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BENTHAM SCIENCE

Computational Docking Studies of Novel Heterocyclic Carboxamides as Potential PI3Kα Inhibitors

Kamal Sweidan[a,*], Diana Al Sahlawi[a], Jörn Engelmann[b], Heba Abdel-Halim[c] and Ghassan Abu[d]

[a]*Department of Chemistry, The University of Jordan, Amman 11942, Jordan*

[b]*Faculty of Pharmacy, Al-Zaytoonah University of Jordan. Amman, 11733. Jordan*

[c]*High Field Magnetic Resonance Center, Max Planck Institute for Biological Cybernetics, Tübingen. Germany*

[d]*Faculty of Pharmacy and Medical Sciences, University of Petra, Amman, Jordan*

Abstract: Drugs comprising a heterocyclic system show widespread therapeutic impact such as antimicrobial, antidepressant, antihypertensive, and anticancer activity. We describe herein computational studies that support the promising biological activity of four new compounds (5, 6, 10 and 13). The wild-type and mutant phosphatidylinositol-4,5-bisphosphate 3-kinase α (PI3Kα) proteins were used as models to explore the potential interaction of the designed molecules with this important kinase involved in the growth regulation of cancer cells. The results of our studies showed that the verified compounds ought to fit into the kinase domain of wild-type and mutant PI3Kαs It is predicted that they interact with S774, K802, Y836, V851, S854, T856, Q859. and DY33 that are known to be key binding residues for active inhibitors both in wild-type and mutant PI3Kαs. Docking scores infer the selectivity of compounds 5, 6 and 10 toward the mutant PI3Kα (H1047R), whereas compound 13 displayed a slightly higher affinity to the wild-type protein. The pharmacophore modeling of PI3Kα inhibitors showed that the explored compounds shared four out of five pharmacophoric points with such inhibitors. Thus, the recently developed four compounds might be recruited as lead structures for the design of new antitumor drugs targeting PI3Kα.

Keywords: Heterocyclic compounds, pharmacophore screening, molecular docking, PI3Kα.

INTRODUCTION

Docking is a computational approach in which ligands are placed in the binding site of a receptor [1]. The aim of ligand docking is to predict the intermolecular interactions between the receptors and their ligands. It is one of the major tools of computational drug design used in drug discovery. Docking includes the prediction of possible ligand binding domains on a protein or enzyme and the prediction of ligand/protein complex interactions [2].

The two major problems of using computational docking methods are: 1) the Inaccurate prediction of ligand receptor free binding energies (binding affinity) and. 2) the flexibilities of ligand and receptor [3]. Many docking programs account for partial or full ligand flexibility, despite the fact that a protein exists in equilibrium of an ensemble of conformational states rather than as one dominant more stable conformation. Thus, protein flexibility is still often disregarded in ligand docking studies [4]. The term flexible docking is referring to the conformational search on the ligands before their docking [5].

The docking protocols are a combination of a search algorithm and a scoring function. The search algorithm covers the relevant conformational space of the ligands, while the scoring function characterizes the binding affinity between the ligand and the protein in term of free binding energy [5, 6] A large number of search algorithms and scoring functions are available [6]. However, the key elements in any docking protocol success are still the ligands and the binding sire where they should be docked.

Recently, we described the synthesis and cytotoxicity evaluation of new benzofuran/benzothiophene carboxamide derivatives (Fig. 1) [7]. We observed cytotoxic effects of selected compounds on a human cervical carcinoma cell line (HeLa) and 3T3 mouse fibroblasts. One derivative, 13, showed an approximately three times higher cytotoxicity in HeLa cells than in 3T3 cells. We now applied computational methods to evaluate further the potential anticancer activity of the recently synthesized compounds. For this purpose, we have chosen the phosphatidylinositol-4,5-bisphosphate 3-kinase a (PI3Kα), a heterodimeric lipid kinase, as a target enzyme for our studies.

