Discovery of Small-Molecule Modulators of the Sonic Hedgehog Pathway

Giannina I. Schaefer,[†,‡] José R. Perez,[‡] Jeremy R. Duvall,[‡] Benjamin Z. Stanton,[†,‡,§] Alykhan F. Shamji,[‡] and Stuart L. Schreiber*,[†,‡]

[†]Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138, United States

[‡]Broad Institute of Harvard and MIT, 7 Cambridge Center, Cambridge, Massachusetts 02142, United States

Ⓢ Supporting Information

ABSTRACT: The Hedgehog signaling pathway is involved in the development of multicellular organisms and, when deregulated, can contribute to certain cancers, among other diseases. The molecular characterization of the pathway, which has been enabled by small-molecule probes targeting its components, remains incomplete. Here, we report the discovery of two potent, small-molecule inhibitors of the Sonic Hedgehog (Shh) pathway,

BRD50837 and BRD9526. Both compounds exhibit stereochemistry-based structure−activity relationships, a feature suggestive of a specific and selective interaction of the compounds with as-yet-unknown cellular target(s) and made possible by the strategy used to synthesize them as members of a stereochemically and skeletally diverse screening collection. The mechanism-of-action of these compounds in some ways shares similarities to that of cyclopamine, a commonly used pathway inhibitor. Yet, in other ways their mechanism-of-action is strikingly distinct. We hope that these novel compounds will be useful probes of this complex signaling pathway.
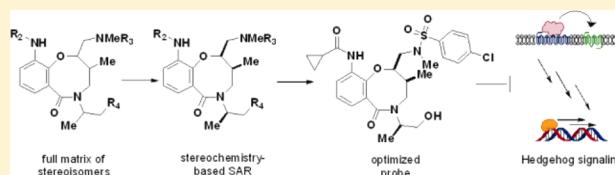
INTRODUCTION

The Hedgehog (Hh) signaling pathway plays an important role in embryonic development and the overall growth and morphology of insects and vertebrates.[1,2] Improper Hh signaling can result in developmental diseases, such as holoprosencephaly.[3] Somatic genomic alterations in genes encoding members of the pathway drive the development and maintenance of several cancers, especially basal cell carcinoma (BCC) and medulloblastoma.[4−7]

The pathway becomes activated when an extracellular secreted protein from the Hh family, most commonly Sonic Hedgehog (Shh), binds patched (Ptch), a transmembrane receptor. In the absence of this binding, Ptch represses the G-protein coupled transmembrane receptor, smoothened (Smo). Formation of the Shh/Ptch complex in some still unknown way derepresses Smo, causing its translocation to the primary cilium where it influences the state of the transcription regulator Gli. Smo enables a release of Gli from a repressor complex comprising Gli and, among others, suppressor of fused (SuFu). The resulting activated form of Gli translocates to the nucleus and activates genes involved in cell proliferation and differentiation.[4,8,9]

Several small-molecule modulators of this complex pathway have been discovered, with many acting on Smo directly. Prominent examples are cyclopamine (a natural product found in *Veratrum Californicum*) and vismodegib (an FDA-approved drug for the treatment of BCC).[10−12] Other inhibitors have been reported to act on Shh (robotnikinin),[13] modulate the

motor protein dynein (ciliobrevin A),[14] or disrupt DNA−Gli interactions (GANT-61).[15]

In addition to this 'canonical' Hh signaling, Hh proteins also promote 'noncanonical' signaling that is Gli-independent.[16−18] Further complexities are evidenced by the findings that different small-molecule inhibitors of Smo can result in different cellular outcomes. For example, vismodegib prevents Smo translocation to the primary cilium, while cyclopamine promotes Smo accumulation in the primary cilium.[19,20]

To enhance our molecular understanding of the pathway, we aimed to discover novel small-molecule probes of Hh signaling. We first performed a cell-based high-throughput screen for novel inhibitors of Gli-induced transcription. We discovered a group of small molecules having compelling stereochemistry-based structure−activity relationships (SAR), which we interpret as indirect evidence for a selective interaction with cellular target(s). Synthetic chemistry to generate analogs resulted in the elucidation of additional building block-based SAR and characterization of the novel Shh pathway inhibitors BRD50837 and BRD9526 with a mechanism-of-action distinct from cyclopamine.

