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Short Communication

In silico binding affinity studies of N-9 substituted 6-(4-(4-propoxyphenyl)piperazin-1-yl)-9H-purine derivatives-Target for P70-S6K1 & PI3K-δ kinases

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ABSTRACT

P70-S6K1 & PI3K-δ kinases are identified to be involved in many physiological processes associated with cancer, therefore many of the inhibitors being designed to target these kinases are in clinical trials. In the current study we have exploited the N-9 substituted 6-(4-(4-propoxyphenyl) piperazin-1-yl)-9H-purine derivatives for their inhibitory properties with the above kinases. We have used an *in silico* docking study with seventeen purine derivatives for their binding affinity calculations. The binding affinities of these small molecules with P70-S6K1 & PI3K-δ were performed using AutoDock Vina. Among all the compounds, PP16 showed highest binding affinity of −14.7 kcal/mol with P70-S6K1 kinase & −17.2 kcal/mol with PI3K-δ kinases as compared to the molecules under clinical trials (PF-4708671 & IC-87114). Docking studies revealed that N-9 coumarine substituted purine derivative could be one of the potential ligands for the inhibition of P70-S6K1 & PI3K-δ kinases. Hence, this compound can be further investigated by *in vitro* and *in vivo* experiments for further validation.

1. Introduction

Ribosomal protein S6 kinase (P70-S6 Kinase1) and phosphoinositide 3-kinase (PI3K-δ) are protein kinases involved in cellular functions such as cell-cycle progression, proliferation and differentiation (Leise and Michael, 2000; Christian et al., 2007). Phase I clinical trials evaluating various PI3K inhibitors have been reported in recent years (Irene and Lillian, 2012). PI3K inhibitors obtained from natural products have been reported in the literature. Despite the various hurdles in identifying the target oriented kinase inhibitors, several quinazolinones, pyrimidinylmorpholines, morpholinothiazoles, pteridines, thiazolidinones, 2-Aminothiazoles based derivatives have been reported to show prominent PI3K inhibition in terms of IC_{50} values (Michael and Jennifer, 2009). In recent years many companies like Novartis, Genentech and Sanofi are involved in the development of PI3K inhibitors, which are being evaluated for clinical trials (Wennan et al., 2017; Khurum et al., 2013). Although the researchers have synthesised many inhibitors but have failed to identify structural features around the ATP-binding site of the targeted enzyme. (Ipsita and Mahitosh, 2012). Therefore have failed to enter into clinical trials.

P70-S6 Kinase is made up of 2 chains namely alpha and beta chains with amino acid length of 327. S6 kinase has two isoforms p85 (S6K1) and p70 (S6K1). The binding site is at 123 and the active site is at 218. It is activated by mTORC1; isoform Alpha I and isoform Alpha II are sensitive to rapamycin, which inhibits activating phosphorylation at Thr-412 (Kim et al., 2009). The activation of kinase requires multiple phosphorylation occurrences on serine/threonine residues. Partially activated kinase domain is phosphorylated in the activational loop which confirms their role in activation. Binding site for nucleotide is from 97 to 105 position in S6 kinase and (Keshwani et al., 2009). P70 S6 kinase/mTOR signaling pathway has been studied in various types of cancers like breast cancer, lung cancer etc (Le et al., 2003; Bostner et al., 2015).

Phosphoinositide-3-kinase (PI3K) phosphorylates PftdIns(4,5) P2 (Phosphatidylinositol 4,5-bisphosphate) to generate phosphatidylinositol 3,4,5-trisphosphate (PIP3). PIP3 plays a key role

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by recruiting PH domain-containing proteins to the membrane, including AKT1 and PDPK1, activating signalling cascades involved in cell growth, survival, proliferation, motility and morphology and mediates immune responses (Clayton et al., 2002; Ali et al., 2004). It is made of an alpha chain consisting of 939 amino acid residues and has two isoforms. PI3K is activated by growth factors and cytokine receptors through a tyrosine-kinase-dependent mechanism and are also activated by RAS (Saudemont et al., 2009). PI3K delta kinase together with PI3K gamma kinase has an important role in inflammation and rheumatoid arthritis (Rommel et al., 2007).

Many purine & pyrimidine based P70-S6K1 & PI3K-δ kinase inhibitors are also under clinical trial. The first S6K1-specific inhibitor to be reported is PF-4708671, which is also pyrimidine based molecule (Laura et al., 2010). Other pyrimidine derivative LY2584702 and its tosylate are selective ATP-competitive P70-S6K1 inhibitor with IC_{50} of 4 nM and are reported to inhibit P70-S6 kinase in patients with solid tumours (Anthony et al., 2014). Further, some purine and pyrimidine based PI3K-δ inhibitors like Pictilisib and IC-87114 are reported to inhibit the enzyme with respect to broader specificity (Fig. 1) (David et al., 2016; Leena et al., 2016; Ingrid and Carlos, 2016).

