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Synthesis and biological evaluation of pyrido[3′,2′:4,5]furo[3,2-*d*]pyrimidine derivatives as novel PI3 kinase p110α inhibitors

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Abstract—4-Morpholin-4-ylpyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidine **2a** was discovered in our chemical library as a novel p110α inhibitor with an IC_{50} of 1.4 μM. By structural modification of **2a**, the 2-aryl-4-morpholinopyrido[3′,2′:4,5]furo[3,2-*d*]pyrimidine derivative **10e** was discovered as a p110α inhibitor with approximately 400-fold greater potency than **2a**. Evaluation of isoform selectivity showed that **10e** is a potent inhibitor of p110β. Furthermore, **10e** showed anti-proliferative activity in various cell lines, including multi-drug resistant MCF7/ADR-res cells, and was effective against HeLa human cervical tumor xenografts in nude mice.

Phosphoinositide 3-kinase (PI3K) is an enzyme that catalyzes phosphorylation of the 3-hydroxyl position of phosphatidylinositides (PIs) and plays a crucial role in mitogenic signal transduction.[1–4] PI3K is negatively regulated by the lipid-phosphatase PTEN, which is one of the most commonly mutated proteins in human cancers,[5–7] and therefore PI3K inhibitors are considered to be potential anti-cancer agents (Fig. 1).

Among the various subtypes of PI3K identified to date,[8–11] class Ia PI3Ks (p110α, p110β, and p110δ) are known to play critical roles in cell growth and survival.[12] The *PIK3CA* gene that encodes p110α is amplified and overexpressed in ovarian and other cancers,[13,14] and is mutated in a variety of cancers.[15–18] Therefore, inhibitors of class Ia PI3Ks, and particularly PI3K p110α, are likely to be useful in cancer treatment.

Several non-selective PI3K inhibitors have been identified, including wortmannin and LY294002,[19–21] but few isoform-specific PI3K inhibitors are available.[22,23] We have reported that the thieno[3,2-*d*]pyrimidine derivative **1** is a highly potent and selective p110α inhibitor, but it is not effective in vivo because of a poor

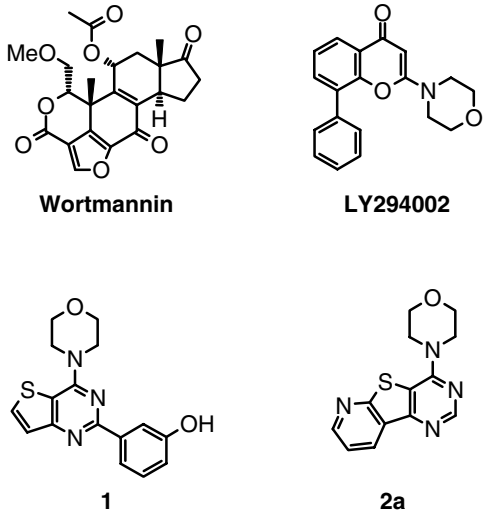

Figure 1. Structures of PI3K inhibitors.

