Cloud Publications
International Journal of Advanced Bioinformatics and Computational Biology
2012, Volume 1, Issue 1, pp. 1-11, Article ID Med-30

Research Article | Open Access

Molecular Docking Studies on Pyrazolopyrimidine and their Derivatives as Human Phosphoinositide 3-Kinase Inhibitors

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Publication Date: 26 December 2012

Article Link: http://medical.cloud-journals.com/index.php/IJABCB/article/view/Med-30

Abstract PI3K (Phosphoinositid-3-Kinase) is the mostly used protein as a target for the ovarian cancer. Ovarian cancer is very deadly disease in mostly women. PI3K have three subunit classes such as p110a, p110 beta, p110g which plays very important role in the development in ovarian cancer. P110a is mostly responsible in sever ovarian cancer among all three classes of PI3K. We worked to find the best possible inhibitors for p110a of PI3K. Crystal structure of p110a subunit PI3K, which has no complex with any other molecule taken from structural database (PDB). In-Silico drug designing approaches follow for molecular docking studies using AutoDock 3.05. The docked complexes were validated and enumerated based on the AutoDock Scoring function to pick out the best inhibitors based on docked Energy. Thus from the entire 70 ligand compounds which were Docked, we got 5 best derivatives of pyrazolopyrimidine with optimal docked Energy (pyrazolo pyrimidine, 5a: -22.64 kcal/mol; pyrazolo pyrimidine, 13: -21.43kcal/mol; pyrazolo pyrimidine, 5e: -21.88kcal/mol; pyrazolo pyrimidine, 10: -18.77kcal/mol, pyrazolo pyrimidine, 25:-16.5kcal/mol). Further the five best-docked complexes were analyzed through Python Molecular Viewer software for their interaction studies.

Keywords *AutoDock, Hyperchem, Ovarian Cancer, PI3K, p110a, Python*

1. Introduction

Cancer begins due to the uncontrolled growth of cells in body part. Cancer cell growth is different from normal cell growth. In recent years, ovarian cancer is main topic or target of research to find the therapeutic development. The region behind this is that ovarian cancer is on fifth ranked in cancer deaths among women. A woman's risk of getting invasive ovarian cancer during her lifetime is about 1 in 71 [1]. Currently therapeutics development has involvement of identification of novel target for ovarian cancer and its drugs. Target must involve in the signaling pathways controlling cell cycle progression, gene transcription, motility, apoptosis and cell metabolism [2]. Current evidence has suggested PI3K/AKT pathway as a novel target for development of anti-therapeutic drugs for ovarian cancer.

The phosphoinositide 3- kinase (PI3K) pathway is consider to have a vital role in wide range of cancer such as breast, ovarian, myeloid leukaemia, lung cancer. PI3K is responsible for conversion of 3 position of the inositol ring of P1 (4, 5) P2 into P1 (3, 45) P3, which is involved in survival of signalling. PI3K family classified into class I-III [3]. The class IA of PI3K comprises the p110a, p110B, p110g isoform, associated with activated Receptor Tyrosine Kinase (RTKs). In ovarian cancer, PI3KCA gene encoding P110A emerged as an attractive target [4]. PI3Ks act as membrane tethers for protein kinase B (PKB) and Phospholipids-Dependent Kinase (PDK1) [5]. The class IA of PI3K has p110a and P85a subunits with SH2 domains which are responsible for the activation of RTKs. PI3K pathway relies on its downstream effectors kinase AKT [3]. So many inhibitors have already discovered to inhibit the PI3K pathway signalling process. Most used inhibitors are Wortamannin, LY294002 [6]. Wortamannin inhibit the PI3K subunit p110a catalytic isoform, but it has a drawback that it's soluble in organic solvents but not in water. LY294002 is potential inhibitor for PI3K, it is flavonoid derivative. Wortamannin or LY294002 alone may inhibit cell proliferation and apoptosis in cancer cell with the inhibition of the PI3K-AKT pathway [3]. In this study, we targeted the p110a subunit of PI3K protein as therapeutic target. Molecular docking or In-Silico docking approach is used to find out the more effective inhibitors to therapeutic treatment by the structure based drug designing for PI3K. So we are concentrating on the docking approach to analysis of effective treatment for ovarian cancer.

