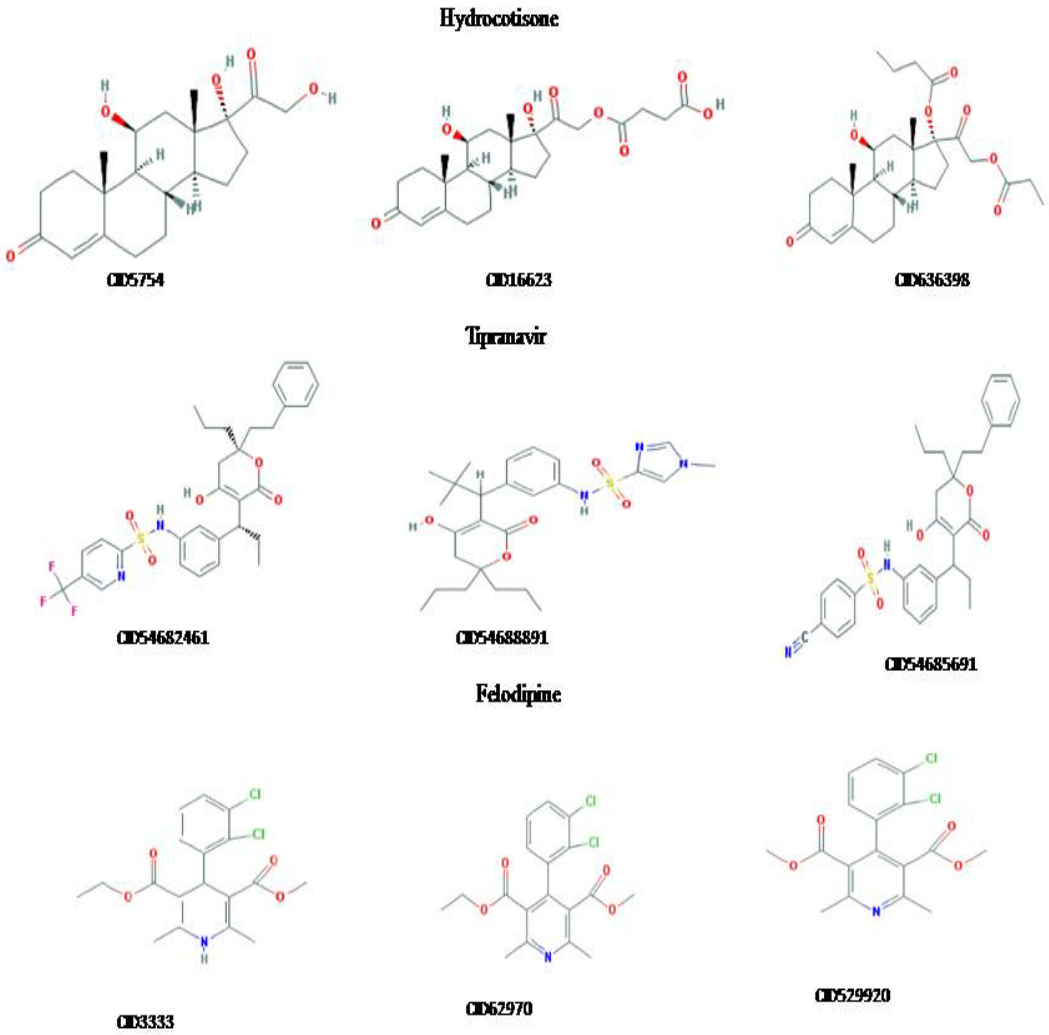
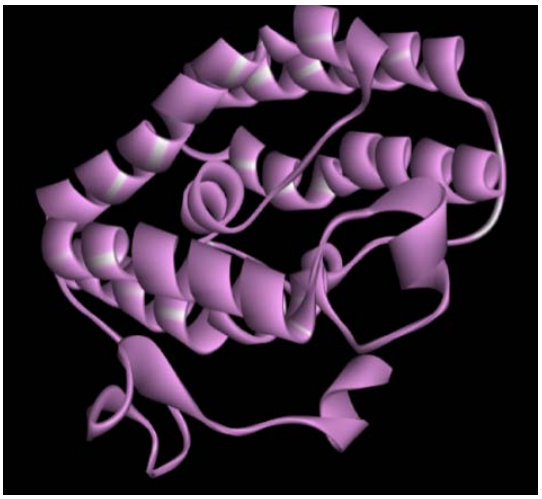


Fig. 1: Analogs for different ligands of Phage encoded protein (D6ZPG9).

