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Exploring the PI3Kα and γ binding sites with 2,6-disubstituted isonicotinic derivatives

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ABSTRACT

A homology model of the p110α catalytic subunit of PI3Kα was generated from the p110γ crystal structure. Using this model, an isonicotinic scaffold was designed for chemically exploring the PI3Kα and γ binding sites. A focused library of derivatives was synthesized and tested. The morpholine acids **5a** and **5b** proved to be the most potent analogs.

Phosphatidylinositol 3-OH kinases (PI3Ks) are dual specific lipid and protein kinases that influence multiple cellular processes including cell growth, proliferation, survival and motility by generation of 3-phosphoinositides.[1] PI3Ks are arranged into three classes I, II and III depending on their structure, regulation and substrate specificity and class I is divided into subclasses IA (α, β and δ) and IB (γ) based on their mode of activation.[2] Aberrant activity of the class I enzymes is observed in various pathological states and inhibition of PI3Ks provides opportunities for treatment of inflammation, immune diseases and cardiovascular disorders.[3] The *PIK3CA* gene encoding the p110α catalytic subunit is frequently mutated in many cancers and PI3Kα is identified as an important chemotherapeutic target.[4] Although several classes of compounds that inhibit the class I PI3Ks have been reported, development of inhibitors selective for PI3Kα is still a major challenge.[5,6] The goal of this work was to build a computer model of PI3Kα based on the X-ray structure of p110γ and develop a chemical scaffold for easy generation of chemical libraries in order to explore differences between the PI3Kα and γ active sites.

The morpholinylchromone LY294002 is widely used as a non-selective PI3K inhibitor. Its X-ray structure with p110γ shows H-bonds of the chromone C=O with Lys833, the morpholine oxygen with Val882 and phenyl at the entrance of the binding pocket facing the solvent.[7] The rigid structure of LY294002 (two rotatable bonds) permits defining its binding mode but does not provide enough conformational freedom for exploration of subtle differences in the active sites of the α and γ isoforms of PI3K.[5] Analysis of the literature on PI3K inhibitors led us to conclude that the six-membered ring of γ-pyrone acts as a pharmacological spacer.[8–12] Accordingly, we chose the 2,6-disubstituted isonicotinic acid as a scaffold that would allow more coverage of conformational space than LY294002 and less than its structural analog TGX126[9] (Fig. 1A). In addition, the COOH in our scaffold extends one carbon further into the phosphate binding area of ATP than the carbonyls of LY294002 or TGX-126. Thus, derivatization of 2-chloro-6-methyl isonicotinic acid provides easy access to three potential libraries (Fig. 1B). In this study 6-substitution was limited to Ar = 4-F–2-MePh and X = O, NH based on literature data.[9]

To evaluate the binding of these isonicotinic acid derivatives we built (Modeller 6v2)[13] and refined (YASARA dynamics—AMBER99 force field)[14] a homology model[15] of p110α based on its sequence and the high resolution structure of p110γ–inhibitor complex (1e7u) and used it for docking with CAChe[16] (flexible ligand—flexible protein). The docking procedure was validated by modeling LY294002 in the p110γ binding site which matched the X-ray structure (1e7v) with RMSD 0.095. Docking of LY294002 and isonicotinic derivative **5b** into our p110α model revealed an orientation similar to p110γ depicting H-bonds with Lys802 (833, p110γ) and Val851 (882, p110γ); Figure 1C–E.