2. Materials and Methods

2.1 Target Identification

At first we identified the recent approachable target for the treatment of ovarian cancer. Target identified by the metabolic pathway which is responsible for the cause of ovarian cancer in human being and mainly in women.

We found the P110A subunit of PI3K [7] protein as very effective therapeutic target which plays a crucial role in development of ovarian cancer by metabolic pathway as shown in (figure 1).

We retrieve the sequence of PI3K protein from the protein database at expasy [8]. After sequence retrieval, find the related PDB ID [9] to the p110a subunit of PI3K protein from PDB database. 2RD0 (PDB ID) was analyzed as a most suitable pure crystal structure of p110a which have not any complex with any other molecule. We add up the hydrogen in the 2RD0 structure using the Pymol software to making the protein more stable.

2.2 Active Site Analysis

Q-SiteFinder method used to ligand binding site prediction; it uses the interaction energy between the protein and a simple Vander Waals probe to locate energetically favourable binding sites. Energetically favourable probe sites are clustered according to their spatial proximity and clusters are then ranked according to the sum of interaction energies for sites within each cluster. It also generates predicted sites with the lowest average volumes of the methods examined in this study.

2.3 Ligand Generation and Optimization

The 3D structures of ligands used in this study were downloaded from pubchem compound database and binding database [10]**.** The downloaded ligands in the pdb format were first converted to the hin (Hyperchem) format using OpenBabel [11]**.** All modeling procedures, including energy minimization and molecular dynamics, were also performed using the HyperChem [12]. Energy calculations were carried out using the AMBER force field. Further the optimize ligands in hin format were converted to the PDB (Protein Databank) format using OpenBabel.

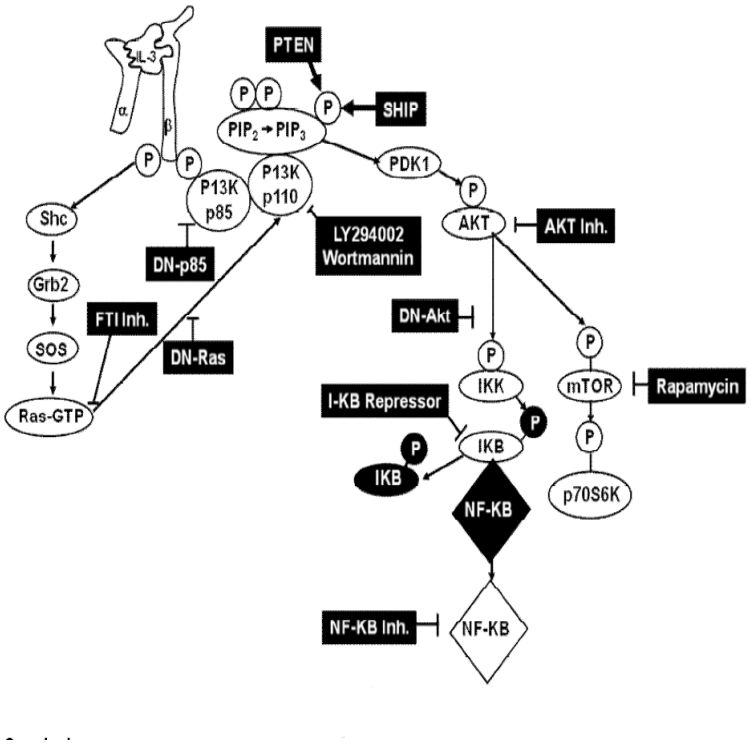

Figure 1: *Metabolic Pathway of PI3K Kinase Involved in the Ovarian Cancer*

2.4 Virtual Screening

Virtual screenings of the entire 70 ligand compounds against of PI3K protein structure, determined by comparative homology modeling, and were done using molecular docking program AutoDock3.05 [13]**.** The Kollman charges and the solvation term were then added to the protein structure using the AutoDock tool [14]**.**

A grid-box was generated that was large enough to cover the entire protein catalytic site and accommodate ligands to move freely. AutoDock3.05 and a Lamarckian Genetic Algorithm (LGA) were used for protein-fixed ligand-flexible docking calculations. Thirty search attempts (ga_run parameter) were performed for each ligand. The maximum number of energy evaluations before the termination of LGA run was 250000 and the maximum number of generations of the LGA run before termination was 27000. Other docking parameters were set to the software's default values. After docking, the ligands were ranked according to their docked energy as implemented in the AutoDock 3.05 program.

