Discovery of GSK2126458, a Highly Potent Inhibitor of PI3K and the Mammalian Target of Rapamycin


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ABSTRACT Phosphoinositide 3-kinase α (PI3Kα) is a critical regulator of cell growth and transformation, and its signaling pathway is the most commonly mutated pathway in human cancers. The mammalian target of rapamycin (mTOR), a class IV PI3K protein kinase, is also a central regulator of cell growth, and mTOR inhibitors are believed to augment the antiproliferative efficacy of PI3K/AKT pathway inhibition. 2,4-Difluoro-N-[2-(methyloxy)-5-[4-(4-pyridazinyl)-6-quinolinyl]-3-pyridinyl]benzenesulfonamide (GSK2126458, 1) has been identified as a highly potent, orally bioavailable inhibitor of PI3Kα and mTOR with in vivo activity in both pharmacodynamic and tumor growth efficacy models. Compound 1 is currently being evaluated in human clinical trials for the treatment of cancer.

KEYWORDS GSK2126458, phosphoinositide 3-kinase α, mammalian target of rapamycin, PI3K/AKT pathway

The phosphoinositide 3-kinase (PI3K) signaling pathway is activated in a broad spectrum of human cancers. Activation of this pathway often occurs indirectly by the activation of receptor tyrosine kinases or indirectly by the activation of receptor tyrosine kinases or the inactivation of the phosphatase and tensin homologue (PTEN) tumor suppressor. Direct activation of PI3K has been demonstrated with the discovery of several activating mutations in the PIK3CA gene itself, the gene that encodes the p110α catalytic subunit of PI3Kα. Several of the mutations found in PIK3CA have been shown to increase the lipid kinase activity of PI3Kα, induce activation of signaling pathways, and promote transformation of cells both in vitro and in vivo. Furthermore, the PI3K pathway is the most commonly mutated signaling pathway in human cancers.

The PI3K family of enzymes is comprised of 15 lipid kinases with distinct substrate specificities, expression patterns, and modes of regulation. In particular, PI3Kα has emerged as an attractive target for cancer therapeutics, and several PI3K inhibitors are currently under evaluation in human clinical trials, including BEZ235 (Novartis), GDC-0941 (Genentech), PX-866 (ProLX), and XL765 (Exelixis). We describe herein the discovery of 2,4-difluoro-N-[2-(methyloxy)-5-[4-(4-pyridazinyl)-6-quinolinyl]-3-pyridinyl]benzenesulfonamide (GSK2126458, 1, Figure 1), a highly potent and selective inhibitor of class I PI3Ks and the mammalian target of rapamycin (mTOR). Compound 1 is being evaluated in a phase I, open-label, dose-escalation study in subjects with solid tumors or lymphoma.

GSK1059615 (2), our first PI3K clinical compound, recently entered a dose-escalation study in patients with refractory malignancies. In follow-up studies, we sought to identify a second inhibitor with improved potency, selectivity, and pharmacokinetic (PK) profiles. Key to our approach for achieving the desired levels of PI3K activity was to pursue structure-based design utilizing crystallography of the more amenable PI3Kβ as a surrogate protein. The inhibitor-bound crystal structure of 2 in PI3Kβ indicated that the thiazolidinedione (TZD) ring formed an interaction with the catalytic lysine (Lys833) within the ATP-binding pocket. However, the structure also showed that larger groups could potentially be accommodated. We reasoned that filling the empty space in the enzyme pocket could lead to inhibitors with improved potency and potentially selectivity, and this was the basis for the initial strategy to identify alternates to the TZD moiety.

Synthesis of these derivatives began with conversion of 6-bromo-4-chloroquinoline (3) to the corresponding 4-ido...
intermediate 4, followed by installation of the 4-pyridyl group under standard palladium-catalyzed cross-coupling conditions to provide quinoline 5 (Scheme 1). Various aryl (Ar) groups were then attached to the 6-position of the quinoline core using an in situ borylation and palladium-catalyzed cross-coupling to furnish the desired analogs 6.\(^{18,19}\)

