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Computer-Aided Design, Synthesis, and Biological Evaluation of New Indole-2-Carboxamide Derivatives as PI3Kα/EGFR Inhibitors

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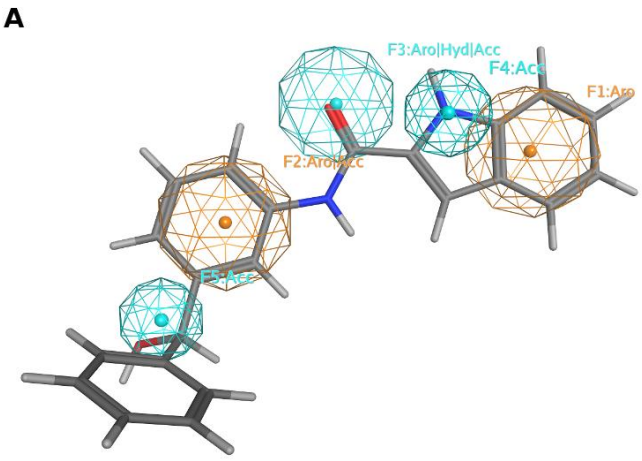
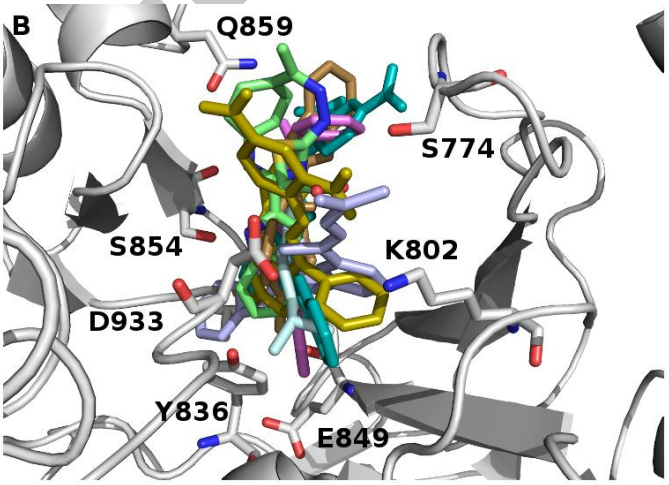


Compound 13 functionalities fit PI3Kα inhibitor pharmacophore model



The structural analogues occupy the kinase domain of PI3Kα

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ABSTRACT

Structure-based drug design and molecular modeling were employed to identify a new series of indole-2-carboxamides as potential anticancer agents. These compounds were synthesized and characterized with the aid of several spectroscopic techniques, such as FT-IR, NMR, and mass spectrometry as well as by elemental analysis. Molecular docking studies confirmed that the newly synthesized compounds accommodate PI3Kα and EGFR kinase catalytic sites and form H-bonding with the key binding residues. The antitumor activity of these new compounds against an array of cancer cell lines (human colon carcinoma (HCT116), leukemia (K562), and breast cancer (MDA231) was evaluated. Results revealed that these compounds were selective against the kinase domain, and none of them showed any inhibitory activity against K562. In addition, results showed that compound **13** exhibited high potency in HCT116 and MDA231 with IC_{50} values of 19 and 15 μM, respectively. Our findings recommend that further optimization of this series would be beneficial for colon and breast cancer treatment.

The chemistry and medicinal study of heterocyclic compounds have been considered a powerful approach to treat a wide range of diseases. Indole (benzopyrrole) system has evolved as an important heterocyclic structure due to its presence in a large number of bioactive compounds; this nucleus was reported as the basic system, as shown in Fig. 1, as antiviral[1-3], antidepressant[4] (**1**), antihyperlipidemic[5] (**2**), and anti-inflammatory agents.[6, 7] Furthermore, other indole derivatives (**3**, **4**) were reported as potent antitumor agents and were extensively studied in treating cancer[8-13] which still remains a major threat to the human life. Similarly, the carboxamide functional group occurs in many drugs; a property that stems from its ability to serve as a hydrogen-bond acceptor through its carbonyl group and as hydrogen-bond donor via its primary/secondary amides.[14, 15]