Keywords: PI3 kinase; p110α; Inhibitor; Anti-cancer agent.
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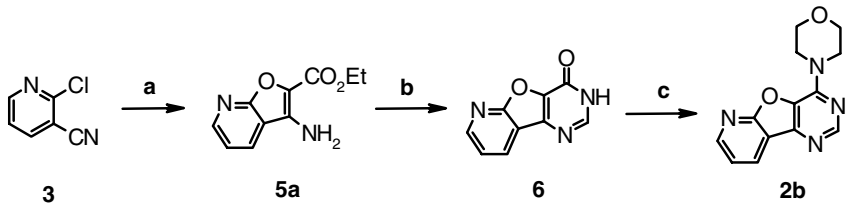
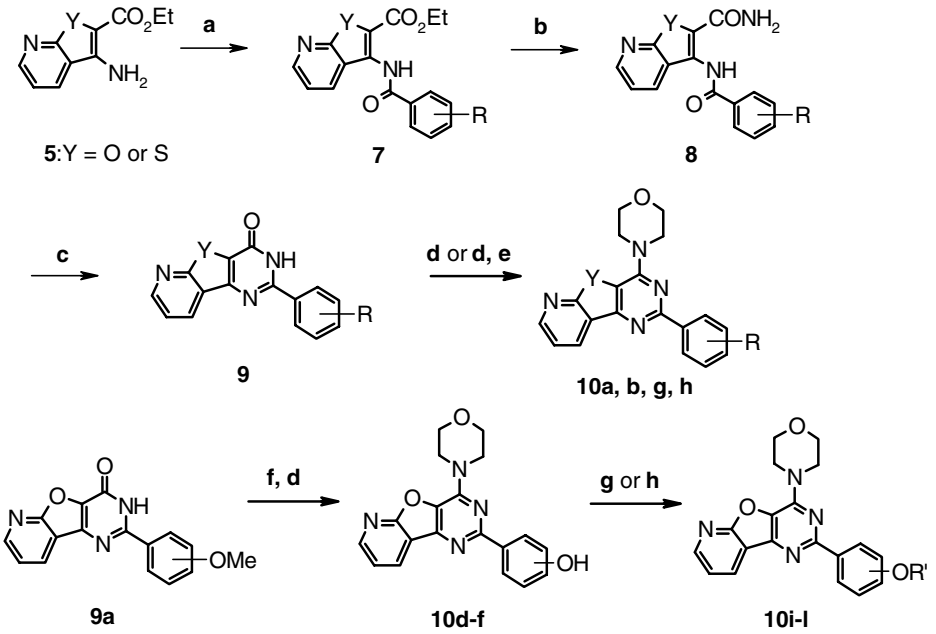
pharmacokinetic profile due to a short half-life of less than 10 min.[24,25] In screening our chemical library, we identified **2a** as another lead compound with structural similarity to **1**. Through structural modification of **2a**, the novel pyrido[3′,2′:4,5]furo[3,2-*d*]pyrimidine **10e** was identified as a potent p110α inhibitor that is also effective in HeLa xenografts in mice. Although biological evaluation of **10e** has been recently reported after our publication of the patent,[26–28] the generation of **10e** and SAR of its derivatives has not yet been described. Herein, we report the synthesis, SAR, and biological data for a series of pyrido[3′,2′:4,5]furo[3,2-*d*]pyrimidine derivatives as novel PI3K p110α inhibitors.

The lead compound **2a** was synthesized according to the literature[29] and its furan analogue, **2b**, was prepared as shown in Scheme 1. Treatment of 2-chloronicotinonitrile **3** with **4** in the presence of DBU afforded a bicyclic acid ester **5a**, which was treated with formamide to give a pyrimidone **6**. Chlorination of **6** with phosphorus oxychloride followed by substitution with morpholine gave the desired product **2b**. The synthesis of compounds **10**, each of which has an aryl group on the pyrimidine ring, is illustrated in Scheme 2. Synthetic methods for the derivatives were generally analogous to those previously described.[24] Acylation of **5** with the appropriate aryl acid chloride, ester hydrolysis, chlorination with $SOCl_2$, and subsequent treatment with NH_4OH afforded the series of amides **8**. Cyclization of **8** under previously reported conditions using MeOH as a solvent[24] provided **9** in low yield due to cleavage of the amide linkage of **8**, while reaction in 2-PrOH instead of MeOH gave cyclized **9** in better yield. Chlorination of **9** with phosphorus oxychloride and substitution with morpholine gave the desired compounds **10a**–**b** and **10h**. The amino derivative **10g** was synthesized from the corresponding nitro derivative via a reduction with iron. Demethylation of **9a** with HBr, acetylation, chlorination, and treatment with morpholine afforded compounds **10d**–**f**. Alkylation of the phenol derivative **10e** gave **10i**–**k**. The acetic acid derivative **10l** was prepared from the corresponding ester derivative by hydrolysis under basic conditions.

Inhibition of p110α was determined by a scintillation proximity assay (SPA), as previously reported,[24,25] which gave similar results to those obtained by a conventional TLC method.[21] The lead compound **2a** had an IC_{50} of 1.4 μM for p110α in the SPA assay. In this assay, LY294002 inhibited p110α with an IC_{50} of 0.63 μM, showing approximately 2-fold greater potency



Scheme 1. Reagents and conditions: (a) $HOCH_2CO_2Et$ **4**, DBU, EtOH, reflux; (b) formamide, reflux; (c) i) $POCl_3$, reflux; ii) morpholine, toluene, reflux.



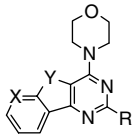
Scheme 2. Reagents and conditions: (a) ArCOCl, Et_3N or pyridine, THF or $CHCl_3$; (b) i) 1 N NaOH, EtOH; ii) $SOCl_2$, reflux; iii) NH_4OH, THF; (c) 2 N NaOH, MeOH or 2-PrOH, reflux; (d) i) $POCl_3$, Δ; ii) morpholine, toluene or neat, reflux; (e) Fe, NH_4Cl, aqueous EtOH, reflux; (f) i) HBr, AcOH, reflux; ii) Ac_2O, AcONa, reflux; (g) R′-Cl, K_2CO_3, DMF; (h) i) $BrCH_2CO_2Et$, K_2CO_3, DMF; ii) 1 N NaOH, MeOH, THF.

than **2a**. Since the morpholino group is important for p110α inhibitory activity in derivatives of **1**,[24] the morpholino group in **2a** was retained and other groups in **2a** were modified with the goal of obtaining p110α inhibitors with greater potency.

