Discovery of Novel and Orally Bioavailable Inhibitors of PI3 Kinase Based on Indazole Substituted Morpholino-Triazines

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S Supporting Information

ABSTRACT: A new class of potent PI3K α inhibitors is identified based on aryl substituted morpholino-triazine scaffold. The identified compounds showed not only a high level of enzymatic and cellular potency in nanomolar range but also high oral bioavailability. The three lead molecules (based on their *in vitro* potency) when evaluated further for *in vitro* metabolic stability as well as pharmacokinetic profile led to the identification of **26**, as a candidate for further development. The IC₅₀ and EC₅₀ value of **26** is 60 and 500 nM, respectively, for PI3K α enzyme inhibitory activity and ovarian cancer (A2780) cell line. The identified lead also showed a high level of microsomal stability and minimal inhibition activity for CYP3A4, CYP2C19, and CYP2D6 at 10 μ M concentrations. The lead compound **26**, demonstrated excellent oral bioavailability with an AUC of 5.2 μ M at a dose of 3 mpk in mice and found to be well tolerated in mice when dosed at 30 mpk BID for 5 days.

KEYWORDS: Cancer, PI3K inhibitors, indazole, morpholinotriazine

IC₅₀ = 60 nM
EC₅₀ = 500 nM
AUC = 5.2 μ M@3mpk
C_{max} = 1.2 μ M@3mpk

Phosphatidylinositol-3-kinase (PI3K) is a lipid kinase and an important component in the PI3K/AKT/mTOR signaling pathway that plays a key role in cell proliferation, growth, survival, and apoptosis as well as protein and glucose metabolism.^{1–3} Class 1 PI3K protein consists of four isoforms known as PI3K α , β , γ , and δ . This classification is based on their sequence homology and substrate preferences. The deregulated activation of PI3K α and its downstream effectors including AKT and mTOR have been linked to tumor initiation and maintenance. Class 1 PI3K protein in general and PI3K α isoform specifically plays a key role in human cancer biology.^{4–7} Aberrantly activated PI3K α protein 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.⁴ Therefore, inhibiting PI3K α is an attractive strategy for cancer therapy. Clinical interest in PI3K inhibitors has been intensifying. In recent years, a large number of compounds have been reported in clinical development phase having strong antitumor activity in animal models.^{8–11}

During our efforts toward the identification and development of class 1 PI3K inhibitors, we identified hit **3** based on the triazine morpholine core.^{12–14} This hit was inspired by the leads reported (Figure 1, compounds **1** and **2**) by Hayakawa et al.¹⁵ It was planned to develop a quick structure–activity relationship (SAR) around identified hit **3**. Based on data reported by others and our internal data, we had ascertained that, the morpholine moiety is critical for activity and potency

Figure 1. PI3K α IC₅₀ of identified hit **3**, compared to literature leads **1** and **2**.

as it interacts with a hinge region backbone NH (Val882) of PI3K protein.^{16–18} Therefore, the morpholino group retained in all subsequent molecules for this study.

Synthesis of the various morpholino–triazines reported in Table 1 was achieved by the general synthetic route outlined in Scheme 1 involving a combination of Suzuki coupling and nucleophilic substitution on morpholine substituted triazine ring.¹⁹

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Table 1

Compound	Ar	R	IC ₅₀ (μM)
4			>20.0
5			>20.0
6			>10.0
7			1.18
8			10.0
9			0.74
10			0.73
11			0.54
12			1.06
13			0.68

*The coefficient of variation (CV %) was found to be <10% for IC₅₀.

Scheme 1^a

^aReagents and conditions: (a) morpholine, acetone/water (4:1), 0 °C, 3 h; (b) amino acid/ester, acetone/NaHCO₃ aq. (1:1), RT, 16 h; (c) aryl borate, Pd(PPh₃)₄, Na₂CO₃, DME/water (4:1), 120 °C, 20 min, MW.

Compounds 4, 5, and 6 illustrate the importance of the phenolic group, as protection, removal, or replacement results in complete loss of activity. This finding is in line with literature reports^{17,18} that highlighted the importance of hydrogen bond donor moieties for potency in PI3K inhibitors having aryl morpholine based structural motifs. Further, compound 3 was

coupled with different amines to produce their corresponding amides (entry 9–12, Table 1) and evaluated for their *in vitro* PI3Kα inhibition activity. The PI3Kα enzyme inhibitory activity of the compounds was determined using a homogeneous time resolved fluorescence (HTRF) assay. The percent inhibition was calculated and plotted against the concentration of the inhibitor to calculate IC₅₀.¹⁹ Interestingly, and as illustrated by compounds 7 through 13, variations of the glycine moiety of 3 does not result in any significant change in activity. Compound 13 even suggests that the glycine moiety may not be interacting with the protein and may potentially be oriented outside the protein into the solvent. This is complementary to literature findings.²⁰ The activity of compound 8 suggests that this loss of activity may be due to steric interaction between the ligand and the protein or the lack of hydrogen bonding interaction between the protein and the ligand. The marginal increase in activity of compound 13 over compound 3 may be due to the either less steric hindrance or less encumbered ability for the formation of the hydrogen bond between the ligand and the kinase.

From this series of molecules, compound 11 was selected for further optimization, primarily based on its potency against PI3Kα. Though, we had initially established the importance of the phenolic group, we needed to find an alternate of phenol. This is due to the generally accepted observation of the poor PK/ADME characteristics imparted to a compound with the presence of a phenol moiety. A small set of new molecules were synthesized as analogues of 11 to find a replacement for phenol (Table 2). A two/three step synthesis, involving a Suzuki coupling with different boronic acids as a key step for diversification was exploited to generate these analogues (Scheme 2).¹⁹ The data from this new set of molecules led to the selection of compound 16 over compound 11 for further optimizations, even though it was 2-fold lower in *in vitro*

Table 2

Compound	Ar	IC ₅₀ (μM)
11		0.54
14		>20.0
15		1.60
16		1.10
17		>20.0

*The coefficient of variation (CV %) was found to be <10% for IC₅₀.

