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Discovery and biological activity of a novel class I PI3K inhibitor, CH5132799

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ABSTRACT

Phosphatidylinositol 3-kinase (PI3K) is a lipid kinase and a promising therapeutic target for cancer. Using structure-based drug design (SBDD), we have identified novel PI3K inhibitors with a dihydropyrrolopyrimidine skeleton. Metabolic stability of the first lead series was drastically improved by replacing phenol with aminopyrimidine moiety. CH5132799, a novel class I PI3K inhibitor, exhibited a strong inhibitory activity especially against PI3Kα (IC_{50} = 0.014 μM). In human tumor cell lines with PI3K pathway activation, CH5132799 showed potent antiproliferative activity. CH5132799 is orally available and showed significant antitumor activity in PI3K pathway-activated human cancer xenograft models in mice.

Phosphatidylinositol 3-kinase (PI3K) plays crucial roles for tumor progression in the survival signaling pathway (PI3K/Akt pathway). This pathway is most commonly activated in human cancer by various factors such as frequent mutation of PI3Kα, overexpression of growth factor receptor tyrosine kinases and inactivation of the PTEN gene. Therefore targeting PI3K is a promising approach for cancer therapy[1] and significant efforts have been made to develop antitumor PI3K inhibitors already.[2] Utilizing the structural information thus made available, we designed and synthesized novel dihydropyrrolopyrimidine derivatives as PI3K inhibitors for cancer therapeutic agents using structure-based drug design (SBDD). A homology model[3] of PI3Kα was used for molecular design and the binding modes of several compounds were confirmed by X-ray crystal structure[4] of PI3Kγ. In this Letter, we wish to report effective SBDD from lead generation to optimization, and the synthesis and biological activities of CH5132799 (**1**), a novel potent class I PI3K inhibitor.

*Design and synthesis:*LY294002 is a well known PI3K inhibitor and its crystal structure complex with PI3Kγ was reported in 2000, indicating that the morpholine ring is essential for the interaction with PI3K at the catalytic site.[4] Using this 3D information, two known PI3K inhibitors possessing a morpholino group, PIramed's PI103 (**2**)[5] and Chiron's inhibitor (**3**),[6] were superimposed onto the binding pocket by modeling tools. Both inhibitors have morpholine, phenol, and pyridine moieties and we presumed that each common moiety plays an important role in their interaction with PI3K. This led us to design a novel dihydropyrrolopyrimidine inhibitor (**4** and **5**), as depicted in Figure 1, whose morpholine and phenol moieties interact with valine 882 and tyrosine 867 of PI3Kγ, respectively, and whose pyridine ring is nearly directed toward that of PI103 by conformational restriction of dihydropyrrolopyrimidine skeleton, although its interaction with PI3K is unclear. Compound **5** strongly inhibited PI3Kα (IC_{50} = 0.0086 μM) and our prediction of the binding mode of its analog (**6**) was confirmed by analyzing the X-ray crystal structure of the PI3Kγ/inhibitor complex (Fig. 2).[7] Hydrogen bonds were observed between morpholine oxygen and valine 882 at the hinge region and between phenol and aspartic acid 841/tyrosine 867 in the affinity pocket.

However, oral bioavailability (BA) of compound **5** in mouse was very low (only 1.6%), mainly due to rapid glucuronidation. The metabolic stability in human liver microsomes was also low ($T_{1/2}$ = 21 min) (Fig. 3). To improve the metabolic stability, we searched for a bioisostere of phenol by docking tools and found an aminopyrimidine moiety as an alternative, which was highly prioritized by FlexSIS virtual docking simulations.[8] Thus we prepared the second lead compound (**7**). It showed 3.8 times less inhibitory activity (IC_{50} = 0.033 μM) against PI3Kα than the first lead (**5**), but 30 times stronger in vitro antiproliferative activity against human breast cancer cell line KPL-4 (IC_{50} = 0.015 μM). It also exhibited good antitumor activity in vivo in a human prostate cancer PC-3 xenograft model (112% tumor growth inhibition (TGI) after oral administration of 100 mg/kg, q.d., of **7** for 11 days) as a result of improved met-

