J Biochem Tech (2012) 3(4):426-430
ISSN: 0974-2328

Taxifolin inhibits GTP binding in wild Apo and mutant (V12G) Apo forms of Ras: Molecular docking and molecular dynamics simulation study

Sharad Verma, Amit Singh, Abha Mishra*

Received: 15 March 2012 / Received in revised form: 14 February 2012, Accepted: 17 September 2012, Published online: 30 October 2012

Abstract

Ras proteins, the inner plasma membrane localized small G proteins, are involved in the transduction of external stimuli to its main effector Raf kinase. Point mutation in the H-Ras p21 (G12V) leads to loss of intrinsic GTPase activity so that Ras-GTP complex continuously relay signal which is associated with human cancers. Activation of oncogenic receptor tyrosine kinases also a prominent cause of continuous signal transduction through wild Ras. Taxifolin, a plant originated polyphenol, is a principal active component of several plants such as *Larix gmelini.* Molecular docking revealed that taxifolin captured GTP binding site in apo Ras (wild/mutant). This interaction might be valuable to target newly synthesized nucleotide unbound Ras. Molecular dynamic simulation revealed that binding of taxifolin was stable at GTP binding site of different Ras forms and may lead to improper functioning of Ras in cancer cells for cancer chemotherapeutics.

Key words: Apo Ras, Ras-GTP complex, Taxifolin, Molecular dynamic simulation, Molecular docking.

Introduction

The Ras family of protooncogenes (H-Ras, N-Ras and K-Ras) codes for small proteins of about 189 amino acids (Valencia et al. 1991). Ras proteins are localized at inner plasma membrane and are involved in the transduction of external stimuli to effector Raf kinase (Barbacid 1987). These proteins bind GDP/GTP and possess intrinsic GTPase activity allowing inactivation following signal transduction in the normal cells (Khosravi-far and Der 1994).

Sharad Verma, Abha Mishra*

School of Biochemical Engineering, Indian Institute of Technology, Banaras Hindu University, Varanasi-221005, India

*Email: abham.bce@itbhu.ac.in

Amit Singh

Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, India

Point mutation in the Ras is one of the most frequent genetic alterations associated with human cancers (Spandidos and Wilkie 1984). Approximately 90% of these activating mutations occur in codons 12 and 59 (Russo et al. 2005). These genetic alterations have been identified in a significant percentage of bladder tumors. Specifically, the mutation replaces the amino acid glycine with the amino acid valine at position 12 (RasG12V) (Oxford and Theodorescu 2003). As a result of these mutational changes, the mutated RasG12V-p21 has a structure that lost its ability to bind with GTPase activating protein (GAP) required for GTP to GDP conversion (Kiaris and Spandidos 1995). These changes keeping the Ras in the GTP-bound (activated) state contributing to a malignant cell phenotype (Bos 1989, Henson and Gibson 2006). There has been a variety of approaches attempted for inhibiting Ras-induced activation of the Raf, ERK mitogenactivated protein kinase (MAPK) signaling cascade and inhibitors of farnesyltransferase (Khazak et al. 2007, Konstantinopoulos et al. 2007, Roberts and Der 2007) . In addition, non-steroidal drug and peptide inhibitors of Ras–Raf interaction have been developed. Sulindac sulfide, an anti-inflammatory drug was shown to decrease the Ras-induced activation of the Raf kinase (Herrmann et al. 1998, Jayakanthan et al. 2009). Apart of these consequences, upstream mutant receptor tyrosine kinases can also turned on Ras wild form via its activator proteins such as sos (son of sevenless) (Patgiri et al. 2011).

Taxifolin is a one of the the principal active component of *Larix gmelini* (Huang et al. 2005) and several other plants such as *Silybum marianum, Acacia sp., Rhododendron sp.* etc. Taxifolin (2-(3,4-dihydroxyphenyl)-2,3 dihydro- 3,5,7-trihydroxy-4H-benzopyran-4-one) is a dihydroflavonol with distinguished antioxidant activity compared to other antioxidants (Audron et al. 2000, Bong-Sik et al. 2000). It can eliminate free radicals in the body, improve the impermeability of capillary vessels and recover their elasticity effectively. It is not embryotoxic and does not lead to malformations, hyper susceptibility or mutations (Wang et al. 2011). In our previous study, we found that taxifolin stably bound to VEGFR-2 kinas nucleotide binding site (Verma et al 2012). Therefore, an attempt was made to explore the potential of taxifolin to modulate Ras signaling by direct binding to the GTP binding site of Ras which is critically required for the signal transduction mediated by GTPase cycle.

