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Scaffold hopping approach to a new series of smoothened antagonists

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ABSTRACT

The hedgehog (Hh) signaling pathway is a key regulator during embryonic development, while in adults, it has limited functions such as stem cell maintenance and tissue repair. The aberrant activity of the Hh signaling in adults has been linked to numerous human cancers. Inhibition of Hh signaling therefore represents a promising approach toward novel anticancer therapies. The Smoothened (Smo) receptor mediates Hh signaling. Here we report a new series of Smo antagonists which were obtained by a scaffold hopping strategy. Compounds from this new scaffold demonstrated decent inhibition of Hh pathway signaling. The new scaffold can serve as a starting point for further optimization.

The hedgehog (Hh) signaling pathway is a key developmental pathway which regulates patterning, growth and cell migration during embryonic development, but in adults it is limited to tissue maintenance and repair. Under normal conditions, the endogenous ligands sonic hedgehog, Indian hedgehog and desert hedgehog bind to their receptor Patched (Ptch), relieving the inhibitory effect of Ptch on Smoothened (Smo). Smo activation triggers a series of events which ultimately lead to specific gene expression mediated by the Gli family transcription factors.[1] Aberrant Hh signaling has been linked to numerous human cancers. Mutational inactivation of the inhibitory pathway components such as Ptch leads to constitutive ligand-independent activation of the Hh signaling pathway, resulting in cancers such as basal cell carcinoma and medulloblastoma.[2] Ligand-dependent activation of Hh signaling is involved in prostate cancer, pancreatic cancer, breast cancer and some blood cancers.[3] Therefore, inhibition of aberrant Hh signaling represents a promising approach toward novel anticancer therapy.[4]

Cyclopamine (Fig. 1), a naturally occurring alkaloid, was the first reported Hh signaling pathway inhibitor,[5] and it was later identified as a Smo antagonist.[6] A cyclopamine derivative, IPI-926 (Fig. 1), which demonstrated better potency, stability and other pharmaceutical properties than cyclopamine, had entered clinical development.[7] Numerous synthetic Smo antagonists had been reported in recent years.[8] The most advanced in the class, GDC-0449 (Vismodegib, Fig. 1), was approved by FDA in January 2012 for the treatment of basal cell carcinoma which was not suitable for operation.[9] This approval showcased the first embryonic pathway inhibitor for the treatment of human cancer. Other Smo antagonists are in different stages of development (Fig. 1).

We have investigated numerous templates in pursuit of novel Smo receptor antagonists. Both GDC-0449[8c] and NVP-LDE225[8h] were the results of optimization campaigns based on initial screening hits. The central amide bonds were reversed, yet both molecules maintained excellent potency. This observation indicated that the central amides were place-holders, unlikely to have participated in significant interactions with the Smo receptor. This assumption was reinforced by Pfizer compound **1**[10] (Fig. 2) and a series of Amgen compounds.[8g] GDC-0449 possessed all sp2-hybridized carbons, resulting in a high melting point (264 °C) and poor solubility (9.5 μg/mL). Enhanced solubility was achieved by adding an *ortho*-chloro group to the right side ring to introduce tilt and reduce planarity of the aryl amide.[8c] It is well documented in the literature that planarity and molecular topology have significant impacts on absorption, metabolism and toxicity.[11] In order to improve the physical–chemical properties, we proposed introducing the saturation ring as the place-holder (introducing sp3-hybridized carbons, reducing planarity), as featured in compound **1**. We also proposed tying the long tail featured

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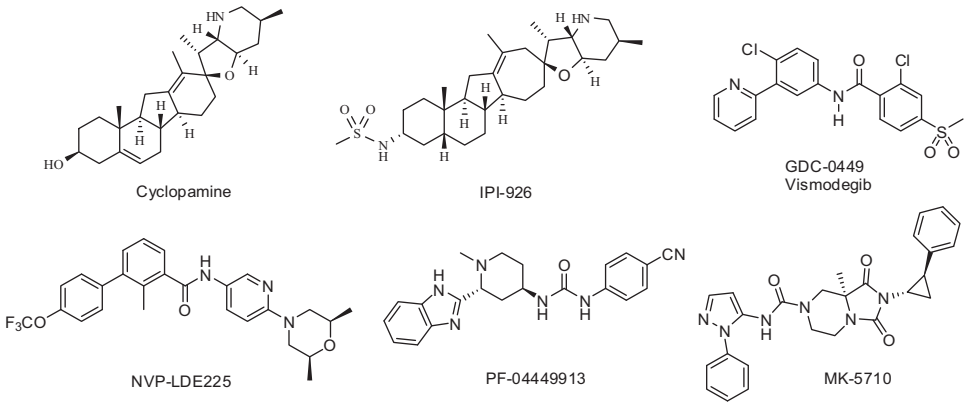


Figure 1. Smoothened antagonists in advanced development.