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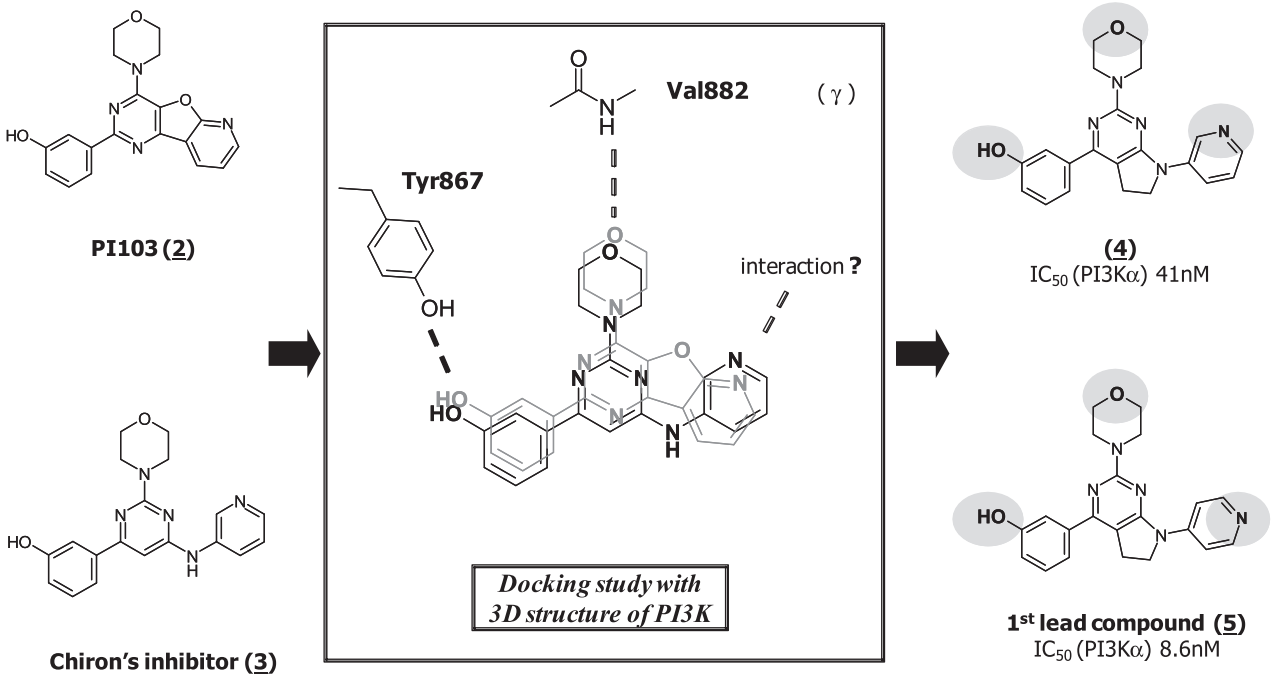


Figure 1. Lead generation from known PI3K inhibitors by docking study.

