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Phosphoinositide-3-kinase inhibitors: Evaluation of substituted alcohols as replacements for the piperazine sulfonamide portion of AMG 511

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ABSTRACT

Replacement of the piperazine sulfonamide portion of the PI3Kα inhibitor AMG 511 (**1**) with a range of aliphatic alcohols led to the identification of a truncated *gem*-dimethylbenzylic alcohol analog, 2-(5-(4-amino-6-methyl-1,3,5-triazin-2-yl)-6-((5-fluoro-6-methoxypyridin-3-yl)amino)pyridin-3-yl)propan-2-ol (**7**). This compound possessed good in vitro efficacy and pharmacokinetic parameters and demonstrated an EC_{50} of 239 ng/mL in a mouse liver pharmacodynamic model measuring the inhibition of hepatocyte growth factor (HGF)-induced Akt Ser473 phosphorylation in CD1 nude mice 6 h post-oral dosing.

Phosphatidylinositol-3-kinases (PI3Ks) are intracellular lipid kinases that catalyze the phosphorylation of the eponymous inositol-containing lipids to generate intracellular secondary messengers (e.g., phosphatidylinositol (3,4,5)-trisphosphate (PIP_3)) in response to external stimulation, such as receptor tyrosine kinase activation in response to growth factor binding.[1] Extensive research over the past decade has revealed the PI3K signaling pathway to be a key regulator of cellular survival, growth, and proliferation.[2] Mutations in components of the PI3K signaling pathway have been estimated to be associated with up to 30% of all human malignancies.[2,3] As a result, significant effort has been directed toward the discovery and development of PI3K inhibitors as potential cancer therapeutics.[4]

Class I PI3Ks—defined both by their dimeric p110/p85 catalytic/regulatory subunit structure and phosphatidylinositol (4,5)-bisphosphate substrate specificity—in particular, have been the focus of these efforts, as these PI3Ks are the most strongly implicated in cancer;[5] aberrant activation of all four p110 isoforms (α, β, γ, and δ) has demonstrated oncogenic potential in in vitro studies.[6] As in vitro studies have also demonstrated the functional redundancy of these p110 isoforms,[7] our efforts in this area have focused on the identification of pan-isoform class I PI3K inhibitors.

In a prior publication,[8] we reported the discovery of AMG 511 (**1**; Fig. 1), a biaryl aminotriazine inhibitor which demonstrated excellent pharmacodynamic and pharmacokinetic effects in murine animal models. We have also previously detailed SAR investigations leading to the identification of the methoxypyridine and piperazinyl sulfonamide fragments of **1**.[8,9] In the present communication, we expand on these studies, providing the results

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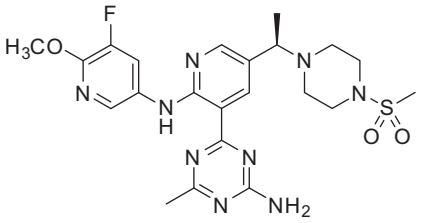


Figure 1. AMG 511 (**1**).

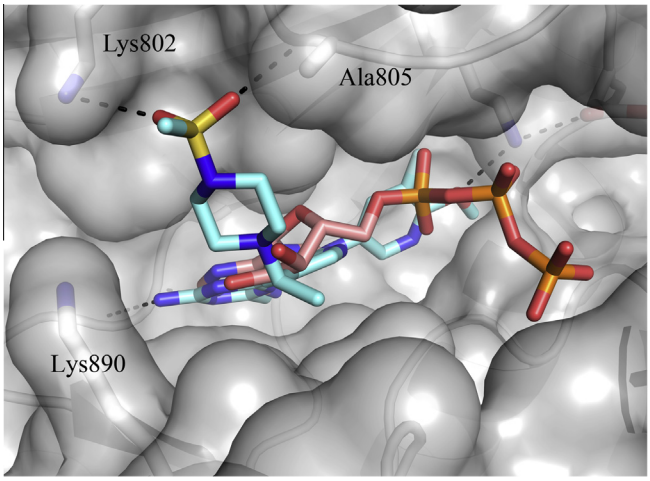


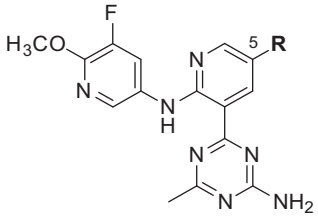
Figure 2. Overlay of X-ray co-crystal structures of AMG 511 (**1**; blue) and ATP (pink) bound to PI3Kγ.[11] Hydrogen-bonding contacts to K802 and A805 are indicated in black.

