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PKI-179: An orally efficacious dual phosphatidylinositol-3-kinase (PI3K)/mammalian target of rapamycin (mTOR) inhibitor

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ABSTRACT

A series of mono-morpholino 1,3,5-triazine derivatives (**8a**–**8q**) bearing a 3-oxa-8-azabicyclo[3.2.1]octane were prepared and evaluated for PI3-kinase/mTOR activity. Replacement of one of the bis-morpholines in lead compound **1** (PKI-587) with 3-oxa-8-azabicyclo[3.2.1]octane and reduction of the molecular weight yielded **8m** (PKI-179), an orally efficacious dual PI3-kinase/mTOR inhibitor. The in vitro activity, in vivo efficacy, and PK properties of **8m** are discussed.

Phosphatidylinositol-3-kinase (PI3K) is a lipid kinase, that is, a central component in the PI3K/Akt/mTor signaling pathway. Currently there are four isoforms of this enzyme known as PI3K α, β, γ, and δ. Among these four isoforms, PI3Kα especially, plays a key role in the biology of human cancer.[1,2] This pathway regulates cell proliferation, growth, survival, and apoptosis.[1–3] The deregulated activation of PI3Kα and its downstream effectors including Akt and mTOR, has been linked to tumor initiation and maintenance. PI3K/Akt/mTOR pathway activation can be caused by loss of PTEN (the phosphatase that regulates PI3K signaling), overexpression, or activation of some receptor tyrosine kinases (e.g., EGFR, HER-2), interaction with activated Ras, over expression of the PI3K-α gene (PIKC3A), or mutations in PIKC3A that cause elevated PI3K-α kinase activity.[1–4] Aberrantly elevated PI3K/Akt/mTOR pathway signaling has been implicated in poor prognosis and survival in patients with various lymphatic tumors, as well as breast, prostate, lung, brain (glioblastoma), skin (melanoma), colon, and ovarian cancers.[1–4] Additionally, PI3K/Akt/mTOR pathway activation contributes to resistance of cancer cells to both targeted anticancer therapies and conventional cytotoxic agents.[5–7] An effective inhibitor of the PI3K/Akt/mTOR pathway could both prevent cancer cell proliferation and induce programmed cell death (apoptosis).[1,2,5] Therefore, several groups[8–13] including our own,[14–17] have embarked on projects to identify potent small molecule inhibitors of the PI3K/Akt/mTOR signaling pathway. Highly mTOR selective ATP competitive compounds have also been reported recently.[18,19] It has been demonstrated that mTOR can also be independently activated by AmpK and LKB pathways, thus providing a strong rationale for developing dual PI3K and mTOR kinase inhibitors. A dual PI3K/mTOR inhibitor could both prevent

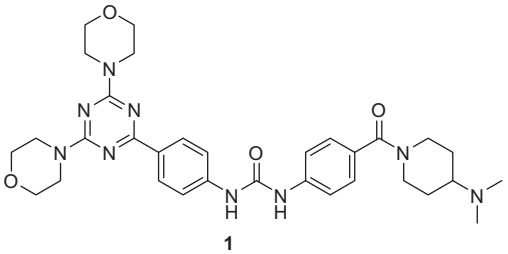

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Figure 1. Structure of PKI-587.

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cancer cell proliferation and induce programmed cell death (apoptosis) by fully suppressing Akt activation.

In a recent Letter[20] we reported the design and synthesis of several bis-morpholino triazine based compounds as potent dual PI3K/mTOR inhibitors. It was also shown by us that PKI-587 (**1**, structure shown in Fig. 1) was highly efficacious and shrunk tumors in several xenograft and orthotopic models. Even though **1** was found to be highly efficacious, it has to be administered intravenously because it was found to have poor plasma levels when administered orally. This could be attributed to several factors such as poor permeability, low *c* log *P* (calculated value 1.24) and high molecular weight (615). Hence efforts have been made to increase the *c* log *P* and to lower the molecular weight. This Letter describes our efforts to alter these values to obtain an orally efficacious compound.