3. Result

3.1 Binding Site Analysis

We identified the eight active site residues in P110 (7) of PI3K (Phosphoinnositide-3- Kinase) with the help of Q-site Finder. ILE800, LEU807, LEU814, TYR836, GLY837, CYS838, ILE848 residues were identified as active site in the p110a. These eight residues are highly conserved in the PI3K family and almost 100% identical among the p110 isoforms .Q-site Finder also confirms the binding affinity pocket having same residues.

3.2 Optimization Results of Inhibitors

Table 1 shows the energies (in Kcal) pyrazolo pyrimidine derivatives inhibitors of PI3K before and after optimization through hyperchem.

Table 1: *Optimization Results of pyrazolo pyrimidine Derivatives Series*

S. No.	Compound Name	Energy before Optimization (Kcal)	Energy after Optimization (Kcal) and cycles	No. of Cycles
1	pyrazolo pyrimidine, 13	550.390930	43.688137	114Cycles and 253Points
2	pyrazolo pyrimidine, 27	106.020340	30.216808	226Cycles and 483Points
3	pyrazolo pyrimidine, 25	89.017464	19.687441	42Cycles and 908Points
4	pyrazolo pyrimidine, 6	73.368983	26.111813	305Cycles and 673Points
5	pyrazolo pyrimidine, 5e	71.775696	40.566872	152Cycles and 336Points
6	pyrazolo pyrimidine, 5a	60.251717	37.519421	149Cycles and 323Points
7	pyrazolo pyrimidine, 10	84.438766	29.913965	250Cycles and 545Points
8	pyrazolo pyrimidine, 1	72.038765	41.335163	144Cycles and 313Points
9	pyrazolo pyrimidine, 1	72.038765	41.335163	144Cycles and 313Points
10	pyrazolo pyrimidine, 5c	74.230705	40.305447	227Cycles and 481Points
11	pyrazolo pyrimidine, 5b	64.657585	30.968548	249Cycles and 545Points
12	pyrazolo pyrimidine, 5d	73.968277	39.915920	210Cycles and 452Points

3.3 Docking Results of Known PI3K Inhibitors

Docking results of known PI3K inhibitors which were retrieved through NCBI Pubchem database are shown in Table 2. It shows the name of PI3K inhibitors, highest docking energy of PI3K inhibitors, Reference RMS, Hydrogen bonds formed in between protein and inhibitors and conformation number along with docking energy. This result indicates the docking of pyrazolo pyrimidine series compounds. In this result pyrazolo pyrimidine, 5a was found to be the best selective known inhibitor of pyrazolo pyrimidine series compounds among different types of inhibitors because it shows maximum docked energy (in -ve).