Inhibition of PI3K\(\alpha\) was measured using a continuous read time-resolved fluorescence resonance energy transfer displacement assay.\(^{20}\) The analogs were also evaluated in a PI3K\(\alpha\)-driven mechanistic cellular assay, which measured the ability of the compounds to decrease intracellular phosphorylation of AKT at S473 (pAKT-S473) in T47D and BT474 cancer cells. Replacement of the TZD with a simple phenyl group (6a) resulted in a dramatic loss in potency (Table 1). The activity was much less attenuated for both pyridine 6b and indazole 6c, although neither showed much improvement in the cellular assay relative to 6a. Molecular modeling overlays indicated that the pyridyl and indazolyl nitrogens of 6b and 6c, respectively, were most likely making distinct interactions with the enzyme. We therefore merged the two heterocycles to form azaindazole 6d, which provided a substantial boost in biochemical and cellular potency. Cocrystallization of 6d with PI3K\(\gamma\) confirmed that the nitrogen at the 2-position of the indazole forms a hydrogen bond with Lys833 and that the pyridyl nitrogen interacts with a conserved active site water molecule (Figure 2).

Although 6d exhibited a promising activity profile, the compound displayed very low aqueous solubility and a poor PK profile, characterized by high clearance, a short half-life, and a lack of oral bioavailability. Opening of the pyrazole portion of the azaindazole to give 2,3,5-trisubstituted pyridine analogs led to the identification of pyridylsulfonamide 6e, which retained the gains made in potency and

### Table 1. TZD Replacement SAR

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>Ar</th>
<th>IC(_{50}) (nM)</th>
<th>pAKT IC(_{50}) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td></td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td>6a</td>
<td></td>
<td>1800</td>
<td>8080</td>
</tr>
<tr>
<td>6b</td>
<td></td>
<td>260</td>
<td>&gt;29,300</td>
</tr>
<tr>
<td>6c</td>
<td></td>
<td>73</td>
<td>2700</td>
</tr>
<tr>
<td>6d</td>
<td></td>
<td>7</td>
<td>76</td>
</tr>
<tr>
<td>6e</td>
<td></td>
<td>10</td>
<td>49</td>
</tr>
</tbody>
</table>

### Table 2. Selected Pyridylsulfonamide Analog SAR\(^{d}\)

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>Ar</th>
<th>IC(_{50}) (nM)</th>
<th>pAKT</th>
<th>DNAUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>6e</td>
<td></td>
<td>10</td>
<td>49</td>
<td>NQ</td>
</tr>
<tr>
<td>6f</td>
<td></td>
<td>1</td>
<td>63</td>
<td>250</td>
</tr>
<tr>
<td>6g</td>
<td></td>
<td>1</td>
<td>45</td>
<td>920</td>
</tr>
<tr>
<td>6h</td>
<td></td>
<td>0.1</td>
<td>7</td>
<td>1100</td>
</tr>
</tbody>
</table>

\(^d\) Units: Dose-normalized area under the curve (DNAUC) (ng h mL\(^{-1}\) mg\(^{-1}\) kg\(^{-1}\)). NQ, not quantifiable.
showed increased solubility. However, no improvement in the PK profile was noted.

We next examined the structure-activity relationship (SAR) of the sulfonamide moiety and found that arylsulfonamides (e.g., 6f) exhibited oral exposure in rats with no loss of inhibitory activity (Table 2). Removal of the 2-amino group to yield 6g further increased exposure ~3-fold. Reversal of the sulfonamide connectivity (6h) led to an increase in biochemical and cellular potency, while maintaining the improved oral exposure.

Reintroduction of a small substituent at the 2-position of the pyridine (e.g., methoxy, methyl, halogen, etc.) resulted in a significant boost in both enzyme and cellular potency (data not shown). We reasoned that this was due to the substituent filling unoccupied space deep within the enzyme pocket, as well as a potential effect on the orientation of the substituent filling unoccupied space deep within the enzyme pocket, as well as a potential effect on the orientation of the enzyme, while the quinoline nitrogen forms an interaction with the hinge (Val882).

