Short Communication:

Molecular docking and dynamic studies of human growth factor receptor-bound protein (Grb) 2 insights to identify novel inhibitors

Sandeep Swargam,[1] Hema Kanipakam,[1] Natrajan Pradeep,[1] M.M Suchitra,[2] J. Rajeswari,[3] A. Umamaheswari[1]
Dpartments of [1]Bioinformatics, [2]Biochemistry, Sri Venkateswara Institute of Medical Science, Tirupati and Department of [3]Biochemistry, Acharya Nagarjuna University, Guntur

ABSTRACT

Background: Human growth factor receptor bound protein-2 (Grb 2) involves in initiation of kinase signaling by Son of Sevenless (SOS) and activates mitogen activated protein kinase pathway. Grb2 overexpress during cancerous condition hence it emerged as a potent target for various cancers.

Material and Methods: Seven pharmacophores were developed from seven co-crystal structures of Grb2 and applied for common pharmacophore hypothesis. Two common pharmacophore hypothesis (CPH) models were screened and hits were applied for docking and free energy [ΔG] calculations.

Results: Two leads were proposed from docking and ΔG analysis. Energy of the system, RMSD, RMSF, hydrogen bonds and water bridges of lead1 was better than the co-crystal ligand during 50 ns molecular dynamics simulations.

Discussion: Two leads are interacting with Src homology 2 (SH2) domain of Grb2 and blocking the function of Grb2.

Keywords: *Neoplasm, GRB2 adapter protein, Molecular docking simulation*

Sandeep S, Hema K, Pradeep N, Suchitra MM, Rajeswari J, Umamaheswari A. Molecular docking and dynamic studies of human growth factor receptor-bound protein (Grb) 2 insights to identify novel inhibitors. J Clin Sci Res 2016;5:252-8. DOI: http://dx.doi.org/10.15380/2277-5706.JCSR.16.07.006.

INTRODUCTION

Mitogen activated protein kinase (MAPK) pathway plays an important role in cellular signaling events in humans. Human growth factor receptor-bound protein 2 (Grb2) receives signal from receptor tyrosine kinases [RTKs- Eg: Epidermal growth factor receptor (EGFR)]. It involves in the downstream cell signalling events like activation of retro virus associated DNA sequence (Ras) and protein kinase B (Akt) with in the cytosol.[1] Grb2 protein comprised of ubiquitous N-terminal proto oncogene tyrosine protein kinase (Src) homology 3 (SH3) - src homology 2 (SH2) - C - terminal SH3 domains. SH2 domain of Grb2 binds with diverse receptor proteins as well as Son of Sevenless (SOS) proteins.[2] Grb2 binds with dimerized EGFR and SH3 domains of Grb2 activates SOS. In turn, SOS mediates activation of RAS proteins, and further Ras activates rapidly accelerated fibrosarcoma (RAF) kinases. Activated RAF induces the activation of mitogen activated extracellular kinase (MEK) 1 and MEK2, which further activates extra cellular regulated kinase (ERK) 1 and ERK2. Activated mitogen activated protein kinase (MAPK) pathway cascade involves in cellular growth and proliferation.[3] Grb2 plays pivotal role during the cancerous condition which involves in continuous tumor progression through MAPK pathway.[4] That is why it emerged as a potent therapeutic target to inhibit the higher levels of Grb2 in cancers.

Received: July 16, 2016; Revised manuscript received: September 12, 2016; Accepted: September 16, 2016.

Corresponding author: Dr A. Umamaheswari, Associate Professor and Coordinator of BIF, Department of Bioinformatics, Sri Venkateshwara Institute of Medical Sciences, Tirupati, India.
e-mail: svims.btisnet@nic.in

Online access
http://svimstpt.ap.nic.in/jcsr/oct-dec16_files/sf.16.07.006.pdf
DOI: http://dx.doi.org/10.15380/2277-5706.JCSR.16.07.006

Gleevec CEP-701 and P27 were reported as inhibitors of Grb2 which stops its function by blocking association of guanine nucleotide exchange factor SOS.[5] Grb2 inhibitors were unclear in preclinical levels and some were under development.[5] The association mechanism of SH2 domain of Grb2 with SOS was crucial step for development of new anti-cancer agents.[6] The present study was implemented with energy based (E) pharmacophore modeling, common pharmacophore hypothesis (CPH), multiple docking, ΔG calculations and molecular dynamics (MD) simulations to identify novel lead molecules to inhibit over expression of Grb2 during angiogenesis.

