Discovery of Chromeno[4,3-c]pyrazol-4(2H)-one Containing Carbonyl or Oxime Derivatives as Potential, Selective Inhibitors PI3Kα

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A series of novel chromeno[4,3-c]pyrazol-4(2H)-one containing carbonyl or oxime derivatives (4a–n, 5a–n) have been synthesized and evaluated their biological activities as phosphatidylinositol 3-kinase (PI3K) inhibitors. Out of them, compound 5l showed the most potent antiproliferative activities against HCT-116 with IC_{50} of 0.10 µM in vitro, and exhibited the most potent activity for PI3Kα with the value of 0.012 µM. Docking simulation of 5l into PI3Kα active site were performed to determine the probable binding model, and it indicated that compound 5l could be optimized as a potential inhibitor of PI3Kα in the further study.

Key words chromeno[4,3-c]pyrazol-4(2H)-one derivative; inhibitor; antiproliferative

Three distinct classes of lipid phosphatidylinositol 3-kinases (PI3Ks), as a family, play a key role in cellular processes by regulating functions such as cell metabolism, proliferation, differentiation, motility, and intracellular trafficking. Class I PI3K, the best studied of the three PI3K classes, are heterodimers consisting of a p110 catalytic subunit and a p85 regulatory subunit, comprising four isoforms (α, β, δ, γ). In recent years, PI3Kα is recognized as one of the most potent targets in the treatment of cancer. All of these enzymes are activated by receptor tyrosine kinases (RTKs) and G-protein coupled receptors (GPCRs) on the cell surface. Subsequent activated by receptor tyrosine kinases (RTKs) and G-protein coupled receptors (GPCRs) on the cell surface. The importance of the PI3Kα is most commonly observed in many cancers. In addition, activated PTEN is commonly lost in many tumors. Therefore, small molecule inhibitors against PI3Kα is necessary to cancer treatment, for PI3K pathway has a key role in cancer. There are many researches about inhibitor of PI3Kα and some inhibitors have entered clinical trials.

Several natural products as PI3K inhibitors are widely known, including first-generation PI3K inhibitors such as wortmannin (Fig. 1), quercetin and myricetin. Second-generation PI3K inhibitors are designed small molecules, including LY294002 (a quercetin analogue) and its analogues, of which LY294002 was proved to be the most potent PI3K inhibitor. Then, GDC0941 as a considerable PI3K inhibitors was reported in 2011. It was a feasible program to design a core region, and our research team already reported a series of 2-alkyl-chromeno[4,3-c]pyrazol-4(2H)-one derivatives containing benzyl and alkyl chain as potential PI3K inhibitors. In our previous studies, we found that there is large space in the active pocket if compounds based-on chromeno[4,3-c]pyrazol-4(2H)-one skeleton as PI3K inhibitors. In continuation to extend our research on chromeno[4,3-c]pyrazol-4(2H)-one derivatives, herein, we introduced acetophenone fragment into the derivatives in order to they can well-bonded with the PI3K protein, and the result of the molecular docking showed they have the lower CDOCKER_INTERACTION_ ENERGY (Table 1). It indicated that these compounds may bond well in the active pocket of PI3K. Therefore, we designed and synthesized a series of 2-(2-oxo-2-phenylethyl)chromeno[4,3-c]pyrazol-4(2H)-one derivatives. In previous reports of 6-bromoirindirubin-3’-oxime (BIO), 3’-oxime substitution played a key role in kinase inhibitory effects. Thus, carbonyl in 2-(2-oxo-2-phenylethyl)chromeno[4,3-c]pyrazol-4(2H)-one derivatives was then replaced by oxime, for this modification might enhance solubility, kinase inhibition and cell permeability. Target compound synthesized from 4-hydroxycoumarin. Upon biological evaluation was carried out, some of the synthesized compounds were found as potential PI3Kα inhibitors. The apoptosis study was also carried out by flow cytometry to show that these compounds induced apoptosis in a dose-dependent manner. Docking simulations were performed using the X-ray crystallographic structure of PI3Kα (PDB code: 3HHM, retrieved from the RCSB Protein Data Bank) in complex with the most potent inhibitor to explore the binding modes of the compound at the active site.

Based on the reported pathway for the synthesis of compound 3, the target compounds (4a–n, 5a–n) were synthesized by routes outlined in Chart 1. First of all, 3-formyl-4-chlorocoumarin (2) was synthesized from 4-hydroxycoumarin under Wilsmeier conditions (phosphorus oxychloride/N,N-dimethylformamide (POCl3/DMF)). Secondly, compound 2 and 80% hydrazine hydrate were dissolved in ethanol, using triethylamine as catalyst, and reacted to get chromeno[4,3-c]pyrazol-4(2H)-one (3). Thirdly, compound 3 and α-bromoacetophenone were allowed to react in aceton to get the de...
sired compounds (4a–n). Furthermore, reaction of compounds (4a–n) with hydroxylamine and pyridine in ethanol afforded compounds (5a–n). Additionally, the structure of compound 4a and compound 5b was further confirmed by X-ray diffraction. The crystal was presented in Fig. 2 gave a perspective view of this compound together with the atomic labeling system.