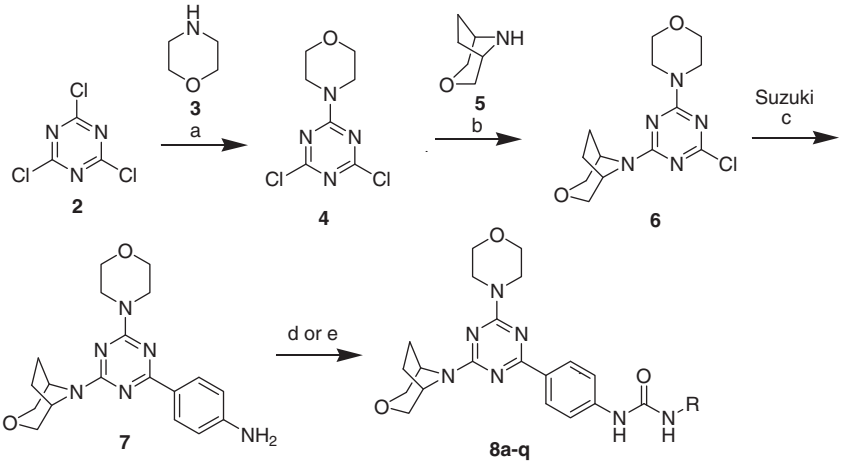
In order to increase the *c* log *P* value, one of the morpholine groups in **1** was substituted with a 'morpholine like' moiety. A bridged-morpholine analog such as 3-oxa-8-azabicyclo[3.2.1]-octane (**5**) was chosen to increase the *c* log *P*. The other morpholine in **1** was kept as it formed a pivotal hinge region hydrogen bond interaction with Val851.[14,16,18,19] The urea appendage of **1** was also kept as a part of the design, since it is involved in vital hydrogen bond interactions with PI3Kα in the solvent exposed region.[14,16,18–20] The molecular weight of **1** was reduced to below 500 by removing the amide portion.

The 1-{4-[4-morpholin-4-yl-6-(3-oxa-8-azabicyclo[3.2.1]oct-8-yl)-1,3,5-triazin-2-yl]-aryl-4-yl} urea derivatives **8a**–**8q** which are exemplified in the present Letter were prepared starting from commercially available cyanuric chloride **2**, as depicted in Scheme 1. Among the three chlorine elements presented in cyanuric chloride, the first chlorine was replaced by using 1 equiv of morpholine and triethylamine at −20 °C to yield **4** in almost quantitative yield. The second chlorine in compound **4** was replaced by 3-oxa-8-azabicyclo[3.2.1]octane (**5**) at room temperature in CH_2Cl_2 to yield **6** in high yield. Suzuki coupling reaction of **6** with 4-aminophenylboronic acid, pinacol ester gave **7**. Compound **7** was reacted with various aryl isocyanates to yield **8a**–**8e**, **8h**, and **8l**. Alternatively, urea derivatives **8f**, **8g**, **8i**–**8k**, **8m**–**8q** were obtained by reacting intermediate **7** with triphosgene and followed by the respective amines. All the final products were purified either by flash column silica-gel chromatography or by preparative HPLC.[21]

All the final compounds **8a**–**8q** were tested in vitro against PI3Kα, PI3Kγ, and mTOR. The IC_{50} values against PI3Kα and PI3Kγ enzymes were determined using a fluorescence polarization format assay.[22] The corresponding mTOR inhibition for the newly synthesized compounds was determined by the protocol outlined by Yu et al.[23]

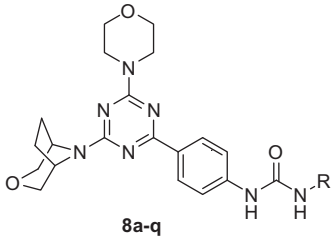
Subsequently the most potent compounds were tested in cell proliferation assays (3 days) MB-MDA-361 (breast cancer cell line with PI3K mutation and Her2+ive overexpression) and PC3mm2 (prostate cancer cell line with mutated PI3Kα and PTEN deletion) cells.[24] The enzyme and cell proliferation assay IC_{50} values are shown in Table 1.