Materials and Methods

Molecular docking

AutoDock 4.0 suite was used as molecular-docking tool in order to carry out the docking simulations (Morris et al. 1998). The crystal structure of Ras wild (pdb id 5P21) and RasG12V mutant (pdb id 2VH5) obtained from RCSB protein data bank. The structures of ligands taxifolin generated from smile strings followed by energy minimization. All the heteroatom was removed except Mg ion. Hydrogen atoms were added to protein crystal structures using autodock program while all non polar hydrogen atoms were merged. Six bonds were made ''active'' or rotatable for the taxifolin. The mode of binding of taxifolin on mutant RasG12V-GTP complex was analyzed using coordinates file (pdb id 2VH5) without removing GTP coordinates. Lamarckian genetic algorithm was used as a search parameter which is based on adaptive local search. Short range van der Waals and electrostatic interactions, hydrogen bonding, entropy losses were included for energy based autodock scoring function (Berendsen et al. 2005, Sudhamalla et al. 2010). The lamarkian GA parameters used in the study were: numbers of run, 30; population size, 150; maximum number of evals; 25000000, number of generation; 27000, rate of gene mutation; 0.02 and rate of cross over; 0.8. Blind docking is carried out using grid size 126, 126 and 126 along the X, Y and Z axes with 0.375 □ spacing. RMS cluster tolerance was set to 2 □. Flexible docking was performed which includes a flexible ligand and a rigid receptor.

Molecular dynamic simulation in water

A 5000 ps MD simulation of the complex was carried out with the GROMACS4.5.4 package using the GROMOS96 43a1 force field (Van Gunsteren et al. 1996, Lindah et al. 2001). The lowest binding energy (most negative) docking conformation generated by Autodock was taken as initial conformation for MD simulation. The topology parameters of all Ras forms were created by using the Gromacs program. The topology parameters of taxifolin were built by the Dundee PRODRG server (Schuttelkopf and Aalten 2004). The complex was immersed in an octahedron box of extended simple point charge (SPC) water molecules (Van Gunsteren et al. 1998). The solvated system was neutralized by adding 6 Na ions (in Apo wild and mutant) while 10 in case of mutant RasG12V-GTP-taxifoli complex. To release conflicting contacts, energy minimization was performed using the steepest descent method of 1000 steps followed by the conjugate gradient method for 1000 steps. MD simulation studies consist of equilibration and production phases. The position-restrained dynamics simulation of the system was carried out at 300 K for 300 ps. Finally, the full system was subjected to 5000 ps MD production run at 300 K temperature and 1 bar pressure. For analysis, the atom coordinates were recorded every 0.5 ps during the MD simulation.

Results and Discussions

Molecular docking

Taxifolin was found to bind at GTP-binding site of wild and mutant Ras apo form with highest negative binding energy -39.22 Kj/Mol and -40.06 Kj/Mol respectively. Free energy of binding is calculated as a sum of four energy terms of intermolecular energy (van der Waals, hydrogen bond, desolvation energy and electrostatic energy), total internal energy, torsional free energy and unbound system energy. The major interactions shown in the Ras-GTP-binding site and taxifolin are the important H-bonds with residues: Gly 31, 116, 119, 146 and Ala 147 (in wild Ras) and Gly 13, Gly 31 and Asp 38 (in mutant Ras) (Fig 1).