PI3 kinases phosphorylate the 3-hydroxy moiety of phosphatidylinositol 4,5-biphosphate (PIP2), generating phosphatidylinositol 3,4,5-triphosphates (PIP3), an essential second messenger regulating the activity of PI3K downstream effectors AKT and mTOR. The activation of PI3K/AKT signaling cascade stimulates cell proliferation, growth, survival, tumor progression, invasion, angiogenesis, and metastasis Atypical PI3K/AKT pathway has been characterized in a number of human cancers. [8]

*Address correspondence to this author at the Department of Chemistry, The University of Jordan, Amman 11942, Jordan; Tel: +96265355000; Ext: 22155, Fax. +96265348932; E-mail: k.sweidan@ju.edu.jo

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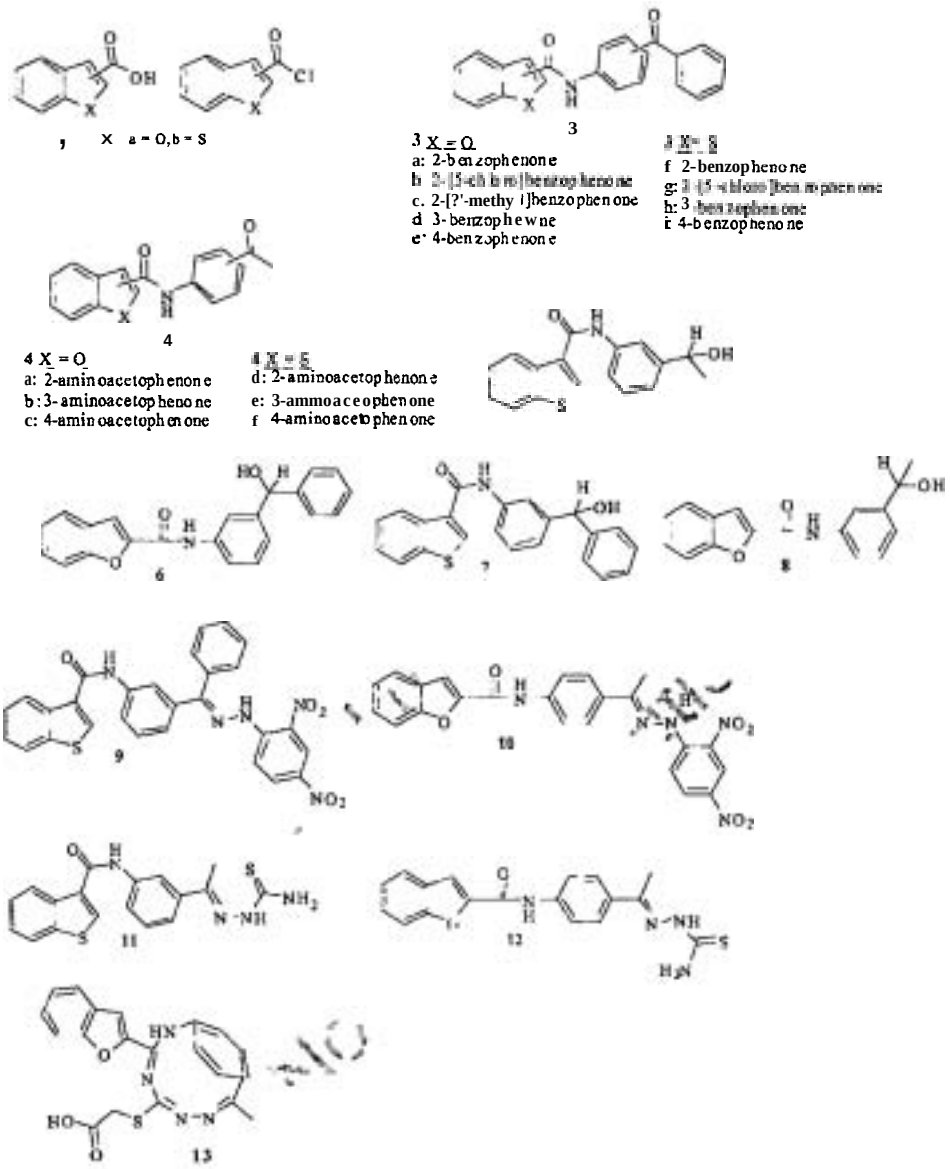

Fig. (1). Synthesized benzofuran/benzothiophene carboxamide derivatives used for the computational docking studies.