As can be seen from Table 1, the initial compound **8a** was found to have potent PI3Kα, γ, and mTOR inhibitory activity; but exhibited moderate potency in MDA361 and PC3mm2 cell proliferation assays. However, these initial results encouraged us to probe structure–activity relationships more systematically to further optimize enzyme and cellular potencies. Substitution of the phenyl group (R = phenyl) in **8a** with 4-F **8b**, 4-methyl **8c** and 4-cyano **8d** groups led to a decrease in both PI3Kα and γ potencies. However, the corresponding mTOR potency was retained. Except for compound **8e**, all the compounds exemplified here were found to have excellent mTOR potency, irrespective of the substituent on the R group. The structural basis for the degree of tolerance of mTOR enzyme to these structural changes is not entirely clear due to a lack of detailed structural information on the mTOR enzyme. Studies using a PI3Kγ homology model revealed[16,20] that the substituents at the 4-position on the R group are solvent exposed. Hence compounds **8f**–**8k** bearing polar entities were prepared to enhance potency and solubility. As can be seen from Table 1, these modifications led to an improvement in PI3Kα enzyme potency. Analogues such as **8f** exhibited good enzyme and cellular potencies. However, poor human and nude mouse microsomal stabilities of compound **8f** ($t_{1/2}$ = 12 min) precluded it from further investigation. In order to improve water solubility, analogues **8h**–**8k** bearing a basic nitrogen atom were prepared. The most potent compound (enzyme activity) in this category was **8h**, which lacked cell potency. Despite good potency, analogues such as **8h**–**8k** had molecular weights >500. Hence, compounds such as **8l**–**8q** bearing polar nitrogen atoms on the aryl group were designed and synthesized. Among the various compounds prepared, analogue **8m** possessed significantly increased potency against PI3Kα, mTOR, and cellular potency against both MDA-361 and PC3mm2 cell lines. This compound had excellent PAMPA permeability (19.5×10^{-6} cm/s at pH 7.4). The solubility of **8m** was poor at pH 7.4 (3 μg/mL), but im-

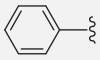
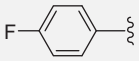
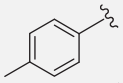
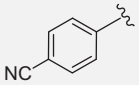
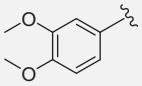
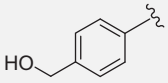
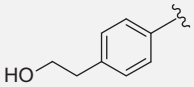
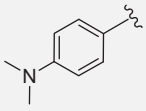
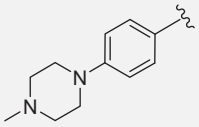
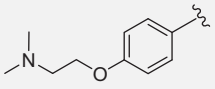
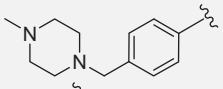
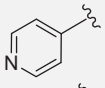
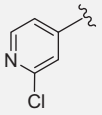
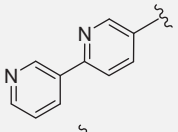
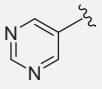


Scheme 1. Reagents and conditions: (a) morpholine (1.1 equiv), Et_3N (2 equiv), acetone, crushed ice −20 °C to 0 °C; (b) 1.1 equiv of **5**, Et_3N (2 equiv),CH_2Cl_2, room temperature; (c) 4-aminophenylboronic acid, pinacol ester (1.2 equiv), $Pd(Ph_3P)_4$ (5 mol %), DME, 2 M Na_2CO_3, 120 °C/30 min, microwave irradiation; (d) RNCO, CH_2Cl_2, room temperature; (e) triphosgene (0.6 equiv), Et_3N (3 equiv), CH_2Cl_2, room temperature, 15 min, then RNH_2 (5 equiv), 2–6 h.

Table 1
In vitro enzyme inhibition and cell proliferation inhibition IC_{50} (nM) values and calculated *c* log *P* values of analogues **8a**–**8q**[a]

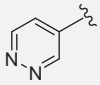


8a-q

Compd	R=	PI3K-α	PI3K-γ	mTOR	MDA361	PC3	*c* log *P*
8a		18	51	2	104	147	2.97
8b		39	154	1.9	98	176	3.09
8c		69	122	1.3	167	301	3.34
8d		86	290	3.3	345	3600	2.79
8e		13.5	358	93.5	402	1246	1.78
8f		7	98	0.43	15	28	1.8
8g		14	74	0.32	22	30	2.03
8h		3.5	717	13.5	>1000	>1000	2.4
8i		21	45.2	1.1	35	40	2.85
8j		14	100	1.85	<30	<30	2.86
8k		21	189.5	0.80	14	31	3.14
8l		17	150	0.50	73	98	2.12
8m		8	74	0.42	22	29	2.12
8n		23	99	1.26	93	118	2.90
8o		40	44	3.45	56	45	2.84
8p		84	240	0.73	110	138	1.45