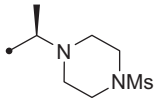
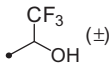
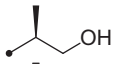
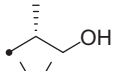
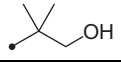
of SAR investigations aimed at addressing potential shortcomings of the piperazine sulfonamide portion of **1**.

Figure 2 depicts an overlay of the X-ray co-crystal structures of AMG 511 and ATP bound to PI3Kγ. The piperazine sulfonamide portion of **1** projects out of the ATP binding site in the ribose-binding region ('ribose pocket') and occupies a lipophilic groove formed by the bottom face of the P-loop, with the sulfonamide oxygens engaging in hydrogen-bonds with Lys802 (ω-amine) and Ala805 (backbone NH). A comparison of **1** with **2**, a compound lacking the ethylpiperazine sulfonamide fragment (Table 1), shows that this moiety provides a 10-fold improvement in enzymatic and cellular potency as well as a significant reduction in oxidative metabolism (as measured by intrinsic microsomal clearance (CL_{int})). Despite these improvements to the core scaffold **2**, we continued to search for alternatives to the piperazine sulfonamide for several reasons: this group added significantly to both molecular weight (327 Da for **2** vs 518 Da for **1**) and to an already high topological polar surface area (tPSA; 109 Å^2 for **2** and 150 Å^2 for **1**).[10] Additionally, metabolite ID studies using human and rat hepatocytes revealed this group to be the major site of metabolism of **1**, with oxidative metabolism of this group accounting for ~70% of all metabolism in rat hepatocytes and >95% of all metabolism in human hepatocytes.

In our efforts to identify an alternative to the piperazine sulfonamide portion of **1**, we chose to focus on small aliphatic substituents bearing an alcohol group. In so doing, we aimed to mimic the hydroxylic functionality present in the ribose portion of the endogenous ligand and, thereby, preserve favorable interactions with loosely ordered water molecules along the solvent-exposed face of the ATP binding site. Initial results from these investigations are found in Table 1.[12] New analogs were initially assayed for PI3K activity against PI3Kα using a modified AlphaScreen® assay.[13,14] A

Table 1
PI3Kα, mTOR, and pAkt activity of inhibitors possessing aliphatic alcohol ribose-pocket binding groups[a]



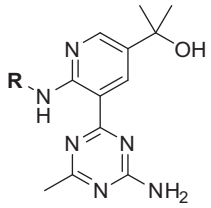
Compd	R	PI3Kα K_i (μM)	mTOR IC_{50} (μM)	U87 pAkt IC_{50} (μM)	HLM/RLM CL_{int} (μL/min/mg)
1		0.004	14.8	0.004	22/20
2		0.048	>22	0.042	99/36
3		0.056	>15	0.044	–/51
4[b]		0.040	>25	0.020	22/45
5[b]		0.048	>25	0.026	<14/<14
6		0.030	2.2	0.034	18/34
7		0.023	7.8	0.013	<14/23
8		0.016	3.7	0.018	39/79
9[b]		0.040	>15	0.031	<14/37
10[b]		0.026	11.1	0.025	14/36
11		0.040	>17	0.037	34/34

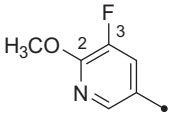
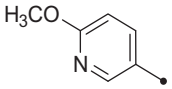
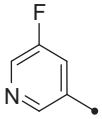
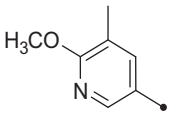
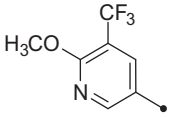
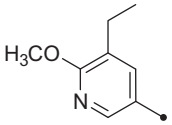
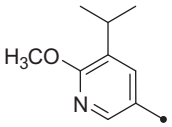
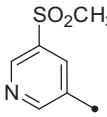
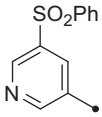
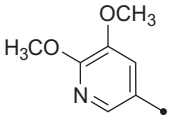
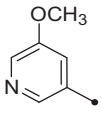
[a] Data represent an average of at least two determinations. '>[Highest concentration tested]' is reported for compounds that failed to inhibit >10% of control activity in one or more replicate measurements. A statistical analysis of this data may be found in the Supplementary material.
[b] Absolute stereochemistry not determined.

