ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Issue 2, 2016

Online - 2455-3891
Print - 0974-2441

Research Article

IN SILICO INTERACTION ANALYSIS OF HERBAL BIOACTIVE MOLECULES WITH PENICILLIN-BINDING PROTEIN IN *STAPHYLOCOCCUS AUREUS*

FLORIDA TILTON[1]*, RANJITH KUMAR M[2], JEYANTHI V[2], LAKSHMI PRIYA K[1]

[1]Biozone Research Technologies Pvt. Ltd., Chennai, Tamil Nadu, India.[2]Department of Microbiology, Asan Memorial College of Arts & Science, Chennai, Tamil Nadu, India. Email: biozone.research@gmail.com

Received: 22 September 2014, Revised and Accepted: 02 November 2014

ABSTRACT

Objective: The present study involves the analysis of mecA gene mediated antibiotic resistance in *Staphylococcus aureus* and screening for potential ligands.

Methods: Swab samples of patients with foot ulcers were collected from hospitals in and around Chennai. The samples were collected in sterile containers containing Enriched Thioglycollate broth transport media and transported to the laboratory within two hours of collection and processed.

Results: The results indicate the interaction capacity of Rosmaric acid with Penicillin binding protein. The 2D interaction results indicate the information about non bonded interactions of ligand and receptor. Best interaction was obtained with Rosmaric acid with a score of 41.16 and 5 h-bond interactions were obtained. The Molecular docking results indicates the interaction of the inhibitors to the antibiotic resistant proteins with a positive fitness.

Conclusion: From the current study, molecular docking approach has proved to be an efficient method for the identification of novel lead compounds against β-lactam resistant *S. aureus* from a broad spectrum of plant compounds and among the studied inhibitors rosmarinic acid was found to be a potent lead molecule.

Keywords: Mec A, PBP (penicillin binding protein), Docking.

INTRODUCTION

Staphylococcus aureus is a common nosocomial pathogen. *Staphylococcal* infection markedly increases the morbidity and mortality in hospitalized patients [1]. The organism has emerged as one of the important and major threat to human community due to rise in antibiotic resistance [2]. Multi-drug resistance in bacteria is usually conferred by enzymes encoded from chromosomal or extrachromosomal genes [3]. *Staphylococcus* causes frequent infections on prosthetic devices, osteomyelitis, and endocarditis [4]. Multi resistance has become a common feature in *S. aureus* [5]. Methicillin-resistant *S. aureus* (MRSA) has now emerged as a widespread cause of community infections. The growing problem in the Indian scenario is that MRSA prevalence has increased from 12% in 1992 to 80.83% in 1999 [6]. It is responsible for a wide spectrum of infections and has a remarkable genetic versatility which allows them for the adaptation to multiple antibiotics. Most of the *S. aureus* isolates are found to be resistant to antibiotics [7] and it has been classified as one of the major public health threats of the 21st century [8]. Therefore, antibiotic options in the treatment against these organisms are extremely limited.

Bacterial membrane protein plays an important role in growth, cell division and maintaining the cellular structure of bacteria. These proteins are major drug targets for controlling the pathogen. Two mechanisms have been reported to be responsible for antibiotic resistance: Structural modification in penicillin-binding protein (PBP) targets and production of β-lactamase [9]. β-lactam antibiotics (penicillin, cephalosporins, carbapenems, and monobactams) target PBP, which are responsible for bacterial cell wall synthesis [10]. It is observed that the resistances shown by *S. aureus* have been increasing to most of the β-lactam antibiotics [11-13]. The broad-spectrum beta-lactam resistance in MRSA strains is due to the presence of PBP [14]. In both Gram-positive and Gram-negative bacteria, PBP is viewed as an attractive target for the development of antimicrobial compounds [13,15]. Therefore, the more research in this field is required to identify new therapeutic agents for controlling infections caused by *S. aureus*. Hence, this study focuses on finding lead compound against PBP in *S. aureus* by performing molecular docking studies against 11 active compounds from 5 medicinal plants.

METHODS

Homology modeling

A homology model was built for PBP of *S. aureus* using MODELLER 9.10 (http://salilab.org/modeller/modeller.html). The three-dimensional (3D) structure of PBP from *Staphylococcus* sp. (PDB ID: 1VQQ) was used as the structural homolog to build the model. The "align2d.py" program has been employed to perform an alignment between the target and template sequence. The rough 3D model was generated using the "model-default.py" program. The rough model was subjected to loop refining using "loop.py" program. The quality of the obtained model was verified using the PROCHEK server (http://nihserver.mbi.ucla.edu/SAVES/).