MATERIAL AND METHODS

Protein preparation

Seven co-crystal structures of Grb2 (3KFJ, 3C7I, 2HUW, 3IMJ, 3IN7, 3IN8, 3IMD) were retrieved from the Research collaboration for structural Bioinformatics (RCSB).[7] Structures were prepared, optimized and minimized using optimized potential for liquid simulations (OPLS)-2005 force field.[8]

Grid generation and docking

Grid was defined around the co-crystal ligand of Grb-2 in glide and applied for glide extra precision (XP) docking.[9]

Generation of e- pharmacophore model and CPH

E-pharmacophore hypotheses in phase was used to generate six built-in types of pharmacophore site, including hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic region (H), negatively charged group (N), positively charged group (P) and aromatic ring (R). The obtained seven e-pharmacophores were applied for CPH.[9-10]

CPH based database screening

The CPH models were applied for pharmacophore based screening in Phase module against in-house library of 21 million compounds downloaded from various small molecule databases.[11] Obtained ligands were processed in Lipinski's filters to have ligands with better drugability using LigPrep[12] with Epik.[13]

Molecular docking and ΔG calculations

The ligands were docked in to grid by applying high throughput virtual screening (HTVS), standard precision (SP) and extra precision (XP) docking modes of rigid receptor docking (RRD).[9] ΔG of the best ranked docking complexes were calculated and compared with crystal ligands. Polarization charge effect of obtained hits with receptor flexibility was checked by quantum polarized ligand docking [QPLD] protocol and ΔG calculations.[9] Further flexibility of receptor with the best hits was analyzed by induced fit docking [IFD] protocol and ΔG calculations.[9,14-16] Absorption, distribution, metabolism, excretion and toxicity (ADMET) properties of the obtained potent hits were calculated using QikProp.

MD simulations

50 ns MD simulations of Grb2-lead1 and Grb2-crystal ligand docking complexes were performed using the Desmond v4.2.[17] Obtained trajectories were used for calculating energy of the system, root mean square deviation (RMSD), root mean square fluctuations (RMSF) and inter-molecular hydrogen bond interactions between ligand and protein.

RESULTS

Preparation of protein structures

Energies of seven prepared co- crystal structures were minimized and ligand binding interactions were analyzed.

Grid generation and XP docking

Crystal ligands were docked in generated grid of Grb2 and obtained docking complexes.