Till today the researchers have been practising to synthesise the analogues and effectively used in inhibitory studies. But alternative strategies need to be used to have better knowledge on affinity of the ligands on a particular receptor through computational studies (Harish et al., 2016; Harish et al., 2013). In particular the docking studies, where the structural aspects are used to selectively target proteins (kinases). This new strategy helps researchers to exploit and identify selective inhibitors with much effective and easier approach (Leonardo et al., 2015; Xuan-Yu et al., 2011; Garrett and Marguerita, 2008; Khaled and Robert, 2013; Sérgio et al., 2006; Jerome de et al., 2016; Kendall et al., 2016).

In silico absorption, distribution, metabolism and excretion (ADME) properties of ligands helps us to predict the pattern of mutagenic and tumorigenic properties, which are used to screen out the synthesized ligands for their further usage as potent leads (Mohd Sajid et al., 2013). A molecular docking study require less cost, time and significantly helps high throughput screening without compromising in the quality of lead innovation (Mohammad Hassan et al., 2016). Dorzolamide was the first drug unambiguously used in human therapy, which was resulted from structure-based drug design (Arun and Sandra, 2014).

With the aid of this molecular docking study, we have identified the PI3K-δ & P70-S6K1 inhibitors of purine based compounds, which are reported to induce apoptosis in MCF-7 cancer cells earlier (Fig. 2) (Manjunath et al., 2016). Molecules synthesised in our lab possess phenyl piperazine at C-6 position and different substituents at N-9 position. We have kept phenyl piperazine scaffold constant at C-6 position of purine with a hope to obtain enhanced anticancer activity as reported in the literature (Chopra et al., 2014; Sun et al., 2015).

Purine derivatives having C-6 phenyl piperazine substitution are reported to inhibit PI3K-δ & P70-S6K1 kinases effectively (Neel et al., 2011; Patrick, 2016). Based on these observations, in the present work we have docked N-9 substituted 6-(4-(4-propoxyphenyl) piperazin-1-yl)-9H-purine derivatives to get more potent PI3K-δ & P70-S6K1 kinase inhibitors.

2. Materials and methods

2.1. Extraction of receptor from PDB

Three dimensional (3D) structures of the proteins were retrieved from Protein Data Bank (PDB) with following PDB codes 3A60- P70-S6 Kinase1 & 4XE0-PI3K-δ kinase (Fig. 3) (Berman, 2008). 3D structures of receptor obtained from the PDB were bound to a ligand molecule. Hence, to remove the bound ligand molecule, whole sequence of protein leaving behind the ligand sequence was selected using PYMOL software. Further, the selected sequence was copied and the 3D structure of P70-S6K1 was saved in PDB file format.

2.2. Extraction of ligand

The synthesised and characterised molecules (**PP04-PP21**) were drawn using the ChemDraw software. The obtained ligand molecule was saved as MOL files and was converted to PDB file to obtain 3D structure using the PYMOL software.

2.3. Preparation of molecules for docking

The receptor molecule retrieved from PDB was added with the polar hydrogen atoms and assigned with the partial atomic charges using Auto Dock Vina (Trott and Olson, 2010) as the retrieved