Although the pyridine ring provided a means for sequential introduction of nucleophiles into 2- and 6-positions (benzylic bromide vs 2-Cl pyridine reactivity), it also created problems for synthesis of key intermediates **2** and **4** (Fig. 2). The issue of polybromination during synthesis of monobromide **2** was solved by selective reduction of the mixture with diethyl phosphite improving its yields from 40% to 75%.[17] Conversion of **2** into **3** proceeded in high yields under standard conditions (85–90%). Nucleophilic displacement of Cl in **3** proved to be sensitive to the nature of the amine and a significant number of amines required excess amounts (>5 equiv) and microwave heating. Under these conditions, the amines reacted with the COOEt faster resulting in formation of complex mixtures of mono and bis-substituted derivatives (e.g., **12** and **13**) that were directly transesterified to esters (**4a,b**) or hydrolyzed to the respective carboxylic acids (**5a,b**, g–i). Mixed anhydrides provided selective N-acylation of N,O-nucleophiles without need for protection of the COOH (**6** and **7**). The Suzuki reaction was used for synthesis of 2-aryl derivatives

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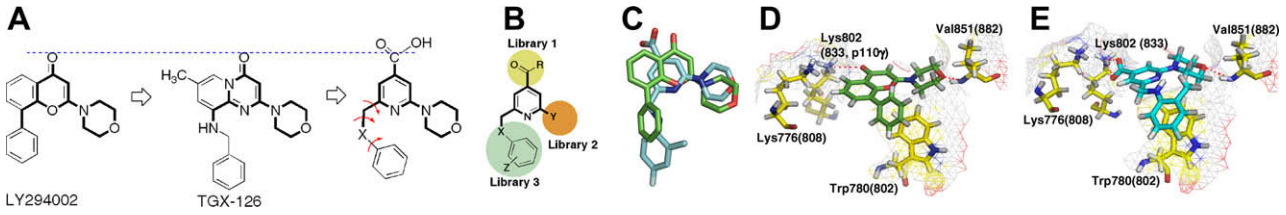


Figure 1. (A) 2,6-Disubstituted Isonicotnic acid scaffold. (B) Opportunities for modification. (C) The p110α docked pose of isonicotinic derivative **5b** (cyan) shows a small anti-clockwise shift for the H-bonding groups and the Ph group extending further than LY294002 (green). Docking of LY294002 (D) and **5b** (E) in the p110α homology model viewed from the same angle.

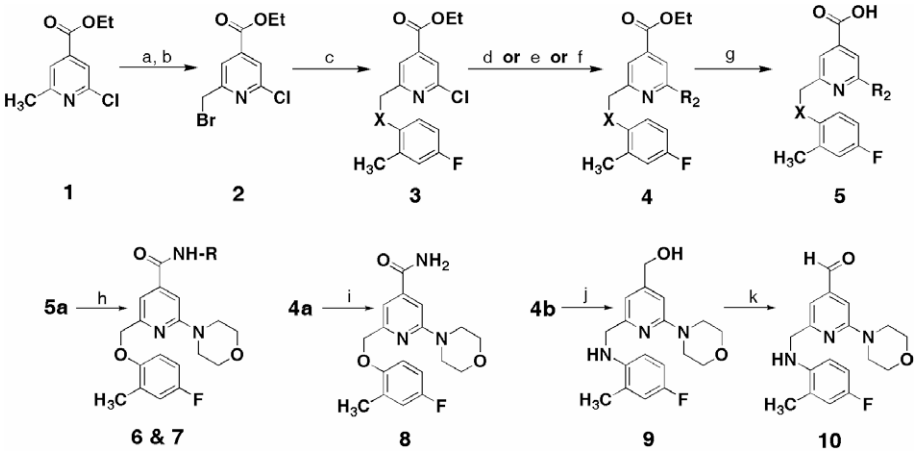


Figure 2. Synthetic scheme. Reagents and conditions: (a) NBS, AIBN, CCl_4, reflux; (b) Diethyl phosphite, DIEA, THF, 0–4 °C; (c) 4-F–2-MePhOH [or] 4-F–2-MePhNH$_2$, K_2CO_3, DMF, rt; (d) NHR^{3}R^4, MW, 200–250 °C then H_2SO_4/EtOH (**4a–b**) or (g) (**5a–b**, **g–i**); (e) HNR^{5}R^6, 1,4-dioxane, 80 °C then (g) (**5c–f**); (f) Ar-B(OH)$_2$, Pd(dppf), 2 M Na_2CO_3, 1,4-dioxane, 80 °C then (g) (**5j–n**); (g) KOH, EtOH/H_2O; (h) ethylchloroformate, NMM, Et_2O, H_2N–R, rt; (i) NH_3/MeOH, 60 °C; (j) $LiAlH_4$/Et_2O; (k) Dess–Martin, DCM, rt.

