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Discovery of novel hedgehog antagonists from cell-based screening: Isosteric modification of p38 bisamides as potent inhibitors of SMO

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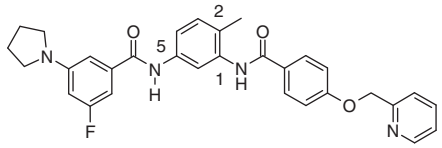
ABSTRACT

Cell-based subset screening of compounds using a Gli transcription factor reporter cell assay and shh stimulated cell differentiation assay identified a series of bisamide compounds as hedgehog pathway inhibitors with good potency. Using a ligand-based optimization strategy, heteroaryl groups were utilized as conformationally restricted amide isosteres replacing one of the amides which significantly increased their potency against SMO and the hedgehog pathway while decreasing activity against p38α kinase. We report herein the identification of advanced lead compounds such as imidazole **11c** and **11f** encompassing good p38α selectivity, low nanomolar potency in both cell assays, excellent physiochemical properties and in vivo pharmacokinetics.

The Hedgehog pathway affects numerous biological processes, such as cell differentiation and proliferation, and particularly embryogenesis, where the pathway regulates embryo patterning. During embryogenesis, Hedgehog pathway typically is activated by the secreted Hedgehog ligands, which directly bind to their receptor protein, 12-pass transmembrane protein Patched1 (Ptch1),[1] and inhibit its repression of the G protein-coupled receptor-like protein Smoothened (SMO).[2] Recently it has been reported that primary cilia plays a pivotal role in Hedgehog pathway.[3] Under basal conditions, Ptch1 is localized to the primary cilium and SMO is sequestered in endosomes;[4] Hedgehog ligands induce Ptch1 movement out of and consequently SMO trafficking into primary cilium. The pathway activation ultimately leads to the modulation of Gli zinc-finger transcription factors, permitting transcription activation of Hedgehog-responsive genes whose expression is crucial for tissue patterning, growth and differentiation and tissue homeostasis.

The linkage of the Hedgehog pathway to diseases, such as cancer, is established, and a number of different oncogenetic mechanisms related to the Hedgehog pathway have been identified. Autocrine or paracrine Hedgehog signaling was found in certain neoplasms, such as small-cell lung cancers and pancreatic adenocarcinomas.[5] Ligand-independent Hedgehog target gene expression can also lead to tumorigenesis, exemplified by Gorlin's syndrome patients who are heterozygous for Ptch1mutations and susceptible to basal cell carcinomas, medulloblastomas, and rhabdomyosarcomas.[6] Oncogenic mutations in SMO and Su(fu) have also been identified.[7] Pharmacological inhibitors of the Hedgehog pathway should have great therapeutic value. Particularly, the SMO antagonist cyclopamine is known to block tumor progression in a variety of mouse cancer models.[8] More recently, known SMO antagonist Vismodegib (GDC-0449) demonstrated good response rate in basal cell carcinoma patients and has since been approved by FDA to treat metastatic basal cell carcinoma and locally advanced basal cell carcinoma.[9]

To identify Hedgehog pathway inhibitors, we screened a subset of over 40,000 compounds against a cell-based Gli-Luciferase assay[10] and a selection of active compounds from the Gli-Luciferase assay in a C3H10T1/2 Hedgehog-dependent differentiation assay.[11] A series of bisamide compounds, including compound **1a** (Fig. 1), were among the initial hits identified. In both Hedgehog pathway assays, this series of compounds offered attractive potency and



1a

Figure 1. Structure of a bisamide hit identified from the cell-based Gli-Luciferase assay.

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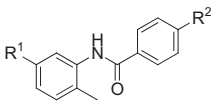
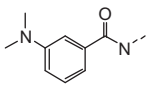
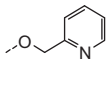
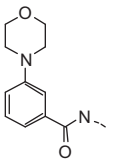
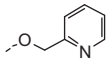
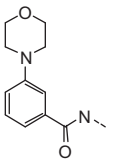
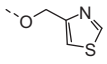
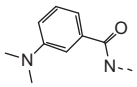
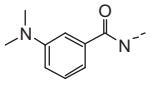
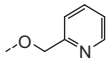
Table 1
Activities of bisamide compounds against Hedgehog pathway and p38α