The desired compounds (4a–n, 5a–n) were tested against four cell lines: HCT-116, A549, Huh7 and HL60. All data were presented in Table 2. The results showed that most of the synthetic compounds displayed remarkable antiproliferative effects on the four cell lines. Among compounds (4a–n, 5a–n), compound 5l showed the best inhibitory activity (IC50 = 0.10, 0.31, 0.87, 0.13 µM for HCT-116, A549, Huh7, HL60, respectively), which was superior to the positive control LY294002.

From the data listed in Table 2, the compounds (5a–n) that contain the oxime showed a remarkable improvement versus its counterpart (4a–n) containing the carbonyl in the inhibition of HCT-116 cell proliferation. It is noteworthy that compound 5l exhibited a 44-fold improvement compared with compound 4l in the inhibition of HCT-116 cell proliferation. These comparisons indicated that the oxime moiety was a key contributor to the potent antiproliferative activities of the compounds (5a–n).

Among compounds (5a–n), the compounds 5j and 5l bear-

Table 1. Binding Energy of Compounds with PI3Kα

| Compounds | Binding energy \( \Delta G_b \) (−kcal/mol) | Compounds | Binding energy \( \Delta G_b \) (−kcal/mol) |
|-----------|--------------------------------------------|-----------|--------------------------------------------|
| 4a        | −41.1195                                  | 5a        | −47.7256                                  |
| 4b        | −40.4394                                  | 5b        | −48.2233                                  |
| 4c        | −42.3012                                  | 5c        | −48.7303                                  |
| 4d        | −49.5995                                  | 5d        | −56.0358                                  |
| 4e        | −43.2467                                  | 5e        | −51.0145                                  |
| 4f        | −41.4545                                  | 5f        | −49.7636                                  |
| 4g        | −44.9009                                  | 5g        | −49.3267                                  |
| 4h        | −45.3174                                  | 5h        | −52.1426                                  |
| 4i        | −47.4145                                  | 5i        | −52.4405                                  |
| 4j        | −48.3688                                  | 5j        | −54.6955                                  |
| 4k        | −44.6799                                  | 5k        | −49.8198                                  |
| 4l        | −48.545                                   | 5l        | −57.7501                                  |
| 4m        | −46.9464                                  | 5m        | −53.3411                                  |
| 4n        | −45.3309                                  | 5n        | −55.2235                                  |
| LY49002   | 41.9934                                   |           |                                           |

Reagents and conditions: (a) DMF, POCl3, 60°C, 6 h. (b) Ethanol, Et3N, 80% hydrazine hydrate, 20°C, 14 h. (c) Acetone, \( \alpha \)-bromoacetophenone, Et3N, 0°C, 8–12 h. (d) Ethanol, hydroxylamine hydrochloride, pyridine, sodium acetate, 80°C, 15–20 h.

Chart 1
LY294002 was described by Yin et al.

Table 2. Antiproliferative Activities in Vitro of Target Compounds (4a–n, 5a–n) against Four Cancer Cell Lines