RESULTS AND DISCUSSION

We first screened 21 753 compounds in a cell-based assay using Shh light II cells. These cells are derived from NIH/3T3 cells by cotransfection with a Gli-responsive Firefly luciferase
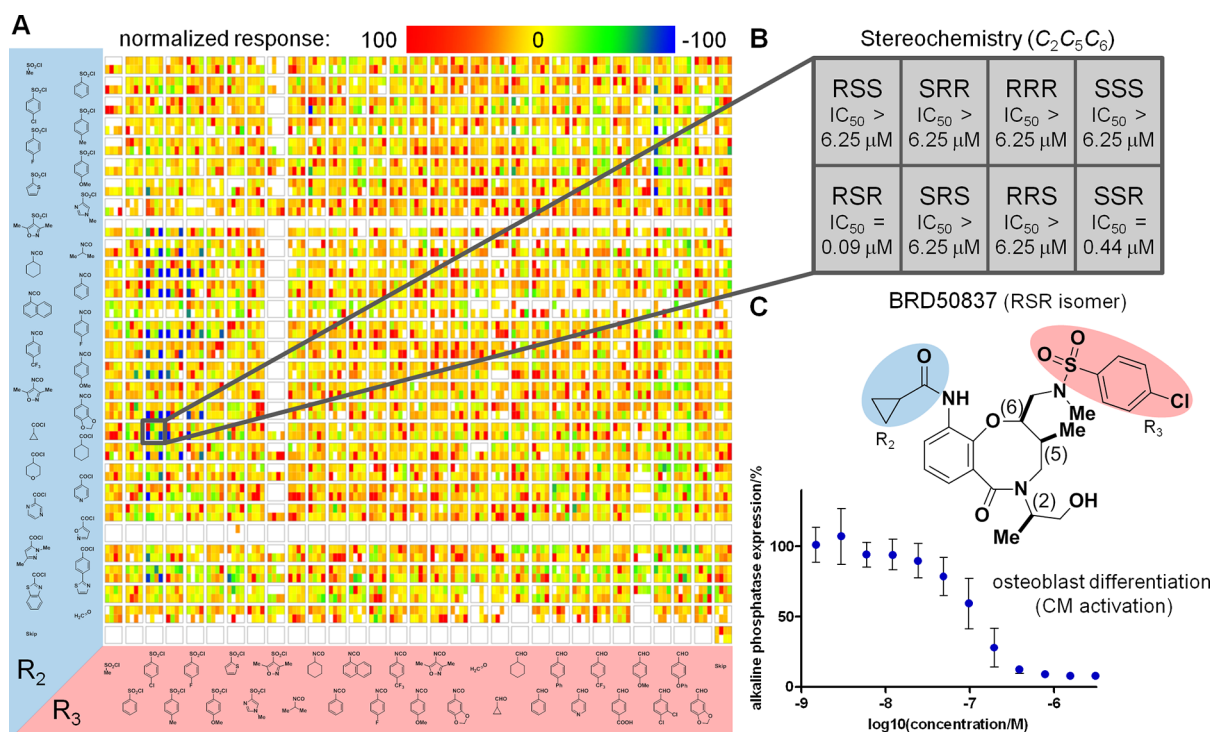
Figure 1. (a) Primary screening data are displayed as percent luciferase activity in Shh light II cells. Each small block displays a heat map associated with eight stereoisomeric compounds having the same skeleton and appendages. The overall panel A displays a near-complete matrix of multiple skeletons (only one shown in C) and building blocks used for R_2 and R_3 (displayed on the left (y-axis) and bottom (x-axis)). In the heat maps, blue represents inhibition (−100), yellow represents no activity (0), and red represents activation (100) normalized to DMSO control. Empty cells represent compounds not tested. Values shown are from testing compounds twice in single dose. (b) The highlighted block represents the dose−response data of eight stereoisomers of a primary subject of this report (BRD50837). (c) Dose−response curve of BRD50837 in C3H10T1/2 cell differentiation assay and structure of BRD50837 highlighting positions of building block attachment. All values shown are generated from three independent experiments run in duplicate (values are calculated average ± SD).

reporter.[10,21] All compounds were screened in duplicate at a single concentration. Screening positives (mean inhibition ≥65%) were retested in dose, and their toxicity was assessed using CellTiter-Glo to measure cellular adenosine triphosphate (ATP) levels as a surrogate for viability (Figure S1a-b). A total of 390 hits were identified and advanced for further investigation.