(*continued on next page*)

Table 1 (*continued*)

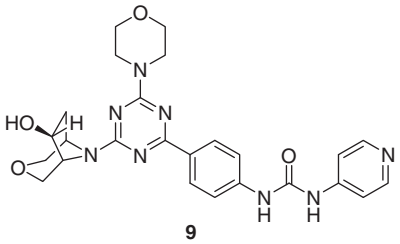
Compd	R=	PI3K-α	PI3K-γ	mTOR	MDA361	PC3	*c* log *P*
8q		28	149	1.55	36	47	1.32

[a] The values are an average of at least two separate determinations with a typical variation of <±30%.

proved at pH 3.0 (60 μg/mL). Stability evaluation in nude mouse, rats, and human microsomes revealed that **8m** is very stable ($t_{1/2}$ >30 min) in nude mouse and rats; but moderately stable in human ($t_{1/2}$ = 14 min). Metabolite identification studies revealed that the ethylene bridge on the bridged-morpholine group was the primary site of metabolism. This metabolite was isolated by incubating compound **8m** with human liver microsomes and its structure was determined to be **9** (Fig. 2). Since compound **9** was formed as the major metabolite, its pharmacological potency against PI3Kα, γ, and mTOR enzymes and tumor cells was determined. As can be seen from Table 2, compound **9** is an active metabolite. Further studies on this active metabolite are in progress.[25]

Based on its enzyme and cellular potencies and its pharmaceutical profile, analogue **8m** was chosen for further evaluation.[26] First, we explored whether **8m** exhibited activity over other PI3K isoforms in addition to PI3Kα and γ. The IC_{50} values against PI3K-β, δ and the two most common mutant forms of PI3Kα (E545K and H1047R) were 24, 77, 14, and 11 nM, respectively. In addition, **8m** was selective when tested against a panel of 361 kinases; none of them were inhibited at an IC_{50} below 50 μM. Pharmacokinetic studies of **8m** in nude mouse, rat, monkey, and dogs after oral administration (10 mg/kg) showed good oral bioavailability (98% in nude mouse, 46% in rat, 38% in monkey, and 61% in dog) and a high half-life (>60 min). Finally, the potential for cardiac effects and drug–drug interactions was explored. Inhibitory concentrations of **8m** against hERG and various Cytochrome P450 isoforms (1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4) were >30 μM. The only CYP isozyme that was inhibited below 30 μM was CYP 2C8 with an IC_{50} of 3 μM.

Oral administration of **8m** (50 mg/kg) to nude mice bearing MDA361 tumor xenografts resulted in good inhibition of PI3K signaling, lasting up to 8 h, as evidenced by the lack of phosphorylation of Akt T308 (shown in Fig. 3) and good inhibition of mTORC1 signaling, as evidenced by the lack of phosphorylation of S6K and its downstream substrate S6 (not shown). Phosphorylation of the



9

Figure 2. Structure of compound **9**.

Table 2
IC_{50} vales in nM for the metabolite **9**[a]

PI3Kα	PI3Kγ	mTOR	PC3mm2	MDA-361
4	33	0.8	80	32

[a] The values are an average of at least two separate determinations with a typical variation of <±30%.

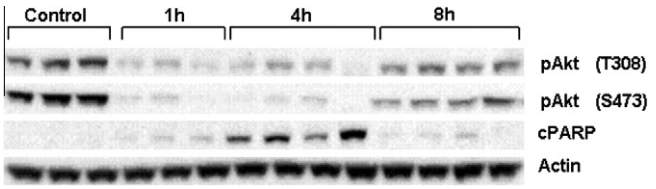


Figure 3. In vivo biomarker inhibition by 8m at 50 mg/kg in MDA361 tumors grown in nude mice.

mTORC2 substrate, Akt (S473) was also significantly inhibited (shown in Fig. 3). Evidence for induction of apoptosis and cell death is seen by the appearance of cleaved PARP at 4 h (shown in Fig. 3).