Table 2: *Docking Results of pyrazolo pyrimidine Series Compounds*

S. No.	Inhibitor Name	IUPAC Name	Molecular Formula	IC50 (nM)	Docked Energy (Kcal/mol)	Ref RMS
1	pyridofuropyrimidine derivative, 2	(3-(4-morpholin-4-ylpyrido [3, 2:4, 5] furo [3, 2-d] pyr...)	C19H16N4O3	3.6	-10.82	145.83
2	pyrazolo pyrimidine, 13	1-[4-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-ylpyrazolo[3, 4-d]pyrimidin-6-yl]phenyl]-3-methylurea	C29H34N8O2	14.0	-21.43	140.76
3	pyrazolo pyrimidine, 27	methyl 4-[6-[4-[[4-(4-methylpiperazin-1-yl)phenyl]carbamoylamino]phenyl]-4-morpholin-4-ylpyrazolo[3,4-d]pyrimidin-1-yl]piperidine-1-carboxylate	C34H42N10O4	15.0	-15.92	126.67
4	pyrazolo pyrimidine, 25	methyl 4-[4-morpholin-4-yl-6-[4-(pyridin-4-ylcarbamoylamino)phenyl]pyrazolo[3,4-d]pyrimidin-1-yl]piperidine-1-carboxylate	C28H31N9O4	18.0	-16.5	131.98
5	pyrazolo pyrimidine, 6	1-methyl-3-[4-(4-morpholin-4-yl-1-piperidin-4-ylpyrazolo[3, 4-d]pyrimidin-6-yl)phenyl]urea	C22H28N8O2	23.0	-11.47	126.83
6	pyrazolo pyrimidine, 5e	3-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-ylpyrazolo[3, 4-d]pyrimidin-6-yl]phenol	C27H30N6O2	31.0	-21.88	
7	pyrazolo pyrimidine, 5a	3-(4-morpholin-4-yl-1-piperidin-4-ylpyrazolo[3,4-d]pyrimidin-6-yl)phenol	C20H24N6O2	36.0	-22.64	
8	pyrazolo pyrimidine, 10	1-methyl-3-[4-[4-morpholin-4-yl-1-[1-(pyridin-3-ylmethyl)piperidin-4-yl] pyrazolo[3,4-d]pyrimidin-6-yl]phenyl]urea	C28H33N9O2	41.0	-18.77	135.42
9	pyrazolo pyrimidine, 1	3-[4-morpholin-4-yl-1-[1-(pyridin-3-ylmethyl)piperidin-4-yl]pyrazolo[3, 4-d]pyrimidin-6-yl]phenol	C26H29N7O2	47.0	-15.89	127.83
10	pyrazolo pyrimidine, 1	3-[4-morpholin-4-yl-1-[1-(pyridin-3-ylmethyl)piperidin-4-yl]pyrazolo[3, 4-d]pyrimidin-6-yl]phenol	C26H29N7O2	47.0	-16.51	135.81
11	pyrazolo pyrimidine, 5c	[4-[6-(3-hydroxyphenyl)-4-morpholin-4-ylpyrazolo[3, 4-d]pyrimidin-1-yl]piperidin-1-yl]-phenylmethanone	C27H28N6O3	50.0	-12.5	136.61
12	pyrazolo pyrimidine, 5b	1-[4-[6-(3-hydroxyphenyl)-4-morpholin-4-ylpyrazolo[3, 4-d]pyrimidin-1-yl]piperidin-1-yl]ethanone	C22H26N6O3	69.0	-10.72	129.11
13	pyrazolo pyrimidine, 5d	[4-[6-(3-hydroxyphenyl)-4-morpholin-4-ylpyrazolo[3, 4-d]pyrimidin-1-yl]piperidin-1-yl]-pyridin-3 ylmethanone	C26H27N7O3	86.0	-11.39	126.33

3.4 Interaction Studies Results for Protein phosphoinositide-3-kinase with pyrazolo pyrimidine Derivatives through Python Molecular Viewer