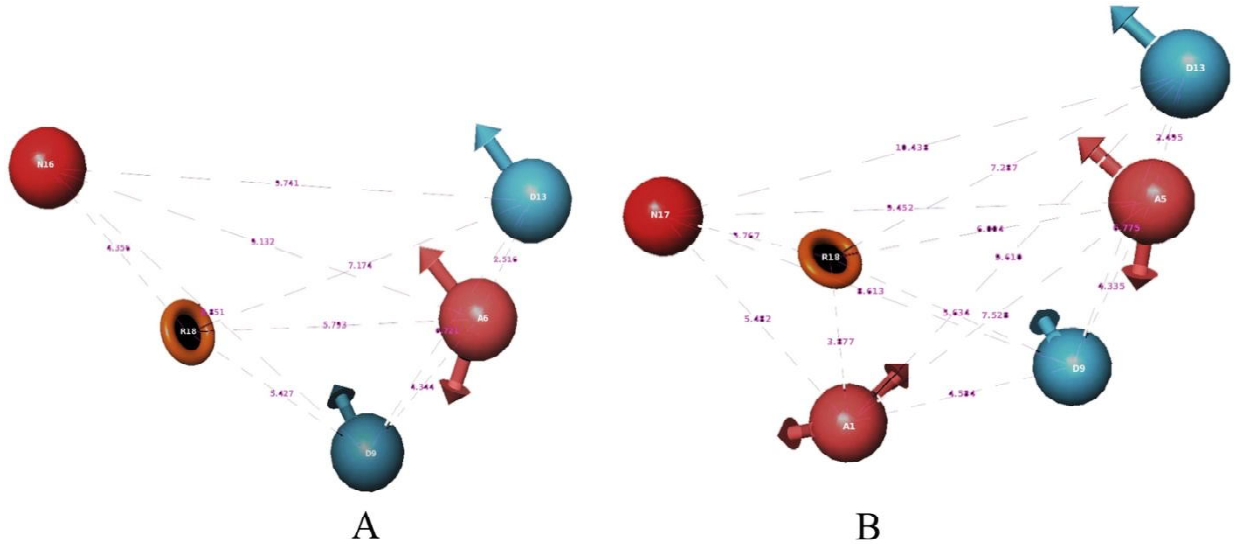

Figure 1: E-pharmacophores of human Grb2 (A) ADDRN (B) AADDRN
Grb2 = Growth factor receptor-bound protein; hydrogen bond acceptor (A), hydrogen bond donor (D), hydrogen bond donor (D), aromatic ring (R), negatively charged group (N) (ADDRN); hydrogen bond acceptor (A), hydrogen bond acceptor (A), hydrogen bond donor (D), hydrogen bond donor (D), aromatic ring (R), negatively charged group (N) (AADDRN)

E-pharmacophore generation and CPH

CPH models [hydrogen bond acceptor (A), hydrogen bond donor (D), hydrogen bond donor (D), aromatic ring (R), negatively charged group (N) (ADDRN)] and [hydrogen bond acceptor (A), hydrogen bond acceptor (A), hydrogen bond donor (D), hydrogen bond donor (D), aromatic ring (R), negatively charged group (N) (AADDRN)] (Figure 1A and B) were obtained from seven Grb-2 e-pharmacophores based on the energies of pharmacophore sites and fitness scores.

Screening of CPH models and ligand dataset preparation

5000 structural analogues were obtained for CPH models from in house library. These hits were processed in Lipinski's filters and 3500 compounds taken as a dataset for further docking analysis.

Multiple docking analysis

Docking studies were carried out with 3500 compounds against Grb2 and applied for HTVS, SP and later with XP modes of docking. 1000 compounds were obtained from HTVS process with score of 6.0 kcal/mol^{-1} and 4 hydrogen bonds. They were subjected to another round of docking by SP. Top compounds (250) from SP were subjected to XP docking. Top 50 hits were applied for molecular mechanics-generalized Born surface area (MM-GBSA) calculations. Comparison of ΔG score of top ranked hits with co-crystallized ligands (Figure 2A) showed two hits were having better ΔG score (Table 1). Top ranked two leads from RRD, ΔG analysis were applied for QPLD and ΔG analysis (Figure 2B). In IFD also the two leads showed similar interactions like RRD, QPLD. Lead1 formed one hydrogen bond with Ser88, Ser96, two hydrogen bonds with Arg67, Lys109, Arg86 and π- cation interactions with Arg67, Lys109, Leu120, whereas lead2 formed one hydrogen bond with Arg67, Arg86, Ser98, Lys109; π- cation interactions with His107, Phe108. Thus the obtained leads had better RRD, QPLD, IFD and ΔG score than crystal ligands (Table1).

Molecular dynamics simulations

During 50 ns MD simulations, Grb2-lead1 and Grb2-co-crystal ligand docking complexes