LanthaScreen™ FRET assay[15] was used to monitor the effect of ribose pocket modifications on inhibition of the structurally related kinase, mTOR, to determine whether new analogs retained the PI3K-selectivity of compound **1**. Functional activity was subsequently confirmed by measuring the inhibition of Akt S473 phosphorylation in U87 MG cells.[16]

As seen in Table 1, introducing a hydroxymethyl group into analog **2** failed to improve PI3Kα inhibitory activity (compound **3**; K_i = 56 nM), as did further α-methylation or α-trifluoromethylation of the hydroxymethyl substituent (compounds **4**–**6**). *gem*-Dimethylation of the hydroxymethyl fragment, however, resulted in 2–3-fold enhancements in both PI3Kα activity and U87 pAkt IC_{50} (compound **7**). Compound **7** additionally demonstrated significantly enhanced microsomal stability relative to the unsubstituted analog, **2**. Although replacement of the piperazine sulfonamide portion of **1** with the 2-hydroxypropyl fragment also led to slightly enhanced inhibition of mTOR, compound **7** remained highly PI3K-selective, with greater than 300-fold selectivity for PI3Kα. The cyclopropyl analog of **7**, compound **8**, preserved the properties of the *gem*-dimethyl analog, retaining potency in both enzymatic

Table 2
PI3Kα, mTOR, and pAkt activity of inhibitors bearing varied affinity pocket substituents[a]



Compd	R	PI3Kα K_i (μM)	mTOR IC_{50} (μM)	U87 pAkt IC_{50} (μM)	HLM/RLM CL_{int} (μL/min/mg)
7		0.023	7.8	0.013	<14/23
12		0.089	0.79	0.048	<14/19
13		0.087	2.5	0.097	<14/15
14		0.118	7.8	0.049	<14/ 22
15		0.090	>25	0.146	n.a.
16		0.469[b]	15.8[b]	0.470	n.a.
17		0.153	>25	0.414	n.a.
18		0.801	>16	1.16	<14/<14
19		0.224	6.7	0.766	n.a.
20		0.030	10.6	0.010	<14/<14
21		0.089	2.1	0.071	17/19

[a] Data represent an average of at least two determinations, unless otherwise noted. '>[Highest concentration tested]' is reported for compounds that failed to inhibit >10% of control activity in one or more replicate measurements. A statistical analysis of this data may be found in the Supplementary material.
[b] Data from a single measurement.

(K_i = 16 nM) and cellular assays (IC_{50} = 18 nM), while again demonstrating modestly enhanced mTOR activity (IC_{50} = 3.7 μM). Compound **8**, however, demonstrated significantly reduced microsomal stability relative to **7**. Homologated versions of the hydroxyethyl analogs **4** and **5** (i.e., compounds **9** and **10**) generally mirrored the activity of their non-homologated counterparts. Homologation of hydroxypropyl analog **7**, however, led to reduced activity in both enzymatic (K_i = 40 nM) and cellular assays

Table 3
Pharmacokinetic parameters for **1**, **7**, and **20**[a]

Compd	CL (L/h/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	%*F*	F_u	Efflux ratio[b]
1	0.45	1.7	3.3	57	0.08	9.7
7	1.1	1.7	3.1	37	0.06	2.4
20	1.3	4.2	4.8	n.a.	n.a.	25

[a] Male Sprague-Dawley rat (iv: 1 mg/kg, DMSO, $n = 3$; p.o.: 2 mg/kg, 1% Pluronic F68, 2% HPMC, 15% HPβCD, pH = 2.2, $n = 3$).
[b] Measured in LLC-PK1 cells stably over-expressing rat P-gp. 5 μM compound, 0.1% BSA.