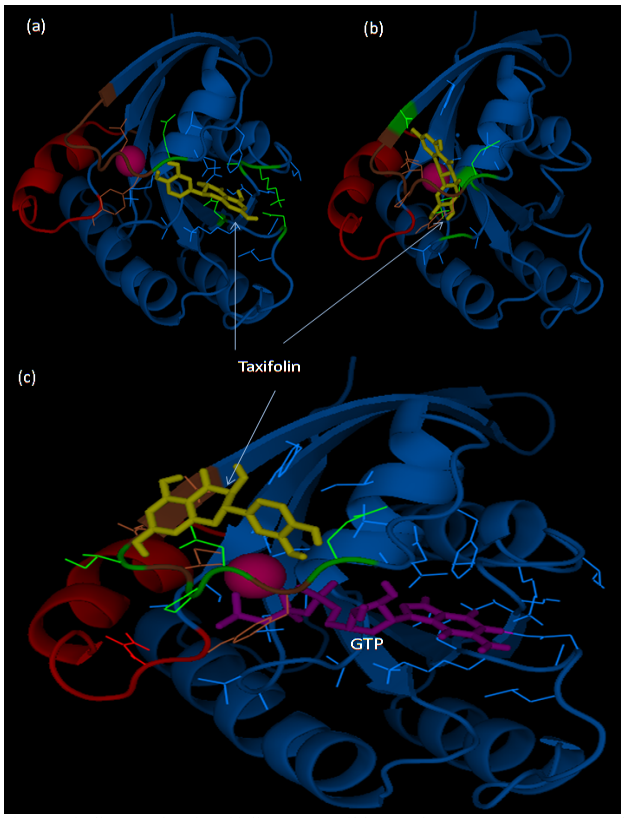

Fig. 1. Binding mode of taxifolin on (a) wild Ras, (b) mutant Ras G12V and (c) mutant Ras G12V-GTP complex (pink sphere- Mg^{+2}, brown - Switch I, red – Switch II and green- taxifolin interacting residues, yellow-taxifolin)

These results suggest that taxifolin have very high affinity for GTP-binding site of Ras apo forms (GTP unbound) and probably act as competitive inhibitor. This feature of taxifolin can be used to target newly synthesized Ras in cellular system and to intermediate state during GDP to GTP transition. GTP binding to apo form is a two step process in which the first interaction between Ras and GTP is very week (Grand and Owen 1991, john et al. 1990, Zhang and Matthews 1998). These findings supported by the very less binding energy (-19.44 Kj/Mol) of GTP (14 rotable bonds) for mutant Ras (pdb id 2VH5) obtained by autodock using similar procedure as described in section 2.1. The triphosphate moiety of GTP was found to interact by H-bonding with residues: Gly 13, Val 14, Gly 15, and Lys 16, Asp 33 and Thr 35 along with coordination with Mg^{+2} while sugar moiety formed H-bond with Asp 33 and Asp 38. Nitrogen base not contributed to H-bonding during initial interaction (Fig 2). The main cause of low affinity is the very high torsional free energy of GTP which increases the total binding energy (less negative). Molecular docking analysis evidenced that taxifolin has approximate 2 times more affinity for mutant Ras as compared to GTP for initial interaction. In case of Ras-GTP complex taxifolin occupies site nearby to GTP-binding site with binding energy -25.74 Kj/Mol (Fig 1(c)). Taxifolin was found to interact by H-bonds with switch I residues (Shima et al. 2010): Gly 31, Asp 33, Pro 34 and Ile 36.

Molecular dynamic simulation in water

The Ras-taxifolin complex with the largest negative binding energy obtained using Autodock was used for carrying out MD simulation. All-atom MD simulations represent a convenient method to investigate differences in motions of residues/atoms that are led to structural and chemical changes (Morra et al. 2009). We have

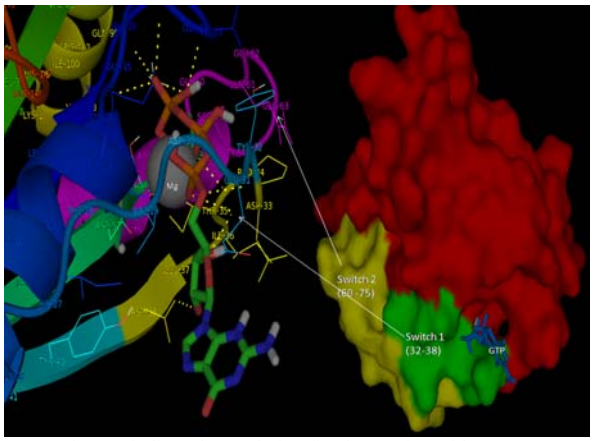

Fig. 2. Binding of GTP during first interaction with Ras (G12V)

