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Inhibition of PI3 Kinase Gamma Enzyme by Novel Phenylpyrazoles

Sukumar Bepary,[†] In Kwon Youn,[†,*] Hee-Jong Lim, and Ge Hyeong Lee[*]

Korea Research Institute of Chemical Technology, P.O. Box 107, Yuseong-gu, Daejeon 305-600, Korea
[*]E-mail: ghlee@krict.re.kr
[†]Pai Chai University, Department of Chemistry, Daejeon 302-160, Korea. [*]E-mail: yink@pcu.ac.kr
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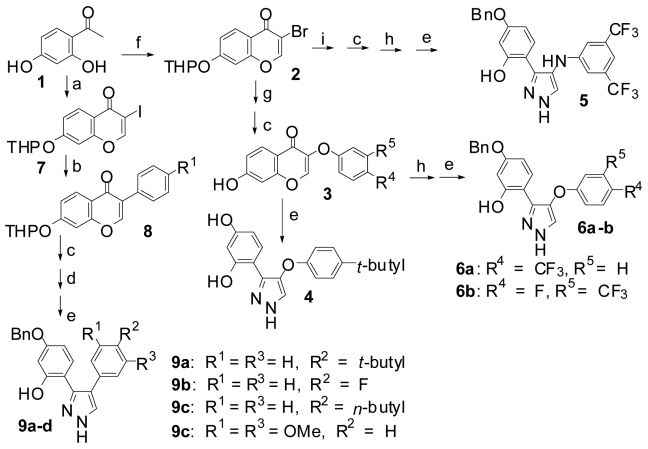
Phosphatidylinositol-3-kinase gamma (PI3Kγ), with its catalytic subunit P110γ and regulatory subunit p101, shows relatively restricted tissue distribution, but abundant in myeloid cells.[1,2] This isoform has been found to be utilized by the BCR-ABL fusion oncogene, implicated in chronic myeloid leukemia, cell proliferation and drug resistance,[3] and is also known to be a Ras effector,[4] thereby indicating specialized roles in the human pathological changes.

Increasing evidence suggests that PI3Kγ enzyme is involved in inflammatory processes and immune system functions.[5-10] Chronic inflammatory diseases such as, Crohn's disease and Barrett's esophagus appeared to increase the risk of developing tumors.[11] Tumors induce host inflammatory responses and thereby stimulate angio-genesis,[12-15] immunosuppression[16-20] and tumor metastasis.[21] Myeloid cells may differentiate into tumor-associated macrophages (TAMs) or tumor-associated neutrophils (TANs), thereby promoting tumor growth[22-25] and relapse after therapy.[26] Thus, targeting tumor inflammation could provide substantial therapeutic benefit to cancer patients.

Again activated PI3Kγ promotes chemotaxis and polarization of neutrophils in response to GPCR ligands, such as chemokines.[7,27,28] Research also showed that chemo-attractants stimulating structurally diverse GPCRs, Receptor Tyrosin Kinases (RTKs) and type I cytokine receptors, all activate myeloid cell integrin α4β1.[28] Since the integrin family of adhesion proteins also plays key roles in inammation,[29-31] this relationship brings the PI3Kγ under consideration for further study. Accordingly it was found[28,32] that PI3Kγ is necessary and sufficient to activate the myeloid cell integrin α4β1. Thus PI3Kγ inhibition seems to offer therapeutic benefits in chronic arthritis.

Our studies were centered in discovering novel inhibitors against PI3Kγ isozyme and accordingly, a novel backbone, selected by High Throughput Screening (HTS) of 7500 compounds available in the Chemical Bank of Korea Research Institute of Chemical Technology (KRICT), was further derivatized for developing the desired Hits and Leads and herein we report the details of our observations.

The syntheses of different samples have been summarized in Schemes 1 to 3. As shown in Scheme 1, 1-(2,4-dihydroxy-phenyl)ethanone (**1**) after phenolic OH group protection was treated with DMF-DMA (Dimethylformamide dimethyl-acetal) followed by iodine to get the 4*H*-chromen-4-one