For the past few years, our research group has been interested in the design and synthesis of small molecular weight inhibitors that target phosphatidylinositol 3-kinase (PI3Kα).[14-20] We synthesized two novel scaffolds: *N*-phenyl-4-hydroxy-2-quinolone-3-carboxamides[17] as selective (H1047R) PI3Kα inhibitors and derivatives of benzoin[19] as PI3Kα inhibitors. In addition, we have recently described the synthesis and cytotoxicity evaluation of new benzofuran and benzothiophene carboxamide models,[21] and employed molecular docking studies for novel heterocyclic carboxamides as potential PI3Kα Inhibitors.[22]

In continuation of our ongoing search of new heterocyclic derivatives targeting PI3Kα inhibition, we employed an adopted pharmacophore model[16] of PI3Kα inhibitors to tailor the indole core nucleus with functionalities that match the finger print of active PI3Kα inhibitors (Fig. 2). Moreover, we assigned the crystal structures of PI3Kα and EGFR as target enzymes having kinase domain to explore the structural basis of binding of this series and identify structural features that are required to induce an inhibitory activity.

Phosphatidylinositol 3-kinase (PI3Ks) enzymes are lipid kinases that phosphorylate the hydroxyl group at the 3 position of the inositol ring yielding phosphatidylinositol 3,4,5 triphosphates (PIP_3). PIP_3 regulates many cellular processes such as cell proliferation, survival, motility, and apoptosis. On the other hand, PI3Ks are negatively regulated by phosophoinositide phosphatases such as phosphatase and tensin homolog (PTEN) which dephosphorylate the 3 OH position of PIP_3.[24, 25] PI3Ks are divided into three classes (I, II, and III) based on their substrate interaction and coding sequence. Class IA PI3K has PI3Kα isoform encoded by PIK3CA gene. PI3Kα is amplified, overexpressed, and mutated in a variety of human tumors including breast, prostate, cervix, gastric, colon, and endometrium tumors.[26] Cancer derived "hotspots" somatic mutations were observed in PI3Kα coding sequence and are clustered in helical (E542K and E545K) and kinase (H1047R)

domains leading to change in protein conformation and gain in lipid kinase activity.[27-32]

Tyrosine kinases phosphorylate tyrosine residue in a panel of different substrates and are classified as a receptor tyrosine kinase (RTK) and non-receptor tyrosine kinase (NRTK). [33] Epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase that modulates cell signaling cascade, induces cancer cell proliferation and growth, promotes angiogenesis and metastasis, and causes antiapoptotic effect. [34-37] In cancer cells, the kinase activity of EGFR is impaired by different oncogenic mechanisms; EGFR point mutation, gene amplification, and overexpression.[38]

EGFR overexpression is observed in patients with metastatic non-small-cell lung cancer (NSCLC) corresponding to poor prognosis.[39] Mutations in the catalytic kinase domain of EGFR (G719C, L858R, and L861Q) accompany lung adenocarcinoma, particularly non-small-cell lung cancer (NSCLC)[40-42], contrarily to mutations in the extracellular domain of EGFR that cause glioblastoma.[40, 42] Around 6% of breast tumors are associated with EGFR overexpression.[43] Therefore, selective targeting of PI3Kα and/or EGFR would offer a beneficial therapeutic approach for cancer treatment. Several chemical core structures have been designed and synthesized targeting PI3Kα[44-50] and/or EGFR[51, 52] and some of these have been reported in clinical studies.[15, 27, 53-59]

We report herein the synthesis of novel compounds containing an indole nucleus recruiting ligand- and structural-based drug design strategies; these compounds, to the best of our knowledge, have not been previously described in the literature. In addition, their antitumor activity against an array of cancer cell lines was investigated *in vitro*.