analyzed the time dependent behavior of MD trajectories for different Ras forms (Ras (unbound), Ras-taxifolin and mutant Ras-taxifolin) including root mean square deviation (RMSD) for all backbone atoms, potential energy profile as well as the average residue fluctuations of the residues (RMSF). RMSD trajectory of backbone of Ras, Ras-taxifolin and RasG12V-taxifolin complex was calculated at every 0.5 ps using its initial structure as a reference. Fig 3(a) shows that the RMSD trajectories were always less than 2.5 Å (0.25 nm) for the entire simulation suggesting the stability of simulation system. The trajectories were equilibrated after about 2500 ps. In all three cases no great difference in trajectory was found. Potential energy profile obtained for all MD simulation production run also showed very stable profile. Ras and Ras-taxifolin potential energy remain stable about the average value of -241550 Kj/Mol while RasG12V-taxifolin stabilized at average value of -257758 Kj/Mol throughout the MD simulation (Fig 3(b)). RMSF analysis revealed that switch I (32-38) and switch II (60-75) residues of mutant Ras–taxifolin complex were fluctuate more as compared to mutant Ras–GTP-taxifolin complex specially 35. The reason behind this outcome might be the bonding between residues and GTP, and taxifolin. Apart of this Val 12 show very low fluctuation in GTP bound form as compared to unbound as γ-Phosphate hindered the motion of residue. In case of wild apo and taxifolin bound form no significant difference in fluctuation in switch I (32-38) and switch II (60-75) residues was found (Fig 3(c)).

Ramachandran plot analysis (http://mordred.bioc.cam.ac.uk /~rapper/rampage.php) of 5000 ps conformation of wild Ras, Ras-taxifolin, mutant Ras-taxifolin and RasG12V-taxifolin complex revealed that taxifolin binding leads to unallowed conformation as compared to wild Ras (taxifolin unbound form). The percentage of residues in favoured and allowed region are 97.4 (unbound), 94.4 (wild Ras-taxifolin) and 96.3 (RasG12V-taxifolin). Further, Ramachandran plot analysis of 3000 ps conformation of RasG12V-GTP-taxifolin complex also favours the above mentioned results. The percentage of residues in favoured and allowed region is 94.9 (Fig 4).

We set out to quantify correlated motions to identify protein regions that move in a concerted fashion depending on the presence of taxifolin. We have performed analysis of the cross-correlation coefficients of wild Ras (apo), wild Ras-taxifolin, mutant Ras-taxifolin and mutant Ras-GTP-taxifolin residues from MD simulations. This approach provides a convenient framework to identify concerted, nonrandom fluctuations (Ichiye and Karplus 1991, Bradley 2008) as a function of the ligand type. The correlation matrix describes the linear correlation between any pairs of backbone atoms as they move around their average position during

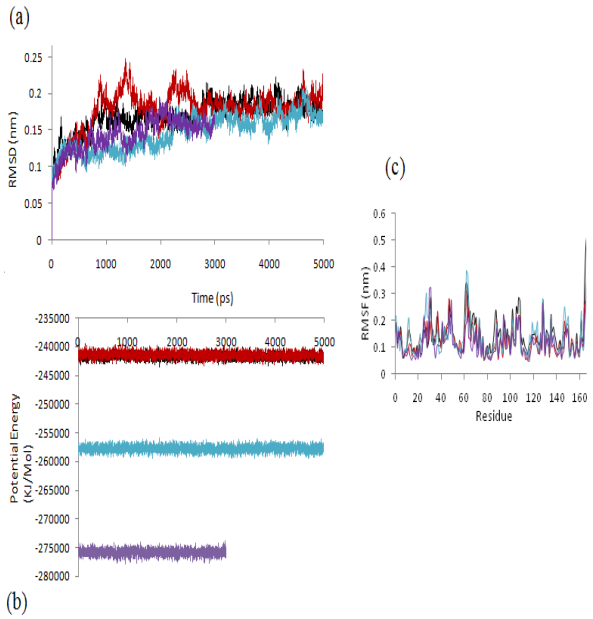

Fig. 3. (a) Plot of root mean square deviation (RMSD) of backbone RMSDs were calculated using the initial structures as templates. The trajectories were captured every 0.5 ps until the simulation time reached 5000 ps (3000 ps for Ras-GTP-taxifolin complex), (b) Potential energy profile during 5000 ps MD simulation (3000 ps for Ras-GTP-taxifolin complex), (c) Root mean square fluctuation (RMSF) of residues during 5000 ps MD simulation (3000 ps for Ras-GTP-taxifolin complex) (Black- unbound wild Ras, Red- wild Ras – Taxifolin, Blue- mutant Ras-taxifolin and Purple- mutant Ras-GTP-taxifolin complex)

