Journal of Advanced Bioinformatics Applications and Research
ISSN 0976-2604.Online ISSN 2278 – 6007
Vol 3, Issue 3, 2012, pp 374-378
http://www.bipublication.com

STRUCTURE BASED INHIBITOR DESIGNING FOR RAC-ALPHA SERINE/THREONINE-PROTEIN KINASE IN HUMAN

Vidyanath Chaudhary

Department of Biotechnology, Jamia Hamdard (Hamdard University), New Delhi
Corresponding author E-mail: vidyanath.chaudhary@gmail.com

[Received-01/09/2012, Accepted-03/11/2012]

ABSTRACT:
The activation of RAC-alpha serine/threonine-protein kinase (an enzyme encoded by AKT1 gene in human) inhibits the phenomenon of apoptosis, one of the major causes for promoting cancer. There is no sufficient information relaying about inhibitor for RAC-alpha serine/threonine-protein kinase in Homo sapiens. Therefore, structure based inhibitor for RAC-alpha serine/threonine-protein kinase in human was designed. In order to design ligand, target kinase, was docked with ATP molecule by Molecular Docking Server and the backbone of hydrogen bonding was taken to sketch ligand structure. The ligand was docked with target kinase using Molecular Docking Server exhibiting high binding affinity than ATP and inhibition. The ligand was also found to be fit with good druggable character according to Lipinski's rule of five. The discussed information thus provides structure based inhibitor for RAC-alpha serine/threonine-protein kinase in human.

KEYWORD: RAC-alpha serine/threonine-protein kinase, ATP, Molecular docking, ligand, Lipinski's Screening.

[I] INTRODUCTION:

RAC-alpha serine/threonine-protein kinase is an enzyme [EC: 2.7.11.1] in human encoded by AKT1 gene which is one of the members of protein kinase family. This protein kinase possess a catalytic subunit which transfers the gamma phosphate to one or more amino acid residues-serine or theornine, in a protein substrate side chain from nucleotide triphosphates (often ATP), resulting in a conformational change in their structure altering protein function [1]. The conformational change in this protein kinase activate itself which further activates phosphatidylinositol 3-kinase leading to the production of Phosphatidylinositol (3,4,5)-trisphosphate (PIP_3), one of the component in downstream signaling pathway to apoptosis [2,4]. The activation of this protein kinase is abrogated by mutations in the pleckstrin homology domain of AKT1 [3]. The activation of serine/threonine kinase AKT1 inhibit the phenomenon of apoptosis by phosphorylating and inactivating components of the apoptotic machinery, one of the major causes for promoting cancer. The serine-threonine protein kinase AKT1 is on inactive state in

serum-starved primary and immortalized fibroblasts [4]. There is no sufficient information relaying about inhibitor for RAC-alpha serine/threonine-protein kinase in Homo sapiens. So, the present study deals with designing of Structure based inhibitor for RAC-alpha serine/threonine-protein kinase in human.

[II]MATERIAL AND METHOD:

2.1 Selection of Target Protein and its Structure Preparation:

Protein Data Bank (PDB) is a repository of 3-D structural data of bio macromolecules (http//www.rscb.org/pdb/). In the present study, the fasta sequence of RAC-alpha serine/threonine-protein kinase in Homo sapiens (Accession: NP_005154.2) was procured from a profile generated from Non-Redundant protein database for proteins exhibiting similarity to the unknown structure using NCBI PSI – BLAST of pleckstrin homology domain of the human protein kinase B (1P6S) .The fasta sequence was processed by CPH models-3.0 server [5] to create PDB and then analyzed protein structures using PyMOL program (figure 1).

2.2 Perparation of Ligand:

In order to design ligand for target kinase, ATP molecule was docked with RAC-alpha serine/threonine-protein kinase using Molecular Docking Server [6, 7, 8], an online tool for Ligand Protein Docking and Molecular Modeling (http://www.dockingserver.com). Binding interactions between ATP and RAC-alpha serine/threonine-protein kinase shows nine significant hydrogen bond interaction namely- N_3-THR_{82}, N_4-GLU_{117}, N_4-VAL_{271}, N_3-TRY_{272}, N_3-TRY_{272}, N_4-TRY_{272}, N_1-ARG_{273}, O_9-LYS_{276} and O_{10}-TRY_{326} (figure2). Thus, hydrogen bonding residues of ATP (N_1, N_3, N_4, O_9 and O_{10}) was taken as the backbone to sketch ligand structure namely- N-{[{[(2R)-2, 3-dihydroxypropyl] amino}methyl]amino}methanimidamide (figure 3). The chemical structure was drawn in Molecular Docking Server using Jmol program and docked again with RAC-alpha serine/threonine-protein kinase. Both the receptor and ligand were optimized for proper geometry and the best ligand pose was found to be with lowest free energy of binding -4.79 kcal/mol. The obtained complex showed six hydrogen bonds between ligand and target kinase- N_2-THR_{82}, N_2-GLU_{117}, N_2-TRY_{272}, N_3-ARG273, $_{N4}$-ARG_{273} and N_1-ASP_{292} (figure4).