The PI3Kα subunit p110α coding gene (PIK3CA) is mutated, amplified and overexpressed in numerous human cancers. Somatic mutations in PIK3CA cluster in the helical (E542K, E545K) and kinase (H1047R) domains of PI3Kα increase the lipid kinase activity and snmulate the AKT signaling pathway. These mutations were observed in ≈ 30% of prostate, cervix, and endometrium tumors (Catalog of Somatic Mutations in Cancer, www.sanger.ac.uk/genetics/CGP/cosmic) [9]. In addition, PI3Kα activity has been shown to be amplified in tumor samples of cervical carcinomas and cervical carcinoma cell lines (HeLa) [10], whereas its acrivity is comparably low in 3T3 cells [11]. The activation of PI36 pathway is negatively regulated by the lipid phosphatase and tensin homolog (PTEN), and mutations in PTEN or irregularity of PTEN function or expression exists in human cancers [12]. The oncogenic potential of PI3Kα and its regulator (PTEN) makes PI3Kα a promising target for anticancer drug design.

In continuation of our former work, we report herein the computational studies to evaluate the potential of the new benzofuran/benzothiophene carboxamide derivatives as specific PI3Kα inhibitors.

Fig. (2). The kinase domains of 2RW (grey colored) and 3HMM (yellow colored) harboring the explored compounds embedded with some of key binding residues (for clarity purpose). The compounds and residues are depicted in stick model. H-atoms are hidden for clarity purpose.

RESULTS AND DISCUSSION

In order to identify the structural basis of binding for the synthesized molecules with the binding clefts of wild-type (wt) and mutant (mut) PI3Kα, we carried out docking studies of our experimentally tested compounds. A Glide docking approach [13] was applied to wt PI3Kα (PDB ID 2RD0) [14] and mutant PI3Kα (H1047R) (PDB ID 3HHM) [15], as well as the generated mutants E542K, E545K, and the doubly mutated E542K/E545K PI3Kα (mutations in the hehcal region of PI3Kα outside of the catalytic domain). The binding affinity of the PI3Kαs/ligand complex was expressed in terms of binding free energy (ΔG); docking scores (kcal/mol). The more negative the docking score, the better the binding and the more favorable the complex. A docking score of -2 kcal/mol as well as a difference of 2 kcal/mol were considered significant.

Our Glide docking results of the synthesized molecules against the wt (2RD0) and mutant PI3Kα proteins (H1047R, E542K, E545K, E542K/E545K) showed that all compounds bind to ihe PI3Kα kinase catalytic domain (Fig. 2). Most of the tested compounds formed hydrogen bonds (H-bonds) with D933, N920, S919, Q859, S854, V851 (via backbone NH), E849, Y836, D810, K802, A775, and 5774 (Tables 1 and 2).

Compound 13 showed a relative high docking score for wt PI3Kα (-6.40 kcal/mol) indicating a good affinity to the binding cleft of the kinase (Table 1). However, its binding to the mut PI3Kα (H1047R) seemed to be slightly less effective. In contrast, compounds 6 and 10 displayed a relative low docking score for the wt PI3Kα, bur a significantly better docking score for the mut PI3Kα (H1047R) (Table 1). These computational data deduced that these compounds might have a preferential selectivity against the H1047R PI3Kα. Notably compounds 3b and 3c showed considerable affinity towards mutant H1047R PI3Kα whereas 11 favored native PI3Kα. Therefore, the docking scores suggest that their backbone could be optimized to get better cytotoxic activity.

Recent experimental [14] and computational data [16-17] confirmed the importance of binding of the tested compounds to these residues, in particular of the binding to Asp933 [18]. Sabbah et al. [19] identified the structural differences between wt and mutant H1047R PI3Kα; particularly residues Q859, S854, Y836, and S774 could be exploited to design active PI3Kα inhibitors. Moreover, H-bond interactions with the scaffolds of S774, S854, and Q859 might be targets for selective inhibition of mutant H1047R PI3Kα [20].

