Docking Study to Predict the Efficacy of Phosphatidylinositol 3-Kinase α Inhibitors

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***Abstract*—The phosphatidylinositol 3-kinase (PI3K) family comprises lipid kinases that cross-link signals between living cells and their surroundings. PI3Ks are classified into several groups and isoforms with specific characteristics and functions. Genes encoding PI3Ks are mutated in several types of cancer, and their isoforms have varying capacity in promoting cell signaling and cancer progression. Many compounds have been introduced as PI3Kα inhibitors, but not all of them have the same inhibitory effects. For successful PI3K-related biomedical experiments, it is vital to select the most specific and potent compounds with the highest inhibitory effects for targeting this kinase. In this study, we investigate 28 well-recognized PI3Kα inhibitors through predicting their specificity and potency using the docking software AutoDock Vina. Our data showed that PF 05212384 had the highest docking score (−9.2 kcal/mol), and 3-methyladenine had the lowest docking score (−4.8 kcal/mol). Our data also showed different types of interactions and bonds formed between the inhibitors and protein residues. In conclusion, PF 05212384 and AZD 6482 compounds are the best candidates for targeting PI3Kα. In addition to hydrophobic interactions in the PI3Kα binding pocket, the formation of hydrogen bonds between these inhibitors and binding pocket residues was confirmed.**

***Index Terms*—AutoDock Vina, Cancer cell, Cell signaling, Docking, Phosphatidylinositol 3-kinase, Phosphatidylinositol 3-kinase α.**

I. Introduction

The phosphatidylinositol 3-kinase (PI3K) family is a group of lipid kinases that interlink signals between living cells and their surroundings. These kinases affect downstream targets that modulate signaling pathways involved in cell proliferation, growth, survival, vesicle transport, cytoskeletal rearrangement, motility, and metabolism (Vivanco and Sawyers, 2002; Luo, et al., 2003; Engelman, et al., 2006; Liu, et al., 2009; Hancock, 2010). PI3Ks are activated in response to growth factors and cytokines through cell surface receptors (Peso, et al., 1997; Laurino, et al., 2005; Dudu, et al., 2012). After activation, PI3Ks generate phosphatidylinositol-3,4,5-trisphosphate (PIP3) through the addition of a phosphate group to phosphatidylinositol-4,5-bisphosphate (PIP2), and this process is an important step in initiating activation of their downstream targets (Whitman, et al., 1988; Auger, et al., 1989). Phosphatase and tensin homolog acts as a negative regulator of PI3K by dephosphorylating PIP3 to PIP2 (Chalhoub and Baker, 2009; Hollander, et al., 2011). Depending on the characteristics of their structure and specificity toward substrates, PI3Ks are classified into three groups: Class I PI3K, Class II PI3K, and Class III PI3K (Fruman, et al., 1998; Katso, et al., 2001). Class I, which we are mostly interested in, is subdivided into IA and IB. Class IA PI3Ks are heterodimers with the catalytic subunit p110 of one of the following isoforms: p110α, p110β, or p110δ. These isoforms are encoded by the PIK3CA, PIK3CB, and PIK3CD genes, respectively (Engelman, et al., 2006; Katso, et al., 2001). PI3K Class IA members also have the regulatory subunit p85 of the p85α, p55α, p50α, p85β, or p85γ isoform. The first three isoforms are encoded by PIK3R1 and are generated by differential splicing, and the last two isoforms are products of the PIK3R2 and PIK3R3 genes, respectively (Songyang, et al., 1993; Vanhaesebroeck, et al., 2001). Class IB PI3Ks are also heterodimers, but unlike Class IA, their catalytic subunit is the p110γ subunit integrated with regulatory isoforms p101 and p87. The p110γ subunit is encoded by the PIK3CG gene, and the p101 and p87 subunits are encoded by the PIK3R5 and PIK3R6 genes, respectively (Ueki, et al., 2002; Okkenhaug and Vanhaesebroeck, 2003; Amzel, et al., 2008). PIK3CA is frequently mutated in different types of cancer (Samuels and Waldman, 2010; Mangone, et al., 2012; Wang, et al., 2013; Schmidt, et al., 2018). Anomalies in p110α are associated with increased enzymatic activity of PI3K and promote oncogenesis in affected cells (Bader, et al., 2005; Echeverria, et al., 2015). Due to their roles in promoting cancer cell survival, transformation, proliferation, and migration, PI3Ks are considered promising targets in cancer therapy (Vivanco and Sawyers, 2002; Hausler, et al., 1998; Vanhaesebroeck, et al., 2010). Since different PI3K