Table 4

Compound	Microsomal Stability (%parent @ 2hr)			Cyp Inhibition (IC ₅₀ in μ M)			Mouse Pharmacokinetics @ 3mpk			
	HLM	DLM	MLM	CYP3A4	CYP2C19	CYP2D6	T _{max} (hr)	C _{max} (nM)	AUC (nM/hr)	T _{1/2} (hr)
26	100.7	83.8	45.6	>15	>10	>10	1.00	1244	5246	4.86
28	99.0	ND	27.3	>15	>10	>10	1.33	1664	5849	4.34
29	80.7	ND	24.8	>15	>10	>10	0.83	535	1618	3.15

the role of the spacer and the spatial orientation of the terminal amide. To explore this further, several other analogues were synthesized using an aromatic ring spacer between the triazine and terminal amide (compounds **21–24**). All of the new analogues demonstrated improved enzymatic and cellular potency. Replacement of the bridging nitrogen (compound **22**) with oxygen (compound **25**) led to a 4-fold loss in enzymatic as well as cellular potency, but this loss was recovered by moving the amide group from the *meta*- to the *para*-position on the phenyl ring (compound **26**) resulting in the best compound in this series. Replacing the aromatic ring spacer of **26** with a cyclohexyl ring (compound **28** and **29**) resulted in compounds equipotent to **26**. Hence, compounds **26**, **28**, and **29** were identified as the optimized leads based on their enzymatic and cellular potencies. These three leads were further evaluated for their *in vitro* metabolic stability with human liver microsome (HLM), dog liver microsome (DLM), and mouse liver microsomes (MLM). The three leads were incubated with isolated liver microsomes of the three species for 2 h. The concentration of the parent compound was monitored by HPLC at four time points (0, 0.5, 1, and 2 h, respectively). All three molecules (**26**, **28**, and **29**) showed encouraging level of microsomal stability particularly in HLM. The data of 2 h time point is shown in Table 4 and a detailed study report is provided as Supporting Information.

Compounds **26**, **28**, and **29** were screened further for their cytochrome P450 inhibition activity. All three leads showed no or minimal inhibitions for activity of CYP3A4, CYP2C19, and CYP2D6 at 10 μ M concentrations. All three molecules were also evaluated for oral bioavailability in mice. Each was dosed orally at 3 mpk to Swiss mice to evaluate their pharmacokinetic profile and determine the key parameters such as C_{max}, AUC, t_{max}, and t_{1/2}. The results are summarized in Table 4. Compounds **26** and **28** showed better PK profile compared to **29** with higher plasma exposure of >5 μ M at 3 mpk dosing. On the basis of its potency and pharmacokinetic profile, **26** was selected as the molecule of choice for further evaluation

Compound **26** was evaluated *in vivo* for dose ranging pharmacokinetic study in mice at three different doses of 1, 3, and 10 mpk. A summary of the pharmacokinetic parameters are shown in Table 5. The plasma exposure of **26** demonstrated a reasonable dose response exposure with escalating dose from 1 to 3 to 10 mpk.

Table 5. Dose Ranging Pharmacokinetics Study of **26** by Oral Administration in Mice

PK parameters	dose (oral)		
	1 mpk	3 mpk	10 mpk
t _{max} (h)	1.00	1.00	2.33
C _{max} (nM)	360	1243	1798
AUC (nM·h)	2117	5246	9791
elimination t _{1/2} (h)	6.93	4.86	6.87

The lead **26** was also screened for hERG activity using the hERG-lite assay from Chantest Corporation.¹⁸ The objective of the study was to estimate *in vitro* effects of **26** on surface expression of the hERG potassium channel. Both wild type (WT) and the single-point mutant (SM) channel G601S were examined. Predicted hERG liability was indicated as channel block and/or trafficking inhibition. On the basis of the hERG lite assay data, the IC₅₀ of the hERG liability determined by the patch clamp method was predicted. Overall, the predicted hERG liability of **26** was found to be >10 μ M implying that **26** is not expected to have hERG liability.

The *in vivo* multiday dose tolerability of **26** was evaluated in Swiss mice.¹⁹ The lead **26** was dosed at 30 mpk BID for 5 consecutive days and monitored for effect and changes in body weight, body temperature, body coat, and level of physical activity. The animals did not show any significant body weight loss (Figure 2), hyper- or hypothermia, change in body coat or lowered physical activity. This clearly indicates that **26** is well tolerated by mice.

Figure 2. Effect of **26** on mice bodyweight when dosed at 30mpk BID oral for 5 days.

In conclusion, our efforts toward development of class I PI3K inhibitors led to identification of a small molecule **26** as a potent and orally bioavailable inhibitor of PI3K α protein. The lead **26** was found to have excellent metabolic stability, no hERG liability with appreciable oral exposure, and tolerability. Efforts for the development of **26** are in progress and will be summarized in a future publication.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acsmedchemlett.5b00322](https://doi.org/10.1021/acsmedchemlett.5b00322).

Experimental details of chemical synthesis as well as characterization and *in vitro* and *in vivo* screening of all compounds (PDF)

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Notes

The authors declare no competing financial interest.

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(19) Please see Supporting Information for experimental details. This includes chemical synthesis, characterization, and *in vitro* and *in vivo* screening of all compounds.

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