It is worth noting that H-bmding of compounds 5, 6 and 10 to S774, S854, and Q859 could explain their preferential affinity against mutant H1047R (Figs. 3 and 4). Fig. (3) shows that compound 10 adopts different binding conformations in wt and mutant H1047R PI3Kα and forms H-bonds with Q859 (NH arnide side chain) and T856 (OH) of wt PI3Kα. Contrarily, its U-shaped conformation in mutant H1047R favors H-bond with S774 (OH), V851 (NH backbone), and Q859 (NH amide side chain).

The binding mode of compounds 5 and 6 in PI3Kαs binding clefts showed that these compounds miss H-bond interactions with the scaffolds of key binding residues and thus explains their lower affinity to the wt PI3Kα. Interestingly, their H-bonds with S854 (OH) of H1047R Increased their binding affinity Moreover, the binding domain of PI3Kαs was enclosed with hydrophobic residues (W780, I800, 1848, V850, V851, I932, and M922) which explained the binding patterns of compounds 5 and 6 in PI3Kαs. The adopted binding mode of compound 13 in PI3Kαs showed that it formed H-bond with K802 (NH_2) of wt PI3Kα and 5774 (OH) of the mutant H1047R protein (Fig. 4).

However, comparing the docking scores of the synthesized molecules against the generated mutant proteins (E542K, E545K, and double E542K/E545K PI3Kα) showed that these compounds have comparable affinities to these helical mutants as to that of the wt PI3Kα (Table 2). This was due to the position of these residues relative to the kinase domain. The PI3Kα catalytic kinase domain resides in residues region (699-1094). The mutant H1047R was embedded in this region and therefore it displays better differences in affmity compared to the wt PI3Kα. On the other hand, the considerable distance of the helical mutant fmm the kinase domain weakens the influence of these mutants on the binding affinity and consequently shows comparable affinity to that of the wt PI3Kα.

In order to get further details about the functionalities which are responsible for activity of the synthesized compounds 5, 6, 10 and 13, we screened them against an adopted pharmacophore model of PI3Kα inhibitors [19]. We found that these compounds match four out of five pharmacophoric points (Fig. 5). As shown in Fig. (5D), the structurally different compound 13 harbors one aromatic ring (F1), one aromatic or H-bond acceptor (F2), one aromatic or hydrophobic or H-bond acceptor (F3), and one H-bond acceptor (F4). In contrast, compounds 5, 6 and 10 contained the H-bond acceptor function F5 instead of F4. This describes the affinity of all four compounds toward PI3Kαs kinase domain. Furthermore, the accommodation of 5, 6 and 13 in the kinase binding site could explain their cytotoxic effect

Table 1. Docking scores (kcal/mol) and H-bond interactions between eompounds and PI3Kαs (WT and MUT H1047R).

MUT PI3Kα (H1047R)		WT PI3Kα (2RD0)		
Binding Residues	Docking Score	Binding Residues	Docking Score	Compound
D933	-6.84	V851	-6.60	1a
Q859	-7.27	V851	6.78	1b
NA	-6.71	NA	-7.04	2a
M811	-6.94	V851	-7.61	2b
NA	-6.18	NA	-6.98	3a
Q859	-6.38	Q859	-4.33	3b
V851,Q859	-6 8:	V851, Q859	-5.05	3c
NA	-6.63	NA	-5.96	3d
NA	-3.68	K802	-3.89	3e
NA	4.40	NA	-5.53	3f
Q859	-525	NA	-5.30	3g
V851	-6.37	NA	-6.10	3h
D933	4.97	K802	-4.27	3i
V851,Q859	7.15	V851	-691	4a
V851	-5 82	NA	4.00	4b
V851	-4.80	K802	-4.32	4c
NA	-6.01	NA	-6.60	4d
	-5.38	NA	4.14	4e
NA	-5.75	NA	-6.89	4f
S854	-6.69	NA	-5.91	5
V851,S854	-7.25	NA	4.82	6
E849	-6.18	D933	-7.55	7
V851	-7.50	D933	-637	8
NA	-4.87	S909	-3.70	9
V850, Q859,S774		Q859, Thr856	-3.67	10
NA	-4.12	D933	-7.19	11
V8S1	-6.26	D933, Y836	-5.44	12
S774	-5.40	K802	-6.40	13

NA: not available; no H-bond interaction.