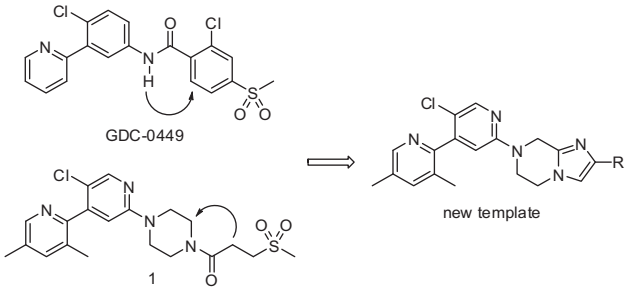
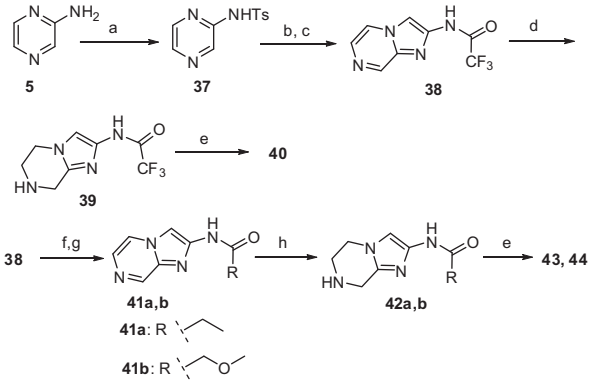


Figure 2. Scaffold hopping approach to a novel series of smoothened antagonists.

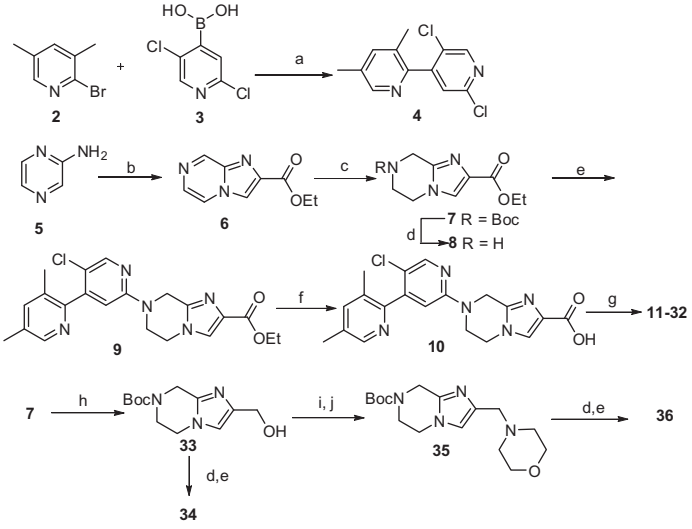
in compound **1** to decrease rotatable bonds as a way to enhance absorption and metabolic stability.[12] The scaffold hopping strategy led us to a novel template shown in Figure 2.

The general synthetic route used to prepare compounds **9**–**36** was shown in Scheme 1. Commercially available pyrazin-2-amine **5** was reacted with ethyl 3-bromo-2-oxopropanoate in DME at room temperature and then refluxed in EtOH to give intermediate **6**. Hydrogenation of intermediate **6** under Pd/C with Boc_2O/EtOH provided intermediate **7**. Removal of the Boc-protecting group of



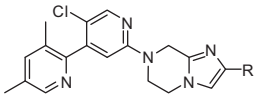
Scheme 2. Reagents and conditions: (a) TsCl, pyridine, rt, 80 min, 72%; (b) 2-iodoacetamide, DMF, rt, 28 h, 64%; (c) trifluoroacetic anhydride, DCM, reflux, 2 h, 97%; (d) 10% Pd/C, H_2, MeOH, rt, 10 h, 25%; (e) Compound **4**, CsF, DMSO, 120 °C, 48 h, 18–26%; (f) NH_4OH, MeOH, 70 °C, 6 h, 44%; (g) corresponding acid, HATU, DIPEA, DMF, rt, 24 h, 44–52%; (h) PtO_2, H_2, MeOH, rt, 48 h, 73%.