First, modifications on the tricyclic ring of **2a** were investigated (Table 1). Replacement of sulfur with oxygen at Y in **2a** resulted in a 3-fold increase in p110α inhibitory activity (**2b**: IC_{50} = 0.56 μM), whereas the pyrazole derivative **2c** (Y = NH) was inactive against p110α. Introduction of a phenyl group at R of the thiophene derivative **2a** retained similar p110α inhibitory activity (**10a**: IC_{50} = 1.7 μM), whereas the furan derivative **10b** with a phenyl ring at C2 was about 10-fold more potent than **2a** (**10b**: IC_{50} = 0.16 μM). The benzothienopyrimidine derivative **2d** (X = C) showed comparable potency to **2a**; however, introduction of a benzene ring at C2 (**10c**) eliminated the p110α inhibitory activity.

The 3-hydroxy group on the benzene ring of **1** was previously found to be important for p110α inhibitor activity.[24] Considering the structural similarity between **1** and **10b**, introduction of a hydroxy group on the benzene ring of **10b** was investigated (Table 2). The 2-hydroxy derivative **10d** showed about a 3-fold decrease in potency compared with **10b**. As expected, the 3-hydroxy derivative **10e** and the 4-hydroxy derivative **10f** were approximately 45- and 6-fold more potent p110α inhibitors compared with the unsubstituted **10b** (**10e**, **10f**: IC_{50} = 3.6, 26 nM, respectively). Since the hydroxy group at the 3-position on the benzene ring of **10e** was especially effective in producing p110α inhibitory activity, other hydrophilic substituents at C3 on the benzene ring were introduced, based on the possibility of a hydrophilic binding pocket in the binding site. The 3-amino derivative **10g** showed almost the same p110α activity compared with **10b**. Introduction of a 3-methoxy group at C3 on the benzene ring of **10b** retained the same p110α inhibitory activity, but most compounds with ether substituents at this position showed increased activity. Among these compounds, the morpholinoethoxy derivative **10j** and the carboxylic acid derivative **10l** were potent p110α inhibitors, with IC_{50} values of 16 and 24 nM, respectively. Preliminary studies indicated that the inhibitors are ATP competitive but further work is required to investigate the inhibition kinetics in more detail.

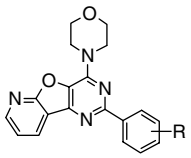
Table 1. Inhibition of p110α by tricyclic derivatives



Compound	X	Y	R	IC_{50}[c] (μM)
				p110α
LY294002[a]				0.63
2a[a]	N	S	H	1.4
2b[a]	N	O	H	0.56
2c[a]	N	NH	H	>30
10a[a]	N	S	Ph	1.7
10b[a]	N	O	Ph	0.16
2d[b]	C	S	H	2.4
10c[a]	C	S	Ph	>30

[a] Free base.
[b] HCl salt.
[c] IC_{50} values represent means of at least two separate determinations with typical variations of less than ±20%.

Table 2. Inhibition of p110α by pyridofuropyrimidine derivatives



Compound	R	IC_{50}[d] (μM)	
		p110α	A375
10b[a]	H	0.16	NT
10d[a]	2-OH	0.40	>30
10e[b]	3-OH	0.0036	0.33
10f[b]	4-OH	0.026	>30
10g[c]	3-NH_2	0.14	3.87
10h[b]	3-OMe	0.10	15.78
10i[c]	3-(Piperidinoethoxy)	0.17	9.67
10j[c]	3-(Morpholinoethoxy)	0.016	12.32
10k[c]	3-(Thiomorpholinoethoxy)	0.079	11.76
10l[b]	3-OCH_2CO_2H	0.024	>30

NT, not tested.
[a] Free base.
[b] HCl salt.
[c] 2HCl salt.
[d] IC_{50} values represent means of at least two separate determinations with typical variations of less than ±20% for the p110α enzyme and A375 cell proliferation assays.

The inhibitory activities of these derivatives were evaluated against serum-induced proliferation of A375 human melanoma cells. The 3-hydroxy derivative **10e** showed a potent anti-proliferative effect in vitro with an IC_{50} of 0.33 μM, whereas compounds **10f**, **10j**, **10k**, and **10l**, which had relatively potent p110α inhibitory activities in the enzyme assay, did not show cellular activity; it can be speculated that this may be due to poor permeability through the cell membrane.