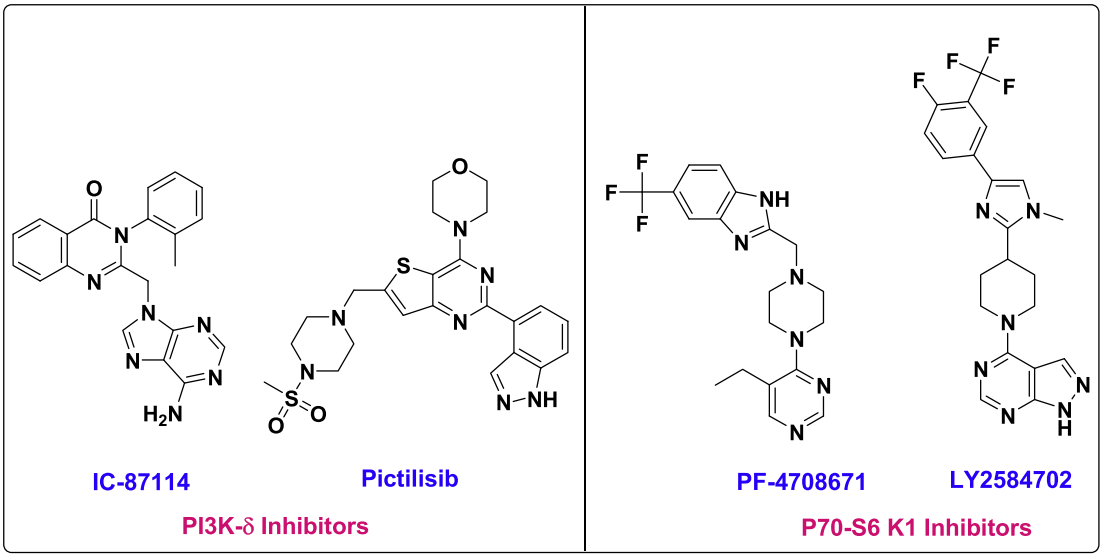

Fig. 1. Purine & Pyrimidine based PI3K-δ & P70-S6K1 inhibitors in clinical trials.

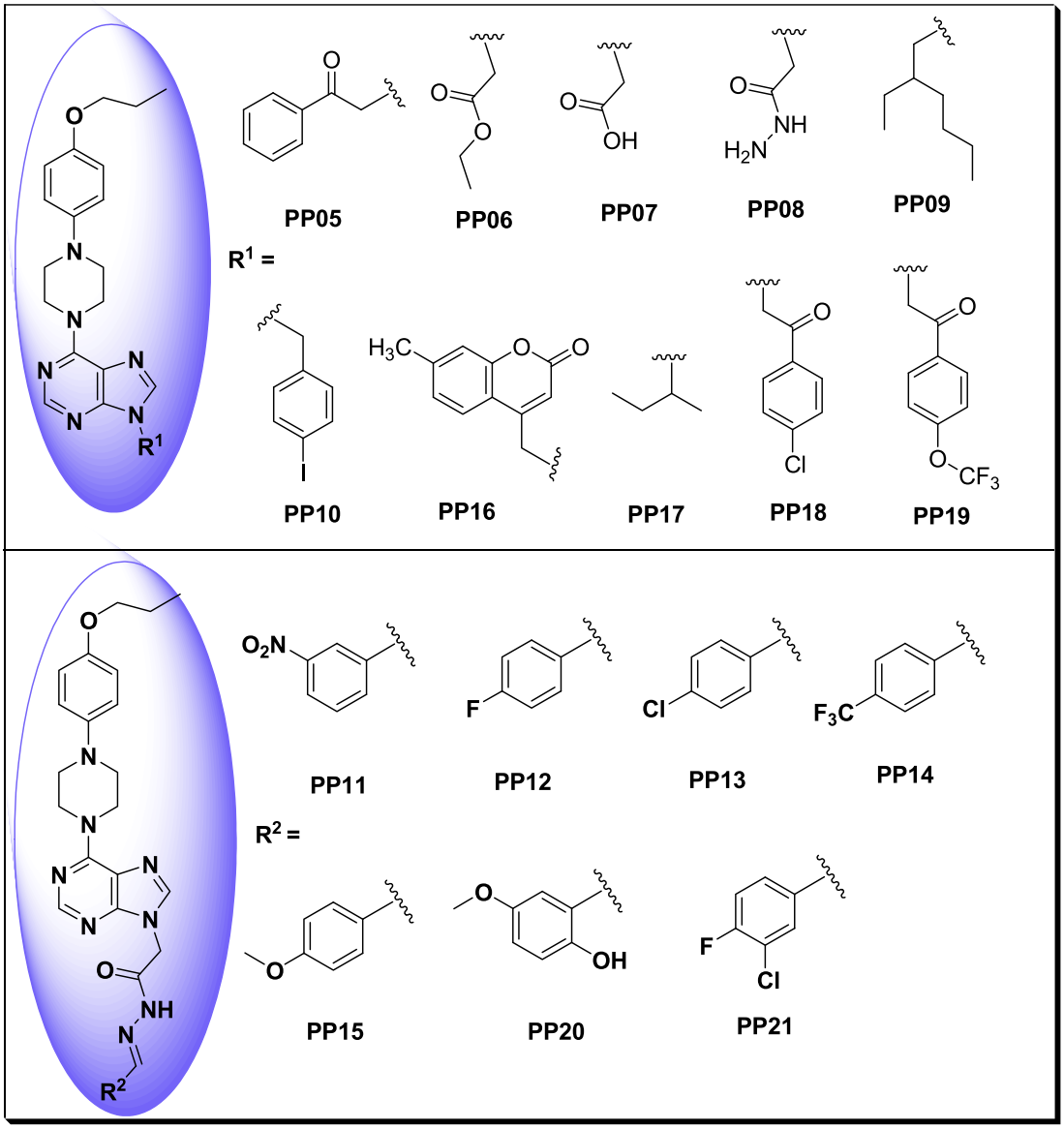

Fig. 2. Structures of the compounds screened for docking against P70-S6K1 & PI3K- δ kinases.