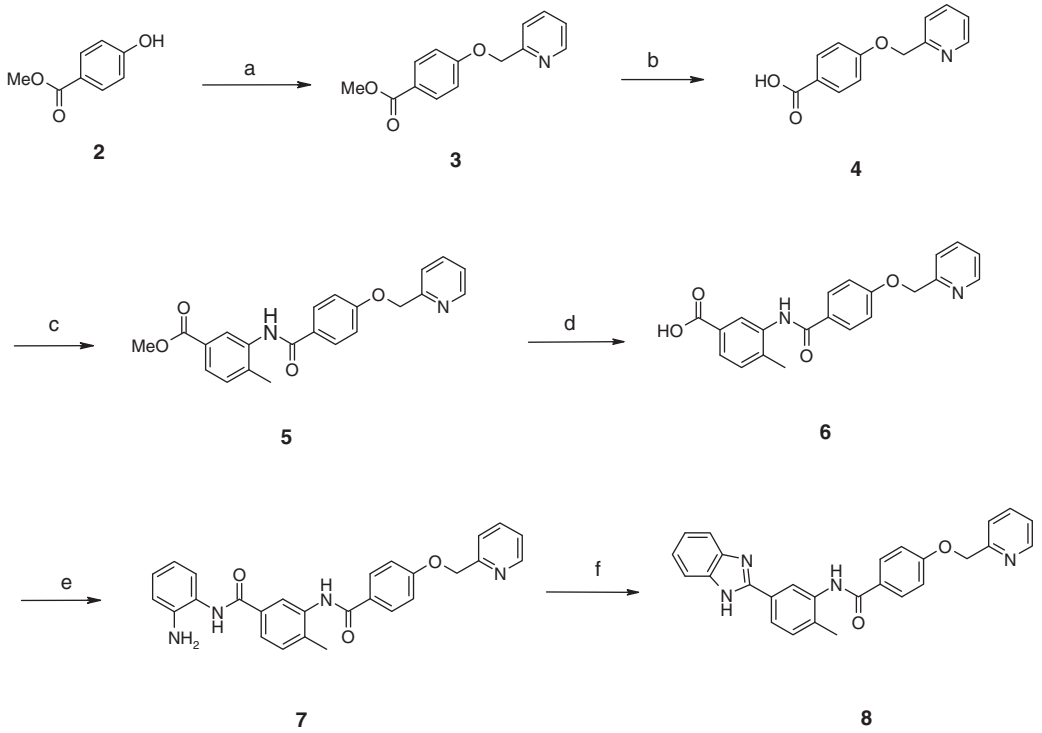
	R^1	R^2	Firefly EC_{50} (μM)	p38α Enz IC_{50} (μM)	Solubility (μM)	Hu PPB (% free)
1a			0.45	0.025	<1	<1
1b			0.45	0.03	1.4	3.6
1c			>3	0.017	<1	2
1d			3.2	0.166	<1	<1
1e			1.1	0.051	<1	<1

preliminary SAR. Herein we report our lead optimization of this series to improve its potency and selectivity against p38-type kinases.

The synthesis of the bisamide compounds (e.g., **1a**) was reported previously.[12] Compound **1a** offered considerable potency in the firefly reporter assay against the Hh pathway with IC_{50} = 0.45 μM, and it appeared to be selective over the renilla firefly assay[13] (IC_{50} >30 μM). The biophysical properties of this series are poor in general; aqueous solubility of compound **1a** is ~1 μM and its strong protein binding in plasma gave smaller than 1% free fraction. In addition, one of the main potential concerns as a Hedgehog pathway inhibitor is that the bisamide series are known as potent p38 MAP kinases inhibitors (p38α IC_{50} = 0.025 μM for compound **1a**). The initial screening results in the firefly reporter assay for this series indicated a wide tolerance of various functionalities on the phenyl ether side of the molecule, but those structural variations did not meaningfully attenuate p38α activity. The data for a selection of compounds and their activities in Hh pathway firefly reporter and p38α kinase assays are summarized in Table 1.

It is noteworthy that the 2-pyridyl group on the right side of the molecule appeared to be optimal for Hh pathway inhibition, and other heteroaryls or alkyl groups reduced potency. Further installation of solubilizing basic amine groups on the right hand side of the molecule failed to attenuate their activities on p38 kinase, and it further reduced their potency on Hh pathway inhibition. Some improvement on solubility was observed, although with limited significance (data not shown).

Structural optimization efforts focused on the modifications of C-5 substitution on the middle phenyl group. It was reasoned that reducing the molecular weight from the left hand side of molecule could potentially improve both potency and the physical properties of this series of compounds.



Scheme 1. Synthesis of benzimidazole amide compound **8**. Reagents and conditions: (a) 2-(bromomethyl)pyridine, K_2CO_3, acetonitrile/water (80%); (b) NaOH (1 N), MeOH, reflux (90%); (c) 3-amino-4-methylbenzoic acid methyl ester, HATU, DIPEA, DMF (99%); (d) NaOH (1 N), MeOH, reflux (91%); (e) 1,2-diaminobenzene, HATU, DIPEA, DMF (93%); (f) AcOH, reflux (99%).