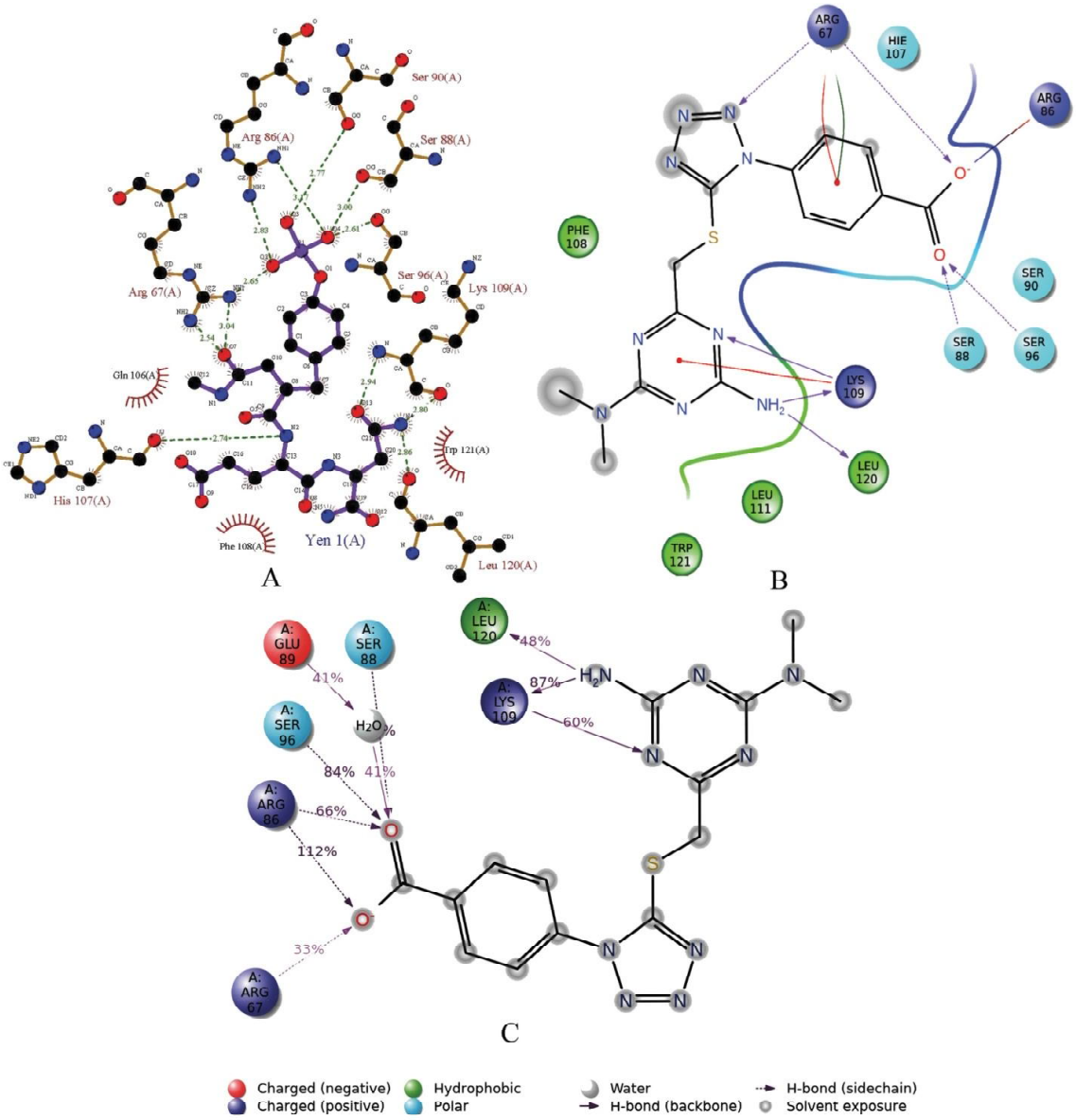

Figure 2: Docking interactions; **(A)** Interactions of Grb2 with crystal ligand; Lead 1 interactions with Grb2 in **(B)** QPLD and **(C)** MD simulations; QPLD = Quantum polarized ligand docking; MD = Molecular dynamics

Table 1: Docking scores and ΔG scores of proposed lead molecules and crystal ligands with Grb2