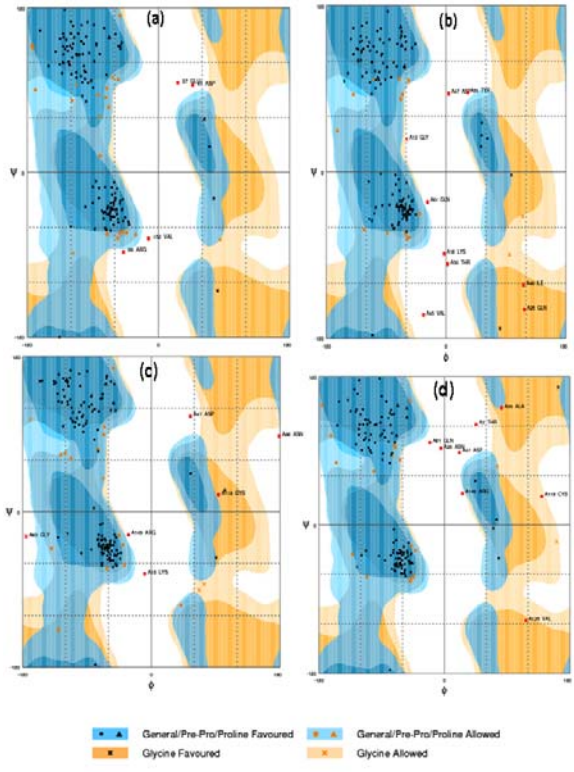

Fig. 4. Ramachandran plot for different forms of Ras: (a) Ramachandran plot for the wild Ras apo, (b) Ramachandran plot for the wild Ras-taxifolin complex; (d) Ramachandran plot for mutant Ras-taxifolin complex, (d) Ramachandran plot for mutant Ras-GTP-taxifolin complex

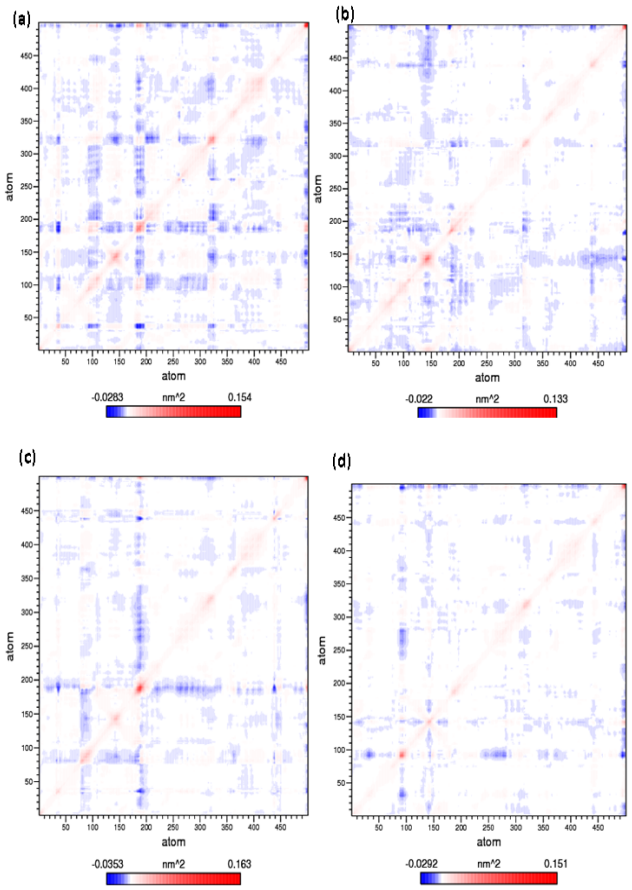

Fig. 5. Cross-Correlation matrices calculated considering the motion of backbone atoms around the average position: (a) Cross-Correlation Matrix for the wild Ras apo, (b) Cross-Correlation Matrix for the wild Ras-taxifolin complex; (d) Cross-Correlation Matrix for mutant Ras-taxifolin complex, (d) Cross-Correlation Matrix for mutant Ras- GTP-taxifolin complex (color code: red corresponds to coordinated motion of the atom pair along the same direction, whereas (color code: blue corresponds to a negative correlation indicates motion in opposite directions).

dynamics. At a qualitative level, a positive correlation between two atoms reflects a concerted motion along the same direction, whereas a negative correlation indicates an opposite direction motion (Morra et al. 2009).

