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INSILICO RECEPTOR AND LIGAND BASED APPROACH FOR IDENTIFYING THE R GROUPS OF SELECTED PI3K SCAFFOLD.

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ABSTRACT

The scaffold hopping is an important process in Computer Aided Drug Designing (CADD).

We have described an insilico methodology for selecting R groups through various receptor and ligand based approaches. This approach has been discussed in this article for PI3K scaffold of 4-thieno [3, 2-d] pyrimidin-4-ylmorpholine. The selected R groups were further ranked on the basis of interaction energy. The interaction energy results were further compared with IC50 values reported in literature.

The present studies will throw light on the selection of various R groups at Insilico level without synthesis and invitro studies at initial phase of drug designing. This approach will be helpful in resource optimization during drug discovery.

Key words: docking, PI3K

INTRODUCTION

Computational approaches are already acting as a fuel for enhancing the speed of drug discovery. There is further need has to come to club the different Insilico approaches to accelerate the speed of drug discovery process.

The identification of potential R groups for any selected scaffold is basic need in drug designing field. In general process, pharma companies decide on the impact of functional groups by invitro studies as a proof of concept. This general procedure is time consuming and involve avoidable synthesis and testing.

PI3K, a target which has already has proven its importance as anticancer target, has been selected for our studies. The impact of this pathway is such that almost all big pharma companies such as Novartis, GSK, and Genentech etc are in race of designing potential inhibitors. Their efforts have resulted in almost 20-30 inhibitors which are at various stages of clinical phases.

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PI3K is further split into three classes -Class I, II and III as given in Fig 1, based on their primary structure, mode of regulation, substrate specificity, tissue distribution and mapping inside the cell [1]. Class-I is the most important target.

Classification of PI3K Family

Class I is a heterodimer protein responsible for the production of Phosphatidylinositol 3-phosphate (PI(3)P), Phosphatidylinositol (3,4)-bisphosphate (PI(3,4)P2), and Phosphatidylinositol (3,4,5)-triphosphate (PI(3,4,5)P3 **.** It consists of a regulatory unit and a catalytic unit. It is further divided into Class IA and Class IB based on their sequence similarity and mode of activation pathway.

Class IA: consists of any one of the 'catalytic' subunits (p110α, p110β, or p110δ) complexes with any one of the 'regulatory' subunits (p85α, p85β or p55γ). There are five different classes of regulatory subunits - p85α, p85β or p55γ encoded by the PIK3R1 gene, and p85β and p55γ, encoded by PIK3R2 and PIK3R3.

P110α or PI 3-Kinase alpha plays an important role in proliferation, survival and regulation of the potential oncogene PKB.PIK3CA gene encode for human p110α [2]. It is composed of 85 kDa regulatory subunit and a 110 kDa catalytic subunit. It phosphorylates phosphatidylinositol (PtdIns),

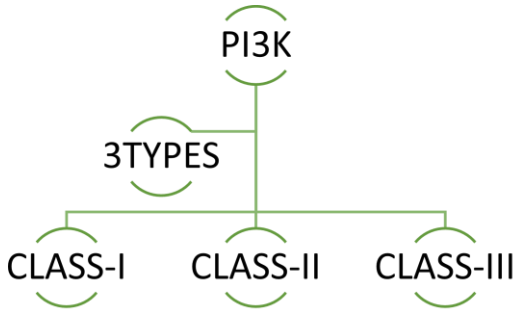


Fig 1. PI3K classification

PtdIns4P and PtdIns (4, 5) P2 [3]**.** The most frequent mutations in cancer constitutively activate PI3Kα and may drive the oncogenic transformation.

p110β - This gets triggered by insulin via the insulin receptor to initiate a cascade of events that control cell growth and metabolism. This is encoded by PIK3CB gene [4-5].

p110δ- This enzyme in human is encoded by PIK3CD gene .This is expressed in leukocytes (WBC).