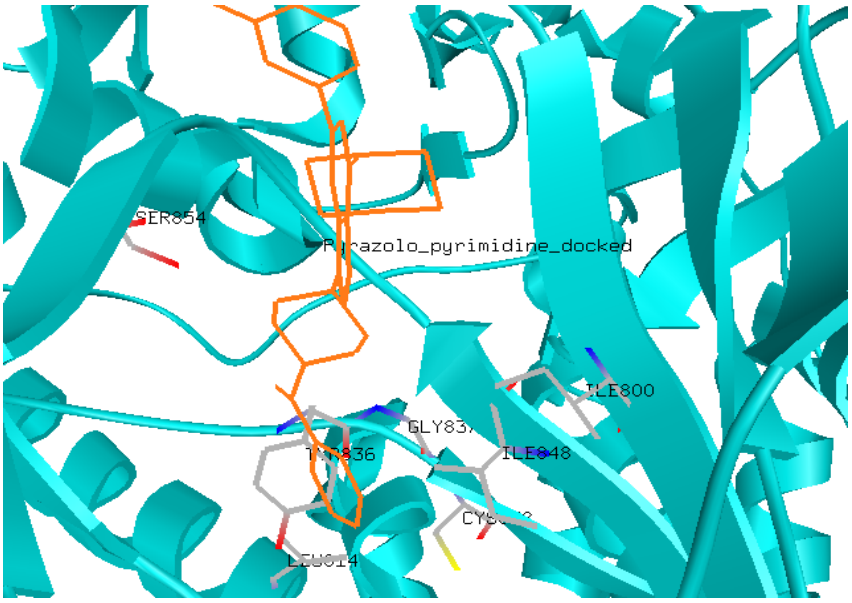

Figure 2: *Docking pose of pyrazolo pyrimidine 5a, into the binding site pocket of protein phosphoinositide-3-kinase*

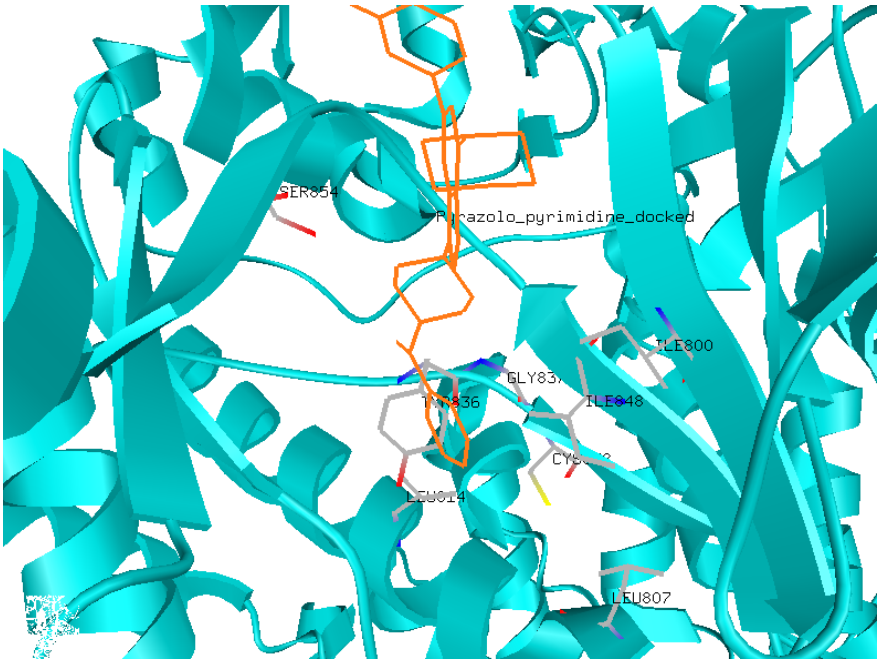

Figure 3: *Docking pose of pyrazolo pyrimidine 5e, into the binding site pocket of protein phosphoinositide-3-kinase*