Both the primary screen and multiple dose-retest data revealed a striking correlation between activity and stereochemistry of members of a library of the screening collection. These compounds were initially synthesized using the build/couple/pair strategy of diversity-oriented synthesis (DOS).[22,23] As a consequence, all possible stereoisomers of each structural type are included in the collection. The compounds in the library screened include ~6700 compounds with varying eight-membered rings that are formed by nucleophilic aromatic substitution reactions. Based on the primary screening data, two of the eight stereoisomers of several compounds having the same eight-membered ring skeleton were active, the RSR and the SSR isomers, with the sole difference being the configuration of the extra-annular methyl group (Figure 1). These initial findings were confirmed in a second biological assay that measures Hh-induced differentiation of C3H10T1/2 cells into osteoblasts.[24] Retesting all eight stereoisomers of several hit compounds in dose using Shh-conditioned medium-induced C3H10T1/2 cells confirmed that BRD50837 selectively blocks the Hh pathway in cells (Figures 1b and S1c).

All hits were tested in the secondary differentiation assay using C3H10T1/2 cells to substantiate their on-pathway

activity (Tables 1 and S1). Additionally, to rule out gross toxicity as source of signal, all compounds were tested in a viability assay using CellTiter-Glo as a means to estimate cellular levels of ATP (Table S1). BRD50837 displayed high potency with an EC_{50} of 0.09 μM. A PubChem search of other assays wherein BRD50837 (CID 44499307) was screened revealed that, as of May 27, 2013, BRD50837 had been tested in 31 different assays but only scored in our initial screen, suggesting that it is not broadly active. Compared to other similarly potent compounds, BRD50837 showed good phosphate buffered saline (PBS) solubility (64.3 μM) and was thus chosen as a starting point for further experimentation.

To elucidate additional building block-based SAR, we synthesized novel analogs, varying the attachments on the aniline and the extra-annular amine as well as removing the extra-annular alcohol. BRD50837 (7) and additional novel analogs 8−19 were synthesized using an abbreviated synthetic pathway relative to the previously reported solid-phase synthesis (Schemes 1 and S1). For the synthesis of 7−16, intermediate 4a was synthesized from 1 and 2a as previously reported.[22,23] Subsequently, the nitrobenzene was reduced to an aniline that was acylated with acyl chlorides to yield anilides 5. After deprotection of the extra-annular nitrogen with hydrogen fluoride (HF)/pyridine and addition of a sulfonyl chloride, the para-methoxybenzyl ether (PMB) group was removed with 2,3-dichloro-5,6-dicyanobenzo-quinone (DDQ), resulting in the final compounds.

To synthesize analog 18, 2b was used instead of 2a as a starting material, and the final PMB deprotection step was

Table 1. EC$_{50}$ in C3H10T1/2 Cells and PBS Solubility of Analogs of BRD50837

compound	R$_2$	R$_3$	R$_4$	EC$_{50}$ (µM)	solubility (µM)[a]
7 (BRD 50837)			—OH	0.09	64.3
8		"	"	0.22	-
9		"	"	0.29	-
10		"	"	0.91	-
12		"	"	0.08	1.1
20		"	"	0.03	5.5
21		"	"	0.08	3.9
18		"	—H	4.03	-
13	"		—OH	0.95	-
14	"		"	1.45	-
15 (BRD 9526)	"		"	0.06	57.4
17	"		"	3.37	-
22	"		"	0.58	-
23	"		"	0.45	-
24	"		"	2.86	-

[a]Solubility was measured for compounds that were considered for subsequent experimentation based on their EC$_{50}$.

omitted. Analog **17** was synthesized by preparing intermediate **5a** as before, removing the PMB group with DDQ, deprotecting the *tert*-butoxycarbonyl (Boc) group with