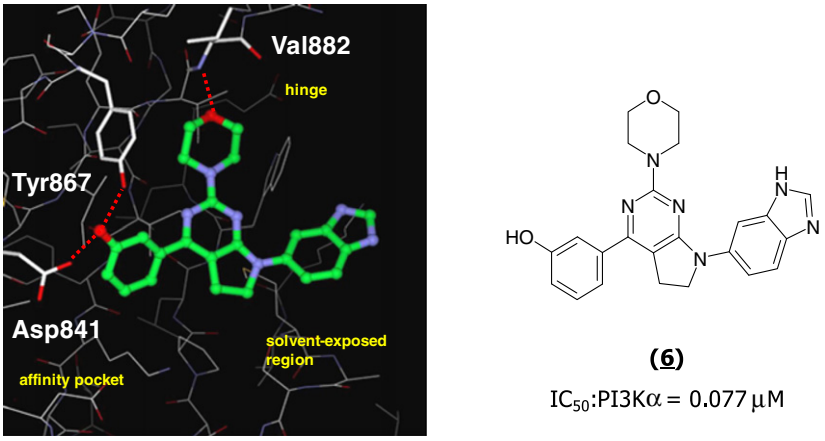
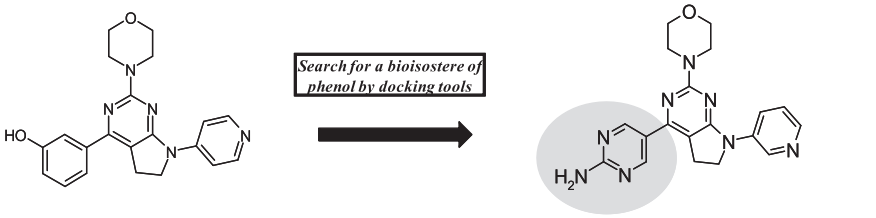


Figure 2. Crystal structure of PI3Kγ with **6**.



PI3Kα IC_{50} 0.0086 μM
KPL-4 IC_{50} 0.45 μM
in vitro glucuronidation $T_{1/2}$ 11min
Liver microsome stability $T_{1/2}$ 21min (human)
F=1.6% (mouse)

PI3Kα IC_{50} 0.033 μM
KPL-4 IC_{50} 0.015 μM
in vitro glucuronidation $T_{1/2}$ >60min
Liver microsome stability $T_{1/2}$ 71min (human)
F=41% (mouse)
TGI(PC-3) 112% at 100mg/kg
(po, q.d. x 11)

Figure 3. Profiles of compounds **5** and **7**.

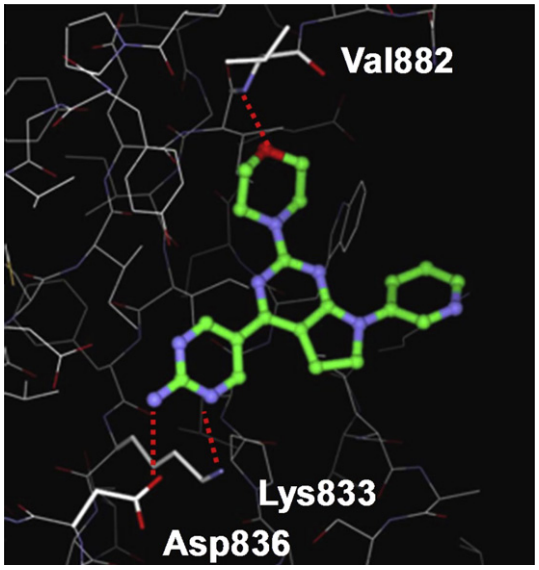


Figure 4. Crystal structure of PI3K with **7**.

abolic stability and oral BA (41% in mouse) (Fig. 3). It was confirmed that compound **7** binds ATP binding sites of the enzyme by X-ray crystal structure analysis of the PI3Kγ/inhibitor complex.[9] Hydrogen bonds were observed between morpholine oxygen and valine 882 and between aminopyrimidine and aspartic acid 836/lysine 833 (Fig. 4). With the aim of further improving the physicochemical and ADME profiles, we modified the right-hand side of the molecule (pyrido-3-yl moiety), and finally identified CH5132799 (**1**) as a clinical candidate that showed excellent oral BA (101% in mouse), human liver microsomal stability and in vivo antitumor activity in the PC-3 xenograft model (TGI: 101% at 25 mg/kg, po, q.d. × 11 days). Its X-ray crystal structure showed almost the same binding mode as that of compound **7** (Fig. 5).[10] An additional hydrogen bond between aminopyrimidine and aspartic acid 841 was observed.