Analysis of correlation matrices of backbone revealed that taxifolin binding reduces anticorrelation motion in wild Ras as compared to unbound form. Mutant form showed significantly less correlation as compared to wild form. In mutant Ras-GTP-taxifolin most of backbone atoms of residues not showed any type of correlation motion (positive or negative). Switch I (32-38) and Switch II (60-75) which are responsible for GAF binding show positive correlation in Ras (unbound), negative correlation in Ras–taxifolin, negative correlation mutant Ras-taxifolin and very mild correlation in mutant Ras-GTP-taxifolin complex (Fig 5).

Conclusion

Continuous signaling by mutated RTKs or by mutated Ras itself leads to uninterrupted downstream cascade which is responsible for cancer phenotype. Molecular docking revealed that taxifolin captured GTP binding site in apo Ras (wild/mutant). This interaction might be valuable to target neo apo Ras and intermediate nucleotide unbound form during GDP to GTP exchange. These results were first time reported in present study which would be valuable in further study of Ras inhibition for more effective cancer chemotherapeutics.

Acknowledgement

One of the authors (Sharad Verma) is thankful of Council of Scientific and Industrial Research (CSIR), India for providing Senior Research Fellowship.

References

Audron M, Regina K, Jonas A, Narimantas N (2000) Inhibition of phthalocyanine-sensitized photohemolysis of human erythrocytes polyphenolic antioxidants: Description of quantitatives structure–activity relationships. Can Lett 157:39–44.

Barbacid M (1987) Ras genes. Annu Rev Biochem 56:779-827.

Berendsen HJC, Van der Spoel D, Van Drunen R (1995) GROMACS - a message-passing parallel molecular-dynamics immplecations. Comp Phys Comm 91:43–56.

Bong-Sik Y, In-Kyoung L, Jong-Pyung K, Sung-Hyun C, Gyu-Seop S, Ick-Dong Y (2000) Lipid peroxidations inhibitory activity of some constituents isolated from the stem bark of Eucalyptus globulus. Arch Pharmacal Res 23:147–150.

Bos JL (1989) ras oncogenes in human *cancer*: a review. Cancer Res 49:4682-4689.

Bradley MJ, Chivers PT, Baker NA (2008) Molecular dynamics simulation of the Escherichia coli NikR protein: equilibrium conformational fluctuations reveal interdomain allosteric communication pathways. J Mol Biol 378:1155–1173.

Grand RJA, Owen D (1991) The biochemistry of ras, p21. Biochem J 279:609-631.

Henson ES, Gibson SB (2006) Surviving *cell* death through epidermal growth factor (*EGF) signal* transduction pathways: Implications for cancer therapy. Cell Sig 18:2089-2097.

Herrmann C, Block C, Geisen C, Haas K, Weber C, Winde G, Moroy T, Muller O (1998) Sulindac Sulfide inhibits Ras signaling. Oncogene 17:1769-1776.

Huang ZH, Fang GZ (2005) Study on purifying arabinogalactan from Larix gmelini Rupr. by column chromatography. Chem Indus Forest Prod 25:125–128.

Ichiye T, Karplus M (1991) Collective motions in proteins-a co-variance analysis of atomic fluctuations in molecular dynamics and normal mode simulations. Proteins 11:205–217.

Jayakanthan M, Wadhwa G, Mohan TM, Arul L, Balasubramanian P, Sundar D (2009) Computer-aided *drug design* for cancer-causing H-Ras *p*21 mutant protein. Lett Drug Des Disc 6:14-20.