Scheme 1[a]
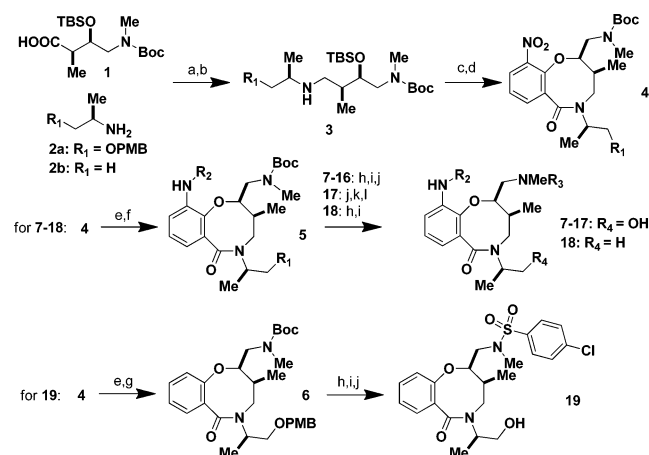
[a]Reagents and conditions: (a) PyBOP, DIPEA, DCM, rt, 52−83%. (b) borane dimethyl sulfide complex, THF, 65 °C, 88−89%. (c) 2-fluoro-3-nitrobenzoic acid chloride, NEt$_3$, DCM, rt, 92−100%. (d) CsF, DMF, 85 °C, 100%. (e) 10% Pd/C, H$_2$, EtOH, 35 °C, 100%. (f) R$_2$Cl, 2,6-lutidine, DCM, rt. (g) NaNO$_2$, NaHSO$_3$, EtOH/AcOH, rt, 48%. (h) TBSOTf, 2,6-lutidine, DCM, rt, then HF/pyridine, THF, rt. (i) R$_3$Cl, 2,6-lutidine, DCM, 0 °C, 68% over 3 steps (**18**). (j) DDQ, pH 7 buffer/DCM, rt, 6−59% over 4 steps (**7**−**16**), 49% over 4 steps (**19**). (k) TFA, DCM, rt. (l) 4-chlorobenzaldehyde, sodium triacetoxyborohydride, DMF/2% AcOH, rt, 10% over 4 steps (**17**).

trifluoroacetic acid (TFA) and forming the tertiary amine **17** by reductive amination with *para*-chlorobenzaldehyde. Compound **19** was prepared by treating intermediate **4a** with sodium nitrate and sodium bisulfate, which resulted in the deaminated product **6**. Subsequent deprotection of the Boc group with HF/pyridine, addition of the sulfonyl chloride and removal of the PMB group with DDQ yielded **19**. All compounds were purified by column chromatography (30 min, 0−100% ethyl acetate in hexanes) and if necessary by HPLC purification (SI, synthetic procedures).

Both new and previously synthesized analogs were tested in C3H10T1/2 cells using Shh-conditioned medium-induced differentiation as readout of Hh signaling (Tables 1 and S1). Viability for all compounds was tested in this system as well, using CellTiter-Glo as a measure of ATP levels (Table S1). Changes on the aniline moiety of the compound were tolerated, but activity was optimal for saturated ring systems (BRD50837, **12**, **20**). The cyclopropyl derivative (BRD50837) proved to have better solubility in PBS than the cyclohexyl derivatives (**12**, **20**) making it the more favorable candidate. Complete removal of the aniline moiety (**19**, Table S1) resulted in a loss of activity. Ureas instead of amides also showed activity but were less soluble (**21**, **25**, **26**, Table S1).

Changing the sulfonamide building block from *para*-chlorobenzene sulfonyl chloride to *ortho*- or *meta*-chlorobenzene sulfonyl chloride (**13**, **14**) resulted in reduced activity. An additional chlorine in the ortho position of the sulfonamide building block (BRD9526 (**15**)) did not impact the activity, suggesting that the chlorine in the para position is interacting with a putative cellular target, while that in the ortho position is not. This was also reinforced by the previous observation that the compound lacking the chlorine entirely had reduced activity (**24**). When testing the previously synthesized compounds it was additionally shown that the chlorine derivative is more active as compared to the fluorine and methyl derivatives (**22**,

23). Having an additional methylene in the sulfonyl chloride also resulted in a decrease of activity (**16**, Table S1). Preparing the tertiary amine (**17**) rather than the sulfonamide resulted in a decrease of activity, demonstrating a possible electronic or spatial requirement for the sulfonamide connector. This was also reflected by additional tertiary amines tested in previous SAR studies (**27, 28**, Table S1).