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isoforms have diverse capacities in cellular signaling and cancer progression, applying inhibitors that target individual isoforms may produce better therapeutic outcomes in treating cancer (Vanhaesebroeck, et al., 2010: Wang, et al., 2015). Many compounds have been introduced as PI3Kα inhibitors, but not all of them have the same antitumor effects. It is convenient – especially for pre-clinical and clinical studies – to distinguish the most specific and potent chemical that delivers the highest inhibitory effect. Accordingly, in this study, we tried to uncover the best commercially available inhibitor(s) for targeting PI3Kα through predicting their specificity and potency using the docking software AutoDock Vina. This could help to improve treatment drugs in the future by cutting down the list of available ones to save more time, resources, and lives.

II. Computational Methods

A. Preparation of Phosphoinositide 3-kinase and Inhibitors

The crystal structure of PI3Kα was retrieved from the RCSB Protein Data Bank (PDB) with the code PDB-ID of 2RD0 (Gilliland, et al., 2000). There were four sections of missing residues in the 2RD0 crystal structure. Templates for the missing sections were adopted from the following PDB-ID entries: 3HHM, 3HIZ, 3ZIM, and 4JPS. All of the non-standard amino acid residues were removed separately from these structures, and PI3Kα was prepared as the receptor and the inhibitors as ligands using Discovery Studio 4.1 (Dassault Systèmes, 2010). The overall PI3K model was built by the homology modeling module with Modeller 19.9 (Sali and Blundell, 1993). Molecular Graphics Laboratory (MGL) Tools 1.5.6 were used to prepare the protein structure for molecular docking. Polar hydrogens were added to the protein structure and saved in the "PDBQT" file format (MGLTools, 2017). In the present study, a total of 28 PI3K inhibitors were investigated (Table I). The inhibitors were selected based on their specificity for PI3Kα and were obtained from the suppliers Tocris Bioscience (www.tocris.com) and Sigma-Aldrich (www.sigmaaldrich.com). They can also be tracked using their unique compound identification number through the PubChem website (https://pubchem.ncbi.nlm.nih.gov) (Kim, et al., 2019). Some of the two-dimensional (2D) structures were constructed by ChemDraw Pro 12.0 software and were saved in the "mol" file format (PerkinElmer, 2009). The 2D structures of the inhibitors were converted to three-dimensional structures using Discovery Studio 4.1. Finally, all of the structures were converted to the "PDBQT" file format with the Open Babel graphical user interface (O'Boyle, et al., 2011).

B. AutoDock Vina

The inhibitor docking site on PI3Kα was defined by establishing a cube that covered the area of the docking stage. The established cube was mainly defined through the manipulation of a colored box at the X, Y, and Z axes. The volume of the cube can be as large or as small as required; however, there is an exponential increase in computation time as the volume of the box increases. For this study, we used the dimensions of 25 Å × 25 Å × 25 Å to cover the inhibitor binding site with a grid point with 1.0 Å spacing and center grid boxes of 60.127, 62.455, and 114.509 in the X, Y and Z axes, respectively. Once the docking area was introduced, the coordinates of the grid box were written in a configuration file (a text document file) which fed into AutoDock Vina 1.1.2 software through a command line (Trott and Olson, 2010). The configuration file also specified the inhibitor (ligand) molecule and the PI3K (receptor) docking, and the broadness of the search can be set between 1 and 8, where 8 is the most comprehensive search. AutoDock Vina was run on the Microsoft Windows 8.1 operating system with four central processing units (1.7 GHz), and all PI3Kα inhibitors were docked into the PI3Kα (PDB-ID: 2RD0) protein (Trott and Olson, 2010). Three runs were performed for every single inhibitor.