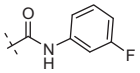
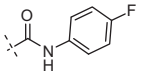
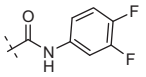
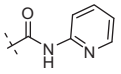
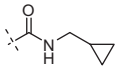
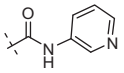
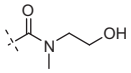
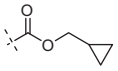
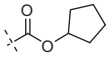
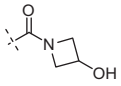
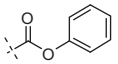
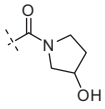
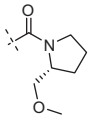
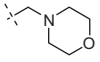
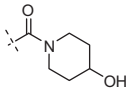
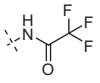
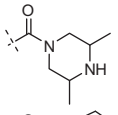
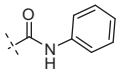
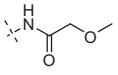
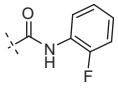
intermediate **7** in the presence of HCl/EtOAc led to intermediate **8**, which was used to displace the 2-position chlorine of bipyridine **4** to yield ester **9**. Compound **4** was obtained by Suzuki coupling of



Scheme 1. Reagents and conditions: (a) $Pd(dppf)Cl_2$, $K_3PO_4{\cdot}3H_2O$, dioxane, H_2O, 110 °C, 16 h, 41%; (b) ethyl 3-bromo-2-oxopropanoate, DME, rt, 2.5 h, then EtOH, reflux, 2.5 h, 27%; (c) 10% Pd/C, EtOH, Boc_2O, H_2, 24 h, 63%; (d) HCl/EtOAc, rt, 3 h, 83–95%; (e) compound **4**, CsF, DMSO, 120 °C, 48 h, for **9**, 85%; for **34**, 16%; (f) LiOH, THF, H_2O, rt, 12 h, 70%; (g) HATU, DIPEA, DMF, rt, 16 h, for **11**–**28**, 26–72%; alcohol, $SOCl_2$, reflux for **29** (45%) and **30** (36%); DCC, HOBt, DMAP, DCM, 6–36 h for **31** (25%) and **32** (66%); (h) $LiAlH_4$, THF, 0 °C, 30 min, 70%; (i) MsCl, TEA, DCM, rt, 30 min; (j) morpholine, Na_2CO_3, ACN, 100 °C, 2 h, two steps, 43%.

Table 1
SAR of designed compounds



Compound	R	hSMO IC_{50}[a] (μM) ± SEM	Compound	R	hSMO IC_{50}[a] (μM) ± SEM
9		0.69 ± 0.13	**24**		0.087 ± 0.016
10		>10	**25**		0.44 ± 0.02
11		2.7 ± 1.4	**26**		0.42 ± 0.16
12		1.3 ± 0.09	**27**		0.27 ± 0.04
13		0.52 ± 0.03	**28**		0.20 ± 0.07
14		4.1 ± 2.1	**29**		0.38 ± 0.21
15		0.27 ± 0.03	**30**		0.27 ± 0.17
16		0.79 ± 0.36	**31**		0.74 ± 0.46
17		>10	**32**		0.96 ± 0.14
18		>10	**34**		4.1 ± 0.39
19		0.55 ± 0.06	**36**		0.91 ± 0.23
20		1.2 ± 0.46	**40**		1.8 ± 0.70
21		1.5 ± 0.70	**43**		0.89 ± 0.03
22		0.30 ± 0.01	**44**		0.65 ± 0.10
23		0.35 ± 0.16		Vismodegib	0.023 ± 0.016[b]

[a] Inhibition of luminescence signaling in NIH3T3-GRE-Luc reporter gene assay with 10 nM SAG as the Hh pathway agonist. Data are expressed as geometric mean values of at least two runs ± the standard error measurement (SEM).
[b] Vismodegib was run as standard in each assay. Data are expressed as geometric mean values of seven runs ± the standard error measurement (SEM).

2-bromo-3,5-dimethyl pyridine and 2,5-dichloropyridin-4-ylboronic acid with $Pd(dppf)Cl_2$ and $K_3PO_{40}{\cdot}3H_2O$ in dioxane and water. Hydrolysis of ester **9** in the presence of $LiOH/THF/H_2O$ yielded acid **10**, which was coupled with corresponding amines or alcohols to provide amides **11**–**28** or esters **29**–**32**, respectively. In order to obtain compounds **34** and **36**, intermediate **7** was selectively reduced with $LiAlH_4$ to give alcohol **33**, which was de-protected and then used to substitute 2-position chlorine of bipyridine **4** under CsF/DMSO at 120 °C to provide compound **34**. The alcohol **33** was mesylated and then substituted with

morpholine to give intermediate **35**, which was de-protected and then used to substitute 2-position chlorine of bipyridine **4** to provide compound **36**.