Removing the extra-annular alcohol (**18**) resulted in a loss of activity, showing that the alcohol, which originally was used as a point of attachment in solid-phase synthesis, is necessary.

The original DOS pathway also yielded compounds having eight-membered rings where the aniline moiety is in the para instead of the ortho position, and nine-membered rings where the aniline moiety is also in the para position.[22] BRD50837s analogs having these structural elements were not active (**29, 30**, Table S1).

We prioritized BRD50837 and BRD9526 (**15**) for further experimentation as both displayed good EC_{50}s (Figure 2a) and
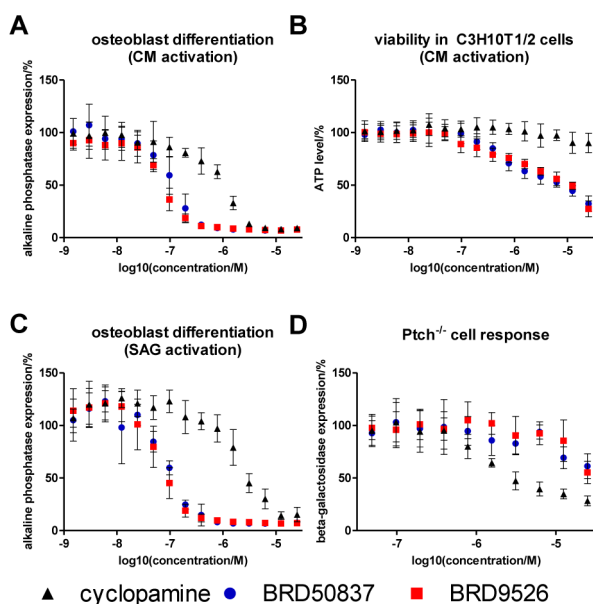

Figure 2. All values are shown and generated from three independent experiments run in duplicate (values are calculated average $\pm$ SD). (a) Inhibition of Shh-conditioned medium (CM) induced differentiation of C3H10T1/2 cells by BRD50837, BRD9526, and cyclopamine after 48 h. (b) Viability of CM-induced C3H10T1/2 cells in response to 48 h treatment by BRD50837, BRD9526, and cyclopamine. (c) Inhibition of SAG-induced differentiation of C3H10T1/2 cells by BRD50837, BRD9526, and cyclopamine after 48 h. (d) β-galactosidase expression response of Ptch$^{-/-}$ cells to 48 h treatment with BRD50837, BRD9526, and cyclopamine.

similar PBS solubility (Table 1). Neither showed significant toxicity based on measuring ATP levels as a surrogate for growth or viability (Figure 2b). Reduction of Gli1 expression in C3H10T1/2 cells by 1 μM treatments of these two compounds was confirmed (Figure S2a).

To understand the mechanisms-of-action of these compounds, we tested the response to BRD50837 and BRD9526 in several epistasis experiments and a competition assay in comparison to cyclopamine, a commonly used Smo inhibitor. We first tested the compounds in C3H10T1/2 cells that were treated with SAG (a small-molecule activator of Smo) rather than Shh-conditioned medium to activate the Hh pathway (Figure 2c).[21] Like cyclopamine, the compounds suppressed

SAG-induced differentiation, suggesting a mechanism-of-action involving modulation of a step in the signaling cascade at or following Smo signaling. In parallel, we tested the compounds in Ptch$^{-/-}$ cells, mouse embryonic fibroblasts that contain a β-galactosidase reporter gene instead of the Ptch gene after the Ptch promoter.[10] Lacking the repressor Ptch, the Hh pathway is constitutively active in these cells. In this assay cyclopamine maintained its inhibition of the pathway, but BRD50837 and BRD9526 had no effect (Figure 2d). When viewing the overall pathway as a linear set of response nodules, these results are apparently in contrast to the previous observation; they suggest that the compounds act at the level of Ptch or a step upstream of Ptch signaling. However, we also identified compounds from the original screen with responses similar to cyclopamine in Ptch$^{-/-}$ cell-based, SAG/C3H10T1/2 cell-based, and BODIPY-cyclopamine displacement assays (data not shown), which gave us confidence that the assays accurately measure compound/activity profiles.