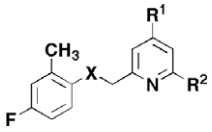
(**5j–n**). All tested compounds were >99% pure except for **10** which was 90% pure.[18]

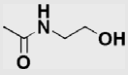
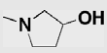
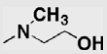
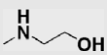
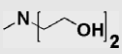
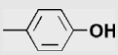
As seen from Table 1 and Figure 3 the parent carboxylic acids **5a** and **5b** inhibited PI3Kα and γ in the low micromolar range with sixfold selectivity for PI3Kα. Compound **5a** containing the H-bond acceptor phenyl ether oxygen is equipotent to **5b** with the H-bond donor/acceptor aniline nitrogen (Table 1). Conversion of COOH to non-ionizable derivatives **6**, **7**, **8**, **9** and **10** with H-bonding capability allowed further exploration of the ATP-phosphate binding region. All compounds from this series proved to be less potent than the parent acids **5a**,**b**. The least drop in potency (fourfold) was observed for alcohol **9** suggesting that the acids **5a**,**b** interact with the active site by H-bonding and not by salt bridge formation. The esters **4a**,**b** showed less inhibition than the corresponding carboxylic acids **5a**,**b** probably due to their limited solubility (Fig. 3).

The active sites within 4 Å of ATP or LY294002 ligands for all four isoforms of PI3Ks have very similar sequence with a difference of 7 amino acids between the α and γ isoforms and 5 or 3 between β and δ relative to α.[10] The active sites of PI3Kα and γ show the largest difference not only in sequence but also in polarity and H-bonding properties of amino acids next to the H-bonded oxygen of morpholine—non-polar Ala885 in p110γ corresponds to the polar H-bonding Ser854 in p110α. The same difference exists between Ala805 and Ser774. Since it was logical to assume that addition or removal of H-bonding fragments to the morpholine template might impact interaction with either PI3Kα or γ isoforms, we synthesized a set of flexible (**5g**–**i**), semi-rigid (**5c**–**f**) and rigid (**5j**–**n**) analogs of morpholine in **5b**. Replacement of the morpholine oxygen with NH (**5c**) or CH_2 (**5d**), its complete removal (pyrrolidine derivative **5e**) or removal with addition of OH group (3-OH pyrrolidine **5f**) led to a significant drop in potency (Table 1 and Fig. 3) indicating lack of direct interaction with Ser854. The observed decrease in potency (similar to that for LY294002)[8] for protonated **5c** and neutral non-basic **5d** was most likely caused by loss of H-bonding with the backbone NH. While conversion of the hydrophilic H-bond acceptor oxygen in morpholine (**5b**) into hydrophilic H-bond donor $^+NH_2$ in **5c** did not affect selectivity, the replacement of $^+NH_2$ with the isosteric non-polar CH_2 (**5d**) considerably reduced potency at PI3Kα but not at PI3Kγ (Fig. 3). Compounds **5d**,**e** showed similar potency and selectivity (Fig. 3), that is, the 4-CH_2 in piperidine is not essential. At the same time, addition of 3-OH (**5f**) to the pyrrolidine **5e** which enables polar interactions does not affect the activity at PI3Kγ but increases activity at PI3Kα (Fig. 3). This biological profile agrees with the above difference in properties of the Ser/Ala pairs and potential involvement of the hydrophobic Ile881 in p110γ versus corresponding Val850 in p110α located in the morpholine binding pocket.