Compound **4** was synthesized from γ-butyrolactone. Condensation with 3-methoxybenzoyl chloride gave ketolactone (**8**). Compound **8** was condensed with amidine followed by chlorination by $POCl_3$ which gave dichloropyrimidine derivative **10**. Palladium catalyzed amination with 3-aminopyridine afforded **11a** as a white solid. Compound **4** was obtained by treatment of **11a** with sodium thioethoxide. Compounds **5** and **6** were synthesized analogously using 4-aminopyridine and 1*H*-benzimidazole-5-ylamine instead of 3-aminopyridine, respectively (Fig. 6). Compound **7** was synthesized as shown in Figure 7. Cyclization of **14** (Fig. 8) with 3-aminopyridine in NaH/THF gave **15** in 88% yield. Suzuki coupling with boronate **13**, derived from 2-amino-5-bromopyrimidine in two steps, followed by deprotection of the 4-methoxybenzyl group of **16** in TFA, afforded compound **7** as a white solid in good yield. The synthesis of CH5132799 (**1**) is outlined in Figure 8. A pyrimidine derivative (**18**) was prepared from morpholin-4-carboxamidine hydrobromide and 2-oxotetrahydrofuran-3-carboxylic acid methyl ester[11] (**17**) using sodium methoxide as a base. Tri-chlorination of **18** with phosphoryl chloride in toluene gave pyrimidine trichloride (**14**) as a white solid in good yield. 6,7-Dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine derivative (**19**) was successfully synthesized from **14** and methanesulfonamide in the presence of potassium carbonate in NMP. Suzuki–Miyaura cross coupling of **19** and 2-amino-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidine was conducted using potassium phosphate and dichlorobis(triphenylphosphine)palladium(II) in DMF to give CH5132799 (**1**) as a colorless powder in 93% yield.

*Biological activities of CH5132799:*CH5132799 selectively inhibits class I PI3Ks, especially PI3Kα (IC_{50} = 0.014 μM) but showed less inhibition of class II PI3Ks, class III PI3K and mTOR and also no inhibitory activity (IC_{50} >10 μM) against 26 protein kinases[12] (Table 1). In human tumor cell lines with PI3K pathway activation by mutation, CH5132799 showed potent antiproliferative activity [HCT116(CRC): IC_{50} = 0.20 μM, KPL-4(BC):[13] IC_{50} = 0.032 μM, T-47D(BC): IC_{50} = 0.056 μM, SK-OV-3(Ovarian): IC_{50} = 0.12 μM] as shown in Table 1. In KPL-4 cells, effective suppression of phosphorylation of AKT was observed.[12] CH5132799 exhibited good oral BA in mouse, rat, monkey and dog (*F*: 54.2–101%, Table 2). In a human breast cancer (KPL-4: PI3Kα H1047R) xenograft model in mice, oral treatment with CH5132799 (12.5 mg/kg, q.d.) showed strong tumor regression as shown in Figure 9. The strong regression was maintained during the 6 week administration, even in the intermittent dosing schedule (q.d., 2 weeks on/1 week off; q.d., 5 days on/2 days off), suggesting that a flexible administration schedule can be applicable in the clinic.

In summary, we successfully designed and discovered novel PI3K inhibitors by SBDD. CH5132799 is an orally available, potent class I PI3K inhibitor which showed significant antitumor activity in PI3K pathway-activated human cancer xenograft models in mice and promises to provide clinical benefits.

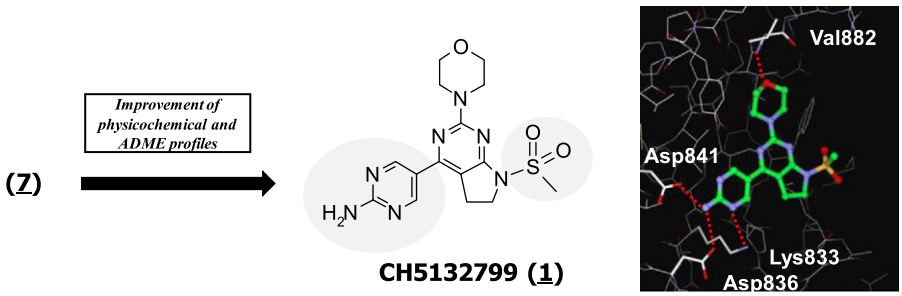


Figure 5. Structure and profiles of CH5132799 (**1**) and crystal structure with PI3K.