Leads and crystal ligands	RRD kcal/mol	ΔG kcal/mol	QPLD kcal/mol	ΔG kcal/mol	IFD kcal/mol	ΔG kcal/mol
Lead 1	-10.712	-50.76	-13.802	-56.700	-15.13	-73.17
Lead2	-10.237	-50.02	-12.97	-54.87	-13.52	-70.85
3KFJ	-10.14	-48.15	-11.14	-50.10	-10.07	-51.0
2HUW	-10.85	-49.85	-11.85	-51.27	-11.58	-50.9
3IMD	-10.99	-48.99	-12.13	-51.09	-10.89	-50.7
3C7I	-10.06	-47.85	-11.95	-50.47	-11.19	-50.05

RRD = Rigid receptor docking; IFD = Induced fit docking; ΔG = Free energy calculations

Table 2: Molecular dynamics simulations of lead1 and co-crystal ligandwith Grb2

	Total energy (kcal/mol)	RMSD (Å)		RMSF (Å)		Hydrogen bond interactions	Water mediated interactions	Hydrophobic interactions
		Protein	Lead	Cα-Protein	Lead			
Lead1	-39805	2.4	0.3-2.0	3.0	1.0	Arg67, Arg86, Glu89, Ser88, Ser96, His107, Lys109 and Leu129	Arg67, Glu89, Ser90 and Leu120	Trp121
Crystal ligand	-37805	2.4	0.6-2.0	3.0	1.6	Arg67, Arg86, Ser88, Glu89,Ser90, Ser96,His107, Lys109andLeu 129	Arg67, Glu89, Ser90 and Leu120	Trp121

RMSD = root mean square deviation; RMSF = root mean square fluctuations

showed total energies of -39805 kcal/mol and -37805 kcal/mol respectively. RMSD of lead1 was 0.3 - 2.0 Å and protein was 2.4 Å whereas RMSD of crystal ligand was 0.6 - 2 Å and protein was 2.4 Å. Average RMSF in lead1 docking complex; Cα-protein was 3 Å and lead1 was 1Å; whereas RMSF of co- crystal structure of Cα-protein is 3 Å and co-crystal ligand was 1.6 Å. Lead1 showed similar RMSD with crystal ligand and very low fluctuations when compared with crystal ligand. Lead1 formed seven hydrogen bond interactions with ligand binding site (SH2) residues of Grb2 namely Arg67 (33%), Arg86 (100%), Glu89 (82%), Ser88 (98%), Ser96 (84%), Lys109 (87%) and Leu120 (48%) in 10486 trajectories (Figure 2C). Co-crystal ligand formed hydrogen bond interactions with Arg67 (90%), Arg86 (90%), Glu89 (43%), Ser88 (88%), Ser90 (30%), Ser96 (63%), His107 (98%), Lys109 (90%) and Leu120 (99%). Lead1 and co-crystal ligand formed similar water mediated interactions with SH2 domain residues such as Arg67, Glu89, Ser90, Leu120 and Arg142 which increase the electrostatic effect and results in strong binding affinity towards the Grb2 protein. Water molecules increase entropy gain in the complex formation[18] which also provides strong binding affinity. Lead1 and co-crystal ligand also formed hydrophobic interactions with SH2 region residue Trp121. The torsion angles were found to be stable within allowed range of fluctuations (Table 2).

DISCUSSION

Abnormal cell signaling and autophosphorylation of tyrosine kinase leads to many types of cancers. In the present era of cancers research the most studied and validated pathway is EGFR/RAS/RAF/MEK/ERK pathway. The pathway mediates intra cellular signaling functions involved in cell growth, migration and proliferation. In normal condition, activated EGFR activates downstream protein, RAS through the adaptor proteins like SOS and C3G proteins. The RAS family comprises of HRAS, NRAS and KRAS proteins. The first RAS effector pathway is RAF-MEK-ERK pathway and it controls the cell survival, cell growth, cell differentiation and cell transformation. Further effector proteins belong to RAF family (RAF-1, A-RAF, and B-RAF) which activate and phosphorylate by different protein kinases. Activated RAF phosphorylates MEK which in turn phosphorylate and activate extracellular signal–regulated kinases; 1 and 2 (ERK1/ 2). ERK regulates the many downstream signaling proteins that affect the cell metabolism.