2.3 Lipinski 5 Screening:

This screening method was applied to analyze the Drug likeness of the proposed ligand. Lipinski's rule of 5 [9] is an essential screening method for rational drug design (Ekins and Rose, 2002; Miteva et al., 2006; Smith et al., 2004). It states that poor absorption or permeation are more likely when a ligand molecule comply with two or more of Lipinski's rule of five i.e. molecular mass less than 500 Dalton, high lipophilicty (expressed as lopP less than 5), less than 5 hydrogen bond donors, less than 10 hydrogen bond acceptors and molar refractivity should be between 40-130. The Ligand of the present study has well qualified in Lipinski's filter (http:// www.scfbioiitd.res.in/utility/LipinskiFilters.jsp)

[III] RESULT AND DISCUSSION:

The Docked complex of RAC-alpha serine/threonine-protein kinase in Homo sapiens with ATP was found to have 0.61 kcal/mole free energy of binding (ΔG) (Table 1). The lowest positive ΔG value there by means binding affinity of ATP with protein kinase was good. The present study was initiated to explore the possibility to develop an inhibitor mimicking the above mentioned interaction. Hence, Structure based drug design approach was implemented to develop the inhibitor. The derived Structure based inhibitor was docked with RAC-alpha serine/threonine-protein kinase and was found to have very low free energy of binding i.e. -4.79 kcal/mol (Table 1). The gradual decrease in ΔG

is attributed by total intermolecular interaction energy and other energy (Table1) between ligand and target kinase. This leads to highly efficient binding affinity with target kinase, leading to the lesser requirement for the inhibition. This inhibitor form hydrogen bond with theronine of target kinase and inhibit the phoshorylation of theronine by ATP. Thus, unphoshorylated theronine causes inactivation of target kinase and stop downstream signaling pathway to apoptosis. We also screened the docked ligand for Lipinski's rule of 5, which in turn proved to be a qualified drug (Table 2). The drug designed in the present study is unique and is based on the protein kinase's interaction with ATP.

[IV] CONCLUSION:

On the basis of structure based drugs design, a ligand was prepared by mimicking the interaction of RAC-alpha serine/threonine-protein kinase with ATP. The docking score i.e free energy of binding, inhibition constant, dispersion/repulsion(vdW), hydrogen bonding (Hbond) and desolvation energy, electrostatic energy, total intermolecular energy etc. obtained by docking the ligand with target protein kinase, enforce the evidence that ligand work as inhibitor for the target protein kinase. Further, Lipinski's screening presents the ligand as proposed drug for preventing cancer development.

[V] ACKNOWLEDGEMENT:

The author thanks IT Department of Jamia Hamdard, for providing facilities to carry out this work and also acknowledge Department of Biochemistry and Biotechnology of University of Science and Technology, for their constant encouragement.

[VI] REFERENCE:

[1]. Salam Pradeep Singh, Sarangthem Vivek, Rajib Lochan Bezbaruah, Madhumita Barooah (2012), Prediction of the three-dimensional structure of serine/threonine protein kinase pto of Solanum lycopersicum by homology modeling, Bioinformation 8(5):212-215.

[2]. Leevers SJ, Vanhaesebroeck B, Waterfield MD (1999). "Signalling through phosphoinositide 3-kinases: the lipids take centre stage". Current Opinion in Cell Biology **11** (2): 219–25.

[3]. Thomas F. Franke, Sung Yang, tung O. Chan, Ketaki Datta, Andrius Kazlauskas, Deborah K. Morrison, david R. Kaplan and Philip N. Tsichilis (1995), the protein kinase encoded by akt proto-oncogene is a target of pdgf-activated phosphatidylinositol 3-kinase, Cell, Vol. 81, 725-736.