Table 1: The percentage of residues of modeled structure present in the allowed region of Ramachandran plot as predicted by SAVS with its similarity.

Target name	Target id	Target length	Template	Template length	Identity	Ramachandran Plot %		
						MFR	AAR	GAR
Phage encoded protein	D6ZPG9	201	3PWQ(C)	304	31.0%	91.4	5.9	2.2

***MFR**:Most favoured regions, **AAR**: Additional allowed regions, **GAR**: Generously allowed regions*



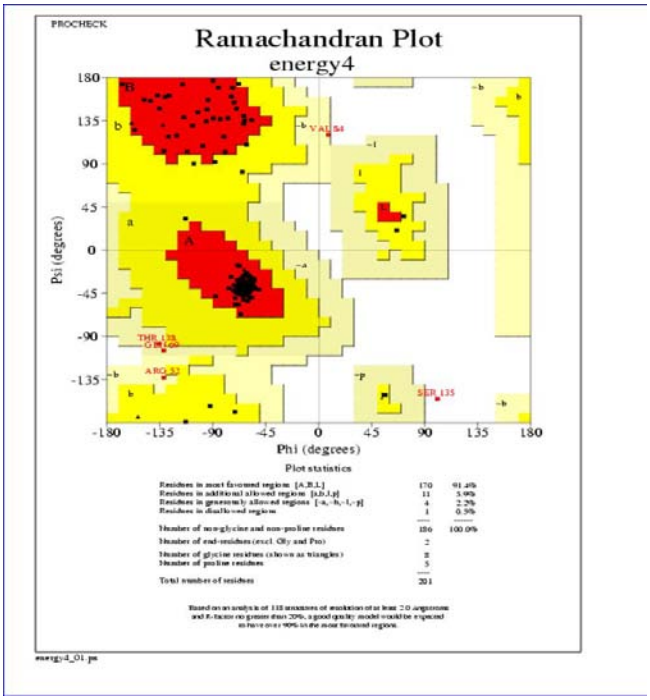


Fig. 2: Final structure of modeled protein and its Ramachandran plot

Receptor Ligand Docking

The Felodipine molecule had the best analog compounds 5-O-ethyl 3-O-methyl4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate, 3-O-ethyl 5-O-methyl4-(2,3-dichlorophenyl) -2,6dimethylpyridine-3,5-dicarboxylate and dimethyl 4-(2,3-dichlorophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate with dock score of 40.7, 34.639 and 39.126 respectively.

The Hydrocortisone molecule had the best analog compounds (8S,9S,10R,11S,13S,14S,17R)-11,17-dihy droxy-17-(2-hydroxyacetyl)-10,13- dimethyl-2,6,7,8,9,11,12,14,15,16- decahydro -1Hcyclopenta[a]phenanthren-3-one, 4-[2-[(8S,9S,10R,11S,13S,14S,17R)-11,17-dihydroxy-10,13-dimethyl-3-oxo-2,6,7,8,9,11,12,14,15,16-decahydro-1H-cyclopenta[a]phenanthren-17-yl]-2-oxoethoxy]-4-oxobutanoic acid and [(8S,9S,10R,11S,13S,14S,17R)-11-hydroxy-10,13-dimethyl-3-oxo-17-(2-propanoyloxyacetyl)-2,6,7,8,9,11, 12,14,15,16-decahydro-1Hcyclopenta [a]phenanthren-17-yl] butanoatewith dock score of 37.687, 36.149, and 25.971 respectively.