Further evaluation of compound **10e** was performed, since this was the most potent p110α inhibitor in the series and was effective as an anti-proliferative agent in vitro. To examine selectivity for p110α, the inhibitory activity of **10e** was evaluated against other PI3K isoforms. As shown in Table 3, **10e** was also a potent inhibitor of p110β (class Ia), which is also a potential target for cancer treatment.[12] Although **10e** showed excellent selectivity for p110α over p110γ (class Ib), the selectivity for p110α over PI3K C2β (class II) was not very high. It is of interest that the thienopyrimidine derivative **1**, which is structurally similar to **10e**, is a more isoform-selective p110α inhibitor than **10e**. Concerning selectivity against protein kinases, **10e** was inactive at 100 μM against protein kinases such as KDR, PKA, PKCα, and cyclin E/CDK_2. Recent publications reported that **10e** also inhibited other kinases such as DNA-PK or mTOR.[27,28] Although we have not yet determined the

Table 3. Selectivity of **10e** against PI3Ks and protein kinases

Compound	IC_{50}[c] (μM)							
	p110α	p110β	p110γ	PI3K C2β	KDR	PKA	PKCα	Cyclin E/CDK_2
LY294002[a]	0.63	0.34	1.6	2.1	NT	NT	NT	NT
10e[b]	0.0036	0.0030	0.25	0.010	>100	>100	>100	>100
1[b]	0.0025	0.016	0.66	0.22	91	3.4	466	28

NT, not tested.
[a] Free base.
[b] HCl salt.
[c] IC_{50} values represent means of at least two separate determinations with typical variations of less than ±20%.

Table 4. Inhibition of human tumor cell proliferation in vitro by **10e**

Compound	IC_{50}[b] (μM)				
	A375 (Melanoma)	HeLa (Cervix)	A549 (Lung)	MCF7 (Breast)	MCF7 ADR-res (Breast)
10e[a]	0.33	0.54	0.54	0.18	0.13

[a] HCl salt.
[b] IC_{50} values represent means of at least two separate determinations with typical variations of less than ±20%.

inhibitory activity of **10e** and its derivatives against mTOR and DNA-PK, the inhibitory potencies of the compounds in A375 melanoma cell proliferation assay were well correlated with those in a PKB phosphorylation assay (data not shown). This observation suggested that the tumor suppressive actions of **10e** and its derivatives may depend mainly on PI3K inhibition. The involvement of mTOR and DNA-PK inhibition should be further investigated.

Evaluation of the effects of **10e** in several human tumor cell lines showed that **10e** exerted anti-proliferative activity at submicromolar concentrations (Table 4). Notably, **10e** was active in MCF7 ADR-res human breast cancer cells, which display a high level of resistance to doxorubicin and several other anti-cancer agents due to overexpression of P-glycoprotein.[30,31] Finally, the activity of **10e** was evaluated in vivo using a HeLa human cervical tumor xenograft model in mice. The half-life of **10e**, as measured by HPLC/MS/MS, was 4.1 h, when administered intraperitoneally. **10e** significantly suppressed tumor growth by 45% in this model when dosed intraperitoneally at 100 mg/kg daily for 2 weeks, and did not cause any associated weight loss (Fig. 2).

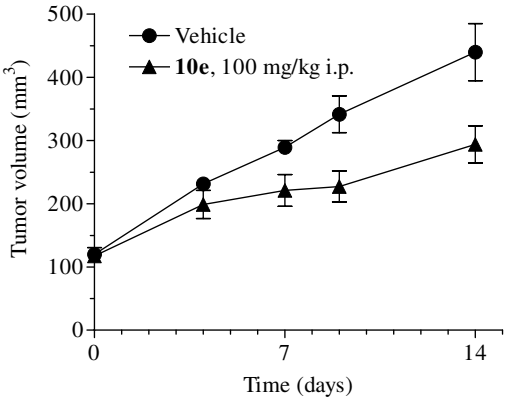


Figure 2. Effect of compound **10e** on the growth of HeLa human cervical tumor xenografts. Compound **10e** (100 mg/kg) suspended in 20% hydroxypropyl-β-cyclodextrin/saline was administered intraperitoneally daily for 2 weeks to nude mice carrying a subcutaneous HeLa xenograft. Error bars show ±SE.

In summary, structural modification of 4-morpholinopyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidine **2a** led to the discovery of **10e** as a p110α inhibitor of approximately 400-fold greater potency than **2a**. Compound **10e** is also a potent inhibitor of p110β and showed selectivity over several tested protein kinases. Furthermore, **10e** exhibited anti-tumor activity against the MCF7 ADR-res tumor cell line, which shows multi-drug resistance to anti-cancer drugs, and was effective in a HeLa human cervical cancer xenograft model without causing weight loss.

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