[4]. Fiona A. Barry and Jonathan M. Gibbins (2002), protein kinase B is regulated in platelets by the collagen receptor glycoprotein VI, Journal Of Biological Chemistry Vol. 277, No. 15, pp. 12874–12878.

[5]. Nielsen M., lundegaard C., Lund O., Petersen TN (2010). CPH model-3.0-Remote homology modeling using structure guided sequence profiles. Nucleic Acids Research, Vol. 38, Doi.10.1093/nar/gkq535.

[6]. Garrett M. Morris, David S. Goodsell, Robert S. Halliday, Ruth Huey, William E. Hart, Richard K. Belew, Arthur J. Olson (1998), Automated Docking Using A Lamarckian Genetic Algorithm And An Empirical Binding Free Energy Function, Journal of Computational Chemistry, Vol. 19, No. 14, 1639-1662.

[7]. Thomas A. Halgren (1996), Merck Molecular Force Field. I. Basis, Form, Scope, Parameterization, and Performance of Mmff94, Journal of Computational Chemistry, Vol. 17, Nos. 5&6, 490-519.

[8]. Zsolt Bikadi , Laszlo Demko , Eszter Hazai(2007), Functional and structural characterization of a protein based on analysis of its hydrogen bonding network by hydrogen bonding plot, Archives of Biochemistry and Biophysics 461 225–234.

[9]. Paul Leeson (2012), Drug discovery: Chemical beauty contest, Nature, Vol: 481: 455–456.

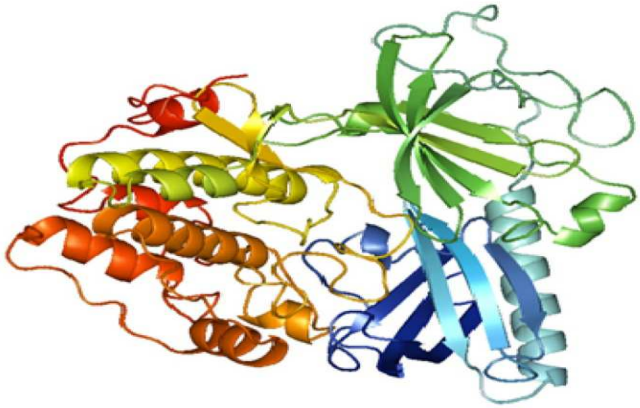

Figure 1: 3-D model of RAC-alpha serine/threonine-protein kinase of Homo sapiens by PyMOL

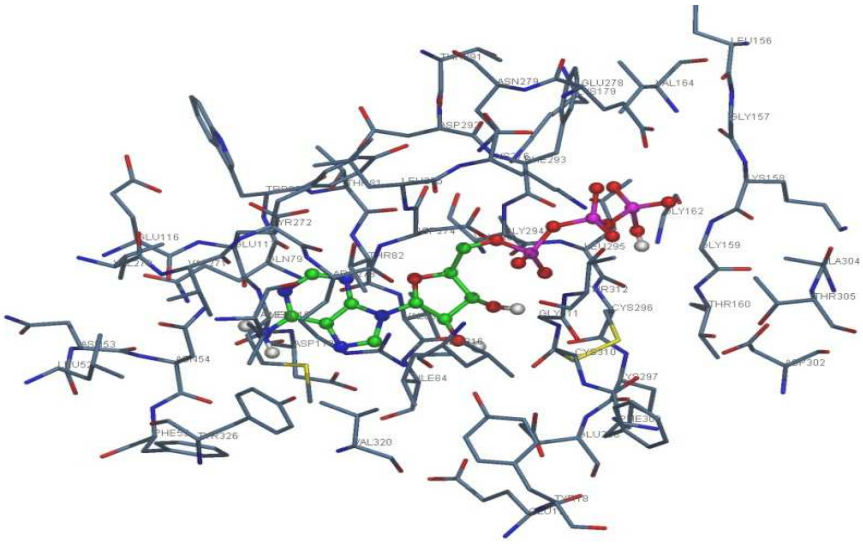

Figure2: Showing docked structure of ATP with of RAC-alpha serine/threonine-protein kinase. The blue cylindrical line represents interacting side chain of protein kinase and ball stick represent ATP molecule