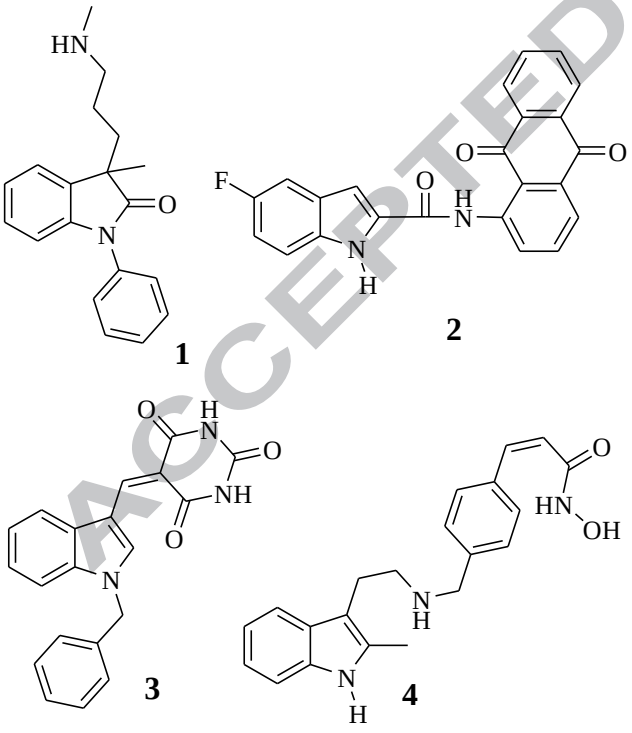


Fig. 1. Chemical structures of some indole-based derivatives.

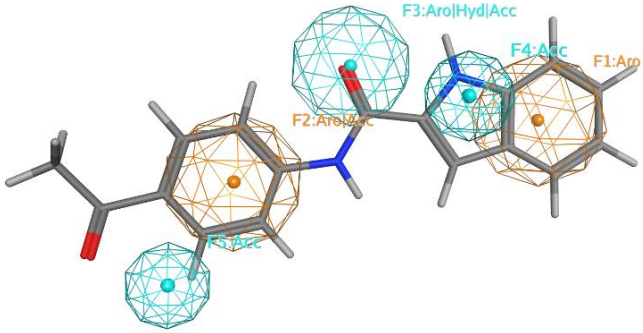
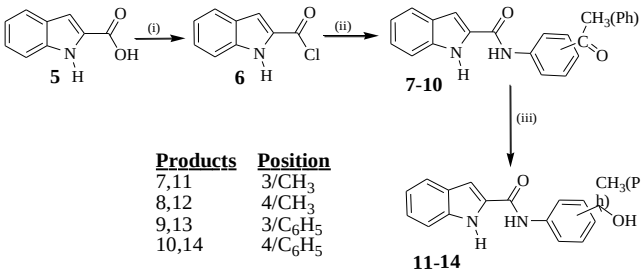


Fig. 2. PI3Kα pharmacophore model with **8**. Aro stands for aromatic rings; Acc for H-bond acceptor; and Hyd for hydrophobic groups. Picture made by MOE.[23]

Indole-2-acyl chloride (**6**) was prepared via reaction of 1*H*-indole-2-carboxylic acid (**5**) with excess thionyl chloride in dry chloroform under reflux; evaporation of the solvent afforded **6** which was immediately used in the next step without further purification. Reaction of **6** with appropriate aminoacetophenone/ benzophenone in presence of pyridine and triethylamine afforded the corresponding caroxamides **7-10.** On the other hand, compounds **11-14** were prepared in good yields by reduction of compounds **7-10** with sodium borohydride ($NaBH_4$) as a chemoselective and simple reducing agent in a polar protic solvent under mild conditions as outlined in Scheme 1. Structures of the newly prepared compounds were determined with the aid of various spectroscopic techniques such as FT-IR and NMR (^1H and ^{13}C), and mass spectrometry and by elemental analysis.



Scheme 1. Reactions and conditions: (i) $SOCl_2$, $CHCl_3$, reflux, 6 h; (ii) Ar-NH_2, base, $CHCl_3$, reflux, 24 h; (iii) $NaBH_4$, dry CH_3OH, r.t, 6 h.