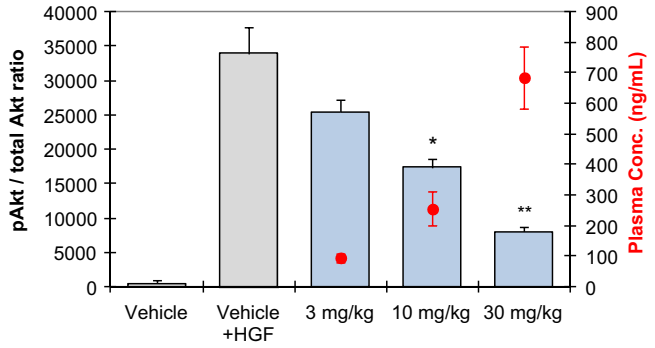


Figure 3. Mouse liver pharmacodynamic assay. Orally dosed **7** reduced liver p(S473)Akt levels in female CD1 nude mice 6 h post-dose. ($N = 3$/group; bars represent average pAkt/total Akt ratio + SE; circles represent average plasma concentration ± SE; $^{*}P$ <0.05 versus vehicle + HGF by ANOVA/Dunnett's Multiple Comparison Test; $^{**}P$ <0.0001 versus vehicle + HGF by ANOVA/Dunnett's Multiple Comparison Test; vehicle = 1% Pluronic F68, 2% HPMC, 15% HPβCD, pH = 2.2.)

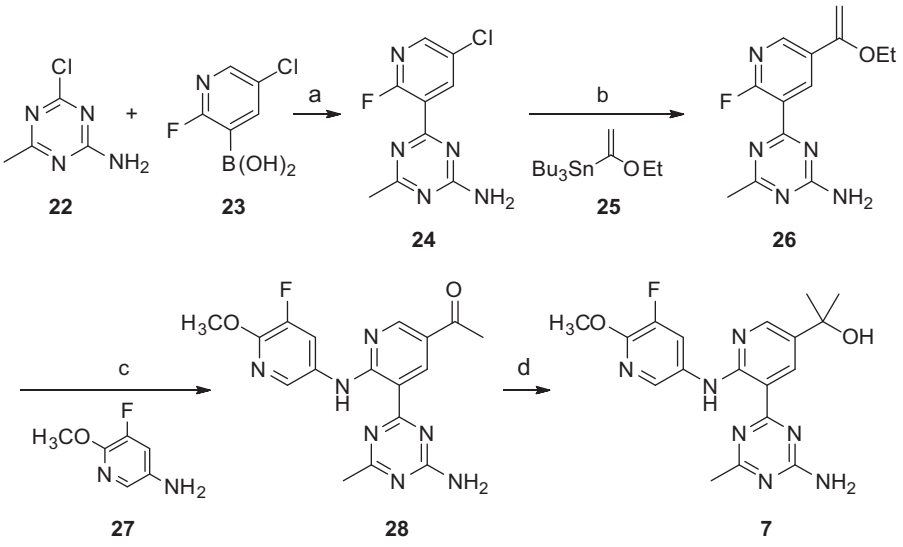
(IC_{50} = 37 nM), as well as in the mTOR FRET assay (IC_{50} >17 μM) (cf., compound **11**).

With an initial screen of small, hydroxylated aliphatic substituents having revealed the isopropoxy group of analog **7** to be the most promising candidate for a piperazine sulfonamide replacement, we next turned our attention to re-optimization of the 2-methoxy-3-fluoropyridyl fragment of **7** to exploit any alteration in the pyridine binding mode brought about by changes to the ribose pocket moiety. As we have detailed in prior publications,[8,9] the methoxypyridyl fragment projects into a generally lipophilic pocket known as the 'affinity pocket,'[17] with the pyridyl nitrogen engaging in a hydrogen bond with an ordered water molecule bridging Asp841 and Tyr867, and the methoxy substituent engaging in a polar interaction with Lys833. Our prior studies revealed the C2 and C3 positions of the pyridyl ring to be the most tolerant of modification without loss of enzymatic activity; accordingly, we targeted these two positions for modification in an effort to enhance the PI3Kα activity of **7**. Results of these investigations are detailed in Table 2.

Removal of either the 3-fluoro or 2-methoxy substituent from compound **7** (compounds **12**, K_i = 89 nM, and **13**, K_i = 87 nM, respectively), led to nearly 4-fold decreases in PI3Kα activity. Likewise, replacement of the 3-fluoro substituent with more sterically demanding substituents (methyl, trifluoromethyl, ethyl, or isopropyl; compounds **14**–**17**) or bulky polar substituents such as methylsulfone (**18**) or phenylsulfone (**19**) produced analogs with reduced PI3Kα activity or pAkt IC_{50} values. A 3-methoxy group was tolerated as a replacement for the 3-fluoro substituent (compound **20**; K_i = 30 nM); however, as in the case of compound **7**, retention of the 2-methoxy group was required to maintain potent enzymatic activity (cf., compound **21**).