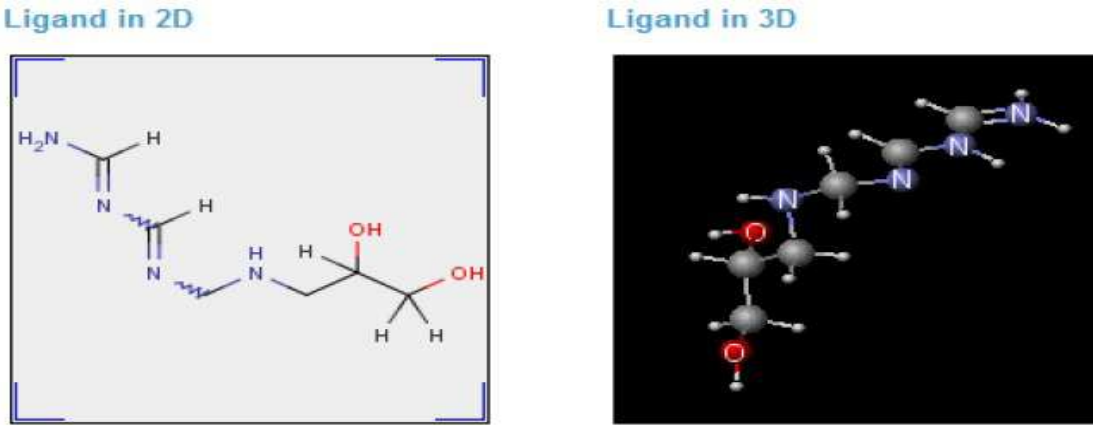

Figure 3: 2D and 3D structure of ligand - N-{[{[(2R)-2, 3 dihydroxypropyl] amino} methyl] amino} methanimidamide designed on the basis of the hydrogen bonding pattern between ATP and RAC-alpha serine/threonine-protein kinase.

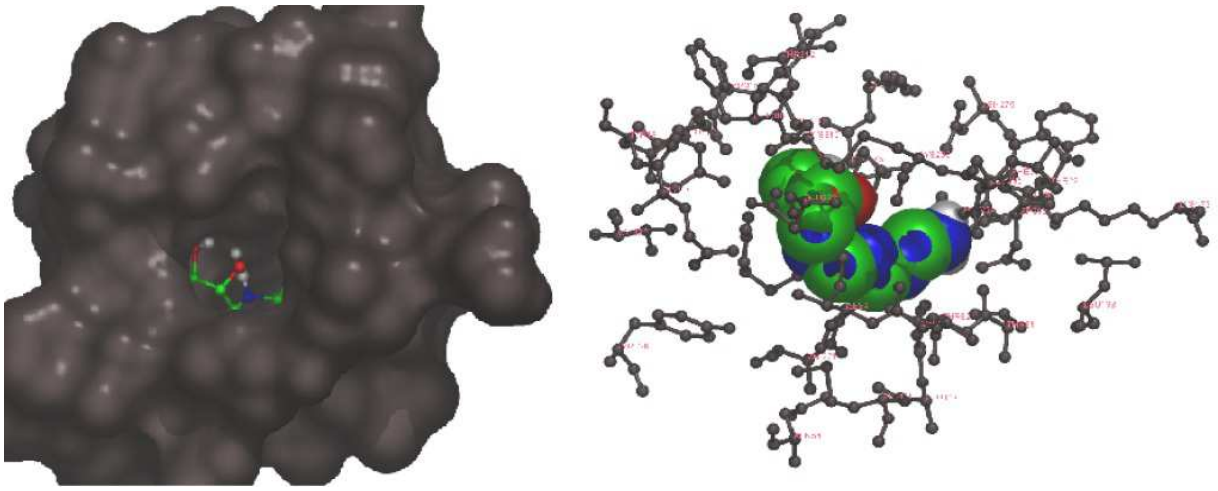

Figure 4: Showing docked structure of ligand with of RAC-alpha serine/threonine-protein kinase. The brown colour represents interacting side chain of target protein kinase and green, blue and red colour represents designed ligand.

	ATP	Ligand
Estimated Free Energy of Binding	+0.61 kcal/mol	-4.79 kcal/mol
Estimated Inhibition Constant, Ki		309.26uM
vdW+Hbond+Desolv Energy	-3.20 kcal/mol	-4.56 kcal/mol
Electrostatic Energy	+0.34kcal/mol	-2.11 kcal/mol
Total Intermolecular Energy	-2.86kcal/mol	-6.67 kcal/mol

Table 1: Value of docking analysis of RAC-alpha serine/threonine-protein kinase with ATP and ligand

Molecular Weight	175.00
Hydrogen Bond Donor	6
Hydrogen Bond acceptor	4
LogP	-2.695
Molar Refractivity	47.221

Table 2: Lipinski's Value of ligand.