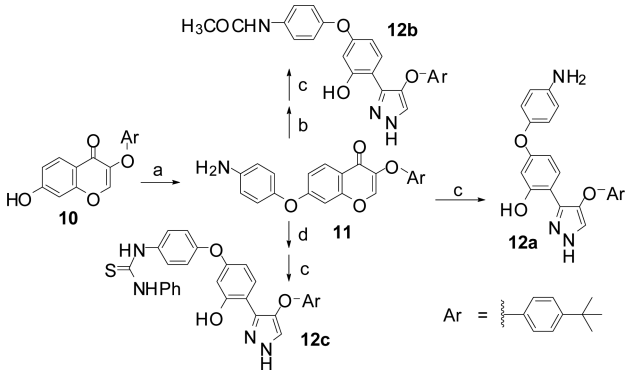


Scheme 1. Reagents and conditions: (a) (i) DHP/PPTS, MC, RT, 4 h, (ii) DMF-DMA, 95 °C, 3 h, (iii) I2, pyridine, RT, 12 h; (b) Respective boronic acid, $Pd(Ph_3P)_4$, Na_2CO_3, Toluene:Ethanol: Water (5:1:2), 130oc (MW), 1 h; (c)Toluene-4-sulfonic acid, MeOH, THF, 60 °C, 1 h; (d) Benzyl bromide, K_2CO_3, DMF, RT, 16 h; (e) NH_2NH_2, Ethanol, Reflux, 5 min; (f) OXONE, 2 N HBr, Et_3N, MC; (g) Respective phenol, $PdCl_2(PPh_3)_2$, K_3CO_3, DMF, 90 °C; (h) Benzyl bromide, K_2CO_3, DMF, RT, 16 h; (i) 3,5-di(trifluoromethyl)aniline, $Pd_2(dba)_3$, (*t*-Bu)$_3$P, Toluene, RT, 4 h.

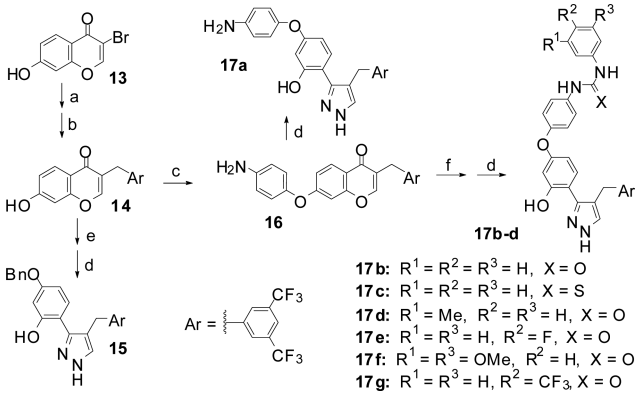
(**7**) that on coupling with organoboronic acids and subsequent *O*-deprotection, *O*-benzylation and reflux with hydrazine offered the samples **9a-d**. Again, **1** after phenolic OH group protection was treated with DMF-DMA followed by bromine to get the bromo-chromone (**2**). On subsequent coupling with phenols and –OH deprotection this chromone gave **3**, which was refluxed with hydrazine to get the sample **4** or was coupled again with benzyl bromide and then refluxed with hydrazine to get the samples **6a-b**. Also, **2** on coupling with 3,5-bis(trifluoromethyl)aniline was subjected to *O*-deprotection, *O*-benzy-lation and then treatment with hydrazine to get sample **5**.

Also, the chromen (**10**) on *O*-arylation followed by reflux with hydrazine gave **12a** (Scheme 2). Also the aniline **11** was then either *N*-acylated or converted to thiourea and then was treated with hydrazine to samples **12b-c**.

In another route, 3-bromo-7-hydroxy-4*H*-chromen-4-one (**13**) after coupling with 3,5-bis(trifluoromethyl)benzaldehyde was hydrogenated to get **14** (Scheme 3), that was then *O*-benzylated and refluxed with hydrazine to get sample **15**.



Scheme 2. Reagents and conditions: (a) 4-iodoaniline, $PdCl_2(PPh_3)_2$, K_3CO_3, DMF, 90 °C (b) Acetic anhydride, reflux, 30 min (c) NH_2NH_2, Ethanol, Reflux, 5 min; (d) Phenylisothio-cyanate, MC, 90 °C, 12 h.



Scheme 3. Reagents and conditions: (a) (i) *iso*-pr-Mg-Cl, THF, –40 °C, 0.75 h; (ii) 3,5-bis-(tri-fluoromethyl)benzaldehyde, THF, –40 °C, 1.6 h; (b) H_2, Pd-C, MeOH, RT, 72 h; (c) 4-iodoaniline, K_3CO_3, $PdCl_2(PPh_3)_2$, DMF, 90 °C; (d) NH_2NH_2, Ethanol, Reflux, 5 min; (e) Benzyl bromide, K_2CO_3, DMF, RT, 16 h; (f) Arylisothiocyanate or Arylisocyanate, MC, 90 °C, 12 h.

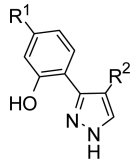
Again **14**, after coupling with 4-iodoaniline, either was refluxed with hydrazine to get sample **17a** or was converted to corresponding phenylurea (or thiourea) and then refluxed with hydrazine to get samples **17b-g**.

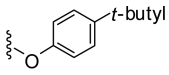
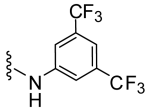
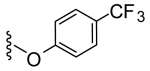
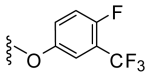
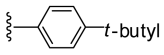
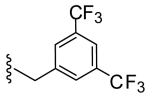
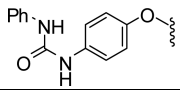
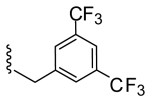
In our study, **4** represented an interesting scaffold when evaluated for the IC_{50} values (Table 1) against the PI3Kγ isozyme according to the 'Millipore protocol for the PI3 Kinase Activity Assay' using 10 μM dose of the ATP *in vitro* taking Wortmannin and TG-100115 as the reference standards. Though these standards have been reported to show the IC_{50} values[33] of 0.009 and 0.083 μM respectively, we got different values (Table 1).