Active site prediction

The possible binding site of PBP was determined using Q site finder (http//bmbpcu36.leeds.ac.uk/qsitefinder). The obtained binding site for PBP from Q site finder was compared to the active sites of the template to determine the residues forming the binding pocket.

Ligand preparation

About 11 compounds with antimicrobial property from *Azadirachta indica*, *Curcuma longa*, *Cuminum cyminum*, *Terminalia chebula,* and *Mentha spicata* (Table 1) were derived from Dr. Duke's Phytochemical and Ethnobotanical Databases. The structures were retrieved using PUBCHEM database. The two-dimensional structures were drawn using ACD Chemsketch (www.acdlabs.com/). The structures were then converted to their 3D geometries, optimized and saved in "MDL mol file" format.

Table 1: Molecular properties of plant derived compounds

S. No.	Ligand	Source	Molecular weight	Molecular formula	H-bond donor	H-bond acceptor
1	Rosmarinic acid	*M. spicata*	360.31	$C_{18}H_{16}O_8$	5	8
2	Rutin	*A. indica*	610.51	$C_{27}H_{30}O_{16}$	10	16
3	Stigmasterol	*C. longa*	412.69	$C_{29}H_{48}O$	1	1
4	Cuminaldehyde	*C. cyminum*	148.20	$C_{10}H_{12}O$	0	1
5	Curcumin	*C. longa*	368.37	$C_{21}H_{20}O_6$	2	6
6	Curlone	*C. longa*	218.33	$C_{15}H_{22}O$	0	1
7	Ellagic-acid	*T. chebula*	302.19	$C_{14}H_6O_8$	4	8
8	Eugenol	*C. longa*	164.20	$C_{10}H_{12}O_2$	1	2
9	Ketoprofen	*C. longa*	254.28	$C_{16}H_{14}O_3$	1	3
10	Menthone	*M. spicata*	154.24	$C_{10}H_{18}O$	0	1
11	Pectin	*T. chebula*	194.13	$C_6H_{10}O_7$	5	7

M. spicata: Mentha spicata, A. indica: Azadirachta indica, C. longa: Curcuma longa, C. cyminum: Cuminum cyminum, T. chebula: Terminalia chebula

GOLD docking simulations

Automated docking studies were performed using the genetic algorithm (GA) GOLD (Version 3.2 CCDC, Cambridge, UK) [16]. The algorithm had been previously validated and successfully tested on a data set of over 300 complexes extracted from the PDB [17]. Ligands rosmarinic acid rutin stigmasterol cuminaldehyde curcumin curlone ellagic-acid eugenol ketoprofen menthone and pectin were docked into the binding site of the receptor (PBP) using GOLD [18]. The GOLD program uses a GA to explore the full range of ligand conformational flexibility and the rotational flexibility of selected receptor hydrogen's. Grid was prepared for the protein with the center and the size of the bounding box set on 10 Å. The coordinates of the enclosing box (x=121 Å; y=87 Å; z=45 Å) were defined starting from the set of active site residues. During docking process, a maximum of 10 different conformations was considered for the drug. The conformer with the highest binding score was used for further analysis [19].

RESULTS AND DISCUSSION

The β-lactam antibiotics resistant natures of the most *S. aureus* strains found in hospitals symbolize a therapeutical challenge for treating serious MRSA infections. PBP is a key enzyme required for the cell wall synthesis of *S. aureus.* Inhibition of these proteins is important for controlling the pathogen.

The 3D structure of PBP, of *S. aureus* has been predicted (Fig. 1) and was subjected to validation using PROCHEK server [20].

The Ramachandran plot shows 84.5% of residues in the most favored region, 13.9% in additionally allowed region, 1.6% residues in generously allowed region and 0% residues in the disallowed region (Fig. 2). Thereby 0% of amino acid in the disallowed region indicates the overall stable conformation of the protein structure.

The inhibiting susceptibility of the compounds was evaluated using their fitness scores generated by the GOLD software. Ligand – receptor interactions were analyzed based on the binding compatibility of ligand in the active site of PBP (docked energy in kcal/mol [fitness]) [19].