The Tipranavir molecule had the best analog compounds N-[3-[(1R)-1-[(2R)-4-hydroxy-6-oxo-2-(2-phenylethyl)-2-propyl-3H-pyran-5-yl]propyl]phenyl]-5-(trifluoromethyl) pyridine-2-sulfonamide, N-[3-[(1R)-1-[(2S)-4-hydroxy-6-oxo-2-(2-phenylethyl)-2-propyl-3H-pyran-5-yl]propyl]phenyl]-5-(trifluoromethyl) pyridine-2-sulfonamide,4-cyano-N-[3-[1-[4-hydroxy-6-oxo-2-(2-phenylethyl)-2-propyl-3H-pyran-5-yl]propyl]henyl] benzenesulfonamide with dock score of 33.512,31.735 and 34.689 respectively.

Docking results of Phage encoded protein (D6ZPG9) with respect to ligand with its analogs as shown in **Table 2** and Receptor ligand interactions between Phage encoded protein (D6ZPG9) and analogs with their distance are shown in **Table 3** and the interactions between the Phage encoded protein (D6ZPG9) and analogs of selected ligands are illustrated in **Fig 3**.

Table 2: Docking Analysis of Phage encoded protein (D6ZPG9) with respect to ligands with its analogs

Ligands	Analogues	PLP1	PLP2	Jain	-PMF	Dock score
Felodipine	5-O-ethyl 3-O-methyl4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate.	56.09	56.27	2.69	-14.84	40.7
	3-O-ethyl 5-O-methyl4-(2,3-dichlorophenyl) -2,6dimethylpyridine-3,5-dicarboxylate.	42.37	34.55	0.82	32.56	34.639
	dimethyl 4-(2,3-dichlorophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate.	45.76	41.18	-0.13	32.51	39.126
Hydrocortisone	(8S,9S,10R,11S,13S,14S,17R)-11,17-dihy droxy-17-(2-hydroxyacetyl)-10,13-dimethyl-2,6,7,8,9,11,12,14,15,16- decahydro -1Hcyclopenta[a]phenanthren-3-one	28.24	32.79	-0.78	51.27	37.687
	4-[2-[(8S,9S,10R,11S,13S,14S,17R)-11,17-dihydroxy-10,13-dimethyl-3-oxo-2,6,7,8,9,11,12,14,15,16-decahydro-1H-cyclopenta[a]phenanthren-17-yl]-2-oxoethoxy]-4-oxobutanoic acid	48.85	45.6	-0.91	21.68	36.149
	[(8S,9S,10R,11S,13S,14S,17R)-11-hydroxy-10,13-dimethyl-3-oxo-17-(2-propanoyloxyacetyl)-2,6,7,8,9,11, 12,14,15,16-decahydro-1Hcyclopenta [a]phenanthren-17-yl] butanoate	35.98	31.03	-1.74	51.47	25.971
Tipranavir	N-[3-[(1R)-1-[(2R)-4-hydroxy-6-oxo-2-(2-phenylethyl)-2-propyl-3H-pyran-5-yl]propyl]phenyl]-5-(trifluoromethyl) pyridine-2-sulfonamide.	36.78	35.36	-2.23	35.7	33.512
	N-[3-[(1R)-1-[(2S)-4-hydroxy-6-oxo-2-(2-phenylethyl)-2-propyl-3H-pyran-5-yl]propyl]phenyl]-5-(trifluoromethyl) pyridine-2-sulfonamide.	20.87	21.65	-4.43	40.12	31.735
	4-cyano-N-[3-[1-[4-hydroxy-6-oxo-2-(2-phenylethyl)-2-propyl-3H-pyran-5-yl]propyl]phenyl]benzenesulfonamide	30.77	26	-3.36	27.96	34.689

Table 3: Receptor ligand interactions between Phage encoded protein (D6ZPG9) and analogs.

Ligand	Analog	Receptor		Ligand	Distance
		Amino acid	Atom	Atom	
	CID3333	LEU54	H27	O	1.82808
Felodipine	CID62970	ILE194	HN	CI1	2.13986
	CID16623	ILE194	HN	CI1	2.14377
	CID5754	GLU196	HS6	OE2	0.996701
Hydrocortisone	CID16623	SER135	H67	O	2.47997
	CID36398	ILE194	HN	O6	2.24238
	CID54682461	TYR191	HN	F3	1.8974
Tipranavir	CID54688891	GLN152	HE21	O	6.16294
	CID54685691	THR138	HG1	N8	2.40917

ADMET properties for the analogs of ligands having better dock score and maximum interaction with the active site residues were analyzed. The plot of polar surface area (PSA) vs logP is shown in **Fig 4**. Based on our analysis, it has been found that the analogs which had maximum dock score have proper logP, Absorption and Blood Brain Barrier value.