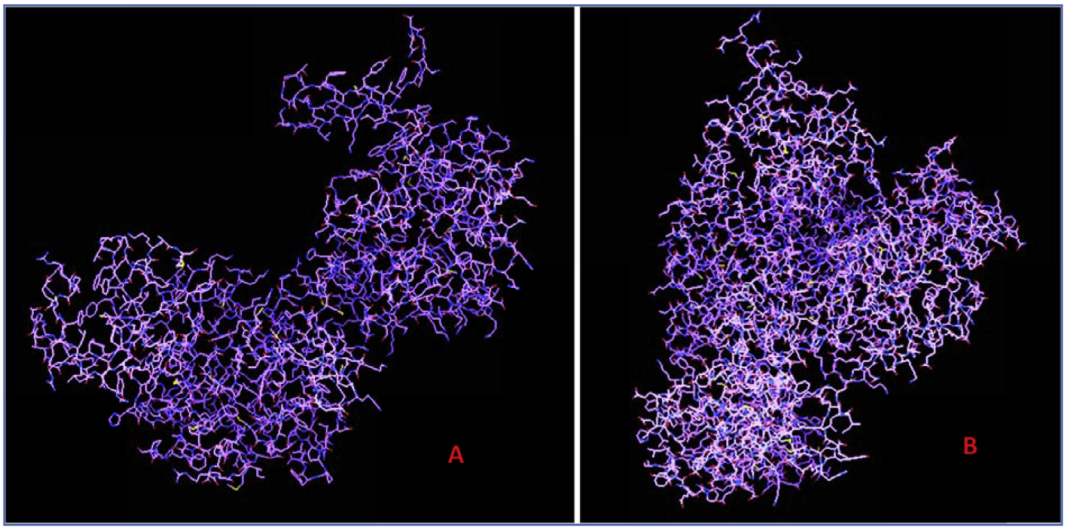

Fig. 3. 3D structures of kinases **(A)** P70-S6K1 (PDB Code: 3A60) and **(B)** PI3K-δ (PDB Code: 4XE0).

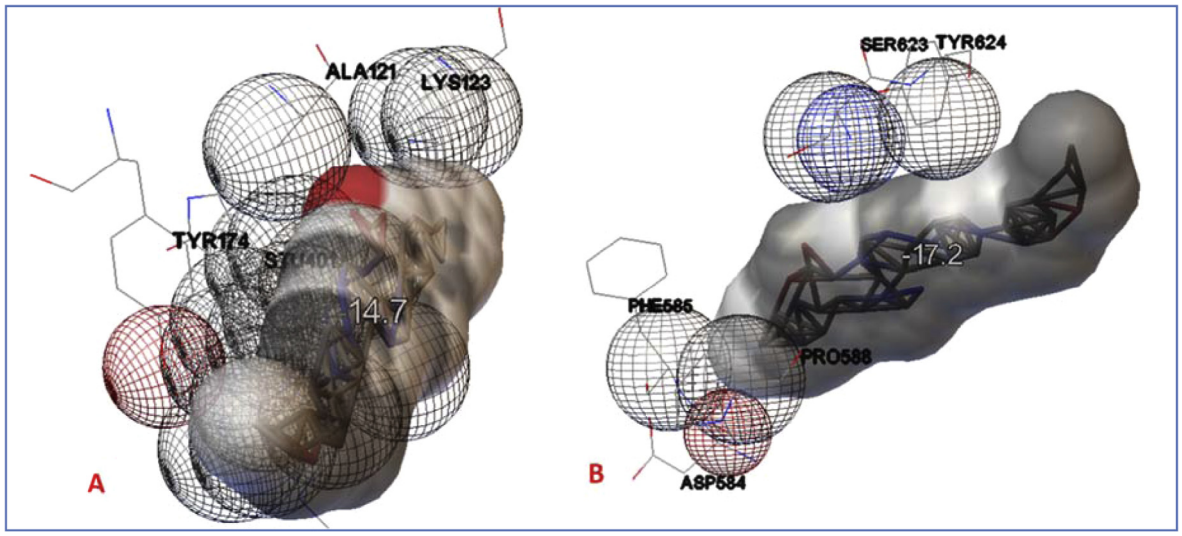

Fig. 4. Potential binding sites of compound PP16 **(A)** with P70-S6K1 kinase and **(B)** with PI3K-δ kinase.