Synthesis of compounds **40**, **43** and **44** was described in Scheme 2. The key intermediate **38** was prepared via a three-step reaction, starting from the tosylation of commercially available pyrazin-2-amine **5** with TsCl to yield intermediate **37**, which was then reacted with 2-iodoacetamide, followed by cyclization with trifluoroacetic anhydride in DCM at reflux temperature. Hydrogenation of intermediate **38** in the presence of Pd/C/MeOH led to intermediate **39**, which was used to substitute the 2-position chlorine of intermediate **4** under CsF/DMSO at 120 °C to yield compound **40**. Hydrolysis of intermediate **38** in the presence of NH_4OH/MeOH followed by coupling with acids provided amides **41a** and **41b**. Hydrogenation of amides **41a** and **41b** in the presence of PtO_2 with MeOH yielded intermediates **42a** and **42b**, which were used to displace the 2-position chlorine of bipyridine **4** under CsF/DMSO at 120 °C to yield compounds **43** and **44**.

The SAR was summarized in Table 1.

The inhibitory effects of the synthesized compounds on the Hh signaling pathway were tested using a cell based NIH3T3-GRE-Luc reporter gene assay with 10 nM SAG as the Hh pathway agonist.[8a,13] The simplest compound of this series of analogues, compound **10**, was virtually inactive (>10 μM). This was not unexpected since the carboxylic acid was a dramatic move away from the usual SAR. Simple amides **11**, **12** and **13** demonstrated moderate to decent potencies (2.7, 1.3, 0.52 μM, respectively). Addition of a polar hydroxyl and capping the NH of the amide did not improve activity (compound **14**, 4.1 μM). However, when secondary amine pyrrolidine was used to form the amide, a compound with respectful activity was achieved (compound **15**, 0.27 μM). Attempts to further improve the activity by applying larger amines or amines with polar functional groups were not successful as compounds **16**–**21** were not as potent as compound **15**. Ethyl ester analogue **9** demonstrated decent potency (0.69 μM), which were further improved when the ethyl was changed to isopropyl and methylcyclopropyl (compound **29** and **30**, 0.38 and 0.27 μM, respectively). Unfortunately, the trend did not hold as larger esters **31** and **32** were less potent (0.74 and 0.96 μM, respectively). Attempts to increase the flexibility of this tail region were made by reducing the amide carbonyl to methylene, but the increased flexibility failed to yield improved potency as both compounds **34** and **36** were less active. Through a different synthetic approach, the 'reversed' amide template was achieved (compounds **40**, **43** and **44**). Trifluoacetamide **40** was moderately active (1.8 μM), while compounds **43** and **44** demonstrated decent activity (0.89 μM, 0.65 μM, respectively). When simple aniline was applied to form the carboxamide, a compound with respectful activity was obtained (compound **22**, 0.30 μM). Addition of an *ortho*-fluorine or a *para*-fluorine had no significant impact on the activity (compounds **23** and **25**, 0.35 and 0.44 μM, respectively), however, addition of a meta-fluorine yielded the most potent analogue (compound **24**, 0.087 μM), which demonstrated activity within reachable range of Vismodegib. Di-substituted fluorine analogue did not help with activity improvement (compound **26**, 0.42 μM). Hetero atoms were incorporated into aniline and both resulting amino-pyridine analogues demonstrated respectful activity (compounds **27** and **28**, 0.27 and 0.20 μM, respectively). Overall, this novel scaffold designed from a template hopping strategy provided numerous active compounds (e.g., **15**, **24**, **28** and **30**, 0.27 μM, 0.087 μM, 0.20 μM and 0.27 μM, respectively). Although the potency was not yet comparable to that of the marketed drug Vismodegib (0.026 μM), these results were nonetheless encouraging and worthy of further investigation in light of the large structural changes involved. Exploration of other regions of this novel template is ongoing in our lab and the results will be reported in due course.

In summary we have utilized a scaffold hopping strategy to design and synthesize a new series of Smo inhibitors. Compounds from this new scaffold demonstrated decent inhibition of hedgehog pathway signaling. The new scaffold can serve as a starting point for further optimization.

Acknowledgments

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