With these puzzling results in hand, we performed two additional assays to characterize the compounds. We first tested the compounds in a competition assay to determine whether they displace BODIPY-cyclopamine in a cellular assay, thus suggesting that they bind Smo in the cyclopamine-binding site.[25] Unlike cyclopamine, both BRD50837 and BRD9526 did not lead to a reduction of BODIPY-cyclopamine binding (Figures 3a and S3), suggesting that BRD50837 and BRD9526 do not interfere with cyclopamine binding.

We next tested the activity of BRD50837 and BRD9526 in SuFu$^{-/-}$ cells. These mouse embryonic fibroblasts lack the pathway repressor SuFu, which leads to constitutively active Hh signaling.[26] It has been reported that Smo antagonists do not inhibit this signaling while the pathway inhibitor GANT-61 does.[15] In our experiments, cyclopamine partially inhibited downstream Gli1 expression (Figure 3b), perhaps due to an off-target activity observed at high concentrations.[27,28] However, another more potent Smo inhibitor (vismodegib)[11] showed no suppression of Gli1 expression, consistent with the existing model of SuFu being downstream of Smo (Figure 3b). BRD50837 and BRD9526, like cyclopamine, partially lowered Gli1 expression at concentrations of 2 and 10 μM (Figure 3b). This partial inhibition may reflect an off-target effect at high concentrations, but it is also possible that these compounds act in a way that influences the pathway at the level of or downstream of SuFu signaling.

The compounds therefore act similar to cyclopamine, a well-characterized pathway inhibitor, in some aspects (SAG/C3H10T1/2 cell-based and SuFu$^{-/-}$ cell-based assays) but seem to have a different mechanism-of-action in other aspects (Ptch$^{-/-}$ cell-based and BODIPY-cyclopamine displacement assays). These data suggest that BRD50837 and BRD9526 may function by mechanisms-of-action that are distinct from cyclopamine and not easily described by traditional linear models of the pathway. Consistent with this notion, BRD50837/BRD9526 repressed Gli1 expression in C3H10T1/2 cells to a lesser extent than cyclopamine when the compounds were tested at concentrations that yield similar responses in Shh-conditioned medium-induced differentiation of C3H10T1/2 cells (1 μM, and 10 μM, respectively, Figure S2a).

■ CONCLUSION

We report here the discovery of BRD50837 and BRD9526, two selective small-molecule inhibitors of the Shh pathway. Though
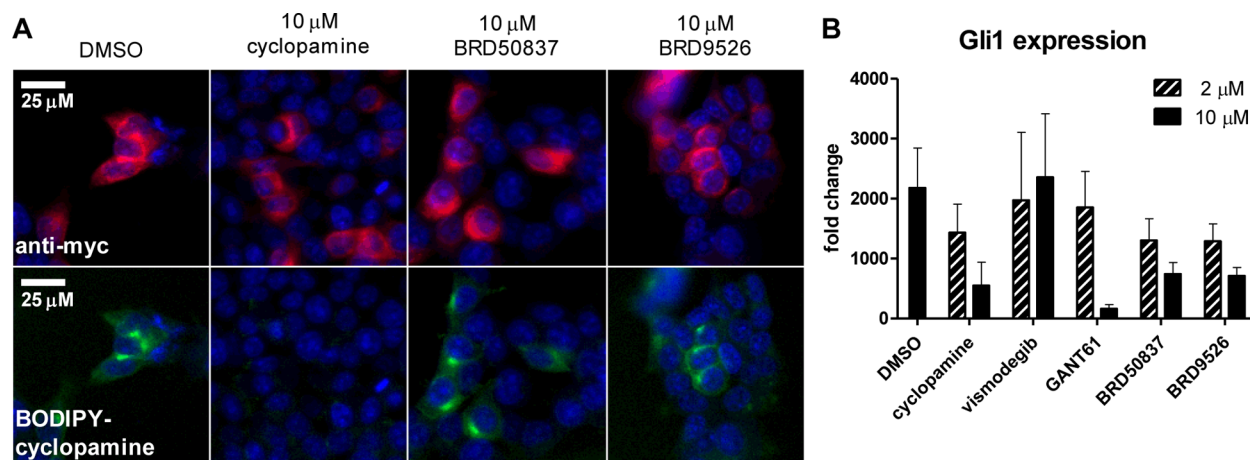
Figure 3. (a) Effects of cyclopamine, BRD50837, and BRD9526 on BODIPY-cyclopamine (10 nM) binding to exogenously expressed Smo. blue (Hoechst 33342), red (anti-myc), green (BODIPY-cyclopamine). (b) Gli1 expression in Sufu$^{-/-}$ cells treated with cyclopamine, vismodegib, GANT61, BRD50837, and BRD9526. All values are shown and generated from three independent experiments run in duplicate (values are calculated average + SD).