The pharmacokinetic properties of two of the most promising analogs to emerge from these studies (compounds **7** and **20**) were subsequently investigated by iv and p.o. dosing in male Sprague-Dawley rats (Table 3). Compound **7** demonstrated a similar pharmacokinetic profile to that of AMG 511 (**1**). Oral bioavailability was slightly reduced (37% vs 57%) and clearance elevated (1.1 vs 0.45 L/h/kg); however, volume of distribution, terminal half-life, and plasma protein binding remained nearly unchanged. Compound **20**, in contrast, demonstrated a slightly extended terminal half-life (4.8 h), with increased clearance (1.3 L/h/kg) offset by an increased volume of distribution (4.2 L/kg). Compound **20** additionally proved to be a fairly strong P-gp efflux substrate.

On the basis of these results, compound **7** was further evaluated in a mouse liver pharmacodynamic model that measured the inhibition of hepatocyte growth factor (HGF)-induced Akt phosphorylation at Ser473 in female CD1 nude mice. In this study, compound **7** was dosed orally at 3, 10, and 30 mg/kg. After 6 h, mice were administered HGF to activate PI3K-dependent Akt phosphorylation in the liver, and five minutes later, phosphorylated (Ser473) Akt levels were determined by an electrochemical luminescence immunoassay. As depicted in Figure 3, compound



Scheme 1. Synthesis of **7**. Reagents and conditions: (a) $PdCl_2(AmPhos)_2$, KOAc, dioxane/water, 100 °C, 38%; (b) $Pd(OAc)_2$, XPhos, CsF, dioxane/DMF, 110 °C, 99%; (c) NaHMDS, THF, 0 °C, 56%; (d) MeMgBr, THF, 0 °C, 34%.

7 significantly suppressed PI3K signaling at 10 and 30 mg/kg, bringing about a dose-dependent decrease in p(S473)Akt. A nonlinear regression analysis established an EC_{50} of 239 ng/mL, comparable to that obtained from compound **1** (EC_{50} = 240 ng/mL) in a prior study.[8] Separate in vitro studies also confirmed compound **7**, like compound **1**, to be a pan-isoform PI3K inhibitor, exhibiting single-digit nanomolar K_i values for the β-, γ-, and δ-isoforms of PI3K.

The synthesis of compound **7** is depicted in Scheme 1 and commenced with the Suzuki coupling of chlorotriazine **22**[9] and commercial fluoropyridine boronic acid **23** to provide the biaryl triazine **24** in moderate yield. Stille coupling of **24** with tributyl(1-ethoxyvinyl)tin (**25**) subsequently furnished enol ether **26** in excellent yield. The affinity pocket fragment (**27**) was introduced by nucleophilic substitution of the 2-fluoro group of **26** using NaHMDS as a promoter, providing acetylpyridine **28** in 56% yield following hydrolysis of the enol ether during chromatographic purification. Compound **7** was subsequently obtained from **28** by the addition of methyl Grignard.

As revealed in these studies, replacement of the piperazine sulfonamide portion of AMG 511 (**1**) with a 2-hydroxypropyl group provided a compound (**7**) that retained the favorable pharmacokinetic properties of AMG 511 and exhibited similar in vivo efficacy (EC_{50} = 239 ng/mL vs 240 ng/mL) in a mouse liver pharmacodynamic assay. Although compound **7** was unable to fully replicate the superb enzymatic and cellular potency of **1**, the activity of the significantly truncated analog (**7**; 385 Da) relative to AMG 511 (**1**; 518 Da) highlights both the efficiency of the 2-hydroxypropyl group as a ribose pocket surrogate as well as the unique effectiveness of the piperazinyl sulfonamide group in the context of the triazinyl pyridine scaffold.[18]

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Supplementary data

Supplementary data (statistical analysis of data presented in Tables 1 and 2, synthetic routes for the preparation of compounds **2**–**21**, and details of in vitro and in vivo assays) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.10.085.

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11. PDB accession codes: 4FLH (AMG 511); 1E8X (ATP).
12. Detailed synthetic procedures for new analogs may be found in the Supplementary material.
13. PerkinElmer Inc., 940 Winter Street, Waltham, MA 02451. See Supplementary material for details.
14. Prior experience with the triazinyl pyridine scaffold (see Refs. 8 and 9) revealed minimal selectivity between p110 isoforms (<10×), and p110α activity was deemed most relevant clinically, given this isoform's association with human cancer (see Ref. 5).
15. Life Technologies, 3175 Staley Road, Grand Island, NY 14072. See Supplementary material for details.
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