Abnormal Ras/Raf/MEK/ERK signaling is activated in human cancers via several different mechanisms. Targeting tyrosine kinase or targeting the downstream signaling modulators such as Grb2 is alternative approach to design drugs. Grb2 is over expressed with SOS1, as the only observed mechanism of oncogenesis.[19] Grb2 is causing breast cancer further suggesting that it can be a valid therapeutic target for pathological process such as the spread of solid tumors through local invasion and metastasis.[20]SH2 and SH3 domains of Grb2 are playing main role in catalytic activity of Grb2 protein. Thus in the present study, structure based pharmacophore modeling studies, docking and molecular docking studies were implemented to block the catalytic activity of Grb2 protein. Seven e-pharmacophore models were generated from the seven available co-crystal structures of human Grb-2 and from them two CPH models (AADDRN and ADDRN)were developed. Co-crystal ligand had "G scores of -38.87 kcal/mol, -42.95 kcal/mol, -40.95 kcal/mol and G score of -9.82 kcal/mol, -10.96 kcal/mol and-10.08 kcal/mol and formed six H-bonds with SH2 domain region residues such as Ser88, Ser96, Arg67(2H), Arg86(2H), Lys109(2H), Leu120in multiple dockings such as RRD, QPLD, IFD. Grb2 in IFD had "G scores of -50.76 kcal/mol, -56.700 kcal/mol, -73.17 kcal/mol and G score of -10.712kcal/mol, -13.802 kcal/mol and-15.13 kcal/mol. Whereas the best lead1 had the lowestfree energywith stable conformation and G score when comparedwith the crystal ligand and lead1 in RRD, QPLD, and IFD. Two leads showed seven hydrogen bonds with most important regions of the SH2 binding site residues.In multiple docking and binding free energy analysis, lead1 formed one additional H-bond with Ser88 andtwo additional ð- cation interactions with Arg67 along with six hydrogen bonds than the crystal structure with Ser88, Ser96, Arg67(2H), Arg86(2H), Lys109(2H) and Leu120. Further Grb2-lead1docking complex had less conformational changes during 50 ns MD simulations run and consistency than the Grb2- crystal ligand docking complex. Lead1 reproduced and maintained all hydrogen bond interactions with Arg67, Arg86, Glu89, Ser88, Ser96, Lys109 and Leu129 which were also observed in XP, QPLD and IFD docking methodologies. Strong binding affinity by electrostatic effect of lead1 towards SH3-SH2 region of Grb2 protein was maintained through water mediated interactions with Arg67, Glu89, Ser90 and Leu120. Therefore, the MD simulations studies revealed that total energy, lower RMSD, RMSF, hydrogen bond, water mediated interactions, hydrophobic interactions and torsion angles of Grb2-lead1 docked complex indicated the lesser structural re-arrangements, lower conformational changes and consistency during the period of 50 nsMD simulations run which is acceptable in physiological environmental condition. Two inhibitor leads with high affinity towardsSH2 domain of Grb2 which in turn prevent formation of Grb2-SOS complex were identified in the present study. The leads would be helpful to inactivate the overexpression of Grb2 and also downstream signaling components of MAPK pathway during tumor formation in cancer condition. The leads showed acceptable range of ADME properties hence they would serve as a starting point for lead optimization, scaffold hopping if synthesized and tested *in vivo* and *in vitro* studies as a potent inhibitors for human Grb2.

ACKNOWLEDGEMENTS

SS is highly thankful to ICMR for sanction of senior research fellowship (BIC/11(09)/2014). Authors are thankful to Department of Biotechnology (DBT), Ministry of Science and Technology for providing infrastructure facility through BIF program (BT/BI/25/001/2006).