Compound 1 shows excellent selectivity over protein kinases (>10,000-fold vs >240 kinases evaluated) with the notable exception of the class IV PI3K family. mTOR, a class IV PI3K protein kinase, is a central regulator of cell growth and exists in two functional complexes, mTORC1 and mTORC2. mTORC2 is proposed to regulate AKT S473 phosphorylation, and its inhibition is believed to augment the antiproliferative efficacy of a PI3K inhibitor by dual inhibition of the PI3K/AKT pathway. The kinase domain of mTOR is homologous to the p110α catalytic subunit of the class I PI3Ks. and is a potent inhibitor of both mTOR complexes with subnanomolar activity (Table 4). Compound 1 is also a potent inhibitor of the class IV PI3 kinase, DNA-PK (IC50 = 0.28 nM).

In mechanistic cellular assays, 1 caused a significant reduction in the levels of pAKT-S473 with remarkable potency (Table 5). Consistent with its activity against both PI3Kα and mTOR, 1 also inhibits phosphorylation of AKT-T308 and p70S6K at low nanomolar concentrations (data not shown). Compound 1 induces a G1 cell cycle arrest and inhibits cell proliferation in a large panel of cell lines, including T47D and BT474 breast cancer lines.

The PK profile of 1 was studied in four preclinical species (mouse, rat, dog, and monkey). The compound showed low blood clearance and good oral bioavailability (Table 6). In addition, 1 had minimal potential to inhibit the human cytochrome P450 isoforms (IC50 > 25 μM vs CYPs 3A4, 1A2, 2C9, 2C19, and 2D6).

In an in vivo setting, 1 exhibited a dose-dependent reduction in pAKT-S473 levels in human BT474 tumors implanted in mice. In the study, designed to measure the magnitude...
Table 6. Preclinical PK Profile of 1<sup>4</sup>

<table>
<thead>
<tr>
<th>species</th>
<th>C&lt;sub&gt;L&lt;/sub&gt;</th>
<th>V&lt;sub&gt;dss&lt;/sub&gt;</th>
<th>T&lt;sub&gt;1/2&lt;/sub&gt;</th>
<th>DNAUC</th>
<th>% F</th>
</tr>
</thead>
<tbody>
<tr>
<td>mouse</td>
<td>10</td>
<td>1.0</td>
<td>2.1</td>
<td>1100</td>
<td>100</td>
</tr>
<tr>
<td>rat</td>
<td>2.3</td>
<td>1.1</td>
<td>6.2</td>
<td>6100</td>
<td>81</td>
</tr>
<tr>
<td>dog</td>
<td>5.8</td>
<td>0.7</td>
<td>1.3</td>
<td>2400</td>
<td>80</td>
</tr>
<tr>
<td>monkey</td>
<td>3.6</td>
<td>0.8</td>
<td>3.5</td>
<td>2300</td>
<td>49</td>
</tr>
</tbody>
</table>

<sup>4</sup> Units: C<sub>L</sub> mL min<sup>-1</sup> kg<sup>-1</sup>, V<sub>dss</sub> L kg<sup>-1</sup>, T<sub>1/2</sub> h; and DNAUC, ng h mL<sup>-1</sup> mg<sup>-1</sup> kg<sup>-1</sup>.

Figure 4. PD effect of 1 in BT474 human tumor xenografts following a single 300 μg kg<sup>-1</sup> oral dose. The ratio of pAKT/total AKT was measured and compared to control. [Blood], concentration of drug in the blood in ng mL<sup>-1</sup>; [NQ], not quantifiable.

Figure 5. Tumor growth efficacy of 1 in BT474 human tumor xenografts as measured by median tumor volume (cu mm). Mice were dosed once daily for 5 days/week (M→F) for 3 weeks (days 17–21, 24–28, and 31–35). Error bars denote the standard error of the mean (SEM).

**SUPPORTING INFORMATION AVAILABLE** Biological assays, biological data, and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

**Accession Codes:** The coordinates for 1 and 6d have been deposited with the RCSB Protein Data Bank under the accession codes 3L08 (1) and 3L54 (6d).

**AUTHOR INFORMATION**

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**ABBREVIATIONS** PI3K, phosphoinositide 3-kinase; mTOR, mammalian target of rapamycin; PTEN, phosphatase and tensin homologue; DNAUC, dose-normalized area under the curve.

**REFERENCES**


(24) Spectroscopic determination of the pKs of 1 was done using Sirius GlypKA-D-PAS.