In order to examine the antiproliferative activities induced by the newly synthesized compounds **7-14** against proteins having kinase domain, we examined the growth inhibition in the colon (HCT-116) and breast cancer (MDA231) cell lines both harboring kinase domains as well as leukemia (K562) cell line as negative control as shown in Table 1. The malignant human colon carcinoma cell line (HCT116) contains both wild-type (WT) and mutant (MUT) (H1047R) PI3Kα produced from a primary tumor tissue culture.[60] The metastatic human breast cancer cell line (MDA-231) was employed as a model for estrogen receptor negative breast cancer; it amplifies and/or overexpresses the gene encoding EGFR resulting in poor clinical prognosis and high recurrence rate. [61-68]

The human myeloid leukemia cell line (K562) was derived from the pleural fluid of a patient with chronic myeloid leukemia in blast crisis.[69] The blast cells in K562 are rich in glycophorin and they might be stimulated to produce fetal and embryonic

hemoglobin in the presence of hemin.[70] K562 cell line represents the primary source of chronic myeloid leukemia cells showing significant indication of malignancy for clinical and experimental studies.[69] Additionally, no overexpression of tyrosine kinase (Bcr -Abl) was observed in K562[71]; thus k562 is considered as a negative control in our experiment. The prospective compounds **7-14** inhibited HCT116 and MDA231 cell lines, both containing kinase domain. Comparably no inhibition was observed in K562 cell line. This provides a proof that this scaffold targets the kinase domain. Compound **13** exerted high potency in both HCT116 and MDA231 cell lines.

Table 1. Growth inhibition IC_{50} (μM) after 24 hours exposure time. Standard deviation (SD) never exceeded 5%.

Compound	IC_{50} HCT116 (μM) ± SD	IC_{50} MDA 231 (μM) ± SD	IC_{50} K562 (μM) ± SD
7	64±3	57±2	>100
8	61±5	50±4	>100
9	55±3	45±2	>100
10	54±2	49±3	>100
11	38±2	34±2	>100
12	37±4	35±2	>100
13	19±1	15±1	>100
14	44±5	38±4	>100

It's worth noting that attaching a methyl or phenyl moiety on either positions 3 or 4 of the aromatic ring of compounds **7-10**, showed similar manner of inhibition in HCT116 and MDA231. This infers that the methyl or phenyl group provided hydrophobic interaction with the backbone of the binding site regardless of steric effect. Interestingly, a better activity was exerted for their structural analogues having an (OH) group instead of (CO) for **11-14**; the IC_{50s} dropped to half of those of **7-10**. This might be due to presence of an OH group that furnishes H-bond donor and acceptor whereas the ketone group provides only H-bond acceptor.

Remarkably, compounds **11** and **12** exhibited comparable inhibition in HCT116 and MDA231. This implies that tailoring the phenyl moiety with hydroxyethyl motif on positions 3 or 4 exerts similar activity against the kinase domain. Surprisingly, the hydroxyphenyl motif on the 3-position of the aromatic ring, as in **13**, appeared to be the most active. On the other hand, **14** showed lower activities compared to **13** which suggests that tailoring the 4-position is not favored. Moreover, tailoring the 3-position is favored for the hydroxyphenyl motif. This finding recommends that position 3 is favored for functionalities bearing H-bond acceptor. Similarly, comparison between **11** and **13** infers that the aromatic ring might be involved in aromatic (π-π) stacking and/or orientation in the binding cleft. Altogether, H-bond acceptor and aromatic ring representing hydroxyphenyl motif are essential for activity. The PI3Kα pharmacophore model with **13** agrees with biological data and declares that core structure of **13** fits the finger print of PI3Kα inhibitor (Fig.3). Moreover, the absence or low activity of this scaffold against K562 provides another clue for the selectivity of this core nucleus towards kinases. K562 harbors esterase, lipid, and acid phosphatase [72-74] and thus it is considered as a negative control.

In order to determine whether growth inhibition of colon and breast cancer cell lines was mediated by an apoptotic pathway, we measured the caspase-3 enzyme activity using the caspase-3 colorimetric assay. Activation of the caspase-3 pathway[75, 76] is a strong indication of apoptosis and can be employed in cellular assays to assess activators and inhibitors of the "death cascade." To determine the apoptotic effects of the compounds under investigation, cells were incubated with 120 μM of each compound for 48 hours and changes in caspase-3 enzyme activities were evaluated. Results indicate a significant increase in caspase-3 activity in response to 48 hours treatment for each compound (Fig.4). Interestingly, the ability of the examined compounds to augment apoptosis was closely relevant to their potency as measured by their IC_{50} values. Compound **13** was the most potent against the two tested cell lines and also appeared to enhance apoptosis more significantly when compared to others.