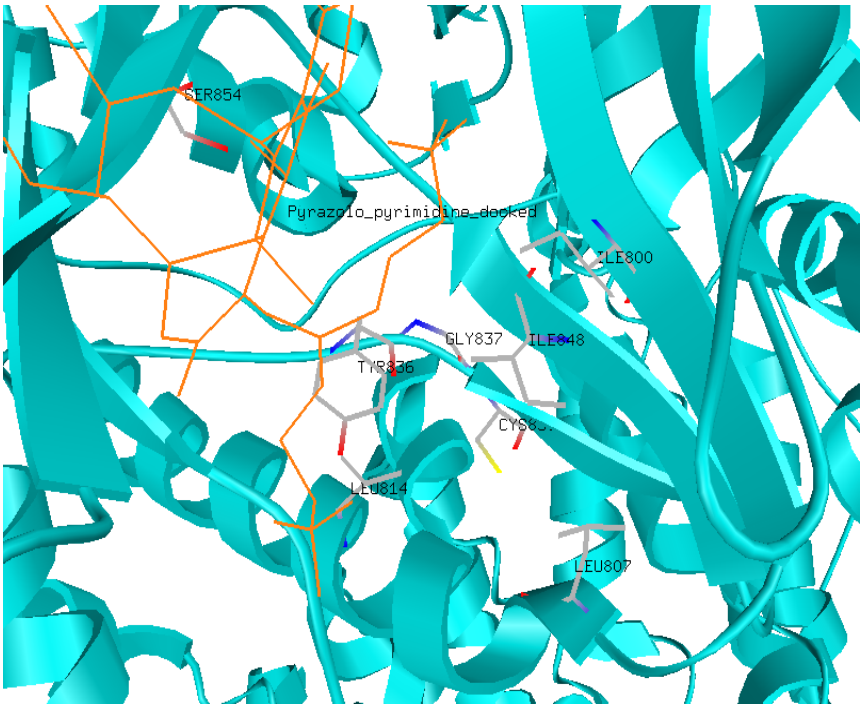

Figure 4: *Docking pose of pyrazolo pyrimidine, 13, into the binding site pocket of protein phosphoinositide-3-kinase*

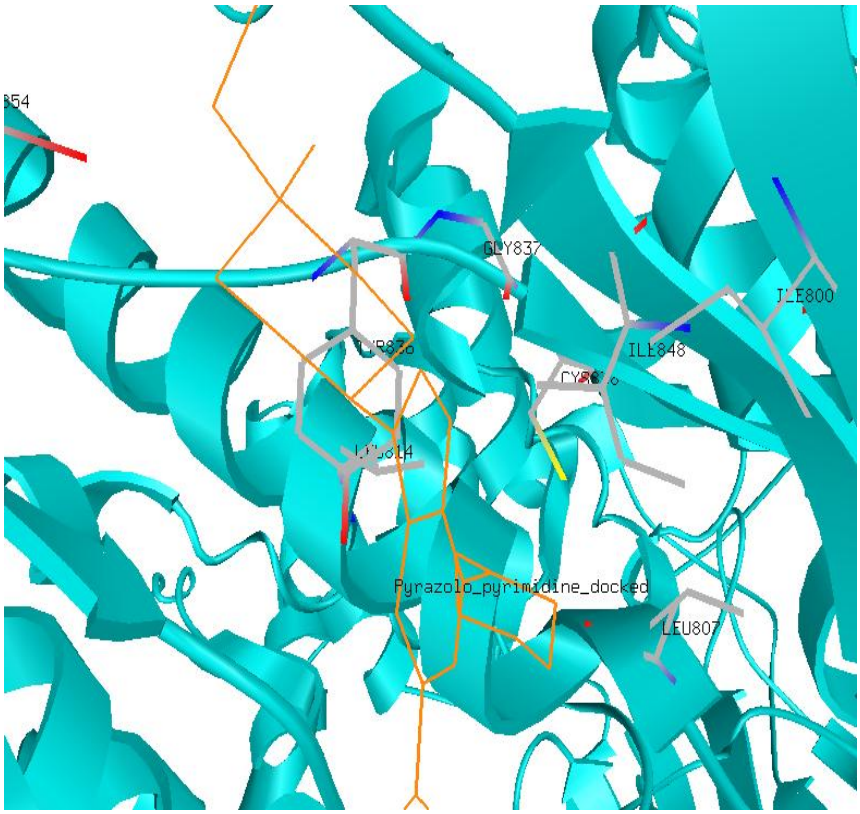

Figure 5: *Docking pose of pyrazolo pyrimidine, 10, into the binding site pocket of protein phosphoinositide-3-kinase*