similar in some respects to traditional pathway inhibitors, the compounds show a distinct pattern of activity in cells perturbed for components of the pathway. The basis for these differences is not yet known, but it hints at the complexity of the pathway. Elucidating the compounds' mechanism-of-action will help to realize their full potential as probes and enable the study of this enigmatic pathway.

EXPERIMENTAL SECTION

For complete experimental information, please refer to the Supporting Information.

ASSOCIATED CONTENT

Ⓢ Supporting Information

Supporting figures, schemes, tables, materials and methods, assay protocols, synthetic procedures, data analysis, and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author
stuart_schreiber@harvard.edu

Present Address
§Howard Hughes Medical Institute, Department of Developmental Biology and Department of Pathology, Stanford University School of Medicine, Stanford, California 94305, United States.

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

REFERENCES

(1) Ingham, P. W.; McMahon, A. P. *Genes Dev.* **2001**, *15*, 3059.
(2) Chiang, C.; Litingtung, Y.; Lee, E.; Young, K. E.; Corden, J. L.; Westphal, H.; Beachy, P. A. *Nature* **1996**, *383*, 407.
(3) Roessler, E.; Belloni, E.; Gaudenz, K.; Jay, P.; Berta, P.; Scherer, S. W.; Tsui, L. C.; Muenke, M. *Nat. Genet.* **1996**, *14*, 357.
(4) Rubin, L. L.; de Sauvage, F. J. *Nat. Rev. Drug Discovery* **2006**, *5*, 1026.
(5) Thayer, S. P.; di Magliano, M. P.; Heiser, P. W.; Nielsen, C. M.; Roberts, D. J.; Lauwers, G. Y.; Qi, Y. P.; Gysin, S.; Fernandez-del Castillo, C.; Yajnik, V.; Antoniu, B.; McMahon, M.; Warshaw, A. L.; Hebrok, M. *Nature* **2003**, *425*, 851.
(6) Ng, J. M.; Curran, T. *Nat. Rev. Cancer* **2011**, *11*, 493.
(7) Teglund, S.; Toftgård, R. *Biochim. Biophys. Acta* **2010**, *1805*, 181.
(8) Peukert, S.; Miller-Moslin, K. *ChemMedChem* **2010**, *5*, 500.
(9) Robbins, D. J.; Fei, D. L.; Riobo, N. A. *Sci. Signaling* **2012**, *5*, re6.
(10) Taipale, J.; Chen, J. K.; Cooper, M. K.; Wang, B.; Mann, R. K.; Milenkovic, L.; Scott, M. P.; Beachy, P. A. *Nature* **2000**, *406*, 1005.
(11) Robarge, K. D.; Brunton, S. A.; Castanedo, G. M.; Cui, Y.; Dina, M. S.; Goldsmith, R.; Gould, S. E.; Guichert, O.; Gunzner, J. L.; Halladay, J.; Jia, W.; Khojasteh, C.; Koehler, M. F.; Kotkow, K.; La, H.; LaLonde, R. L.; Lau, K.; Lee, L.; Marshall, D.; Marsters, J. C., Jr.; Murray, L. J.; Qian, C.; Rubin, L. L.; Salphati, L.; Stanley, M. S.; Stibbard, J. H.; Sutherlin, D. P.; Ubhayaker, S.; Wang, S.; Wong, S.; Xie, M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5576.
(12) Dlugosz, A.; Agrawal, S.; Kirkpatrick, P. *Nat. Rev. Drug Discovery* **2012**, *11*, 437.
(13) Stanton, B. Z.; Peng, L. F.; Maloof, N.; Nakai, K.; Wang, X.; Duffner, J. L.; Taveras, K. M.; Hyman, J. M.; Lee, S. W.; Koehler, A. N.; Chen, J. K.; Fox, J. L.; Mandinova, A.; Schreiber, S. L. *Nat. Chem. Biol.* **2009**, *5*, 154.