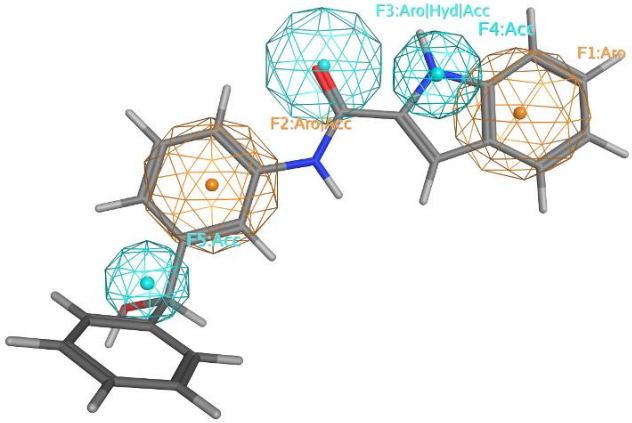


Fig. 3. PI3Kα pharmacophore model with **13**. Aro stands for aromatic rings; Acc for H-bond acceptor; and Hyd for hydrophobic groups. Picture made by MOE.[23]

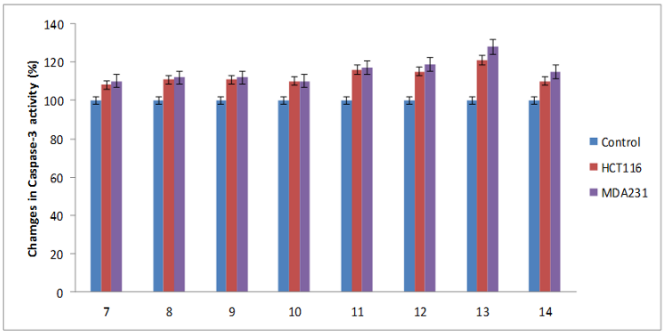


Fig.4. Effects of compounds treatment on caspase-3 activity in HCT116 and MDA231 cells after 48 hrs. Results are the means of two independent experiments. $P < 0.05$ was considered significant.

To explain the anticancer activity of the synthesized compounds against human colon carcinoma (HCT-116) and breast cancer (MDA231) cell lines, we employed the crystal structures of PI3Kα (PDB ID: 2RD0) [77] and EGFR (PDB ID: 3W32) [78] to identify the binding interactions of these compounds in PI3Kα and EGFR kinase domains. The binding site of 2RD0 is composed of M772, K776, W780, I800, K802, L807, D810, Y836, I848, E849, V850, V851, S854, T856, Q859, M922, F930, I932, and D933. Interestingly, similar pattern of binding residues encloses the kinase domain of 3W32: L718, G719, S720, V726, A743, K745, M766, C775, R776, L777, L788, I789, Q791, L792, M793, G796, C797, L844, I853, T854, D855, F856, L858, F997, and L1001. It's worth noting that the hydrophobic and polar residues occupy the binding clefts. Furthermore, the hydrophilic and hydrophobic exposed surfaces of the co-crystallized ligands are in agreement with the surrounding residues (Fig.5). The aromatic residues furnish π-stacking aromatic interaction,

hydrophobic lining provides hydrophobic (van der Waals) interaction, and polar residues offer water accessible surface area through hydrogen-bonding, ion-dipole, and dipole-dipole interactions. Additionally, the polar acidic or basic residues afford an ionic (electrostatic) bonding.