Table 2. Docking scores (kcal/mol) and H-bond interactions between compounds and mutant (E542K, E545K, E542K/E545K) PI3Kαs.

	MUT(E542K/E545K) PI3Kα		MUT(E542K)PI3Kα		MUT(E545K) PI3Kα	
Compound	Docking Score	H-Bond	Docking Score	H-Bond	Docking Score	H-Bond
1a	-7.38	D810, Y836, D933	7.39	D810, Y836, D933	-7.40	D810, Y836, D933
1b	-7 94	E849, V851	-7 87	E849, V851	-7.88	E849, V851

Table 2. contd...

Compound	MUT(E542K/E545K) PI3Kα		MUT(E542K)PI3Kα		MUT(E545K) PI3Kα	
	Docking Score	H-Bond	Docking Score	H-Bond	Docking Score	H-Bond
2a	-6.96	NA	-5 87	D933, Y836	-5.95	NA
2b	-7.71	V851	-7 75	V851	-7.65	V851
3a	-5.20	NA	-5 27	V851	-5.68	NA
3b	-6.02	NA	-5 65	NA	-5.64	NA
3c	-6.12	NA	4.53	NA	4.55	NA
3d	-553	NA	-547		-5 4:	NA
3e	-7.1 1	K802	-5.67	D933, S774	-5.49	K802
3f	-7.05	NA	-4.937	NA	-4.76	NA
3g	4.64	Q859	-4 96	NA	-5.12	NA
3h	-5 83	NA	-5 67	NA	6.14	NA
3i	-3.54	NA	-4 14	K802	-2.50	NA
4a	-6.73	NA	-6 15	V851	-6.09	S854
4b	-5.89	NA	4.57		5.14	NA
4c	-4 21	NA	-3 38	D933, Y836	-340	Y836
4d	-649	NA	-5.63		-6.35	V851, S854
4e	-5.62	NA	-5.71	S773	-5.72	s773
4f	4 5 6	K802	4.68	K802	4 7 1	K802
5	-4 89	NA	-5.67	NA	-5.56	NA
6	-5 47	D933	-5.22	NA	-5.11	NA
7	-7.03	Y836	-6 99	D933	-7.18	D933
8	-5.65	D933	-4.73	D933	-6.49	N853, V851
9	-1.96	Y836, D933	-0.47	NA	-1.28	Y836
10	-4.29	D933	-3 88	V851, Y836	-3.92	Y836
11	-7.01	D933,H917	-5 75	S919, N920	-6.06	N920, S919
12	-6.63	D933	-5 94	D933	-4.82	NA
13	-6.41	NA	-5.89	D933	-6.04	D933

NA: no available; H-bond interaction.

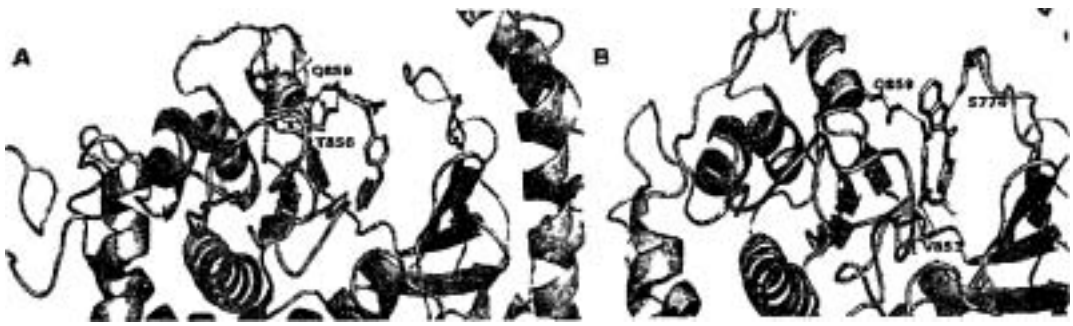

Fig. (3). The binding modes of **10** in PI3Kαs kinase catalytic domain. (A) The binding conformation of **10** m wr PI3Kα shows H-bonds with T856 and Q859. **(B)** The U-shaped conformarion in mutant H1047R forms H-bond with Q859, V851, and S774. Compound **10** and the key binding residues are represented in stick model. The C atoms are depicted in grey; O in red; N in blue color. Hydrogen atoms are hidden for clarity purpose. H-bond is represented in blue dotted line.