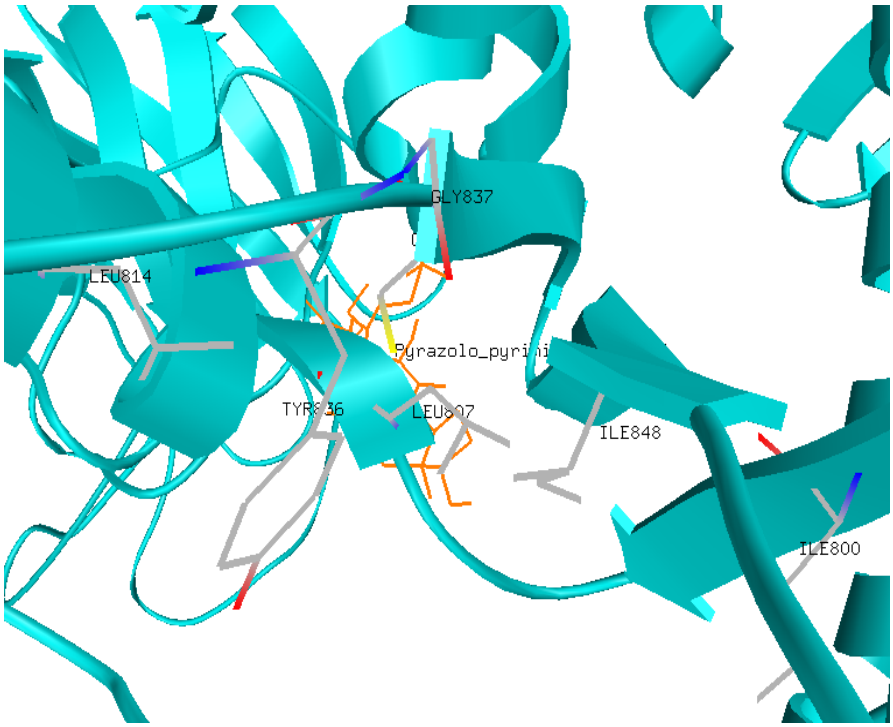

Figure 6: *Docking pose of pyrazolo pyrimidine, 25, into the binding site pocket of protein phosphoinositide-3-kinase*

4. Discussion

3D structure of the entire 70 compounds were generated and subjected to energy minimization using conjugate gradient algorithm through HyperChem7.5 software [15]**.** Docking of these optimized compounds against 2RDO [7] at the catalytic site residues were performed by AutoDock3.05 [12]. Then we analyzed the 13 derivatives of pyrazolo pyrimidine out of 70 docked complexes, we got 5 best docked compounds having lowest docked energy and Root mean square deviation from a reference structures are shown in Table and rest of the data not shown in this work. Five best docked pyrazolo pyrimidine derivative complexes were analyzed through Python Molecular Viewer [16] for their interaction study shown in (Figure 2, 3, 4 and 5). It is evident from this analysis that these 5 pyrazolo pyrimidine derivative out of 13 pyrazolo pyrimidine derivative are the best inhibitors are located in the center of the active site and is stabilized by hydrogen bonding interactions.

Table 3: *Docking results of pyrazolo pyrimidine series compounds*

S. No.	Inhibitor Name	IUPAC Name	Molecular formula	IC50 (nM)	Docked Energy (Kcal/mol)	Ref RMS
1	Pyridofuro pyrimidine derivative, 2	(3-(4-morpholin-4-ylpyrido [3, 2:4, 5]furo[3,2-d]pyr...)	C19H16N4O3	3.6	-10.82	145.83
2	pyrazolo pyrimidine, 13	1-[4-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-ylpyrazolo[3, 4-d]pyrimidin-6-yl]phenyl]-3-methylurea	C29H34N8O2	14.0	-21.43	140.76
3	pyrazolo pyrimidine, 27	methyl 4-[6-[4-[[4-(4-methylpiperazin-1-yl)phenyl]carbamoylamino]phenyl]-4-morpholin-4-ylpyrazolo[3,4-d]pyrimidin-1-yl]piperidine-1-carboxylate	C34H42N10O4	15.0	-15.92	126.67