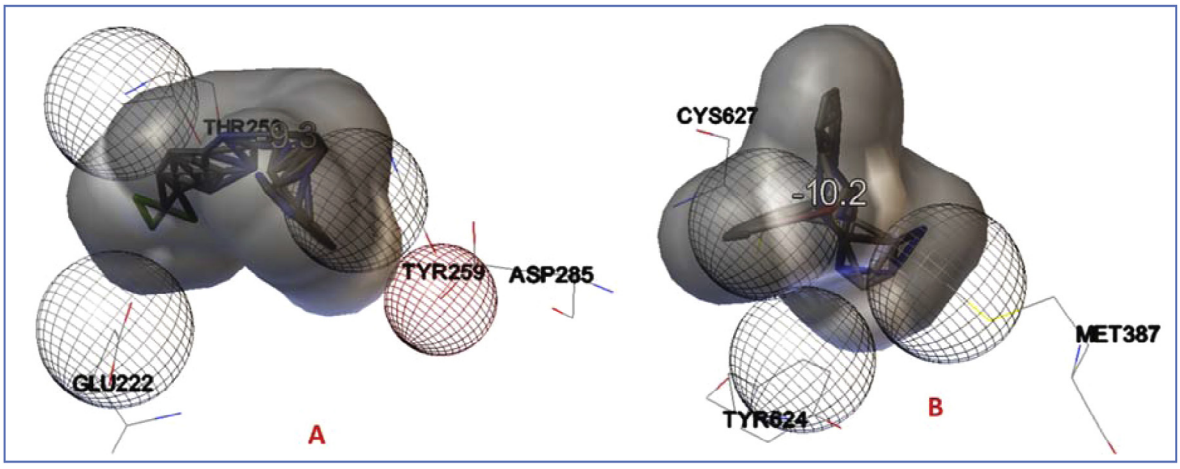

Fig. 5. Potential binding sites of (A) PF-4708671 with P70-S6K1 kinase and (B) IC-87114 with PI3K-δ kinase.

Table 1
Binding affinity of docked molecules & inhibitor PF-4708671 with P70-S6K1 kinase.

Molecules	Binding affinity (kcal/mol)	Molecules	Binding affinity (kcal/mol)
PP05	−12.8	PP14	−14.6
PP06	−13.0	PP15	−13.7
PP07	−12.3	**PP16**	**−14.7**
PP08	−12.6	PP17	−12.8
PP09	−13.7	PP18	−13.4
PP10	−13.9	PP19	−14.0
PP11	−13.1	PP20	−12.9
PP12	−12.8	PP21	–
PP13	−12.6	PF-4708671	−09.3

Table 2
Binding affinity of docked molecules & inhibitor IC-87114 with PI3K- δ kinase.

Molecules	Binding affinity (kcal/mol)	Molecules	Binding affinity (kcal/mol)
PP05	−13.0	PP14	———
PP06	−14.1	PP15	−15.2
PP07	−13.5	**PP16**	**−17.2**
PP08	−13.6	PP17	−13.6
PP09	−15.3	PP18	−15.7
PP10	−15.0	PP19	———
PP11	−14.4	PP20	−15.5
PP12	−14.8	PP21	—————
PP13	−14.3	IC-87114	−10.2

molecule didn't contain any charges. On the other hand ligands were added with Gasteiger charges and rotatable bonds were determined based on the ligand molecules.

Grid map was generated and 1.0 Å spacing was done for ligand binding. Grid dimension was adjusted with differing dimensions for different ligand molecules. Prior to actual docking, map was calculated by Auto Grid. The interaction energy between a particular ligand and receptor was calculated for entire binding site, which is done in discretized manner through a grid (Seeliger et al., 2010). Protein and ligand were embedded in a 3D grid and probe was assigned at each point and interaction energy for each atom of ligand was calculated.

2.4. Docking of receptor and ligand

Automated docking software Auto Dock Vina (Trott and Olson, 2010) was used to determine the binding affinities of ligands with the P70-S6K1 & PI3K-δ kinases. The Binding affinities of all 17