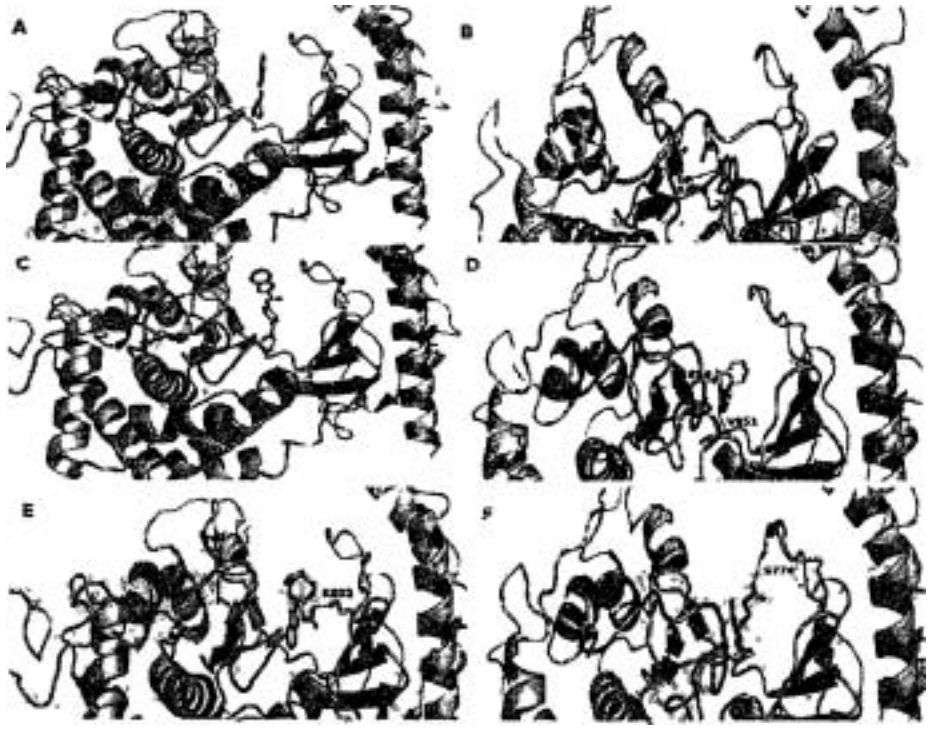

Fig. (4). The protein/ligand interactions of **5, 6** and **13** in the kinase domain of wt and mutant H1047R PI3Kα. **(A)** and **(B)** binding conformations of **5** in wt and H1047R PI3Kα; (C) and @)binding conformationsof 6 in wt and H1047R PI3Kα; **(E)** and **(F) binding** conformations of 13 in wt and H1047R PI3Kα. Hydrogen atoms are hidden for clarity purpose H-bond is represented in blue dotted line.