Evaluation of in vivo efficacy of **8m** was performed in nude mice bearing MDA-361 human breast cancer tumors. Compound **8m** was administered @ 50, 20, 10, and 5 mg/kg, po doses, daily for 40 days (10 mice per group; dose formulation: cci/d5w-la).

In this xenograft model, compound **8m** exhibited pronounced tumor growth arrest when dosed above 10 mg/kg, qd (as shown in Fig. 4). In these studies, compound **8m** was well tolerated, and no significant weight loss of tested animals was observed for all different dosages.

In conclusion, we have shown that an orally efficacious compound can be designed by reducing the molecular weight and altering the *c* log *P* of a lead compound such as PKI-587 (**1**) which is not orally active. Analogue **8m** (PKI-179) is a potent dual PI3K/mTOR inhibitor and exhibits excellent in vitro cell activity and in vivo efficacy in the MDA-361 xenograft model. Its effect on other tumor models is currently under investigation.

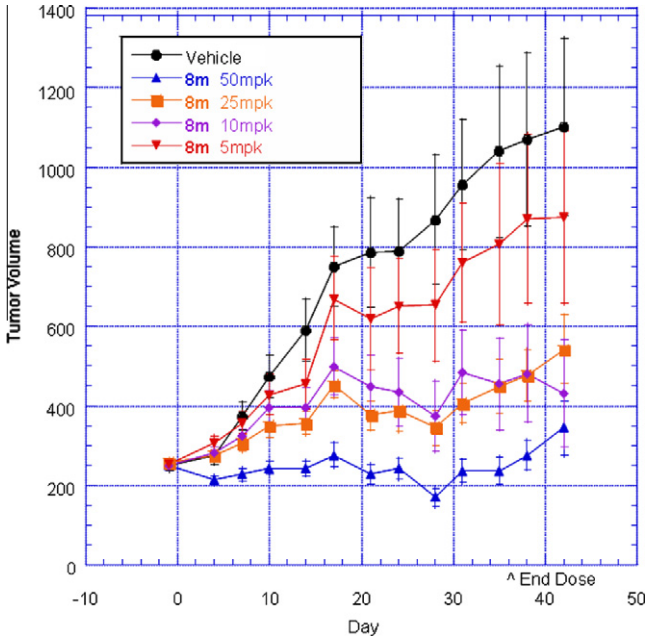


Figure 4. Administration of **8m** for 42 days to nude mice bearing human breast tumor (MDA-361) xenograft dose @ 50, 25, 10, and 5 mg/kg, qd.

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21. The purity of final compounds was determined by analytical HPLC using Prodigy ODS3 column (150 mm × 4.6 mm). Conditions: ACN/H_2O eluent at 1 mL/min flow (containing 0.05% TFA) at 40 °C, 20 min, gradient 5% ACN to 95% ACN, monitored by UV absorption at 215 nm. All final compounds were found to be ⩾95% purity unless otherwise specified. Reverse phase HPLC (preparative HPLC) purifications were performed on a Gilson preparative HPLC system controlled by Unipoint® software using a Phenomenex Gemini® (100 mm × 30 mm).
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26. Analytical data of **8m**: MS (ESI, *m*/*z*) 489 (M+H); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.39 (s, 1H), 10.23 (s, 1H), 8.62 (d, 2H, *J* = 7.6 Hz), 8.32 (d, 2H, *J* = 8.6 Hz), 7.94 (d, 2H, *J* = 7.6 Hz), 7.60 (d, 2H, *J* = 8.6 Hz), 4.82 (s, 1H), 4.63 (s, 1H), 3.90–3.60 (m, 12H), 1.99–1.87 (m, 4H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 165.7, 161.5, 159.1, 151.4, 148.9, 139.3 (2C), 127.8, 127.1 (2C), 115.4 (2C), 110.5 (2C), 68.8, 68.5, 63.6 (2C), 52.3, 52.0, 41.1 (2C), 24.0 (2C); HRMS calcd for $C_{25}H_{28}N_8O_3$ (M+H) 489.2357, obsd 489.2353.