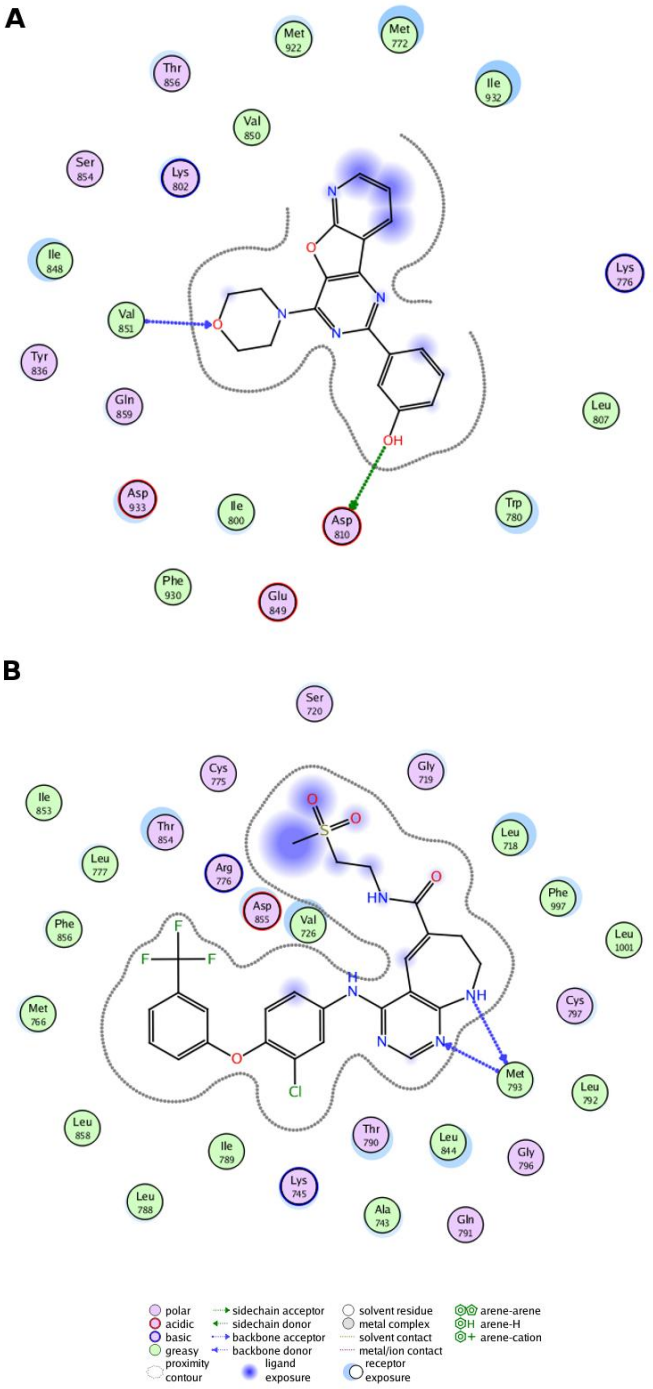


Fig. 5. The binding cleft of (**A**) 2RD0 and (**B**) 3W32 having co-crystallized ligands. Green colored sphere stands for hydrophobic residue, pink colored spheres for hydrophilic (polar) residue, red contour circle represents acidic residue, and blue contour circle represents basic residues.

To determine the structural-basis of binding of tested compounds in the kinase domains of PI3Kα and EGFR, we employed Glide docking[79-81] against the kinase clefts of 2RD0 and 3W32. Our Glide docking data showed that the synthesized molecules accommodate the kinase domains of PI3Kα and EGFR and approved that their binding conformations superpose the co-crystallized docked pose (Fig. 6). The backbones of the prepared compounds form H-bonds with E849, V851, and S854 of 2RD0 (Table 2). Remarkably, these compounds tend to form H-bonds with M793, T854, and D855 of 3W32 (Fig. 7). The significance of these residues and their interaction model has been reported in other computational[14, 16-18, 82] and experimental studies.[77, 78]