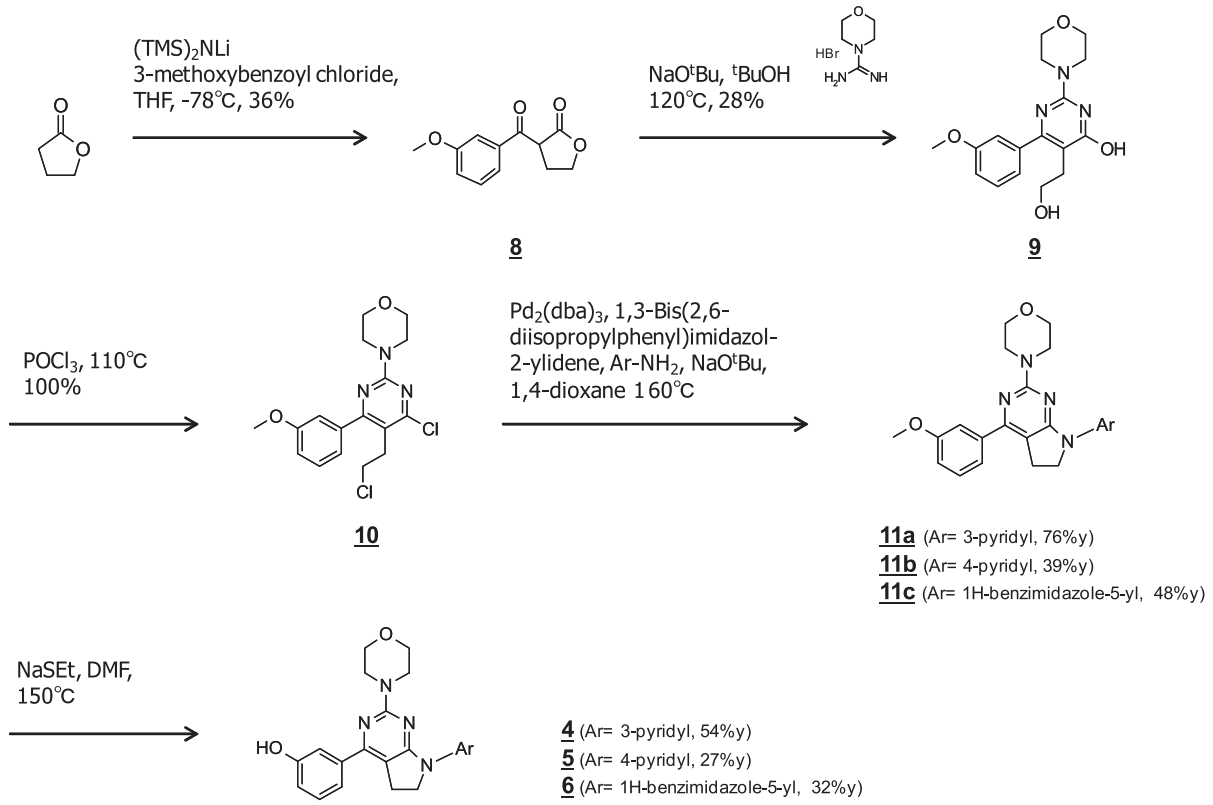


Figure 6. Synthesis of 4, 5 and 6.