4	pyrazolo pyrimidine, 25	methyl 4-[4-morpholin-4-yl-6-[4-(pyridin-4-ylcarbamoylamino)phenyl]pyrazolo[3,4-d]pyrimidin-1-yl]piperidine-1-carboxylate	C28H31N9O4	18.0	-16.5	131.98
5	pyrazolo pyrimidine, 6	1-methyl-3-[4-(4-morpholin-4-yl-1-piperidin-4-ylpyrazolo[3, 4-d]pyrimidin-6-yl)phenyl]urea	C22H28N8O2	23.0	-11.47	126.83
6	pyrazolo pyrimidine, 5e	3-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-ylpyrazolo[3, 4-d]pyrimidin-6-yl]phenol	C27H30N6O2	31.0	-21.88	
7	pyrazolo pyrimidine, 5a	3-(4-morpholin-4-yl-1-piperidin-4-ylpyrazolo[3,4-d]pyrimidin-6-yl)phenol	C20H24N6O2	36.0	-22.64	
8	pyrazolo pyrimidine, 10	1-methyl-3-[4-[4-morpholin-4-yl-1-[1-(pyridin-3-ylmethyl)piperidin-4-yl] pyrazolo[3,4-d]pyrimidin-6-yl]phenyl]urea	C28H33N9O2	41.0	-18.77	135.42
9	pyrazolo pyrimidine, 1	3-[4-morpholin-4-yl-1-[1-(pyridin-3-ylmethyl)piperidin-4-yl]pyrazolo[3, 4-d]pyrimidin-6-yl]phenol	C26H29N7O2	47.0	-15.89	127.83
10	pyrazolo pyrimidine, 1	3-[4-morpholin-4-yl-1-[1-(pyridin-3-ylmethyl)piperidin-4-yl]pyrazolo[3, 4-d]pyrimidin-6-yl]phenol	C26H29N7O2	47.0	-16.51	135.81
11	pyrazolo pyrimidine, 5c	[4-[6-(3-hydroxyphenyl)-4-morpholin-4-ylpyrazolo[3, 4-d]pyrimidin-1-yl]piperidin-1-yl]-phenylmethanone	C27H28N6O3	50.0	-12.5	136.61
12	pyrazolo pyrimidine, 5b	1-[4-[6-(3-hydroxyphenyl)-4-morpholin-4-ylpyrazolo[3, 4-d]pyrimidin-1-yl]piperidin-1-yl]ethanone	C22H26N6O3	69.0	-10.72	129.11
13	pyrazolo pyrimidine, 5d	[4-[6-(3-hydroxyphenyl)-4-morpholin-4-ylpyrazolo[3, 4-d]pyrimidin-1-yl]piperidin-1-yl]-pyridin-3 ylmethanone	C26H27N7O3	86.0	-11.39	126.33

5. Conclusion

PI3K is one of the most recent therapeutic drug targets for ovarian cancer. In this paper, we have analyzed the 5 best derivatives of pyrazolo pyrimidine out of all 13 pyrazolo pyrimidine derivative which are the probable inhibitors against PI3K target protein for treatment of ovarian cancer. Docking results indicate that out of 13 pyrazolo pyrimidine derivatives, there were five inhibitory compounds for PI3K as target for ovarian cancer. As it's well known, hydrogen bonding plays an important role for the structure and function of biological molecules, especially for inhibition in a complex. Thus our study of docking energies of all 13 pyrazolo pyrimidine derivatives confirms that five compounds which shown the best docking energy are pyrazolo pyrimidine, 5a: -22.64kcal/mol, pyrazolo pyrimidine, 5e: -21.88 kcal/mol, pyrazolo pyrimidine, 13: -21.43 kcal/mol, pyrazolo pyrimidine, 10: -18.77kcal/mol, pyrazolo pyrimidine, 25: -16.5 kcal/mol. These are the potential inhibitors for PI3K as target for ovarian cancer forming a hydrogen bonding and with non-bonded interaction to act as a drug candidates yet pharmacological study will yet confirm it to be promising.

Acknowledgments

It gives me immense pleasure to express my gratitude to all who helped me directly or indirectly in writing this research work. At the outset, I bow my head in front of almighty God who bestowed his blessings and enlightened me through all the up's and down's, which have helped me extend many originally hopeless paths of this project.

I am grateful to my supervisor Dr. Sumer Singh and co-supervisor Dr. Susheel Kalia who have imparted their knowledge about the subject to me to complete this project work. This would not have been a successful effort without their blissful insight and guidance.

I thank 'BioBrainz Biotechnology and Bioinformatics Research Center', Lucknow, India for supporting me in reading and checking this manuscript and making the work interesting.

I acknowledge from the bottom core of my heart, a great debt of gratitude to my beloved husband Mr. Praveen Kumar for his constant inspiration without which it would not have been possible to accomplish this task.

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