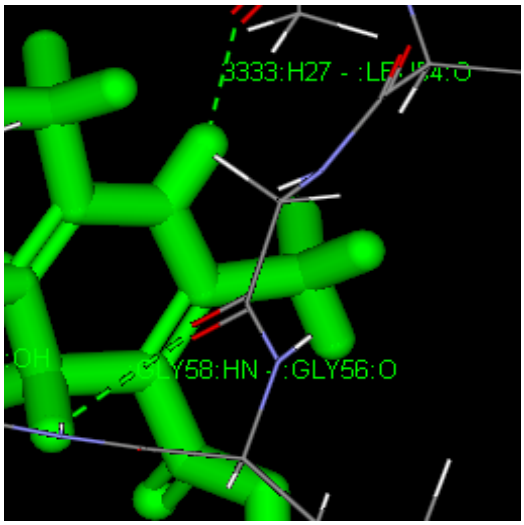


Fig. 3: Docking 5-O-ethyl 3-O-methyl4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate with D6ZS38 of strain TCH8431/19A.

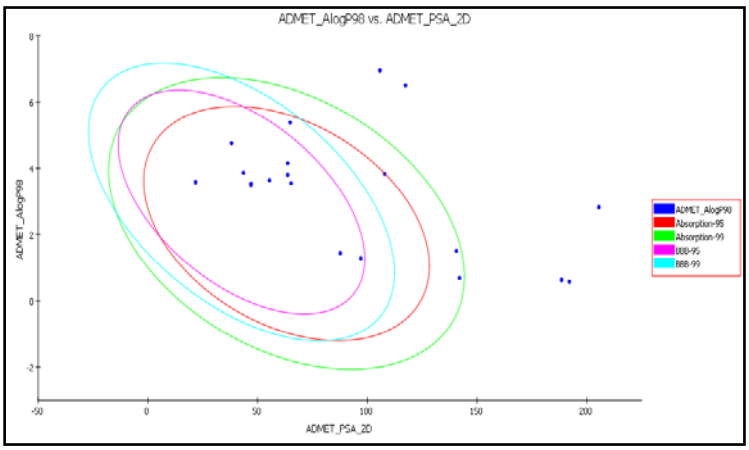


Fig. 4: ADMET plot for ligands.

CONCLUSION

Based on docking studies, we conclude that 5-O-ethyl 3-O-methyl4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate of Phage encoded protein (D6ZPG9) in strain TCH8431/19A with the Dock score 40.7 with 1 Hydrogen bond is the best ligand molecule. ADMET descriptors were also analyzed for the drug candidates. Hence, this protein can be considered as the drug targets and the above mentioned ligand having highest dock score may be considered as the drug candidate.