John J, Sohmen R, Feuerstein J, Linke R, Wittinghofer A, Goody RS (1990) Kinetics of interaction of nucleotides with nucleotide-free H-ras p21. Biochem 29:6058-60765.

Khazak V, Astsaturov I, Serebriiskii IG, Golemis EA (2007) Selective Raf Inhibition in Cancer Therapy. Expert Opin Ther Targ 11:1587-1609.

Khosravi-far R, Der CJ (1994) The Ras signal transduction pathway. Cancer Metas Rev. 13:67-89.

Kiaris H, Spandidos DA (1995) Mutations of ras genes in human tumoursInt. J Oncol 7:413-421.

Konstantinopoulos PA, Karamouzis MV, Papavassiliou AG (2007) Post-translational modifications and regulation of the RAS

super-family of GTPases as anticancer targets. Nat Rev Drug Discov 6:540-555.

Lindah E, Hess B, Van der Spoel D (2001) Gromacs 3.0: A package for molecular simulation and trajectory analysis. J Mol Model 7:306–317.

Morra G, Verkhivker G, Colombo G (2009) Modeling Signal Propagation Mechanisms and Ligand-Based Conformational Dynamics of the Hsp90 Molecular Chaperone Full-Length Dimer. PLoS Comput Biol 5:e1000323

Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ (1998) Automated Docking Using a Lamarckian Genetic *Algorithm and and Empirical Binding Free Energy Function. J Co*mp *Che*m 19:1639-1662.

Oxford G, Theodorescu D (2003) The role of Ras superfamily proteins in bladder cancer progression. J Urol 170:1987-1993.

Patgiri A, Yadav KK, Arora PS, Bar-Sagi D (2011) An orthosteric inhibitor of the Ras-Sos interaction. Nat Chem Biol 7:585–587.

Roberts PJ, Der CJ (2007) Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene 26:3291-3310.

Russo A, Bazan V, Agnese V, Rodolico V, Gebbia N (2005) Prognostic and predictive factors in colorectal cancer: Kirsten Ras in CRC (RASCAL) and TP53CRC collaborative studies. Ann Oncol 4:44-49.

Schuttelkopf AW, van Aalten (2004) DMF PRODRG: A tool for high-throughput crystallography of protein-ligand complexes. Acta Crystallogr 60:1355–1363.

Shima F, Ijiri Y, Muraoka S, Liao J, Ye M, Araki M, Matsumoto K, Yamamoto N, Sugimoto T, Yoshikawa Y, Kumasaka T, Yamamoto M, Tamura A, Kataoka T (2010) Structural Basis for Conformational Dynamics of GTP-bound Ras Protein. J Biol Chem 285:22696–22705.

Spandidos DA, Wilkie NM (1984) Malignant transformation of early passage rodent cells by a single mutated human oncogene. Nature 310:469-475.

Sudhamalla B, Gokara M, Ahalawat N, Amooru ZG, Subramanyam R (2010) Molecular Dynamics Simulation and Binding Studies of beta-Sitosterol with Human Serum Albumin and Its Biological Relevance. J phys Chem 114:9054-9062.

Valencia A, Chardin P, Wittinghofer A, Sander C (1991) The ras Protein Family: Evolutionary Tree and Role of Conserved Amino Acids. Biochem 30:4637-4648.

Van Gunsteren WF, Billeter SR, Eising AA, Hunenberger PH, Kruger PKHC, Mark AE, Scott WRP, Tironi IG (1996) Biomolecular Simulation: The GROMOS96 Manual and User Guide.

Van Gunsteren WF, Daura X, Mark AE (1998) The GROMOS force field.Encyclopedia Comp Chem 2:1211-1216.

Verma S, Singh A, Mishra A (2012) Taxifolin acts as type I inhibitor for VEGFR-2 kinase: Stability evaluation by molecular dynamic simulation. J App Pharm Sci 02:41-46

Wang Y, Zu Y, Long J, Fu Y, Li S, Zhang D, Li J, Wink M, Efferth T (2011) Enzymatic water extraction of taxifolin from wood sawdust of Larix gmelini (Rupr.) Rupr. and evaluation of its antioxidant activity.Food Chem. 126:1178–1185.

Zhang J, Matthews C R (1998) The role of ligand binding in the kinetic folding mechanism of human p21(H-ras) protein. Biochem 37:14881-14890.