III. Results

To validate our methodology, the PI3Kα protein structure (receptor) (PDB-ID: 2RD0) was matched with the PI3Kα isomers (ligands) (PDB-IDs: 3HHM, 3ZIM, 4FAD, and 4FA6)

TABLE I
List of Verified PI3Kα Inhibitors

S. No.	PI3Kα inhibitors	CID	S. No.	PI3Kα inhibitors	CID
1.	KWT (3HHM) (Mandelker, et al., 2009)	5288678	15	LTURM 36	122705988
2.	KKR (3ZIM) (Nacht, et al., 2013)	70699406	16	LY 294002 hydrochloride	11957589
3.	OTA (4FA6) (Le, et al., 2012)	59258964	17	LY 303511	3971
4.	0TB (4FAD) (Le, et al., 2012)	59259014	18	PF 04691502	25033539
5.	3-Methyladenine	135398661	19	PF 05212384	44516953
6.	A66	42636535	20	PI 103 hydrochloride	9884685
7.	AS 252424	11630874	21	PI 828	25181195
8.	AS 605240	5289247	22	PI 3065	24937012
9.	AZD 6482	44137675	23	PP 121	24905142
10.	BAG 956	24882589	24	Quercetin	5280343
11.	CZC 24832	42623951	25	STK16-IN-1	58525066
12.	ETP 45658	25229608	26	TG 100713	17751063
13.	GSK 1059615	23582824	27	TGX 221	9907093
14.	KU 0060648	11964036	28	Wortmannin	3003565

CID: Compound identification number, PI3K: Phosphatidylinositol 3-kinase

using the UCSF Chimera program version 1.10.1 (http://www.cgl.ucsf.edu/chimera/) (Pettersen, et al., 2004). The observed root-mean-square deviation values for these matchings were 0.904, 0.907, 1.174, and 1.174 Å, respectively (Fig. 1).

After successfully completing the docking protocol, the PI3Kα isomers described above were docked with the PI3Kα protein. Our data showed that different energy requirements were needed to perform successful docking. In this regard, KKR was the least energy-demanding chemical, and 0TA was the highest energy-demanding chemical (Table II).

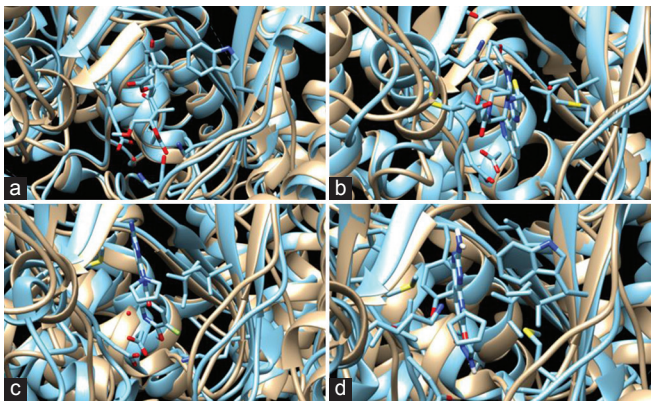

Fig. 1. (a-d) Superimposed structures of PI3Kα proteins and their root-mean-square deviation (RMSD) values for their matchings with PI3Kα protein. (a) 2RD0 (Gold) and 3HHM (Aqua) with RMSD: 0.904 Å; (b) 2RD0 (Gold) and 3ZIM (Aqua) with RMSD: 0.907 Å; (c) 2RD0 (Gold) and 4FAD (Aqua) with RMSD: 1.174 Å; (d) 2RD0 (Gold) and 4FA6 (Aqua) with RMSD: 1.174 Å.

TABLE II
Average Docking Scores of PI3Kα Isomers (Ligands) Docked to PI3Kα Protein

S. No.	PI3Kα inhibitor	1st run	2nd run	3rd run	Av.
1.	KKR	−8.3	−8.3	−8.4	−8.3
2.	KWT	−7.3	−7.3	−7.3	−7.3
3.	OTB	−7.2	−7.2	−7.2	−7.2
4.	0TA	−6.4	−6.4	−6.4	−6.4

PI3Kα: Phosphatidylinositol 3-kinase α

After that, each one of the remaining chemicals (24 inhibitors) was docked onto the PI3Kα protein. Docking scores (kcal/mol) for the inhibitors and the average of three runs were sorted from lowest-energy poses (maximum docked energy in negative) to highest-energy poses (minimum docked energy in negative), as shown in Table III. Our data revealed that the studied inhibitors exhibited different affinities toward the targeted protein. PF 05212384 gave the highest docking score (−9.2 kcal/mol), 3-methyladenine gave the lowest docking score (−4.8 kcal/mol), and the others placed between these two values (Tables III).

IV. Discussion

In this study, the type and potency of interactions between PI3Kα inhibitors and PI3Kα residues were predicted using the AutoDock Vina software. We used AutoDock Vina because it has been previously used in protein-ligand interactions and produced effective docking results (Perryman, et al., 2014: Jaghoori, et al., 2016). Inhibitors' orientation and their conformation were among the most important requirements to fulfill successful fitting between PI3Kα binding sites and the studied inhibitors. In this regard, optimal interactions and best docking scores were considered criteria to understand the fittest conformation among those generated by AutoDock Vina for the studied inhibitors.