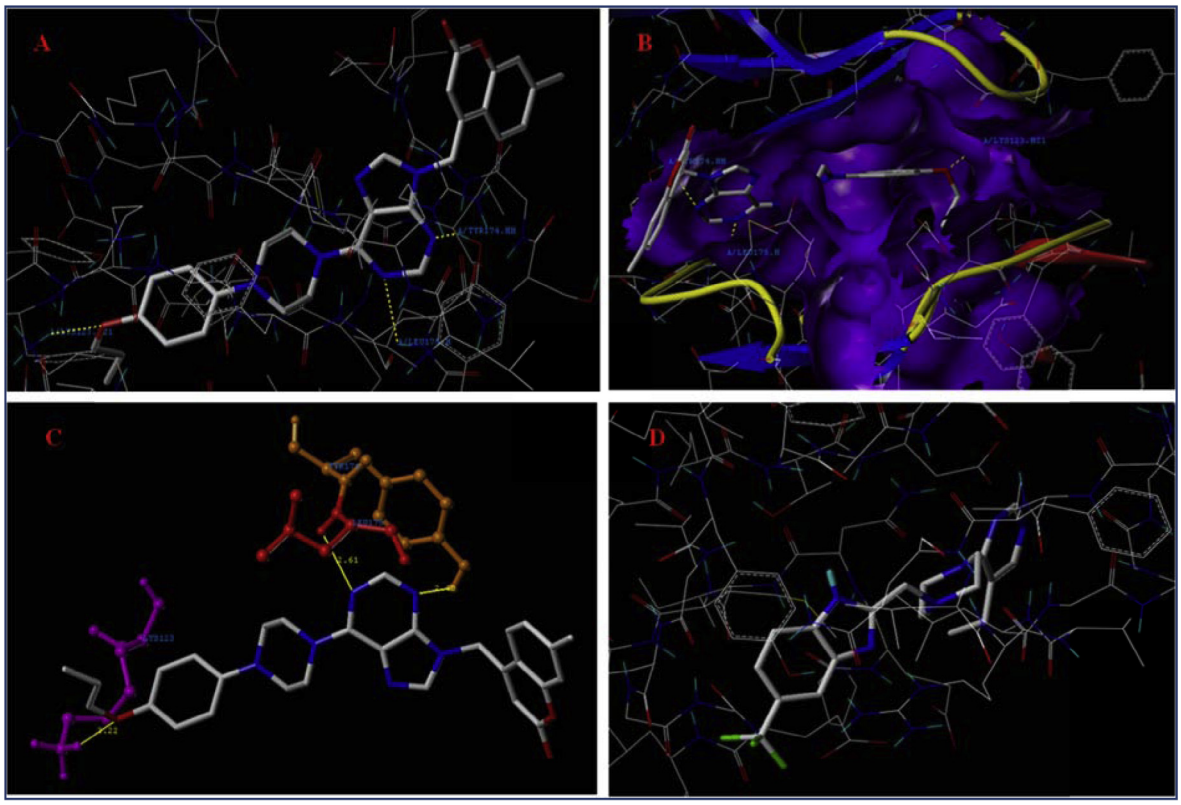

Fig. 6. Interaction of (A-C) compound PP16 & (D) PF-4708671 at the active site of the enzyme P70-S6K1 kinase.

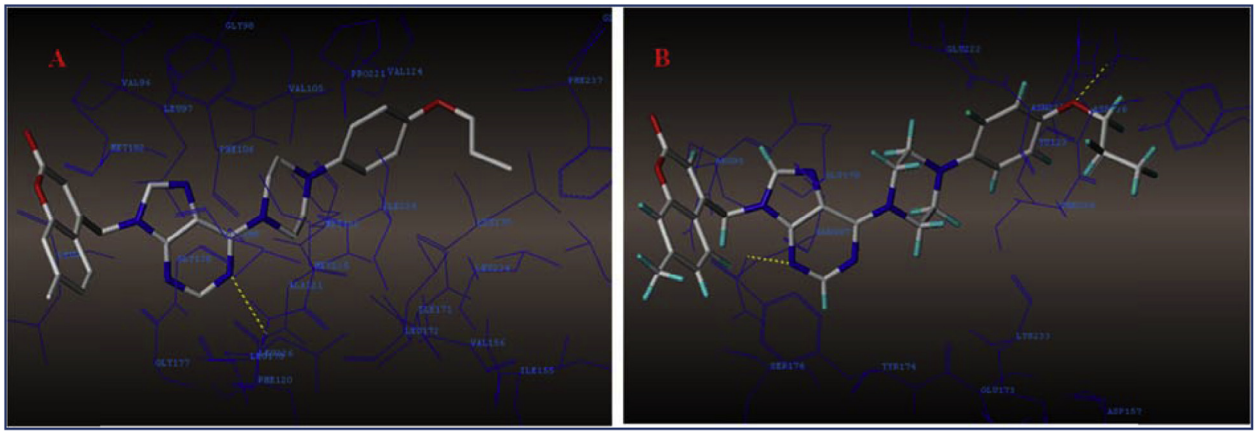

Fig. 7. (A) Hydrophobic and (B) Hydrophilic amino acid surrounded around the PP16 with enzyme P70-S6K1 kinase.

molecules were determined and the calculations of binding free energies were based on hydrogen bonding, electrostatic forces, Van der Waals forces and desolvation effects.