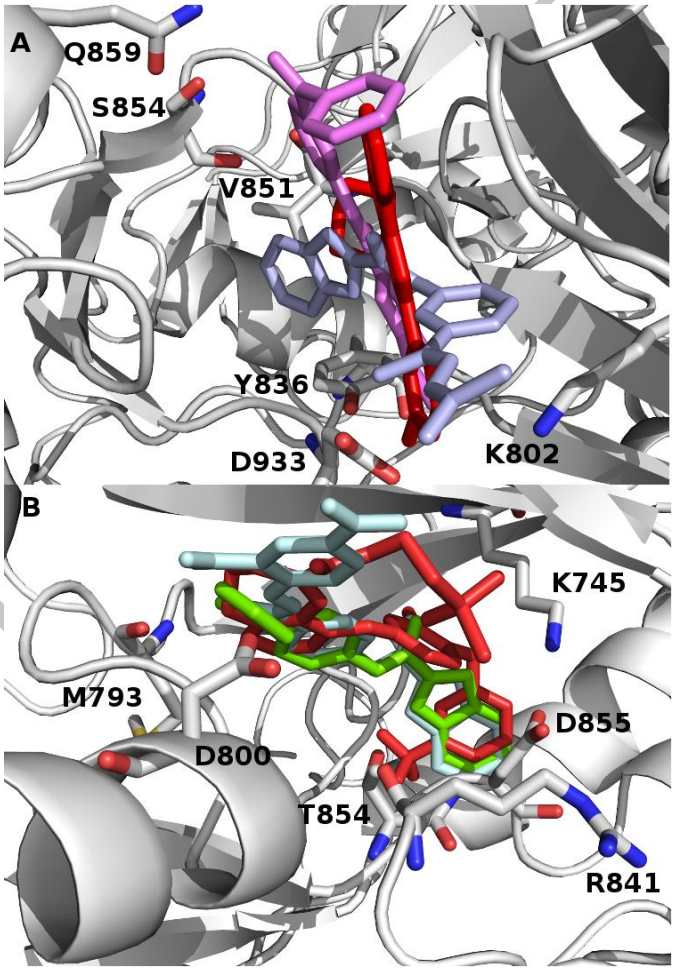


Fig.6. The kinase domains of (**A**) 2RD0 and (**B**) 3W32 accommodate the docked pose of the co-crystallized ligands represented in red color and some verified molecules represented in purple, pink, green, and blue colors. Some of key binding residues are shown for clarity purpose.

Table 2. The Glide docking scores (Kcal/mol) of the synthesized compounds against PI3Kα (2RD0) and EGFR (3W32). NA means (not available).

CPD#	docking score Kcal/mol (PI3Kα)	Binding residues	docking score Kcal/mol (EGFR)	Binding residues
7	-6.89	V851,S854	-7.91	M793, T854
8	-7.13	V851,S854	-7.91	M793, T854
9	-7.71	V851,S854	-7.70	NA
10	-8.13	V851,S854	-7.94	NA
11	-6.56	E849, V851	-8.05	54
12	-6.81	V851,S854	-7.99	M793, T854
13	-7.61	V851,S854	-9.21	D855
14	-8.36	V851,S854	-8.64	M793

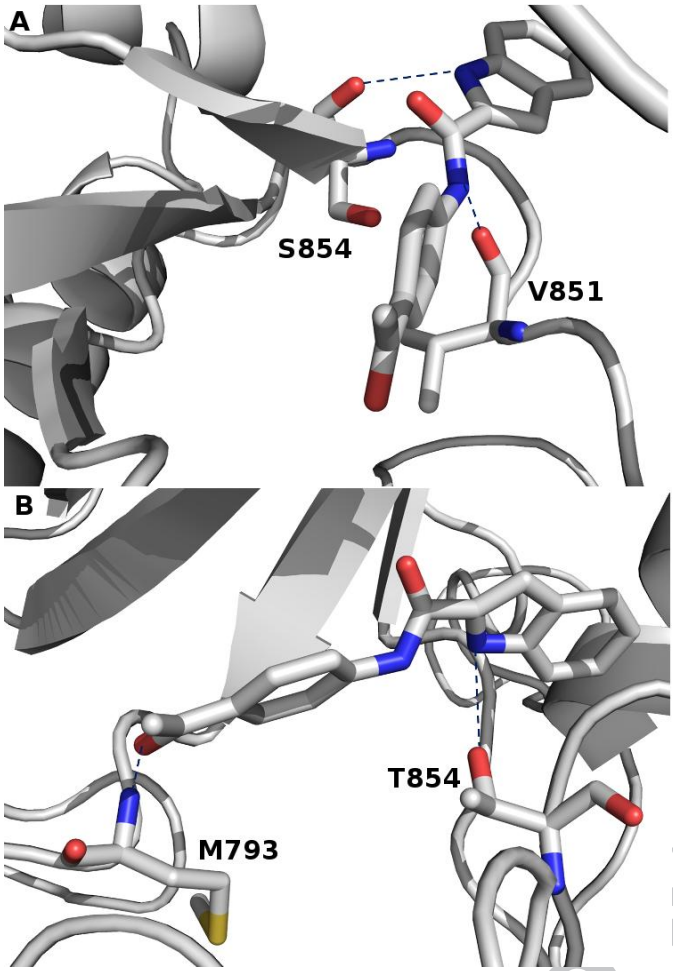


Fig. 7. Binding conformation of **8** in the kinase domains of (**A**) 2RD0 and (**B**) 3W32. H-Bond is depicted in blue dotted line. H atoms are hidden for clarity purpose. Picture made by PYMOL.