The results of docking PI3Kα isomers with the PI3Kα protein recognized KKR as the most potent inhibitor compared with others that are recognized as typical PI3Kα inhibitors (Table II). During the docking of KKR into the PI3Kα binding site (Fig. 2), the formation of hydrogen bonds with SER802, LYS830, and GLN887 and carbon-hydrogen interactions with SER801 was observed. Hydrophobic interactions were also detected between the inhibitor and PI3Kα, causing alkyl interaction formation between protein residues MET800, MET886, VAL878, and MET950 and the inhibitor. The results obtained from the KKR-PI3Kα docking were used later on as a reference to choose the most potent and selective inhibitor(s) from the PI3Kα inhibitors list (Table III). When PF 05212384 docked into the PI3Kα binding site (Fig. 3), it formed

TABLE III
Docking Scores (kcal/mol) for Chemicals Recommended as PI3Kα Inhibitors. Data were obtained through AutoDock Vina 1.1.2 Software

S. No.	PI3Kα Inhibitor	1st run	2nd run	3rd run	Av.	S. No.	PI3Kα Inhibitor	1st run	2nd run	3rd run	Av.
1.	PF 05212384	−9.3	−9.0	−9.2	−9.2	15.	PI 103 hydrochloride	−7.3	−7.4	−7.3	−7.3
2.	AZD 6482	−8.4	−8.4	−8.3	−8.4	16.	LY 294002	−7.2	−7.3	−7.3	−7.3
3.	KKR	−8.3	−8.3	−8.4	−8.3	17.	OTB	−7.2	−7.2	−7.2	−7.2
4.	PI 3065	−8.1	−8.4	−8.2	−8.2	18.	Wortmannin	−7.1	−7.1	−7.1	−7.1
5.	GSK 1059615	−8.1	−8.1	−8.1	−8.1	19.	ETP 45654	−7.1	−7.0	−7.1	−7.1
6.	KU 0060648	−8.0	−8.0	−8.0	−8.0	20.	Quercetin	−7.0	−7.0	−7.0	−7.0
7.	AS 252424	−7.9	−7.9	−7.9	−7.9	21.	A66	−6.9	−6.9	−6.9	−6.9
8.	PP 121	−7.8	−7.8	−7.8	−7.8	22.	TGX 221	−6.9	−6.9	−6.9	−6.9
9.	CZC 24832	−7.8	−7.7	−7.7	−7.7	23.	LY 303511	−6.8	−6.8	−6.9	−6.8
10.	LTURM 36	−7.6	−7.6	−7.6	−7.6	24.	STK16-IN-1	−6.8	−6.8	−6.9	−6.8
11.	TG 100713	−7.6	−7.6	−7.6	−7.6	25.	PI 828	−6.7	−6.7	−6.7	−6.7
12.	AS 605240	−7.4	−7.4	−7.4	−7.4	26.	BAG 956	−6.4	−6.4	−6.4	−6.4
13.	PF 04691502	−7.6	−6.9	−7.6	−7.4	27.	0TA	−6.4	−6.4	−6.4	−6.4
14.	KWT	−7.3	−7.3	−7.3	−7.3	28.	3-Methyladenine	−4.8	−4.8	−4.8	−4.8