Docking of PBP was performed with 11 potential inhibitors (Fig. 3). The final docked conformation for 11 ligands obtained after through docking analysis was evaluated based on the number of hydrogen bonds (H-bonds) formed and bond distance between atomic coordinates of the active site and inhibitor (Table 2).

The H-bond interactions between the 11 inhibitors and PBP along with their bond distances, amino acid involved, atom involved and their corresponding scores are described in Table 2. The inhibitor rosmarinic acid from mint binds with the receptor with the highest GOLD score of 41.16 (Fig. 4), comparatively the inhibitor ketoprofen from *C. longa* binds with a score of 39.4 (Fig. 5) and curcumin an active compound of *C. longa* also binds with a maximum score of 36.02 (Fig. 6) and followed by other inhibitors. Least score of 9.3 was obtained with

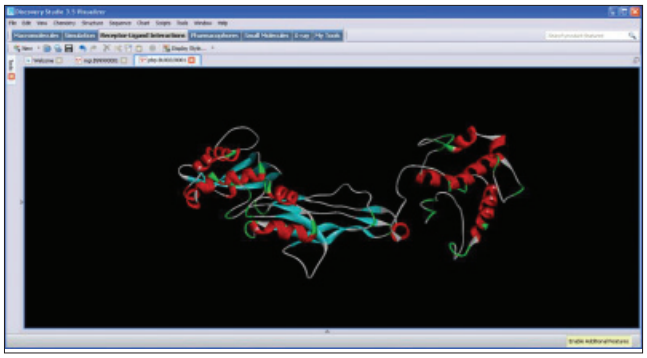

Fig. 1: Tertiary structure of the penicillin-binding protein

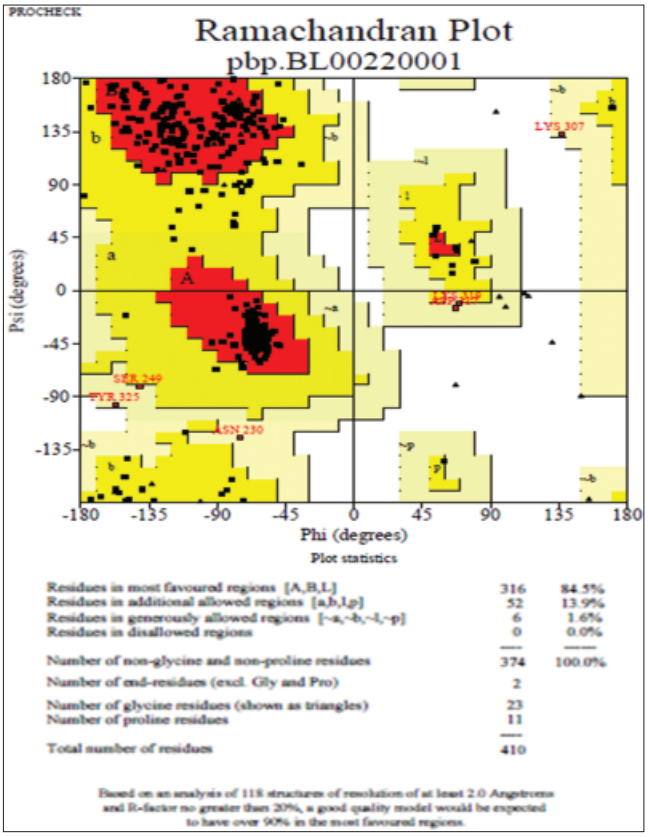

Fig. 2: Three-dimensional structure validation of penicillin-binding protein after loop refinement

vitamin E against the receptor. From the H-bond formations between the 11 inhibitors and the PBP it is evident that rosmarinic acid form five