| Compounds | IC_{50}(\mu M) | CC_{50}(\mu M) |
|-----------|----------------|----------------|
|           | HCT-116 | A549 | Huh7 | HL60 | HCT-116 | A549 | Huh7 | HL60 |
| 4a        | 62.8    | 97.23 | NT^3 | 86.32 | NT^3 |
| 4b        | 66.73   | 78.89 | NT^3 | 77.27 | NT^3 |
| 4c        | 61.05   | 68.41 | NT^3 | 57.68 | 38.74 |
| 4d        | 3.37    | 6.63  | 4.15 | 2.83  | 367.12 |
| 4e        | 49.46   | 84.56 | NT^3 | 52.36 | NT^3 |
| 4f        | 51.70   | 61.43 | NT^3 | 53.28 | 40.59 |
| 4g        | 18.11   | 17.65 | NT^3 | 14.84 | 11.79 |
| 4h        | 25.54   | 32.24 | NT^3 | 31.34 | 24.49 |
| 4i        | 19.21   | 46.13 | NT^3 | 28.56 | 18.72 |
| 4j        | 7.88    | 9.26  | NT^3 | 8.22  | 9.46  |
| 4k        | 32.08   | 27.24 | NT^3 | 29.31 | 26.19 |
| 4l        | 4.48    | 13.33 | NT^3 | 9.27  | 6.81  |
| 4m        | 17.30   | 35.72 | NT^3 | 18.63 | NT^3 |
| 4n        | 17.96   | 31.65 | NT^3 | 42.53 | 62.87 |
| 4o        | 21.45   | 34.27 | NT^3 | 48.43 | 28.35 |
| 4p        | 18.05   | 28.37 | NT^3 | 38.94 | 18.43 |
| 4q        | 18.8    | 39.24 | NT^3 | 22.76 | 15.48 |
| 4r        | 0.21    | 0.62  | NT^3 | 0.91  | 0.15  |
| 4s        | 1.71    | 2.45  | NT^3 | 3.06  | 2.19  |
| 4t        | 15.97   | 37.46 | NT^3 | 24.32 | 11.87 |
| 4u        | 5.04    | 8.53  | NT^3 | 10.26 | 6.64  |
| 4v        | 4.04    | 10.38 | NT^3 | 19.87 | 3.27  |
| 4w        | 2.21    | 6.38  | NT^3 | 11.16 | 2.73  |
| 4x        | 0.36    | 0.77  | NT^3 | 1.53  | 0.61  |
| 4y        | 2.21    | 6.38  | NT^3 | 11.16 | 2.73  |
| 4z        | 0.10    | 0.31  | NT^3 | 0.87  | 0.13  |
| 4aa       | 0.63    | 2.26  | NT^3 | 3.34  | 0.86  |
| 4ab       | 0.98    | 3.25  | NT^3 | 1.82  | 0.87  |

LY294002 51.82* 82.32* 67.18* 18.43* —

Table 3. Enzymatic Activities of Compounds (5a–n) against PI3K

| Compounds | IC_{50}(\mu M) | PI3Kα | PI3Kβ | PI3Kγ | PI3Kδ |
|-----------|----------------|-------|-------|-------|-------|
| 4a        | >10            | >10   | >10   | >10   | >10   |
| 4b        | >10            | >10   | >10   | >10   | >10   |
| 4c        | >10            | >10   | >10   | >10   | >10   |
| 4d        | >10            | >10   | >10   | >10   | >10   |
| 4e        | >10            | >10   | >10   | >10   | >10   |
| 4f        | >10            | >10   | >10   | >10   | >10   |
| 4g        | >10            | >10   | >10   | >10   | >10   |
| 4h        | >10            | >10   | >10   | >10   | >10   |
| 4i        | >10            | >10   | >10   | >10   | >10   |
| 4j        | >10            | >10   | >10   | >10   | >10   |
| 4k        | >10            | >10   | >10   | >10   | >10   |
| 4l        | >10            | >10   | >10   | >10   | >10   |
| 4m        | >10            | >10   | >10   | >10   | >10   |
| 4n        | >10            | >10   | >10   | >10   | >10   |
| 4o        | >10            | >10   | >10   | >10   | >10   |
| 4p        | >10            | >10   | >10   | >10   | >10   |
| 4q        | >10            | >10   | >10   | >10   | >10   |
| 4r        | >10            | >10   | >10   | >10   | >10   |
| 4s        | >10            | >10   | >10   | >10   | >10   |
| 4t        | >10            | >10   | >10   | >10   | >10   |
| 4u        | >10            | >10   | >10   | >10   | >10   |
| 4v        | >10            | >10   | >10   | >10   | >10   |
| 4w        | >10            | >10   | >10   | >10   | >10   |
| 4x        | >10            | >10   | >10   | >10   | >10   |
| 4y        | >10            | >10   | >10   | >10   | >10   |
| 4z        | >10            | >10   | >10   | >10   | >10   |
| 4aa       | >10            | >10   | >10   | >10   | >10   |
| 4ab       | >10            | >10   | >10   | >10   | >10   |

LY294002 0.480 0.985 0.946 1.364

Table 2. Antiproliferative Activities in Vitro of Target Compounds (4a–n, 5a–n) against Four Cancer Cell Lines

Table 3. Enzymatic Activities of Compounds (5a–n) against PI3K

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Fig. 2. Crystal Structure Diagram of Compounds 4a and 5b

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Table 3. Enzymatic Activities of Compounds (5a–n) against PI3K

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...ing double halogen substituent at the meta and para positions of the benzyl group were strongly considered as the most interesting compound with an IC_{50} value of 0.36, 0.10 \mu M for HCT-116, respectively. Comparing the various mono-substituted groups on the acetophenone group, compounds (5a–n) showed antiproliferative activities in order of 3-NO_{2}> 4-CF_{3}>4-F>4-Cl>3-Cl>2-Cl>4-Br>4-OMe>4-CH_{3}>4-H, ...