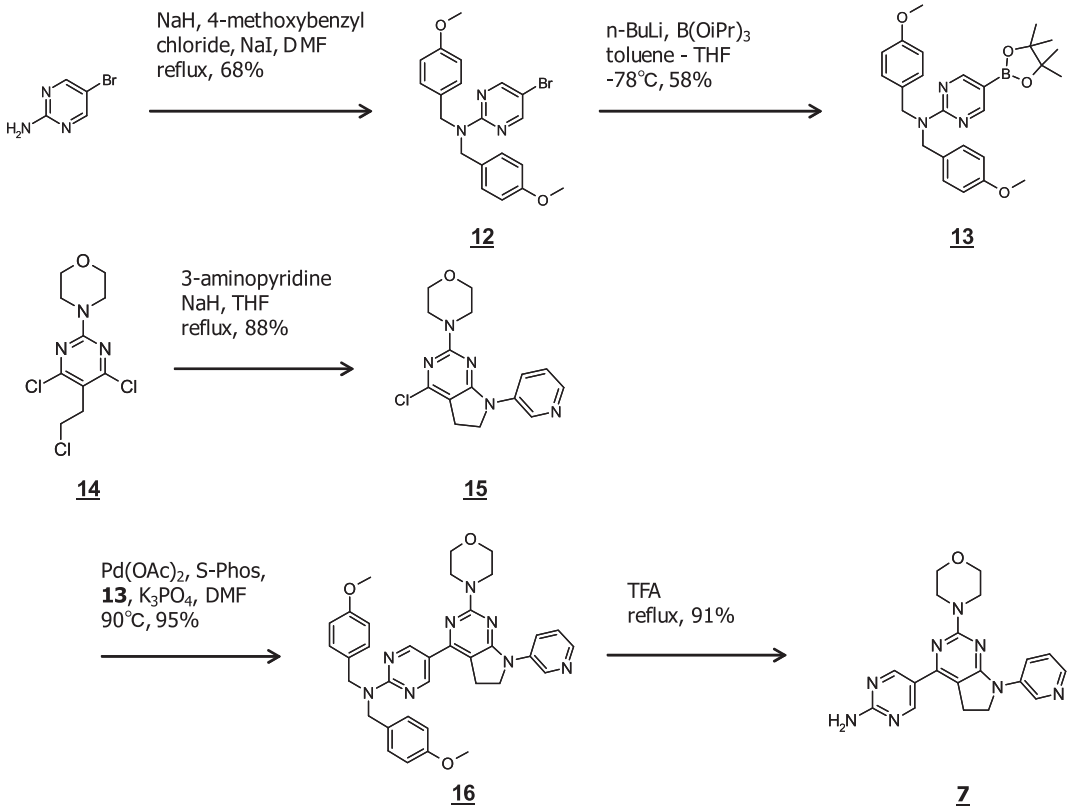


Figure 7. Synthesis of 7.