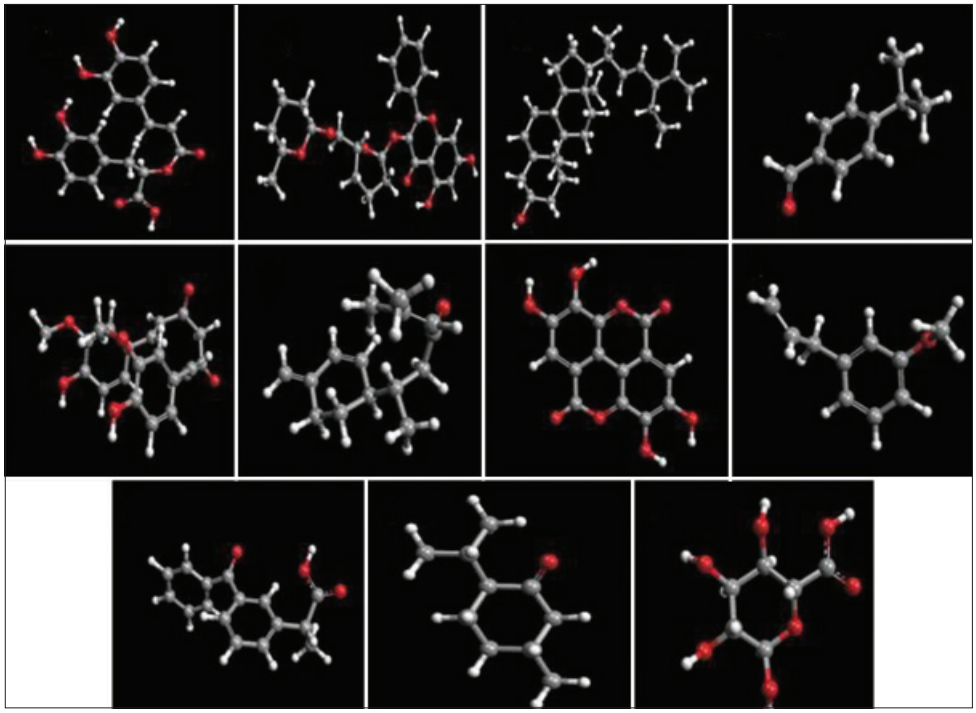

Fig. 3: Three-dimensional structures of rosmarinic acid, rutin, stigmasterol, cuminaldehyde, curcumin, curlone, ellagic-acid, eugenol, ketoprofen, menthone, and pectin

Table 2: Represents the fitness score and the H-bond interactions with ligands and penicillin-binding protein

Ligand name	Atom in ligand	Atom in proteins	H-bond	Score
Curlone				24.18
Eugenol				34.28
Stigmasterol	O25	Glu158:OE2	2.428	10.33
Cuminaldehyde				32.5
Ellagic-acid	O9	Thr204:N	2.604	32.52
	O11	Thr204:N	2.936	
	O20	Tyr184:OH	2.425	
Menthone	O7	Asp214:OD2	2.387	20.18
Pectin	O7	Glu158:OE2	2.897	29.16
	O8	Thr204:OG1	2.619	
	O13	Glu158:OE2	2.997	
Ketoprofen	O10	Ser213:OG	2.242	39.4
Rosmarinic acid	O10	Thr204:N	2.281	41.16
	O23	Ser181:OG	2.521	
	O23	Ser181:N	2.6	
	O25	Thr204:OG1	2.607	
	O26	Glu158:OE2	2.929	
Rutin	O28	Thr204:OG1	2.867	29.38
	O29	Lys203:N	2.38	
	O29	Leu202:N	2.786	
Vitamin E	-	-	-	9.3
Curcumin	O2	Ser181:N	2.276	36.02
	O15	Tyr184:OH	2.388	
	O24	Thr204:OG1	2.816	
	O25	Thr204:OG1	2.831	
	O27	Ser181:OG	3.025	

H-bonds, inhibitor ketoprofen from one H-bonds and curcumin forms five H-bonds.

Although ketoprofen forms a higher interaction score when compared to curcumin from *C. longa*, h-bond interactions clearly indicate that curcumin forms a stable interaction than ketoprofen. Similarly, active molecules rutin of *A. indica*, pectin and ellagic-acid from *T. chebula*

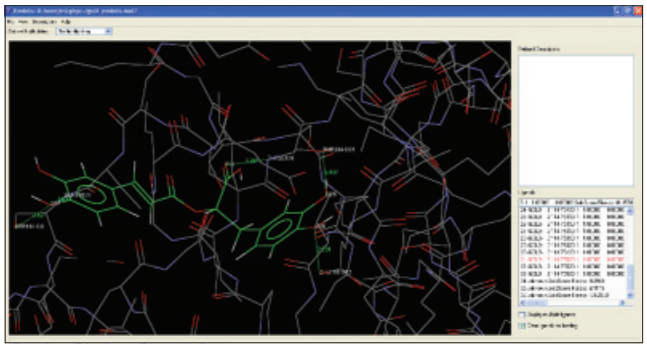

Fig. 4: Penicillin-binding protein of *Staphylococcus aureus* in complex with rosmarinic acid