2.5. Docking of PP16 and receptor for interaction studies

The proteins (P70-S6K1 & PI3K-δ kinases) were prepared for docking by adding polar hydrogen atom with MMFF 94 charges and water molecules were removed. The 3D structure of the ligands was generated by the SKETCH module implemented in the SYBYL program (Tripos Inc., St. Louis, USA) and its energy-minimized conformation was obtained with the help of the Tripos force field using Gasteiger-Huckel (Gasteiger and Marsili, 1980) charges and molecular docking was performed with Surflex-Dock program that is interfaced with Sybyl-X 2.0. (Tripos International, 2012) And other miscellaneous parameters were assigned with the default values given by the software.

3. Results and discussion

P70-S6K1 & PI3K-δ kinases have been involved in various cellular functions. Hence, these kinases are attractive targets for various therapeutic approaches. Therefore initially we have selected nearly 17 molecules (**PP04-PP21**) which were used in *in vitro* experiments (Manjunath et al., 2016). In the current study, we have compared our molecules with the purine and pyrimidine based inhibitors of P70-S6K1 & PI3K-δ kinases which are used in clinical trials for their binding affinity. The binding affinity studies for all

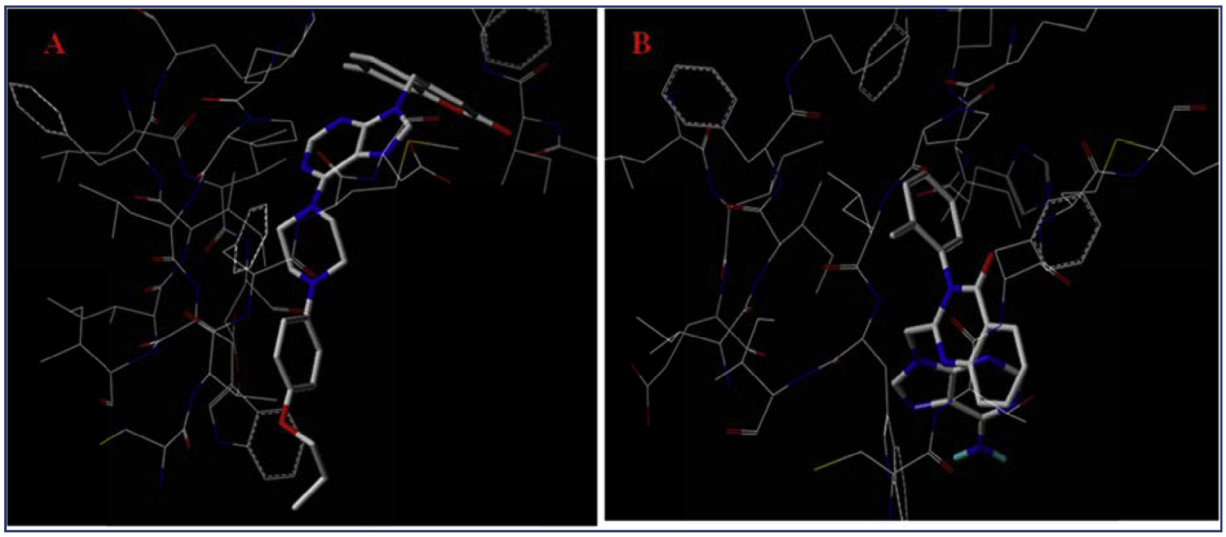

Fig. 8. Interaction of (A) compound PP16 & (B) IC-87114 at the active site of the enzyme PI3K- δ kinase.

Table 3
Surflex Docking score (kcal/mol) of compounds with P70-S6K1 kinase (PDB ID: 3A60).

Compound	C Score[a]	Crash Score[b]	Polar Score[c]	D Score[d]	PMF Score[e]	G Score[f]	Chem Score[g]
PF-4708671	3.82	−0.64	0.00	−1778.96	30.90	−173.88	−19.89
PP16	4.43	−2.10	0.99	−1897.62	−18.84	−206.87	−29.69

Table 4
Surflex Docking score (kcal/mol) of compounds with PI3K-δ kinase (PDB ID: 4XE0).