PI3Kα: Phosphatidylinositol 3-kinase α

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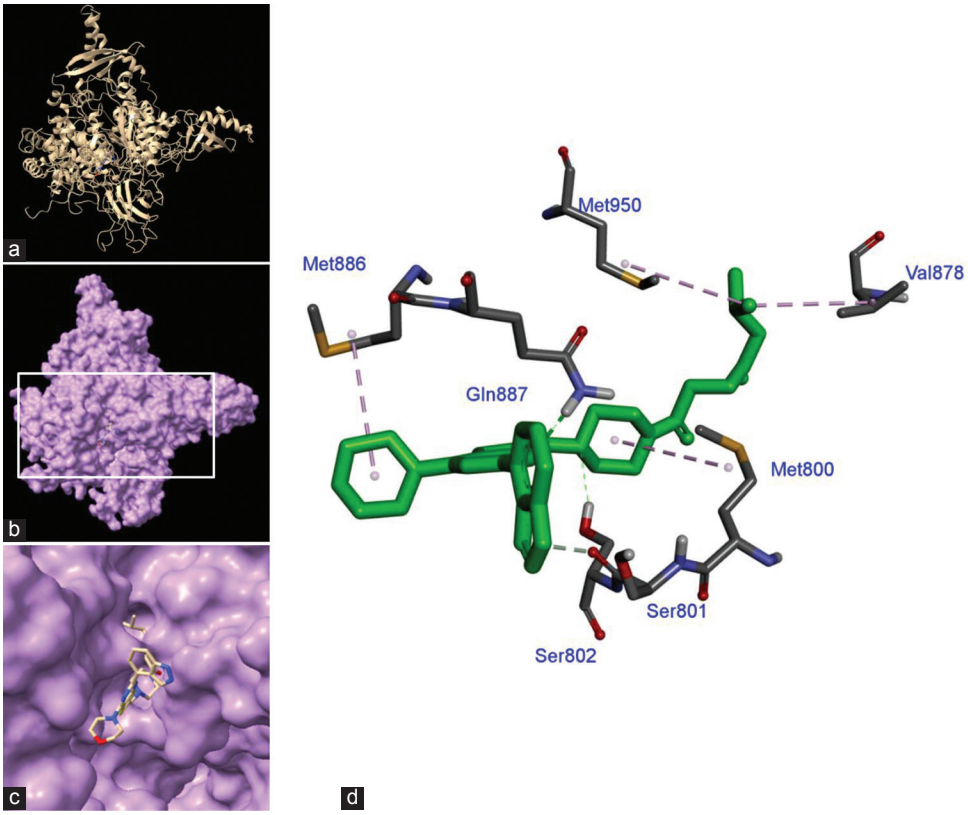

Fig. 2. KKR inhibitor docked to phosphatidylinositol 3-kinase α protein, (a) the protein as ribbons, (b and c) molecular surface and the inhibitor in active site, and (d) protein residues that interact with inhibitor.

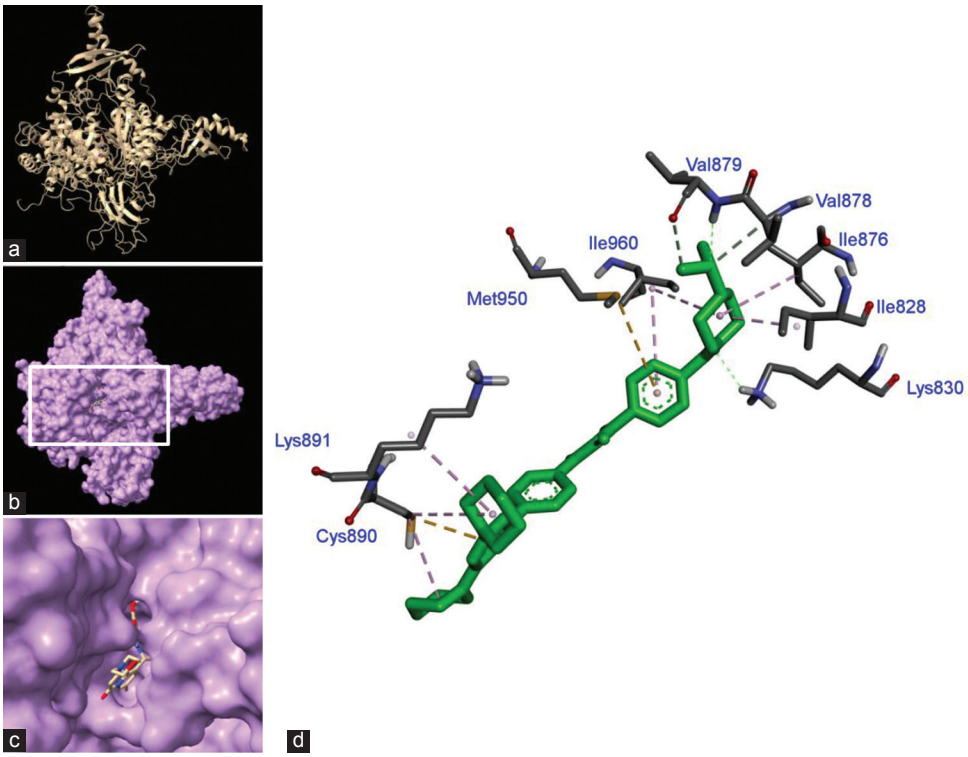

Fig. 3. Inhibitor PF 05212384 (19) docked with phosphatidylinositol 3-kinase α protein, (a) the protein as ribbons, (b and c) molecular surface and the inhibitor in active site, and (d) protein residues that interact with inhibitor.

hydrogen bonds with VAL879 and LYS830. Other non-bonded (pi-sulfur) interactions were formed between sulfur in CYS890 and MET950. Hydrophobic alkyl interactions were also observed between residues ILE828, ILE876, CYS890, LYS891, and ILE960 and the inhibitor. Moreover, a pi-alkyl interaction with ILE960 was also detected.