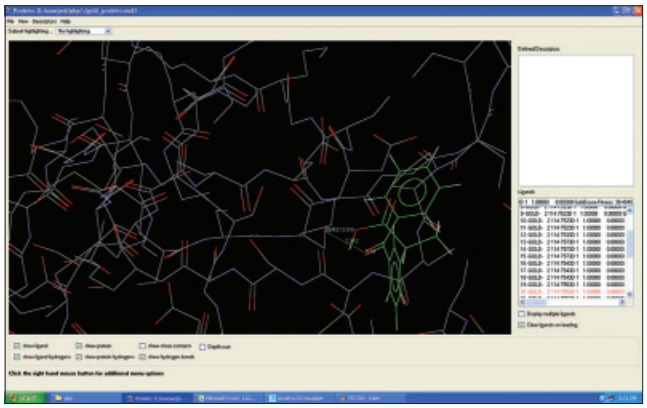

Fig. 5: Penicillin-binding protein of *Staphylococcus aureus* in complex with ketoprofen

forms three H-bond with the receptor with a GOLD score of 29.38, 29.16 and 32.52 respectively. The amino acid residues Thr204, Ser181,

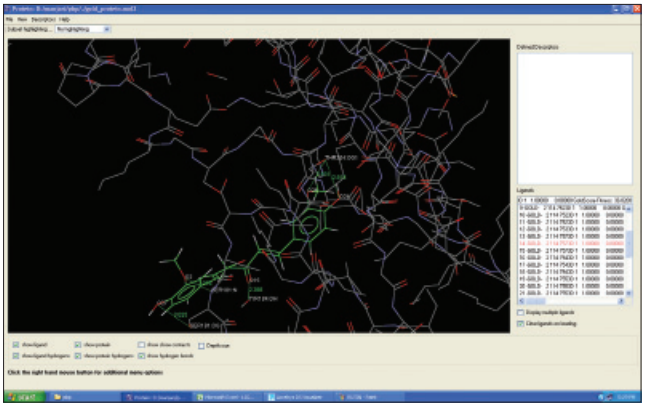

Fig. 6: Penicillin-binding protein of *Staphylococcus aureus* in complex with curcumin

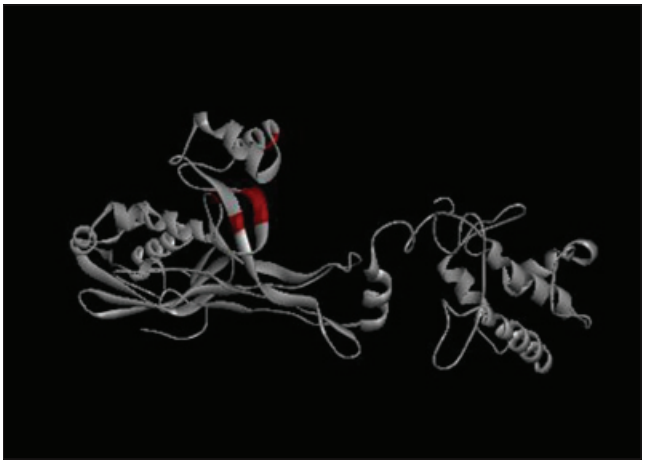

Fig. 7: Red region indicates the ligand binding site of penicillin-binding protein

Glu158, are involved in the interaction with rosmarinic acid. From the H-bond analysis is evident that amino acids GLU158, Thr204, Tyr184, Asp214, Ser213, Ser181, Lys203, Leu202 (Fig. 7) of the receptor are involved in H-bond formation with the 11 active molecules.

CONCLUSION

In recent years, many plants derived compounds are identified with antibacterial activity, but many aspects concerning their mechanisms of actions are still to be predicted. PBP are enzymes required for bacterial cell wall synthesis. Novel targets for PBP is required for the inhibition of bacteria. From the current study, molecular docking approach has proved to be an efficient method for the identification of novel lead compounds against β-lactam resistant *S. aureus* from a broad spectrum of plant compounds. 11 compounds were analyzed against PBP of which, rosmarinic acid showed high binding affinity against PBP. Hence, rosmarinic acid may be a potent lead molecule. It also assesses the various interactions of the drug target with its inhibitors thereby providing insight into the prospects of structure-based drug design.