The moderate potency (IC_{50} value 21.4 μM) of **4** inspired us to move forward for further progress of our research. At first we tried to evaluate the result by adopting a molecular modeling technology where we have docked this compound in the receptor site of PI3Kγ isozyme by using Autodock vina[34] software (Figure 1).

Already researches have showed that amino acid residues like VAL882,[35] ASP964,[35] LYS802,[36] LYS890[37] and MT804[35] are very important for the ligand-receptor interaction. In our

Table 1. IC_{50} values of some selected Phenylpyrazoles for the inhibition of PI3 kinase gamma enzyme



Sample No.	Structure		IC_{50} value (μM)
	R^1	R^2	
Wortmannin			0.03-0.07
TG-100115			0.7-1.0
4	HO–		21.4
5	BnO–		3.7
6a	BnO–		6.8
6b	BnO–		5.7
9a	BnO–		14.0
15	BnO–		5.2
17b			1.1

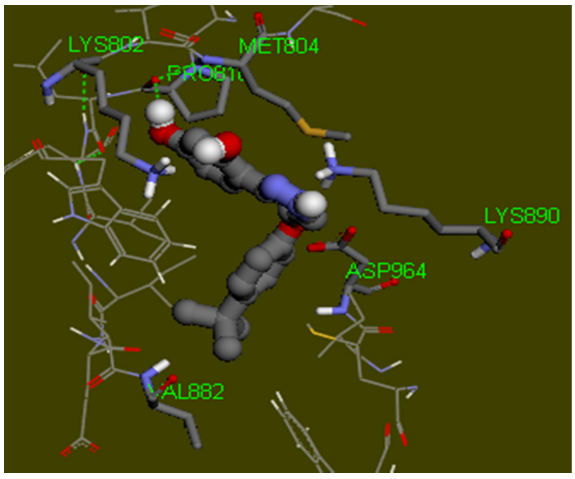


Figure 1. The lowest energy orientation of **4** as observed on docking with Autodock Vina.

docking, although the most feasible lowest energy orientation (Figure 1) of the ligand indicates no hydrogen bonding with these residues, the mode gave some encouraging points of view. The most important is the suitable fit in the pocket with necessary adaptable bending. Besides when considered

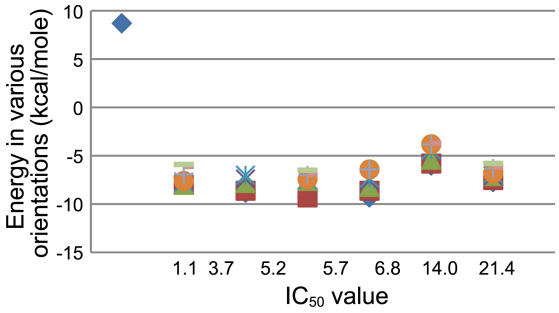


Figure 2. Energy of the ligand in various orientations in the PI3Kγ receptor site as observed on docking with Autodock Vina.

this with some other similar orientations, it was interesting to note that the pyrazole N-H groups were directed to the region with LYS890, LYS833 and ASP964 with one end of the ligand projected to VAL882 and the other projected to LYS802. Even one orientation showed the possibility of crucial hydrogen bonding with VAL882. This encouraging pocket fit and orientation in the receptor site appeared to show some intrinsic binding potential of the scaffold which was justified by the IC_{50} value of **4** when evaluated against the PI3Kγ isozyme.

However, when evaluated some derivatives of **4**, it was noted (See Supplementary content) that relatively non-polar groups (**4** *vs* **6a**) in R^2 position offer relatively higher IC_{50} values as compared to relatively polar groups. Again the phenyl substitution (**9a**) at R^2 seems to make the compound too rigid to fit in the pocket thereby reducing the activity. Similarly, compounds with a -NH- and -CH_2- linker (**5**, **15** and **6a-b**) offered higher inhibitory potency against this isozyme. However it was noted that addition of polar functionality in either of the groups has been postulated to improve the binding potential. Accordingly, we have synthesized some more compounds and compound **17b** was found to be the most potent phenyl pyrazole from our study.

However these compounds (**4**, **5**, **6a-b**, **9a**, **15** and **17b**) were then subjected to the docking again to have idea of binding and molecular orientation using Autodock Vina. While plotting (Figure 2) the IC_{50} values against the corresponding binding affinity values of the different modes, the relationship between the energy and the IC_{50} values was found to be somehow obscure. Also the most potent sample (**17b**) showed very high energy for the affinity in the binding site with just one possible orientation. Thus there remains the scope for the extensive study to find out the most probable interactions responsible for the inhibitory potential of these phenyl-pyrazoles against the PI3Kγ isozyme.

Conclusion

In this study all the compounds we tested were active to inhibit the PI3Kγ isozyme, and thereby it can be concluded that extensive studies with this scaffold may offer some hit and lead compounds in this inhibitor class.

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