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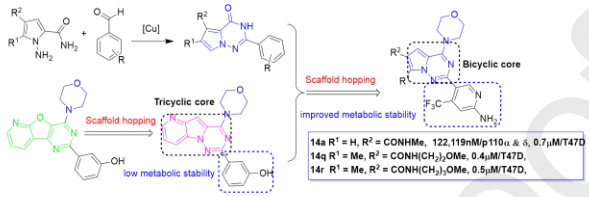

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Hao-Yue Xiang[a,b] , Yan-Hong Chen[b], Yi Wang[b], Xi Zhang[b], Jian Ding[b], Ling-Hua Meng[b*] and Chun-Hao Yang[b*]

[a]*College of Chemistry and Chemical Engineering, Central South University, Changsha, Hunan, 410083, P.R. China*
[b]*State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai, 201203, P.R. China*
∗ Corresponding author. Tel/Fax: +86-21-50806770; e-mail: lhmeng@simm.ac.cn (L. Meng); chyang@simm.ac.cn (C. Yang).

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ABSTRACT

A series of 6-aminocarbonyl pyrrolo[2,1-*f*][1,2,4]triazine derivatives were designed by scaffold hopping strategy. The IC_{50} values of compound **14a** against PI3Ks were measured, showing selective activity against p110α and p110δ with IC_{50}s of 122 nM and 119 nM respectively. All the synthesized compounds were evaluated for their antiproliferative activity against human cancer cells by SRB assay. Compounds **14a**, **14p** and **14q** exhibited potent antiproliferative activity against five types of human cancer cells and the PK property of **14q** was also investigated here.

Cancer, the second common cause of death following cardiovascular diseases, is one of the most challenging global health problems.[1-3] As a result, considerable efforts have been devoted to develop efficient drugs for cancer therapy. For a long time, cytotoxic agents have been used in clinic as the major chemotherapy for most cancers, but limited by a narrow therapeutic index, remarkable toxicities and lack of specificity.[4, 5] Therefore, there is a growing imperative need for further identification of new treatment approaches to cancer. The past decade has witnessed tremendous advance in the molecularly targeted therapies aiming at special targets, with the development of new compounds that interrupt specific molecular abnormalities driving cancer initiation and progression.[6]

Phosphatidylinositol 3-kinases (PI3Ks) are lipid kinases and subdivided into three classes (class I, II, and III) based on their sequence, homology and structural differences.[7] Among of them, Class I PI3Ks comprising four isoforms (p110α, p110β, p110δ, and p110γ), are extensively studied and they can catalyze phosphorylation of the 3'-hydroxyl group of phosphatidylinositol 4,5-biphosphate (PIP2) to generate phosphatidylinositol 3,4,5-triphosphate (PIP3), a potent secondary messenger. Abundant evidences demonstrate that PI3K pathway is hyper-activated in numerous cancer types.[8, 9] Hence, significant efforts have been made to develop inhibitors targeting PI3K signaling pathway for cancer therapies.[10-13] In 1994, the Lilly Research Laboratory developed the first synthetic ATP-competitive pan-PI3K LY294002.[14] Afterwards, a considerable number of PI3K inhibitors based on LY294002 were designed and developed, such as PI-103,[15] BKM120,[16] ZSTK474,[17] GDC-0941,[18] ETP-46992 [19](Fig. 1). All these inhibitors share the aryl-morpholine pharmacophore, which served as a valuable chemical tool for development of many additional PI3K inhibitors.

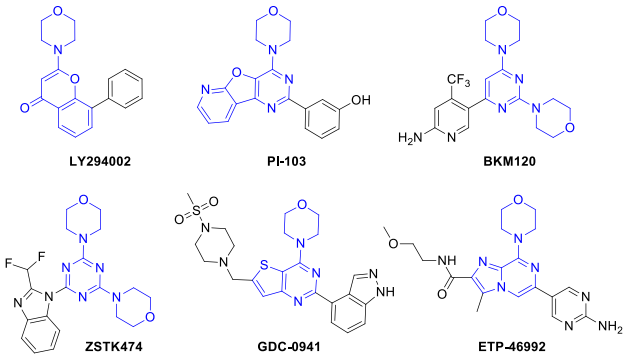


Figure 1. The structure of some aryl-morpholine type PI3K inhibitors

The scaffold hopping is a common and useful method in medicinal chemistry to find novel proprietary hits. Using this strategy, our group previously designed and synthesized series of novel PI3K inhibitors based on PI-103, an aryl-morpholine type PI3K inhibitor. Among the PI3K inhibitors reported by our group, 4-(2-(3-hydroxylphenyl)pyrido[2',3':4,5]pyrrolo[2,1-*f*][1,2,4]triazin-4-yl)morpholine **2** was found to have the IC_{50}