REFERENCES

1. Tsao SM, Hsu CC, Yin MC. Meticillin-resistant *Staphylococcus aureus* infection in diabetic mice enhanced inflammation and coagulation. J Med Microbiol 2006;55:379-85.
2. Harris AD, Samore MH, Lipsitch M, Kaye KS, Perencevich E, Carmeli Y. Control-group selection importance in studies of antimicrobial resistance: examples applied to *Pseudomonas aeruginosa*, *Enterococci*, and *Escherichia coli*. Clin Infect Dis 2002;34(12):1558-63.
3. Chetia H, Sharma DK, Sarma R, Verma A. An *in silico* approach to discover potential inhibitors against multi-drug resistant bacteria producing New-Delhi metallo-β-lactamase 1 (ndm-1) enzyme. Int J Pharm Pharm Sci 2014;6(4):299-303.
4. Haas DW, McAndrew MP. Bacterial osteomyelitis in adults: evolving considerations in diagnosis and treatment. Am J Med 1996;101(5):550-61.
5. Akindele AA, Adewuyi IK, Adefioye OA, Adedokun SA, Olaolu AO. Antibiogram and beta-lactamase production of *Staphylococcus aureus* isolates from different human clinical specimens in a tertiary health institution in Ile-Ife, Nigeria. Am Eurasian J Sci Res 2010;5(4):230-3.
6. Verma S, Joshi S, Chitnis V, Hemwani N, Chitnis D. Growing problem of methicillin resistant *Staphylococci* – Indian scenario. Indian J Med Sci 2000;54(12):535-40.
7. Goldstein EJ, Citron DM, Nesbit CA. Diabetic foot infections. Bacteriology and activity of 10 oral antimicrobial agents against bacteria isolated from consecutive cases. Diabetes Care 1996;19(6):638-41.
8. Lévesque C, Piché L, Larose C, Roy PH. PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrob Agents Chemother 1995;39(1):185-91.
9. Kumar KM, Anitha P, Sivasakthi V, Bag S, Lavanya P, Anbarasu A, *et al*. *In silico* study on penicillin derivatives and cephalosporin's for upper respiratory tract bacterial pathogens. 3 Biotech 2014;4:241-51.
10. Sauvage E, Kerff F, Terrak M, Ayala JA, Charlier P. The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. FEMS Microbiol Rev 2008;32(2):234-58.
11. Sainsbury S, Bird L, Rao V, Shepherd SM, Stuart DI, Hunter WN, *et al.* Crystal structures of penicillin-binding protein 3 from *Pseudomonas aeruginosa*: comparison of native and antibiotic-bound forms. J Mol Biol 2011;405:173-84.
12. Turk S, Verlaine O, Gerards T, Zivec M, Humljan J, Sosic I, *et al.* New noncovalent inhibitors of penicillin-binding proteins from penicillin-resistant bacteria. PLoS One 2011;6(5):e19418.
13. Yoshida H, Kawai F, Obayashi E, Akashi S, Roper DI, Tame JR, *et al.* Crystal structures of penicillin-binding protein 3 (PBP3) from methicillin-resistant *Staphylococcus aureus* in the apo and cefotaxime-bound forms. J Mol Biol 2012;423(3):351-64.
14. Hariprasath B. Molecular docking studies of plant derived compounds. Asian J Pharm Clin Res 2012;5 Suppl 1:87-8.
15. Gogoi RR, Gogoi D, Bezbaruah RL. Virtual screening of compounds from *Tabernaemontana divaricata* for potential anti-bacterial activity. Bioinformation 2014;10(3):152-6.
16. Jones G, Willett P, Glen RC, Leach AR, Taylor R. Development and validation of a genetic algorithm for flexible docking. J Mol Biol 1997;267(3):727-48.
17. Manikandan S, Malik BK. Modeling of human CCR5 as target for HIV-I and virtual screening with marine therapeutic compounds. Bioinformation 2008;3(2):89-94.
18. Girija CR, Prasantha K, Chtan SP, Noor SB, Akheel AS. Molecular docking studies of curcumin derivatives with multiple protein targets for procarcinogen activating enzyme inhibition. J Proteomics Bioinformatics 2010;3(6):200-3.
19. Nissink JW, Murray C, Hartshorn M, Verdonk ML, Cole JC, Taylor R. A new test set for validating predictions of protein-ligand interaction. Proteins 2002;49(4):457-71.
20. Laskowski RA, Mas Arthur MW, Moss DS, Thornton JM. Pro-check: A program to check the stereo-chemical quality of protein structures. J Appl Crystallogr 1993;26:283-91.