Docking study reveals that compounds **7**-**14** exerted comparable binding affinity in the kinase domain of PI3Kα and EGFR. A difference of 1 Kcal/mol in the docking score values against PI3Kα and EGFR implies that these compounds have a tendency toward the kinase domain. Interestingly, the biological data are in agreement with molecular docking and suggest that this scaffold might be a promising kinase inhibitor. Supplementary Fig. 1S shows a positive relationship between the IC_{50s} and Glide docking scores against PI3Kα (r^2 =0.88) and EGFR (r^2 = 0.65). Furthermore, the more negative binding scores of **7**-**14** toward PI3Kα and EGFR infer that this scaffold might serve as a lead for kinase inhibitors.
Notably, the backbones of this scaffold form H-bonds with S854 of PI3Kα. We have reported previously the significance of this residue to target selectively mutant H1047R PI3Kα. [14] This finding prompted us to screen **7**-**14** against a panel of kinases and knockout either allele of human colon cancer cell line to produce only one specific gene in HCT116 to better discuss results based on molecular level.

To evaluate the performance of Glide program, we compare the docked pose of W32 in EGFR (PDB ID: 3W32) to its native conformation in the crystal structure. Fig. **8** shows the superposition of the Glide-generated W32 pose and the native conformation in 3W32.The RMSD for heavy atoms of W32 between Glide-generated docked poses and the native poses was 0.967 Å. Results indicate that Glide docking is capable to identify the native poses in crystal structures and can successfully predict the ligand-binding conformation.

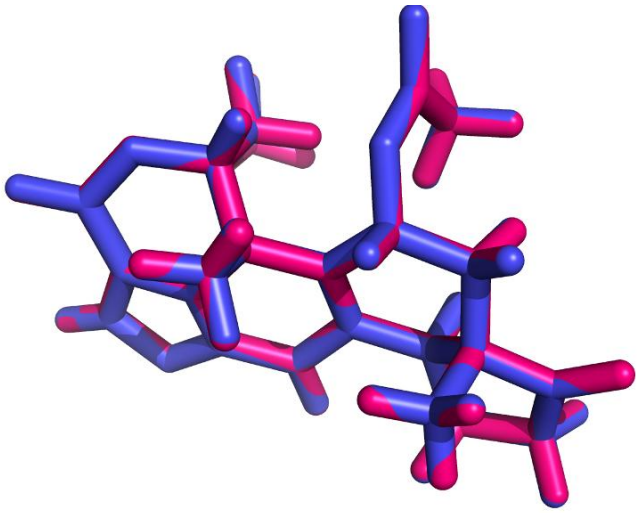


Fig. 8. The superposition of the Glide-docked W32 pose and its native conformation in 3W32. The native coordinates are pink colored and the docked pose is blue. Picture made by PYMOL.

In conclusion, a new class of PI3Kα and EGFR inhibitors having an indole core structure has been identified via recruiting ligand- and structure-based drug design approaches. A series of indole-2-carboxamides have been synthesized and screened *in vitro* against a panel of cancer cell lines: human colon carcinoma (HCT-116), breast cancer (MDA231), and leukemia (K562). Molecular docking illustrated that the synthesized compounds fit PI3Kα and EGFR kinase domains and tend to form H-bonds with key binding residues. Furthermore, comparable binding affinity was observed for kinase harboring proteins (PI3Kα and EGFR). Our biological results showed that none of the synthesized molecules exerted any inhibitory activity in K562. However, compound **13** exhibited high potency in HCT116 and MDA231 with IC_{50} values of 19 and 15 µM, respectively. Our findings recommend that further optimization of this series would be beneficial for colon and breast cancer treatment.

Supplementary Material

Detailed experimental methods, computational approaches, spectroscopic characterization results, as well as additional figures are listed in the supporting information and are available at http://www.elsevier.com

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