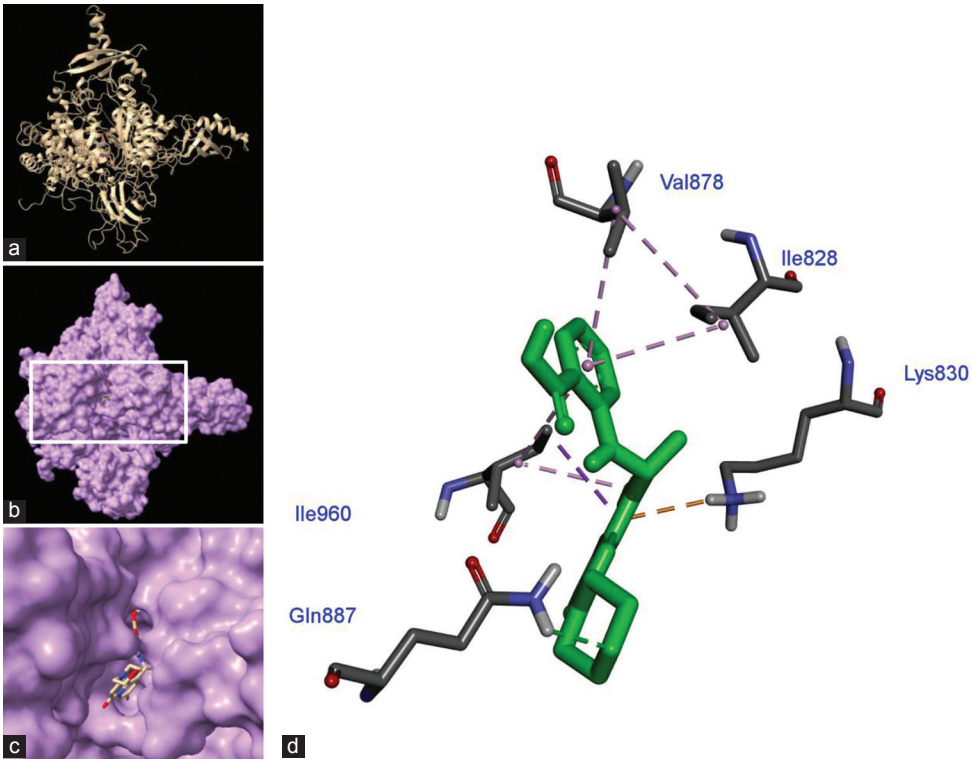

Fig. 4. Inhibitors AZD 6482 (9) docked to phosphatidylinositol 3-kinase α protein, (a) the protein as ribbons, (b and c) molecular surface and the inhibitor in active site, and (d) protein residues interacting residues with inhibitor.

Last but not the least, the inhibitor with the second lowest-energy pose was AZD 6482 (Fig. 4). This inhibitor formed hydrogen bonds with GLN887, an electrostatic interaction with LYS830, and hydrophobic interactions, including alkyl, pi-alkyl, and pi-sigma with ILE960 and pi-alkyl with ILE828 and VAL878.

Previously, other studies were carried out to predict the efficacy of PI3K inhibitors, but our results are not identical to theirs. This is more likely due to their use of different inhibitors and docking software (Sabbah, et al., 2010; Sabbah, et al., 2012; Singh and Bast, 2013; Kawade, et al., 2018).

V. Conclusions

Our analysis showed that among the studied PI3Kα inhibitors, the PF 05212384 and AZD 6482 compounds are the best candidates for targeting PI3Kα. Interaction studies also confirm that these inhibitors interact with PI3Kα by building hydrogen bonds with binding pocket residues in addition to hydrophobic interactions. *In vitro* and *in vivo* studies are needed to confirm our results in regard to the impact of candidate inhibitors on cancer cell survival and migration and their potential toxicity. It is also important to conduct molecular dynamic simulations to reach precise information regarding the dynamic behaviors and stability of the predicted complexes.

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