Class I B : Class IB PI3K differs from the Class I A. It lacks p85 binding domain. This enzyme is further divided into p110γ catalytic unit and p101γ regulatory subunit [6]. The regulatory subunit of p110γ is responsible for the activation of the catalytic subunit downstream of G-proteins, of the protein complex to the membrane surface, where its lipid substrates are located [7-8].

Based on their activity PI3K inhibitors are classified into three categories as given in Fig 2[9-14].

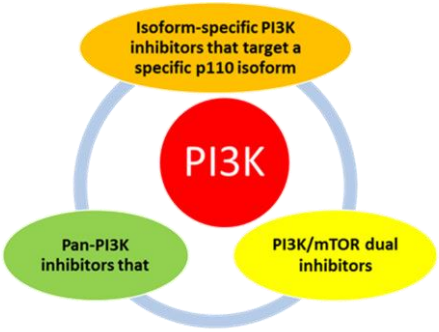


Fig 2 PI3K inhibitors based on their activity

Pan-isoform PI3K Inhibitors

These inhibitors work for all isoforms of Class I PI3K i.e**.** α, β, γ & δ.The most prominent among these is BKM-120, which has entered into Phase -III [14].

There are several other inhibitors such as GDC-0941, GDC-0980, and XL-147 etc.

After selection of our target protein we have selected a scaffold 4-thieno [3, 2-d] pyrimidin-4-yl [15]. GDC-0941, now known as Pictilisib was chosen for our studies. The structure is given in Table 1.The blue color indicates the central scaffold of the molecule. **The red color denotes the R1** group and Green color indicate R-3 group.

Table 1. Structure of scaffold selected based on based on docking studies.

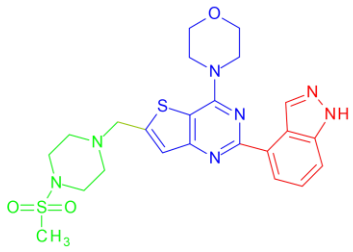
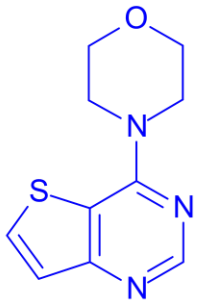
 **Selection of scaffold is based on docking studies.**	**Structure of GDC-0941 (Class-1 Pan PI3K inhibitor)** **Blue color – Identified scaffold (R1).**
 **4-thieno[3,2-d]pyrimidin-4-ylmorpholine**	

Table 2 : Scoring values and their respective interaction energy

Molecule	Ligscore2	Dock score	PMF	R1	R2	R3	Total
GDC-0941	7.43	112.601	133.03	-31.534	-65.100	-36.2856	-132.925
M1	7.39	94.848	125.63	-31.534	-65.100	-32.9373	-129.571
M2	6.67	99.56	113.74	-31.534	-65.100	-22.1247	-118.758
M-3	7.38	98.854	126.3	-31.534	-65.100	-36.8	-141.144
M-4	6.97	103.488	119.35	-31.534	-65.100	-26.4794	-123.113
M-5	6.65	99.857	112.86	-31.534	-65.100	-22.4256	-119.059
M-6	6.63	102.41	117.36	-31.534	-65.100	-17.0401	-113.674
M-7	7.01	104.616	125.02	-31.534	-65.100	-34.4429	-131.076
M-8	6.22	83.713	104.09	-31.534	-18.467	-34.4429	-84.443
M-9	6.72	89.757	113.44	-31.534	-18.467	-36.2856	-86.286
M-10	6.27	88.348	110.9	-31.534	-15.593	-36.2856	-83.412
M-11	6.71	100.017	113.22	-31.534	-1.2523	-36.2856	-69.071
M-12	6.59	100.471	129.14	-31.534	-57.954	-36.2856	-125.773
M-13	7.12	106.384	125.78	-31.534	-65.100	-35.2845	-131.918
M-14	6.37	80.876	114.18	-31.534	-18.467	-35.2845	-85.285

The various Insilico approach such as molecular docking studies, interaction energy calculations and scoring values for selecting the groups are described in the present study.