Compounds with 'opened' morpholine ring (monoethanolamines **5g**,**h** and bis-derivative **12**) had potency similar to **5c**–**f** while the much more polar and flexible diethanolamine **5i** was practically inactive (Fig. 3). The use of rigid flat phenol rings allowed for further exploration of the H-bonding sites in the morpholine binding pocket. Replacement of morpholine in **5b** by 4-OHPh and 3-OHPh (**5l**,**m**) led to only ca. twofold drop in potency compared to **5b**, that is, the presence of the morpholine ring is not vital (Fig. 3) and it can be replaced by an aromatic ring with a polar substituent in 3 or 4 positions. Pyridine mimics properties of both phenol (π-bonding aromatic ring with polar region) and morpholine (H-bond acceptor weakly basic nitrogen) and permits additional examination (**5j**,**k**) of the polar portion of the active site that interacts indiscriminately with the 3- or 4-OH in **5l**,**m**. In contrast to these equipotent 3- and 4-OH derivatives **5l**,**m**, the 4-pyridine **5j** is several times more potent than 3-pyridine **5k** (Fig. 3), that is, the presence of nitrogen in pyridine in the same position as oxygen

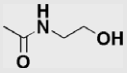
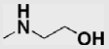
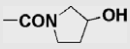
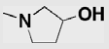
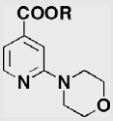
Table 1
Properties and biological activity of the synthesized compounds



No.	R^1	R^2	X	Yield (%)	Mp (°C)	IC_{50} (μM)	
						PI3Kα	PI3Kγ
6			O	50	179–181	⩾50	⩾50
7			O	24	209–211	⩾10	~10
8			O	30	235–237	>10	[c]
4a			O	82	117–119	[c]	[c]
5a			O	53[a]	168–171	7 (±1)	23 (±5)
4b			NH	78	78–80	[c]	[c]
9			NH	38	139–141	20 (±5)	⩾100
10			NH	30	Decomp.	~ 100	~100
5b			NH	44[a]	86–88	5 (±1)	34 (±3)
5c			NH	87[a]	[b]	>100	>100
5d			NH	53[a]	153–155	[c]	>100
5e			NH	43[a]	93–95	[c]	>100
5f			NH	48[a]	[b]	>100	>100
5g			NH	35[a]	[b]	>100	>100
5h			NH	33[a]	[b]	~100	>100
5i			NH	20[a]	[b]	>100	>100
5j			NH	68[a]	186–188	29 (±7)	42 (±3)
5k			NH	56[a]	252–254	>100	>100
5l			NH	55[a]	122–124	~100	~100

(*continued on next page*)

Table 1 (*continued*)

No.	R^1	R^2	X	Yield (%)	Mp (°C)	IC_{50} (μM)	
						PI3Kα	PI3Kγ
5m			NH	47[a]	156–158	~100	42 (±6)
5n			NH	6[a]	227–229	>100	>100
11		Cl	NH	70	191–193	>100	>100
12			NH	61	[b]	>100	>100
13			NH	10	[b]	>100	>100
14a		R = H		26	187–189	36 (±8)	>100
14b		R = Et		21	53–55	51 (±32)	>100
15	LY294002					0.2 (±0.03)	1.2 (±0.3)

IC_{50} values are the mean of three experiments ± SEM.[19]

[a] Isolated yield over two steps from **3**.

[b] TFA salts were very hygroscopic.

[c] Less than 10% inhibition.