against PI3Kα (33.6 nM) comparable to that of PI-103 (16.8 nM) and showed anti-proliferative activity at micromolar concentration inseveral cancer cell lines (Fig. 2).[20] However, it suffered from unfavorable properties like rapid metabolism and poor solubility. To circumvent these problems, we tried to replace the labile 3-hydrophenyl segment with 2-amine-4-trifluoromethylpyrdinyl segment from BKM120. Meanwhile, a parallel scaffold hoping strategy was adopted to replace the tricyclic core of **2** with bicyclic core in order to improve the solubility of these compounds. Recently, a straightforward methodology to 2-substituted pyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-ones was developed in our lab.[21] We thus envisioned applying our method to the design of novel pyrrolo[2,1-*f*][1,2,4]triazine bicyclic PI3K inhibitors library (Fig. 2). In this letter, we focused on investigating the influence of the substituents at the pyrrole ring on the activity of the newly designed compounds to inhibit PI3K kinase and cell proliferation.

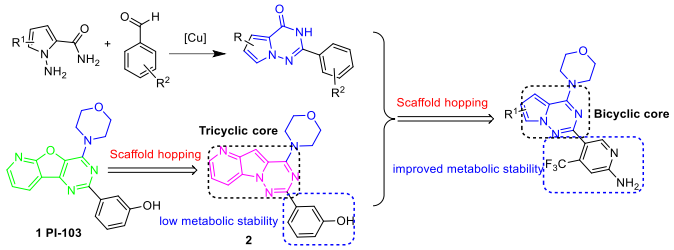
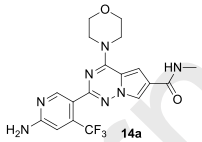


Figure 2. Optimizing strategy.

Initially, a probe compound **14a** was synthesized to evaluate the potential of this novel PI3K inhibitor scaffold. As we expected, **14a** showed potent activity against p110α and p110δ with IC_{50}s of 122 nM and 119 nM respectively, and higher concentration was required to inhihit p110β and p110γ by 50%. Encouragingly, different 6-aminocarbonyl pyrrolo[2,1-*f*][1,2,4]triazine derivatives were synthesized and evaluated in cellular assays.

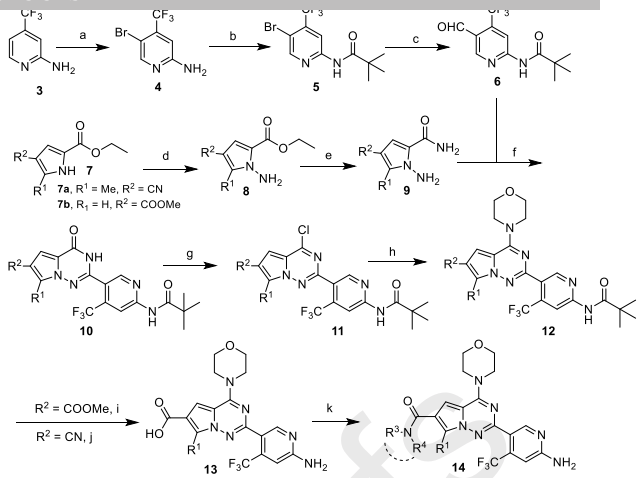
Table 1. Inhibition of the kinase activity of p110α, p110δ, p110β, p110γ by **14a**.[a]



Cpd	IC_{50} [nM]			
	p110α	p110δ	p110β	p110γ
14a	122	119	1293	663
GDC0941	35	9	324	746