REFERENCES

1. Giubellino A, Burke TR Jr, Bottaro DP. Grb2 signaling in cell motility and cancer. Expert Opin Ther Targets 2008;12:1021-33.
2. Lowenstein EJ, Daly RJ, Batzer AG, Li W, Margolis B, Lammers R, et al. The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling. Cell 1992;70:431-42.
3. Suen KL, Bustelo XR, Pawson T, Barbacid M. Molecular cloning of the mouse grb2 gene: differential interaction of the Grb2 adaptor protein with epidermal growth factor and nerve growth factor receptors. Mol Cell Biol 1993;13:5500-12.
4. Daly RJ, Binder MD, Sutherland RL. Over expression of the Grb2 gene in human breast cancer cell lines. Oncogene 1994;9:2723-7.
5. Moeller SJ, Head ED, Sheaff RJ. p27Kip1 inhibition of GRB2-SOS formation can regulate Ras activation. Mol Cell Biol 2003;23:3735-52.
6. Chen CH, Chen MK, Jeng KC, Lung FD. Effects of peptidic antagonists of Grb2-SH2 on human breast cancer cells. Protein Pept Lett 2010;17:44-53.
7. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. The Protein Data Bank. Nucleic Acids Res 2000;28:235-42.
8. Jorgensen WL, Maxwell DS, Tirado Rives J. Development and Testing of the OPLS All Atom Force Field on Conformational Energetics and Properties of Organic Liquids, J Am Chem Soc1996;118:11225-236.
9. Sandeep S, Pradhan D, Pradeep N, Hema K, Siva Krishna V, Umamaheswari A. Structure guided novel lead molecules against ERK proteins: application of multiple docking and molecular dynamics studies. J Biomol Struct Dyn 2015;33134-5.
10. Pradeep N, Priyadarshini IV, Pradhan D, Munikumar M, Sandeep S, Hema K, et al. E-pharmacophore-based virtual screening to identify GSK-3β inhibitors J ReceptSignal Transduct Res 2015;36:1-14.
11. Sandeep S, Hema K, Pradeep N, Suchitra MM, Rajeswari J, Umamaheswari A. Ligand based 3D-QSAR approach of EGFR. Int J Comput Sci, Math and Eng - Special Issue on Computational Science, Mathematics and Biology IJCSME-SCSMB 2016;17-24.
12. Chen IJ, Foloppe N. Drug-like bioactive structures and conformational coverage with the LigPrep/ ConfGen suite: comparison to programs MOE and catalyst. J Chem Info Model 2010;50;822-39.
13. Shelley JC, Cholleti A, Frye LL, Greenwood JR, Timlin MR, Uchimaya M. Epik: a software program for pK(a) prediction and protonation state generation for drug-like molecules. J Comput Aided Mole Des 2007;21:681-91.
14. Hema K, Sandeep S, Pradeep N, Umamaheswari A. In silico agonist for human extracellular superoxide dismutase SOD3. Onl J Bioinform 2016;17:29-40.
15. Priyadarshini IV, Pradhan D, Munikumar M, Swargam S, Umamaheswari A, Rajasekhar D. Genome-based approaches to develop epitope-driven subunit vaccines against pathogens of infective endocarditis. J Biomol Struct Dyn 2014;32:876-89.
16. Pradhan D, Priyadarshini IV, Munikumar M, Swargam S, Umamaheswari A, Aparna B. Para-(benzoyl)-phenylalanine as a potential inhibitor against LpxC of Leptospira spp.: Homology modeling, docking and molecular dynamics study. J Biomol Struct Dyn 2014; 32:171-85.
17. Sandeep S, Priyadarshini IV, Pradhan D, Munikumar M, Umamaheswari A. Docking and molecular dynamics simulations studies of human protein kinase catalytic subunit alpha with antagonist. J Clin Sci Res 2012;1:15-23.
18. Poornima CS, Dean PM. Hydration in drug design.1.Multiple hydrogen-bonding features of water molecules in mediating protein-ligand interactions. Journal of Computer Aided Molecular Design 1995;9:500-51.
19. Zang XP, Siwak DR, Nguyen TX, Tari AM, Pento JT. KGF-induced motility of breast cancer cells is dependent on Grb2 and Erk1,2. ClinExp Metastasis 2004; 21:437–43.
20. Watanabe T, Shinohara N, Moriya K, Sazawa A, Kobayashi Y, Ogiso Y, et al. Significance of the Grb2 and son of sevenless (SOS) proteins in human bladder cancer cell lines. IUBMB Life.2000; 49:317–20.