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REFERENCES

1. Avery OT, MacLeod CM, and McCarty M Studies on the chemical nature of the substance inducing transformation of pneumococcal types. J. Exp. Med 1944;79:137-158.
2. Klein DL Pneumococcal disease and the role of conjugate vaccines. Microb. Drug Resist 1999;5:147-157.
3. Shak JR, Herbert P. Ludewick, Kristen E. Howery, Fuminori Sakai, Hong Yi, Richard M. Harvey, et. al. Novel role for the Streptococcus pneumoniae toxin pneumolysin in the assembly of biofilms, MBio. 2013;4:e00655-13.
4. Cardoso TC, Lopes LM Carneiro AH:A case-control study on risk factors for early-onset respiratory tract infection in patients admitted in ICU. BMC Pulm. Med. 2007;7:12-17.
5. Del Toro MD, Rodriguez-Bano J. Martinez-Matinez L, Pasculal A, Perez-Canoa R, Perea EJ, Muniain MA Epidemiology, chinical features and prognosis of infections due to Stenotrophomonas lmaltophilia. Enferm Infect. Clin. Microbiol. 2006;24:4-9.
6. Orihuela CJ, Gao KP Francis, Yu J, Tuomanen EI. Tissue-specific contribution of pneumococcal virulence factors to pathogenesis. J. Infect. Dis. 2004;190:1661-1669.
7. Orihuela C. J, Gao G, McGee M, Yu J, Francis KP, Tuomanes E Organ-specific models of Streptococcus pneumoniae disease. Scand. J. Infect. 2003;35:647-652.
8. Blue CE, Paterson GK, Kerrm AR, Berge M, Claverys JP, Mitchell TJ et. al. A novel virulence factor of Streptococcus pneumoniae that induces tumor necrosis factor alpha production in the respiratory tract. Infect. Immun. 2003;71:4925-35.
9. LeMessurier KS, Ogunniyi AD, Paton JC Differential expression of key pneumococcal virulence genes in vivo. Microbiology 2006;152:305-11.
10. Nunes S, Sá-Leão R, Carriço J, Alves CR, Mato R, Brito Avô A et. al. Trends in Drug Resistance, Serotypes, and Molecular Types of Streptococcus pneumoniae Colonizing Preschool-Age Children Attending Day Care Centers in Lisbon, Portugal:a Summary of 4 Years of Annual Surveillance, J Clin Microbiol. 2005;43:1285-1293.
11. Robertson GT, Ng WL, Foley J, Gilmour R, Winkler ME Global transcriptional analysis of clpP mutations of type 2 Streptococcus pneumoniae and their effects on physiology and virulence. J. Bacteriol. 2002;184:3508-3520.
12. Blue CE, Mitchell TJ Contribution of a response regulator to the virulence of Streptococcus pnuensoniae is strain dependent. Infect. Immun. 2003;71:4405-4413.
13. Kuo LC, Lai CC. Liao Ch, Hsu Ck, Chang YL, Change CY, Hsueh RR Multidrug-resistant Acinetobacter baumannii bacteremia:clinical feature, antimicrobial therapy and outcome. Clin. Microbiol. Infect. 2007 13:196-198.
14. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, et al. "Gapped BLAST and PSI-BLAST:a new generation of protein database search programs.", Nucleic Acids Res. 1997;25(17):3389-402.
15. Hava DL, Camilli A. Large-scale identification of serotype 4 Streptococcus pneumoniae virulence factors. Mol. Microbiol. 2002;45:1389-1406.
16. Mondal UK, Sen A & Bothra AK Homology modeling of the Cytolethaldistending toxin B gene of Helicobacter hepaticus ATCC 51449 International Journal of Interrogative Biology 2010 10:35-40.
17. Laskoswki RA, MacArthur MW, Moss DS and Thorton JM. PROCHECK:a program to check the stereochemical quality of protein structures. J. Appl. Cryst. (1993).26, 283-291.13.
18. Fiser A, Do RK, Sali A. "Modeling of loops in protein structures", Protein Science. 2000;9:1753-1773.
19. Liang J, Edelsbrunner H, Woodward C."Anatomy of protein pockets and cavities:Measurement of binding site geometry and implications for ligand design". Protein Science. 1998;7:1884-1897.
20. Evensen E, Joseph-McCarthy D, Weiss GA, Schreiber SL, Karplus M. Ligand design by a combinatorial approach based on modeling and experiment:application to HLA-DR4. J. Comput. Aided Mol. Des. 2007;21:395-418.
21. Zheng CH, Zhou YJ, Zhu J, Ji HT, Chen J, Li YW. et al. Construction of a three-dimensional pharmacophore for Bcl-2 inhibitors by flexible docking and the multiple copy simultaneous search method. Bioorg. Med. Chem. 2007;15:6407-6417.
22. Borodina Y,Rudik A, Filimonov D, Kharchevnikova N, Dmitriev A,Blinova V. et al. A new statistical approach to predicting aromatic hydroxylation sites, Comparison with model-based approaches. J. Chem. Inf. Comput. Sci. 2004;44:1998-2009.
23. Venkatapathy R, Moudgal CJ, Bruce RM. Assessment of the oral rat chronic lowest observed adverse effect level model in TOPKAT, a QSAR software package for toxicity prediction. J. Chem. Inf. Comput. Sci. 2004;44:1623-1629.