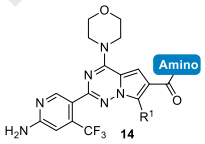
[a] Using PI3-Kinase (human) HTRF™ Assay kit (Cat. 33-017, Millipore)

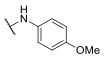
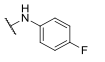
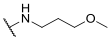
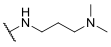
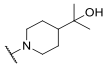
A general synthetic route to prepare the designed compounds **14** was illustrated in Scheme 1.The commercially available **3** was converted into **6** with the conventional steps. Then cyanation of **4** generated **5** with potassium hexacyanoferrate (II) using copper catalysis. Afterwards, the desired aldehyde **6** reacted with **9** that prepared according to the literature[21] to yield the pyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-ones **10**. Chlorination of **10**, followed by morpholine introduction gave **12**. Then deprotection and hydrolysis of **12** in one pot provided the key intermediates **13**. Finally, the carboxylic acids **13** were treated with various amines to give the desired analogs **14**.



Scheme 1. Reagents and conditions: (a) NBS, DCM, rt, 83%; (b) pivaloyl chloride, Et_3N, DCM, ˚C ~ rt, 89%; (c) n-BuLi, DMF, THF, -78 ˚C; (d) NH_4Cl, K_2CO_3, NH_4OH, aliguate-336, NaClO, MTBE, 0 ˚C ~ rt; (e) NH_3 in MeOH, sealed tube, 80 ˚C; (f) $CuCl_2·2H_2O$, DMSO, 100 ˚C; (g) $POCl_3$, DMAP, 110 ˚C; (h) morpholine, THF, reflux; (i) 1M KOH, EtOH, reflux; (j) H_2SO_4, AcOH, H_2O, 100 ˚C; (k) amines, EDCI, HOBT, DIPEA, DMF, rt.

Table 2. Antiproliferative activities of compounds **14** against human rhabdomyosarcoma Rh30 cells.[a]



Cpd	R^1	Amino	IC_{50} (μM)	Cpd	R^1	Amino	IC_{50} (μM)
14a	H		1.9 (2.4±0.5)	**14l**	H		7.8
14b	H		3.2	**14m**	H		7.3
14c	H		3.5	**14n**	H		3.5
14d	H		2.7	**14o**	H		3.6
14e	H		2.7	**14p**	Me		1.3 (1.6±0.3)
14f	H		2.8	**14q**	Me		1.2 (1.7±0.5)
14g	H		>10	**14r**	Me		4.9
14h	H		>10	**14s**	Me		3.5
14i	H		1.9	**14t**	Me		>10
14j	H		3.0	**14u**	Me		3.6
14k	H		6.2	**GDC-0941**			0.8

The antiproliferative activity of the final products **14** were evaluated against human rhabdomyosarcoma Rh30 cells by the sulforhodamine B (SRB) assay. The results were summarized in Table 2. Firstly, compounds **14a-o** without any substituent at the

R^1 position were synthesized and examined. Most of them displayed antiproliferative activity against Rh30 cells at concentrations in the micromolar range. Simple alkyl amides **14a-d** exhibited potent cellular activity, with IC_{50} values ranging from 1.9 to 3.5 μM. Introduction of an oxygen atom into the side chain maintained the antiproliferative activity (compounds **14e**, **f**). However, Compounds **14g-h** bearing a more basic group in the amide chain almost lost their antiproliferative activity, in which the basic side chain seems to be detrimental for cell membrane permeability. Additionally, alkynyl, cyclopentyl and aryl substituents were tolerable (compounds **14i-j** and **14n-o**). Replacing the simple alkyl side chain of the amide segment with six-membered heterocycles, such as morpholine, thiomorpholine and piperazine, resulted in decreased antiproliferative activity (compounds **14k-m**).

Subsequently, a methyl group was introduced at R^1 position of pyrrole ring, wishing to to increase their activity by improving the cell membrane permeability. In line with this, all the compounds with a methyl substituent at R^1 position displayed stronger antiproliferative activity (compounds **14p-q** *vs* **14e-f** and **14r-s** *vs* **14g-h**), especially **14p** and **14q** with IC_{50} values below 2 μM. introducing piperidine cycle with different substituent to the amide segment led to opposite activity (**14t** *vs* **14u**). Therefore, it could be concluded from the results in Table 2 that the introduction of a methyl group was beneficial for increasing the antiproliferative activity of the title compounds and the basicity of the amide chain have significant effect on their cellular activities.