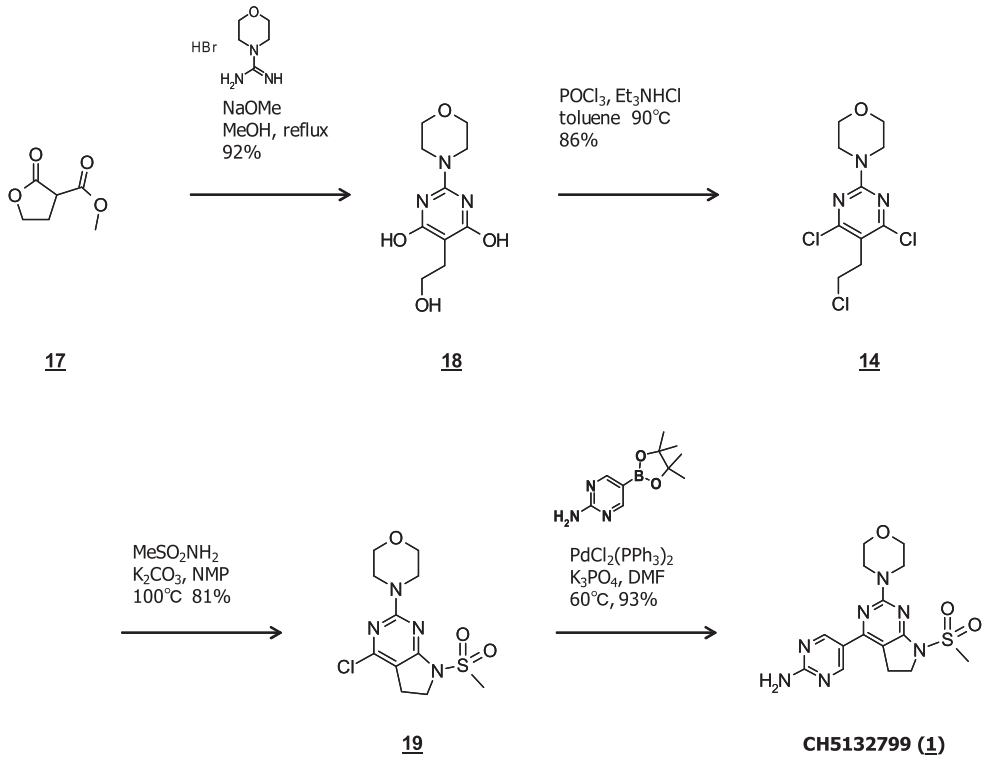


Figure 8. Synthesis of CH5132799 (**1**).

Table 1
Enzyme inhibition profile and antiproliferation activity of CH5132799 (**1**)

Enzyme inhibition profile		Antiproliferation activity	
	IC_{50}(μM)		IC_{50}(μM)
PI3K class I			
PI3Kα	0.014	HCT116 (CRC, PI3Kα H1047R)	0.20
PI3Kβ	0.12	KPL-4 (breast, PI3Kα H1047R)	0.032
PI3Kδ	0.50	T-47D (breast, PI3Kα H1047R)	0.056
PI3Kγ	0.036	SK-OV-3 (ovarian, PI3Ka H1047R)	0.12
PI3K class II		MFE-280 (endometrial, PI3Kα H1047Y)	0.18
PI3K C2α	>10	ME-180 (cervical, PI3Ka E545K)	0.14
PI3K C2β	5.3		
PI3K class III			
Vps34	>10		
PIKK			
mTOR	1.6		
Protein kinase[12]	>10		

Table 2
Pharmacokinetic parameters of CH5132799 in four animals

Species	Dose (mg/kg)	Administration	$T_{1/2}$ (h)	T_{max} (h)	CL (mL/min/kg)	AUC_{inf} (ng h/mL)	Bioavailability (%)
Mouse	2	iv	1.7	—	17.5	1930	—
	1	po	3.8	0.25	—	978	101
Rat	0.5	iv	2.6	—	6.3	1350	—
	1	po	3.5	0.8	—	1790	66.2
Dog	0.25	iv	1.6	—	6.4	660	—
	1	po	3.3	4.5	—	1430	54.2
Monkey	0.25	iv	3.2	—	6.2	692	—
	1	po	6.7	4.0	—	2170	78.4

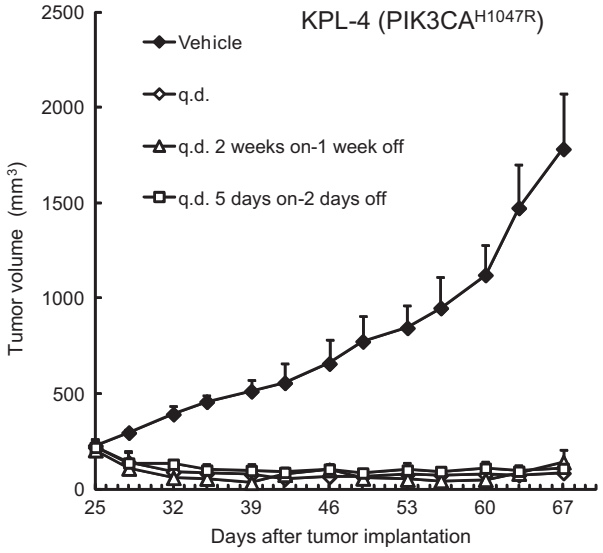


Figure 9. In vivo efficacy of CH5132799 (**1**) in KPL-4 xenograft model CH5132799 was orally administered at 12.5 mg/kg for 6 weeks in three dosing schedules. ♦: vehicle; ◇: q.d; △: q.d., 2 weeks on/1 week off; □: qd, 5 days on/2 days off.

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