MATERIAL AND METHODS

Ligand preparation

14 ligands given Table 2 were drawn. After this it was minimized for reaching the lowest energy conformation and then it was prepared using ligand preparation protocol given in discovery studio

Protein Preparation

The PDB IDs- 3DBS (PI3K gamma) was downloaded from the PDB website and used for molecular docking studies. Protein was subjected to protein preparation protocol. Binding site was predicted based on bound ligand. The prepared ligands were subjected to the molecular docking process using LigandFit module of Discovery studio. This module uses Monte Carlo techniques for generating ligand conformations and docking them in active site using shape –based initial docking method. This process is further evaluated with set of scoring functions such as LIG Score1 , LIG score 2, PLP1, PLP2,Dock Score 2, PMF & JAIN SCORE.

The obtained scoring values are reported in Table 2.

Interaction Energy

The docked Protein structure with their respect-tive ligands were further divided as R1, R2 & R3.

R1 is 4-thieno [3, 2-d] pyrimidin-4-ylmorpholine, and has been kept constant for all docked molecules. Interaction Energy protocol allows to calculate the nonbonded interactions (i.e., the van der Waals term and the electrostatic term) between two sets of atoms in a specified structure or trajectory.

The results of calculated interaction energy are reported in Table 3. Surrounding residues 10 Angs apart were considered for interaction energy calculations.

The number of residues were kept the same for their respective groups.

VALIDATION

Lastly validation of our Insilico approach was carried out by comparing the results with reported IC50 values. Comparative results are reported in Table 3.

RESULTS & DISCUSSION

The potency of each functional groups attached to the selected scaffold was determined using, molecular docking studies, scoring values and interaction energy as given in Table 3.

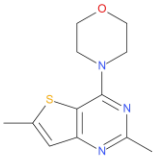
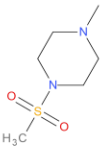
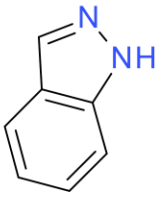
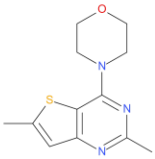
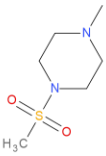
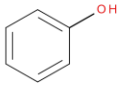
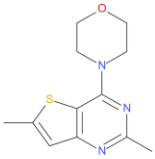
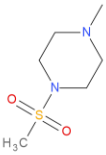
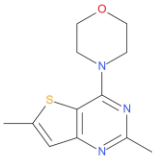
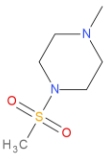
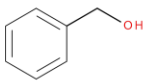
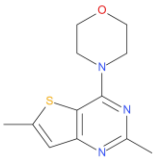
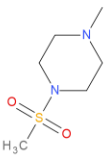
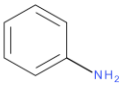
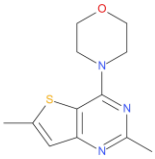
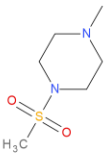
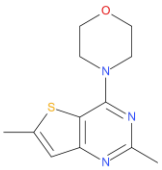
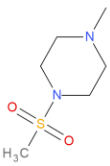
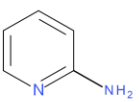
All the groups R1, R2 & R-3 have negative interaction values, which indicates that the designed groups have ability to show potency in terms of experimental studies.