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*(a) IC_{50} values are averaged values determined by at least two independent experiments. Variation was generally 5%.*
which revealed that compounds with electron-withdrawing substituents exhibited better inhibitory activities than those with electron-donating substituents, and the strong electronic-withdrawing substituents on acetophenone group were beneficial for the activity. In addition, compound 5d displayed the good potent antiproliferative activity with IC₅₀ of 0.21 µM, indicated that acetophenone group bearing great steric hindrance play an important role in the antiproliferative activity.

In order to find potent inhibitors, it is important to measure cytotoxicity. Top 12 compounds (4d, l, 5d, e, g–n) were detected for their cytotoxicity on macrophages cells. The pharmacological results of these selected compounds were summarized in Table 2. According to the data, it can be seen that the selected compounds displayed low toxicity.

All new compounds (4a–n, 5a–n) were tested for their PI3K enzymatic activity and results were shown in Table 3.

As shown in Table 3, most of the tested compounds displayed moderate to potent inhibitory activities these four target protein kinases. Additionally, compounds (5a–n) were also much better than their counterpart (4a–n). Particularly, compounds 5j, l and m showed a certain selectivity over PI3Kα, compared with their IC₅₀ in four target kinases from Table 3. It was concluded that compounds 5j, l and m may have potential for further development as selective PI3Kα inhibitors. In particular, compound 5l exhibited most efficient inhibitory activity with IC₅₀ value of 0.012 µM as compared to LY294002 with IC₅₀ value of 0.480 µM. It was concluded that these synthesized compounds may have potential for further development as PI3Kα inhibitors.

A further study comparing the antiproliferative activity against the HCT-116 cell line with the PI3Kα inhibitory activity of the top 12 compounds (4d, l, 5d, e, g–n) was conducted and the result revealed that there was a moderate correlation between PI3Kα inhibition and inhibition for cancer cell, as evidenced in Fig. 3, with a correlation coefficient of 1.1788 and an R² value of 0.8795. In conclusion, the consistency between PI3Kα inhibitory activity of synthesized compounds and their antiproliferative activity against HCT-116 indicate that the potent anticancer activities of synthesized compounds were likely related to their PI3Kα inhibitory activities.

To examine whether compound 5l can induce apoptosis of HCT-116, flow cytometry method of operation was carried on by using Annexin V-phycoerythrin (PE) and propidium iodide (PI) double staining. The results of apoptosis study show that 5l induced apoptosis in a dose-dependent manner in Fig. 4. Moreover, the percentage of early apoptotic cells was...
markedly elevated in a dose-dependent manner from 17.6 to 24.2% at 24 h, as compared to 13.3% of apoptotic cells in the untreated control.

To help understand the potency of studied compounds and guide further structure–activity relationship (SAR) studies, we proceeded to examine the interaction of compound 5l with PI3Kα crystal structure (3HHM pdb). The molecular docking was performed by inserting compound 5l into the active center of the PI3Kα protein. All docking runs were performed by Discovery Studio 3.5. The binding modes of compound 5l and

Fig. 5. (A) Binding Model of Compound 5l with 3HHM; (B) 3D Model of the Interaction between Compound 5l and 3HHM Binding Site of PI3Kα Protein

Fig. 6. The Receptor Surface Model with Compound 5l
PI3κα protein were depicted in Fig. 5. The enzyme surface model was shown in Fig. 6, and the results revealed that the molecule is well filled in the active pocket.

In the binding mode, there are three amino acids VAL851, LYS802 and ILE848 located in the binding pocket playing vital roles in the conformation with compound 5I, which were stabilized by two hydrogen bonds and one π–σ interaction. The π–σ interaction, of which its length was 2.44 Å, was formed between ILE848 and coumarin ring of compound 5I. The oxygen atom on coumarin ring provided one hydrogen bond were formed between VAL 851 and oxygen atoms of oxime (H…N–O: 2.02 Å, 169.85°). In Fig. 6, the enzyme surface model revealed that the small molecule was well embedded in the active pocket. All above, the molecular docking result along with the antiproliferative activity data, suggested that compound 5I is a potential inhibitor of PI3κα.

In summary, a series of novel 2-(2-oxo-2-phenylethyl)-chromeno[4,3-c]pyrazol-4(2H)-one derivatives were synthesized, and these compounds had been tested for their inhibitory activities against four human tumor cell lines which showed good antiproliferative activities. Among them, compound 5I showed the most potent PI3κα inhibition activities (IC_{50}=0.012 µM) and antiproliferative activities (IC_{50}=0.10 µM) for HCT-116. The apoptosis assay results was showed that compound 5I induced apoptosis of stimulated HCT-116 cells. Docking simulation performed to put compound 5I into the PI3κα protein active site to determine the probable binding model, and the result indicated that compound 5I was nicely bound into active site of PI3κα. The result provided valuable information for the design of PI3κα inhibitors for cancer treatment.

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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