(14) Firestone, A. J.; Weinger, J. S.; Maldonado, M.; Barlan, K.; Langston, L. D.; O'Donnell, M.; Gelfand, V. I.; Kapoor, T. M.; Chen, J. K. *Nature* **2012**, *484*, 125.

(15) Lauth, M.; Bergström, A.; Shimokawa, T.; Toftgård, R. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 8455.

(16) Jenkins, D. *Cell. Signaling* **2009**, *21*, 1023.

(17) Polizio, A. H.; Chinchilla, P.; Chen, X.; Manning, D. R.; Riobo, N. A. *Sci. Signaling* **2011**, *4*, pt7.

(18) Polizio, A. H.; Chinchilla, P.; Chen, X.; Kim, S.; Manning, D. R.; Riobo, N. A. *J. Biol. Chem.* **2011**, *286*, 19589.

(19) Wang, Y.; Zhou, Z.; Walsh, C. T.; McMahon, A. P. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 2623.

(20) Wang, Y.; Arvanites, A. C.; Davidow, L.; Blanchard, J.; Lam, K.; Yoo, J. W.; Coy, S.; Rubin, L. L.; McMahon, A. P. *ACS Chem. Biol.* **2012**, *7*, 1040.

(21) Chen, J. K.; Taipale, J.; Young, K. E.; Maiti, T.; Beachy, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 14071.

(22) Marcaurelle, L. A.; Comer, E.; Dandapani, S.; Duvall, J. R.; Gerard, B.; Kesavan, S.; Lee, M. D., IV; Liu, H.; Lowe, J. T.; Marie, J. C.; Mulrooney, C. A.; Pandya, B. A.; Rowley, A.; Ryba, T. D.; Suh, B. C.; Wei, J.; Young, D. W.; Akella, L. B.; Ross, N. T.; Zhang, Y. L.; Fass, D. M.; Reis, S. A.; Zhao, W. N.; Haggarty, S. J.; Palmer, M.; Foley, M. A. *J. Am. Chem. Soc.* **2010**, *132*, 16962.

(23) Chou, D. H.; Duvall, J. R.; Gerard, B.; Liu, H.; Pandya, B. A.; Suh, B. C.; Forbeck, E. M.; Faloon, P.; Wagner, B. K.; Marcaurelle, L. A. *ACS Med. Chem. Lett.* **2011**, *2*, 698.

(24) Spinella-Jaegle, S.; Rawadi, G.; Kawai, S.; Gallea, S.; Faucheu, C.; Mollat, P.; Courtois, B.; Bergaud, B.; Ramez, V.; Blanchet, A. M.; Adelmant, G.; Baron, R.; Roman-Roman, S. *J. Cell Sci.* **2001**, *114*, 2085.

(25) Sinha, S.; Chen, J. K. *Nat. Chem. Biol.* **2006**, *2*, 29.

(26) Svärd, J.; Heby-Henricson, K.; Persson-Lek, M.; Rozell, B.; Lauth, M.; Bergström, A.; Ericson, J.; Toftgård, R.; Teglund, S. *Dev. Cell* **2006**, *10*, 187.

(27) Yauch, R. L.; Gould, S. E.; Scales, S. J.; Tang, T.; Tian, H.; Ahn, C. P.; Marshall, D.; Fu, L.; Januario, T.; Kallop, D.; Nannini-Pepe, M.; Kotkow, K.; Marsters, J. C., Jr.; Rubin, L. L.; de Sauvage, F. J. *Nature* **2008**, *455*, 406.

(28) Zhang, X.; Harrington, N.; Moraes, R. C.; Wu, M. F.; Hilsenbeck, S. G.; Lewis, M. T. *Breast Cancer Res. Treat.* **2009**, *115*, 505.