To further verify the antiproliferative activity of this series of designed compounds, compounds **14a**, **14p** and **14q** were selected to evaluate their antiproliferative activity in other four types of human cancer cells including human breast cancer BT-474, SK-BR-3, T47D cells, and human ovarian carcinoma SKOV-3cells. And these compounds showed weak antiproliferative activity against the human hepatic cell line LO2. The results presented as IC_{50} values were summarized in Table 3. All the three tested compounds showed potent antiproliferative activity against these four human cancer lines as well. And they were more potent against T47D cells than other tested cancer cells with the IC_{50} values in the nanomolar range.

Table 3. Antiproliferative activities of **14a**, **14p** and **14q**

Cpd	IC_{50} (μM)					
	Rh30	BT-474	SK-BR-3	SKOV-3	T47D	LO2
14a	2.4±0.5	2.4±1.1	2.4±0.5	2.3±0.8	0.8±0.1	10.6±0.5
14p	1.6±0.3	1.5±0.0	1.7±0.8	2.1±0.7	0.7±0.2	6.9±0.1
14q	1.7±0.5	1.6±0.4	1.7±0.5	2.8±1.7	0.6±0.2	9.1±0.5

Among these compounds, **14p** and **14q** exhibited the most potent antiproliferative activity against five human cancer cell lines. However, the compound **14p** was more toxic to the human hepatic cell line LO2. As a consquence, compound **14q** was selected to further evaluate its PK properties in rats (Table 4).[22] The mouse PK study revealed **14q** had high clearance, low oral exposure and short half-life. The oral availability of **14q** was low (F = 20%) and probably caused by low absorption. Comparing to our previous reported PI3K inhibitor **1**, despite the solubility and oral availability of **14q** have been improved somewhat, yet its PK profile was not good enough to support itself for its anti-tumor efficacy *in vivo*. Thus further efforts are warranted to be devoted to improve the PK profile of this series of compounds.

Table 4. PK properties of **14q**.

Dose (mg/kg)	$AUC_{0-\infty}$ (ng·h/ml)	Cmax (ng/ml)	MRT (h)	CL (l/h/kg)	$T_{1/2}$ (h)	F
5 (i.v.)	1702	-	0.5	3.0	0.5	-
10 (p.o.)	588	347	1.7	-	1.1	20%

Abbreviations: i.v., intravenous injection; p.o., per oral; AUC, area under the concentration-time curve; Cmax, peak plama concentration of a drug after administratration; MRT, mean residence time; CL, plasma clearance; $T_{1/2}$, elimination half-life; F, bioavailability.

In summary, a series of 6-aminocarbonyl pyrrolo[2,1-*f*][1,2,4]triazine derivatives were designed by scaffold hopping strategy. The synthesized compounds were evaluated against human rhabdomyosarcoma Rh30 cells by sulforhodamine B (SRB) assay. Most compounds displayed potent antiproliferative activity against Rh30 at concentrations in the micromolar range. Selected compounds **14a**, **14p** and **14q** showed antiproliferative activity against other four human cancer lines. Among tested compounds, **14q** exhibited superior antiproliferative activity and thus we investigated its PK property. Despite the PK profile of **14q** was not good enough to support itself for further development as a drug candidate, this modification may provide a useful scaffold for further optimization of PI3K inhibitors. Furthermore, medicinal chemistry efforts are in progress to develop molecules in this series for a potent PI3K inhibitors with improved PK profile and the results will be reported in due course.

Acknowledgments

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22. PK studies were performed on male Sprague–Dawley rats (200–220 g) with four animals. Compound **14q** was administered *via* the oral route at 10 mg/kg or administered *via* the intravenous route at 5 mg/kg respectively. Serial specimens (0.3 mL) were collected *via* the retrobulbar vein and quantified by liquid chromatography–mass spectrometry (LC-MS). PK parameters were calculated from the mean plasma concentration by non-compartmental analyses. The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Shanghai Institute of Materia Medica, Chinese Academy of Sciences. (Shanghai, China).