Compound	C Score[a]	Crash Score[b]	Polar Score[c]	D Score[d]	PMF Score[e]	G Score[f]	Chem Score[g]
IC-87114	4.85	-0.62	1.47	-2242.45	43.47	-179.66	-24.95
PP16	3.89	-1.55	0.00	-2311.14	2.50	-217.25	-30.57

[a] CScore (Consensus Score) integrates a number of popular scoring functions for ranking the affinity of ligands bound to the active site of a receptor and reports the output of total score.
[b] Crash-score revealing the inappropriate penetration into the binding site. Negative numbers indicate penetration.
[c] Polar score indicating the contribution of the polar interactions to the total score. The polar score may be useful for excluding docking results that make no hydrogen bonds.
[d] D-score for charge and Van der Waals interactions between the protein and the ligand.
[e] PMF-score indicating the Helmholtz free energies of interactions for protein-ligand atom pairs (Potential of Mean Force, PMF).
[f] G-score showing hydrogen bonding, complex (ligand-protein), and internal (ligand-ligand) energies.
[g] Chem-score points for H-bonding, lipophilic contact, and rotational entropy, along with an intercept term.

17 molecules and the standard inhibitors in clinical trial were docked by AutoDock Vina. Based on these docking results, the N-9 substituted 6-(4-(4-propoxyphenyl)piperazin-1-yl)-9H-purine derivatives have exhibited better binding affinity compared to the standard.

Compound **PP16** with binding affinity of −14.7 kcal/mol has shown promising binding affinity compared to PF-4708671 (−09.3 kcal/mol) in P70-S6K1 kinase. Further, **PP16** with binding affinity of −17.2 kcal/mol has shown promising binding affinity compared to IC-87114 (−10.2 kcal/mol) in PI3K-δ kinase (Figs. 4-5). Whereas **PP21** has shown no binding affinity with both kinases. Further, **PP14** and **PP19** did not show any binding affinity with PI3K-δ kinase (Tables 1-2). Compound **14, 19** and **21** containing Fluorine atom have shown no binding affinity, even though the Fluorine has been used extensively in drug research as a means of enhancing biological activity (Poonam and Andrew, 2007). This anonymous observation might be attributed due to many interactions like halogen–π interaction, which greatly affects aromatic–aromatic interactions by modulating the electronic nature of the rings. Also the introduction of halogen atoms can negatively affect molecular properties like aqueous solubility (Hans et al., 2012).

Such negative effects are also observed in the literature for a number of cases in which H to F replacement decreases liphophilicity. Few examples with a decreasing logD upon fluorine substitution were observed as few structural patterns correlate with such effects. Hence, proper balance between required lipophilicity and molecular polarity is one of the persistent challenges for medicinal chemists (Hans-Joachim et al., 2004).

As depicted in Fig. 6(A-C), compound PP16 makes three hydrogen bonding interaction with amino acid residues of P70-S6K1 kinase, among them two interactions raised from the nitrogen atoms of adenine ring makes an hydrogen bonding interactions with hydrogen of TYR174 (N------H-TYR174; 2.48 Å) and LEU175 (N------H-LEU175; 2.61 Å). Remaining one bonding interaction is raised from the oxygen atom of terminal propoxy ring with hydrogen of amino acid residue LYS123 (–OC_3H_7------H-LYS123; 2.22 Å). As depicted in the Fig. 6(D) P70-S6 K1 inhibitor (PF-4708671), it does not show any hydrogen bonding interaction at the active site

of the enzyme as it may have other type of interaction apart from hydrogen bonding. Fig. 7(A) and (B) depicts the hydrophobic and hydrophilic interactions of PP16 with surrounding amino acids of P70-S6K1 kinase.

In case of PI3K-δ kinase both PP16 and IC-87114 molecules were docked into the active site of the enzyme, and we found that hydrogen bonding interactions were not present at the active site of the enzyme. Hence, we can assume that these molecules may have different types of interactions at the active site of the enzyme other than hydrogen bonding interaction (Fig. 8(A) and (B)).

The binding affinity, penetration of ligand into the binding site, polar interactions, Van der Waals interactions and hydrogen bonding interaction values for PP16 and standard compounds (PF4708671 and IC87114) under clinical trial are obtained with the help of Surflex docking score (Tables 3-4). After analysing the values obtained, PP16 and standard compounds (PF4708671 and IC87114) values were comparatively nearer to each other. These results indicate that PP16 and standard compounds interaction with both the kinases are more or less similar.

4. Conclusion

In conclusion, N-9 substituted 6-(4-(4-propoxyphenyl)piperazin-1-yl)-9H-purine derivatives exhibit differential affinity towards P70-S6K1 & PI3K-Delta kinases. Among all the docked ligands only the compound with N-9 coumarine substitution (**PP16**) exhibits highest binding affinity for both the kinases. The interactions of PP16 with both kinases are similar to those of standard compound under clinical trial. Therefore, it is evident that the molecule **PP16** could serve as lead molecule for further *in vitro* and *in vivo* studies.

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