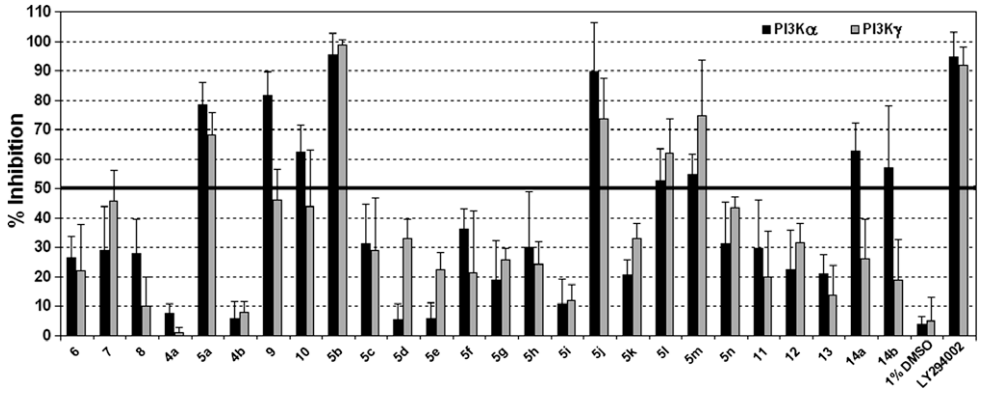


Figure 3. Percent inhibition of PI3Kα and γ by synthesized compounds. Average of three experiments + SD (**4a**,**b** is average of two).[19] All compounds tested at 100 μM; **6** at 50 μM and **7**, **8**, **4a** and **4b** at 10 μM due to limited solubility.

in morpholine is favorable. Binding of pyridine (as a part of the condensed imidazo-quinoline, NVP-BEZ235) in morpholine pocket of p110α has been reported recently.[15c] Substitution of morpholine in **5b** with 4-pyridine (**5j**) does not affect interaction with the PI3Kγ but results in a sixfold drop in potency on PI3Kα (Table 1).

Thus, in accordance with our modeling the above set of 2,6-disubstituted isonicotinic derivatives with limited conformational flexibility demonstrate the importance of morpholine in the 2-position and a polar substituent at the 4-position of the pyridine spacer with the morpholino acids **5a** and **b** being the most potent (Table 1). PI3Kα and γ are not sensitive to the nature of $-CH_2X-$ in the 6-position and morpholine can be replaced with a polar aromatic ring with some loss of potency and selectivity (**5j**, **l** and **m**). The suggested structural modifications led to some discrimination between PI3Kα and γ but less than what was expected based on the difference in their polarity/H-bonding, most likely due to the inherent flexibility of their active sites (exemplified by the latest X-ray data on two structurally different ligands complexed with p110γ[20] Although this paper describes only the PI3Kα and γ isoforms and a limited number of 6-substituents, this scaffold and methodology can be easily applied for mapping the active sites of other PI3Ks.

Acknowledgment

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15. (a) The model proved to be in good agreement with the first X-ray structure of PI3Kα [2rd0, Ref. (b)] published later in December 2007; RMSD 0.942 for 5 Å binding site.; (b) Huang, C.; Mandelker, D.; Schmidt-Kittler, O.; Samuels, Y.; Velculescu, V. E.; Kinzler, K. W.; Vogelstein, B.; Gabelli, S. B.; Amzel, L. M. *Science* **2007**, *318*, 1744; During review of the manuscript one of the referees indicated that a model of p110α kinase domain has been previously reported in: (c) Maira, S.-M.; Stauffer, F.; Brueggen, J.; Furet, P.; Schnell, C.; Fritsch, C.; Brachmann, S.; Chene, P.; Pover, A. D.; Schoemaker, K.; Fabbro, D.; Gabriel, D.; Simonen, M.; Murphy, L.; Finan, P.; Sellers, W.; Garcıa-Echeverrıa, C. *Mol. Cancer Ther.* **2008**, *7*, 1851.
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18. The synthesized compounds were purified by column chromatography or preparative HPLC and/or recrystallized from following solvents: **6**, Hex/EtOAc (1:1); **7**/**8**, acetone; **9**, Hex/EtOAc (3:1); **5n**, triturated with ether; rest of compounds, MeOH/H_2O. Structures were confirmed by NMR and HRMS.
19. The percent inhibition and IC_{50} curves were determined using the Promega Luminescent Kinase Glo™ assay. The IC_{50} values were calculated using GraphPad PRISM V4.0—sigmoidal dose–response curve.
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