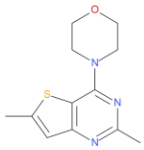
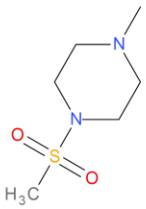
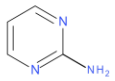
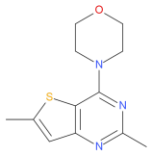
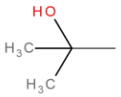
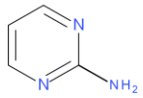
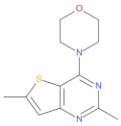
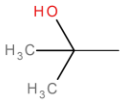
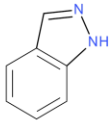
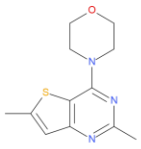
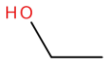
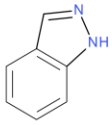
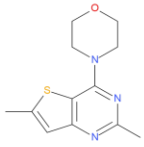
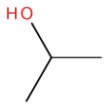
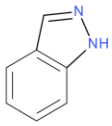
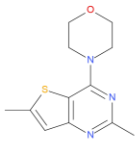
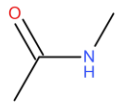
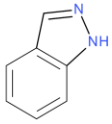
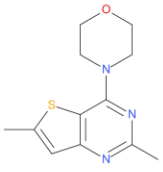
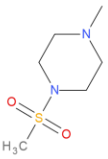
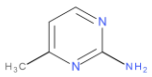
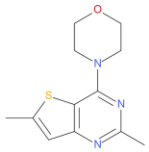
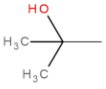
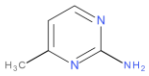
Our next aim was to select among these the best molecules by computational approach. In order to select the molecules, we relied on scoring values and interaction energy.

H-bonds of all 14 docked molecules are reported in Table 4. The calculated interaction energy of selected PI3K scaffold R1, is same for all 14 molecules. R2 values are also similar for GDC-0941, M1 to M7.

GDC-0941, M3, M7 & M13 have the highest

Table 3 Comparison of Insilico approach and IC50

Molecule	Ic50(nm)	Interaction energy			
		R1	R2	R3	Total
GDC-0941	**390**	 -39.534	 -65.100	 -36.2856	-140.919
M1	21	 -39.534	 -65.100	 -32.9373	-137.531
M2	1800	 -39.534	 -65.100	 -22.1247	-126.75
M-3	75	 -39.534	 -65.100	 -36.8123	-141.1447
M-4	1200	 -39.534	 -65.100	 -19.1710	-123.80
M-5	3700	 -39.534	 -65.100	 -17.60	-122.23
M-6	270	 -39.534	 -65.100	 -23.86254	-128.494

M-7	300	 -39.534	 -65.100	 -47.05	-151.684
M-8	330	 -39.534	 --13.733	 -47.05	-100.314
M-9	1300	 -39.534	 13.733 -	 -36.2856	-89.54
M-10	1200	 -39.534	 -15.593	 -36.2856	-91.413
M-11	1100	 -39.534	 -2.2523	 -36.2856	-78.071
M-12	1500	 -39.534	 -57.954	 -36.2856	-133.773
M-13	230	 -39.534	 -65.100	 -39.994	-144.687
M-14	490	 -39.534	 -13.733	 -39.994	-93.216

interaction energy of -132.925, -133.447,-131.674 & -131.918 respectively. Simultaneously we have analyzed the scoring values of these compounds. Lig score2 is 7.43 and Dock score is 112.601 for GDC-

Table 4 H- bond residues

Molecule	H-Bond Residues	Interaction energy	IC50(nm)
GDC-0941	VAL882,TYR867,ASP841,LYS802,ALA805	-132.925	**390**
M1	VAL882,TYR867,ASP841,LYS802,ALA805	-129.571	21
M2	VAL882,LYS802,ALA805	-118.758	1800
M-3	VAL882,TYR867,ASP841,LYS802,ALA805		75
M-4	VAL882,ASP964,ASP841,LYS802,ALA805	-123.113	1200
M-5	VAL882,LYS802,ALA805	-119.059	3700
M-6	VAL882,LYS802,ALA805	-113.674	270
M-7	VAL882,ASP964,ASP841,LYS802,ALA805	-131.076	300
M-8	VAL882,ASP964,ASP841,THR887	-84.443	330
M-9	VAL882,TYR867,ASP841,THR887	-86.286	1300
M-10	VAL882,TYR867,ASP841	-83.412	1200
M-11	VAL882,ASP964,ASP841,THR887	-69.071	1100
M-12	VAL882,TYR867,ASP841	-125.773	1500
M-13	VAL882,ASP964,ASP841,LYS802,ALA805	-131.918	230
M-14	VAL882,ASP964,ASP841,THR887	-85.285	490