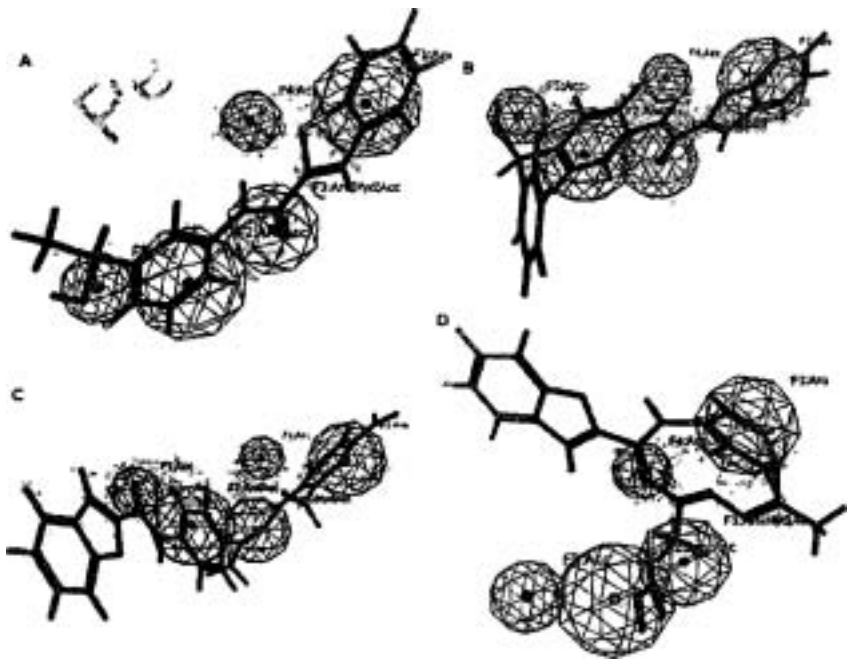

Fig. (5). PI3Kα pharmacophore model with **5A** (EC_{50} **340** mM [7]), **6B** (EC_{50} not determinable [7]), **10C** (not determinable [7]), and 13D (EC_{50} **10** mM [7]). Aro stands for aromatic rings; Acc for H-bond acceptor, and Hyd for hydrophobic groups.

CONCLUSION

We recently developed various heterocyclic carboxamide compounds that displayed promising biological activity [7]. Compounds 5, 6 and 13 shown here, inhibited the growth of immortalized embryonic mouse fibroblast cells (3T3) and human cervical cancer cells (HeLa) in *vitro*. It is of note that compound **13** exerted a selective cytotoxic activity against HeLa cells.

Our computational study displayed that PI3Kα, a kinase overexpressed in cervical cancer cells such as HeLa, represents a cellular target for these synthesized compounds. The Glide docking approaches suggested that these compounds reside in the kinase catalytic domain of wt and mutant PI3Kαs. Compounds 5, 6 and **10** seemed to have a preferential affinity to the mutant H1047R PI3Kα. Their ability to form H-bond particularly with Ser774, Ser854, and Gln859 might explain this behavior. In contrast, the cyclic amidine derivative 13 showed a better binding affinity to wt PI3Kα and an only slightly lower affinity to the mutant PI3Kα. All four compounds shared four out of five pharmacophoric points with typical PI3Kα inhibitors. However, compound **13** also differed in this characteristic from the other compounds by providing a different H-bond acceptor function.

Our data indicate that compound **13** might be a good candidate for further optimization toward a specific antitumor drug and inhibitor of wt PI3Kα. Heterocyclic carboxamides such as 5, 6 and 10, on the other hand, might represent starting points to develop potentially selective inhibitors of the prevalent mutant H1047R PI3Kα.

EXPERIMENTAL

Computational Preparation of Ligand Structures

The tested compounds (ligands) were built based on the coordinates of wortmannin in 3HHM [14] using MAESTRO BUILD module in Schrödinger software [15] and energetically minimized by MacroModel wizard using the OPLS2005 force field.

Computational Preparation of PI3Kα Isoforms

X-ray crystal structures of Apo PI3Kα WT (PDB ID 2RD0) [13] and mutant H1047R PI3Kα (PDB ID 3HHM) [14] were retrieved from the RSCB Protein Data Bank. The homology modeling generated structures of 2RD0 and 3HHM were adopted for this experiment [17]. The coordinates of wortmannin in 3HHM were adopted to 2RD0 and used as the ligand to generate the grid files for docking protocols. The mutants E542K and E545K as well as the double mutants (E542K / E545K) PI3Kα were prepared usmg the MUTATE module in MOE [20]. Then, the protein structures were prepared using the Protein Preparation tool in Schrödinger to maximize the H-bond interactions between residues.

Glide Docking Studies

Five grid files for 2RD0, 3HHM, E542K, E545K, and double E542K/E545K PI3Kα were generated using the Glide Gnd Generation protocol using the bound ligands as centroids. The scaling factor for van der Waals receptor for the non-polar atoms was calibrated to 0.8 to furnish some flexibility. All other parameters were used as defaults [15].

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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