0941. M-3 has 7.38 LIG Score-2 and 126.2 k/cal dock score. M-7 has 7.01 as LIG score-2 and dock score of M-7 is 125.02.

From the X-ray cocrystal structure of PI3Kγ, it was observed that the indazole 2-nitrogen makes a key hydrogen bond with the OH of Tyr 867 and the indazole NH forms an interaction with Asp 841.

Table 4 indicates that the molecules M1, M3 & M7 & M-13 have all required H-bond interactions.

M-2 is docking with the binding site with scoring value of 6.67(Lig score-2) and -118 k/cal interaction energy. R1 & R2 group is similar to the reference GDC-0941. R-3 functional group is different from the reference. But by changing the group both scoring value and interactions have been reduced. TYR867, ASP841, LYS802 & ALA805 H-bonds are missing from M-2. The used Insilico approach has provide an idea that M-2 is not better than M-1 & GDC-0941.The cross validation studies with IC50 (nM) of 1800 has strengthed our prediction.

In case of M-4, scoring values and interaction energies are slightly better than M-3 but not so with reference GdC.-0941. Interaction of R-3 is slightly better in this substitution. The substitution of aniline from pyridine has resulted in gain of ASP964, ASP841 H-bond interactions. This energy gain was also reflected in terms of interaction energy R3 group that has increased from -22.45 to -26.479k /cal. This suggests that the group substitution is not better than GDC-0941 M3, M7 & M13. So we can not expect better invitro results. This hypothesis was further confirmed with the reported IC 50 which is 1200, better than M-2 but not so with GDC-0941 M3, M7 & M13.

In case of M-5, scoring values and interaction energy are not better than previous discussed molecules.

The substitution of benzene is R-3 has forced the molecule to ASP964, ASP841 H-bond interactions. This energy gain was also reflected in terms of interaction energy R3 group that has increased from -22.45 to -26.479. This has led us to believe that this group substitution is not better than GDC-0941 M3, M7 & M13. This hypothesis was further confirmed with the reported IC 50 which is 1200, better than M-2 but not so with GDC-0941 M3, M7 & M13

In case of M-6, R3 group substitution is better than M-5 and M-2.Scoring values are also better with LigScore2 of 7 k/cal and Dock score of 100.881.

M-7 & M-8 have similar R-3 groups. The substitution of pyrimidine 2 amine has increased the interaction energy of group R-3 to -47.05 k /cal compared, to other R-3 groups.

M-8 have different R2 groups, one hydroxyl is able to pick the hydrogen bonds THR-887. The total number of atoms compared to reference are less, as the structure is smaller than other molecules, which is also reflecting in scoring value and interaction energy.

M-9 is having similar R-1 group and R2 group with reference GDC-0941. They differ in R-2 group.

M8 & M-9 structure are highly similar except in R-3 group. In terms of scoring M-9 is better than M-8.

In M-10 R-3 group has interaction energy of -15.593 and scoring value is not better than the reference. The hydrogen bond THR-887 is also missing.

Among M-11 to M-14 ,M-13 & 14 have better R3 group interaction energies .M-13 is better than M-14. Based on scoring values and interaction energy GDC-0941 and M1, M3 & M7 & M-13 have been selected for further processing.

So as per our Insilico approach, we conclude that pyrimidine 2-amine and indazole functional groups are good for R3. 1-methyl-4-methylsulfonyl-piperazine group is good for R-2.

The set of 14 molecules with IC50 values have been compiled (Table 4). The IC50 values indicate that M1, M3 & M7 & M-13 are good and better than reference molecule and are chosen for further studies and evaluation.

This sort of docking studies are going help computational biologist in selecting the R groups.

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