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Discovery of a Potent and Orally Active Hedgehog Pathway Antagonist (IPI-926)

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Recent evidence suggests that blocking aberrant hedgehog pathway signaling may be a promising therapeutic strategy for the treatment of several types of cancer. Cyclopamine, a plant *Veratrum* alkaloid, is a natural product antagonist of the hedgehog pathway. In a previous report, a seven-membered D-ring semisynthetic analogue of cyclopamine, IPI-269609 (**2**), was shown to have greater acid stability and better aqueous solubility compared to cyclopamine. Further modifications of the A-ring system generated three series of analogues with improved potency and/or solubility. Lead compounds from each series were characterized in vitro and evaluated in vivo for biological activity and pharmacokinetic properties. These studies led to the discovery of IPI-926 (compound **28**), a novel semisynthetic cyclopamine analogue with substantially improved pharmaceutical properties and potency and a favorable pharmacokinetic profile relative to cyclopamine and compound **2**. As a result, complete tumor regression was observed in a Hh-dependent medulloblastoma allograft model after daily oral administration of 40 mg/kg of compound **28**.

Introduction

The hedgehog (Hh[a]) signaling pathway regulates cell growth and differentiation during embryonic tissue patterning and plays a pivotal role in tissue homeostasis.[1] Hh-signal transduction is initiated by the binding of Hh protein ligand to its cellular membrane receptor Patched (PTCH), which relieves PTCH-mediated repression of the seven-transmembrane protein Smoothened (SMO). Activated SMO transduces the signal to the GLI family of transcription factors, which translocate to the nucleus to regulate numerous gene products involved in tissue patterning and cell differentiation.

In adulthood, the Hh signaling pathway in normal cells is much less active (Figure 1A). However, an increasing number of reports over the past decade have implicated the Hh pathway in human diseases, especially cancer (Figure 1B).[2−5] The first link between the Hh pathway and cancer came from the discovery that Gorlin's syndrome, an inherited condition, results from an autosomal loss of the gene for PTCH. Children with this condition have multiple physical defects, including a predisposition for cancers such as medulloblastoma and basal cell carcinoma (BCC).[6] Analyses of tumor tissue from sporadic BCC and medulloblastoma have also demonstrated a high incidence of Hh pathway hyperactivation, as indicated by elevated Gli1 protein expression and inactivating mutations in PTCH.[7,8] Subsequent analysis of various tumor samples demonstrated aberrant Hh signaling in breast,[9] esophagus,[10] gastric,[10] medulloblastoma,[11] pancreatic adenocarcinoma,[12] prostate,[13] and small cell lung cancers.[14] Accordingly, targeting the Hh pathway is an emerging therapeutic strategy in cancer.

Several agents acting on various components of the Hh signaling pathway have been described preclinically, and their relevance as novel cancer treatments has been recently reviewed.[15] While most Hh pathway small molecule antagonists target SMO,[16−21] components upstream and downstream of SMO are also being investigated as potential pharmaceutical targets.[22−25] Four Hh pathway antagonists are being explored clinically.[26−29] Herein we report preclinical data that supported the selection of IPI-926 (compound **28**) as a development candidate that has recently entered clinical phase 1.[29]

Cyclopamine (**1**, Figure 2), a natural product isolated from *Veratrum californicum*,[30−32] has been a significant pharmacological tool to validate the Hh pathway in cancer. Cyclopamine directly acts on SMO[33] and inhibits tumor growth in several murine models of pancreatic,[12] medulloblastoma,[34,35] prostate,[13,36,37] small cell lung,[14] and digestive tract[10] cancers. However, the clinical development of cyclopamine as a therapeutic in cancer is hampered by cyclopamine's poor solubility, acid sensitivity, and weak potency relative to other reported small-molecule Hh antagonists. Compound **3**[17] (Figure 2) is 10- to 20-fold more potent than cyclopamine but has essentially the same limitations as cyclopamine from solubility and chemical stability standpoints. Chemical modifications have improved the pharmaceutical profile of cyclopamine. In one study, the addition of carbohydrate moieties on the piperidine nitrogen resulted in the identification of

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[a]Abbreviations: BCC, basal cell carcinoma; BODIPY, 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene; HPBCD, 2-hydroxypropyl-β-cyclodextrin; Hh, Hedgehog; Hic1, hypermethylated in cancer 1; MTD, maximum tolerated dose; Ptch, Patched; Smo, Smoothened; SAR, structure−activity relationship; SuFu, Suppressor of Fused.

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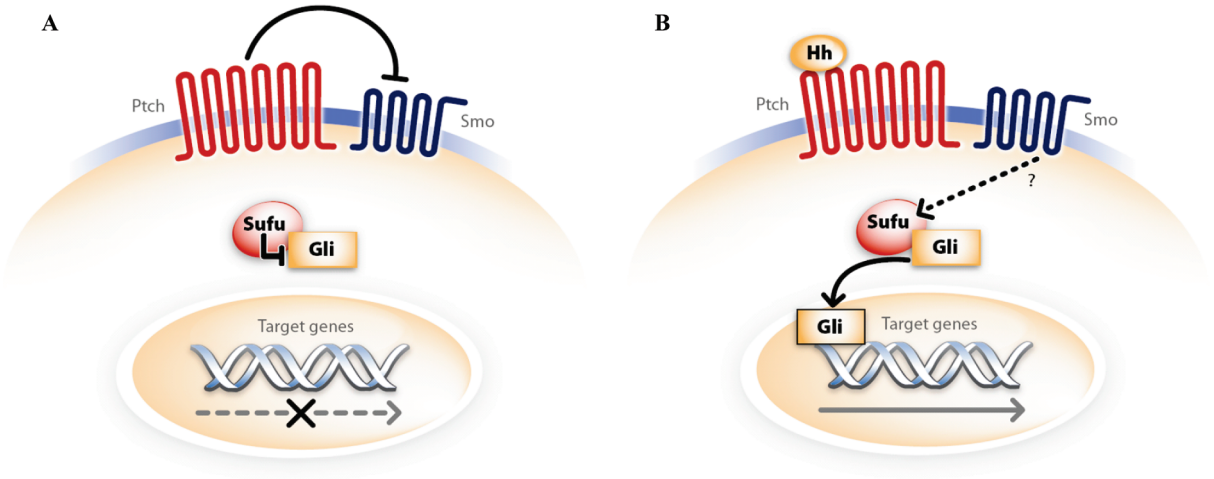


Figure 1. Schematic of the Hedgehog signaling pathway: (A) in the absence of ligand, which represents the situation on most normal cells during adulthood; (B) in several types of cancer cells, for which the pathway is activated by the presence of ligand and/or loss-of-function of PTCH and/or gain-of-function of SMO.

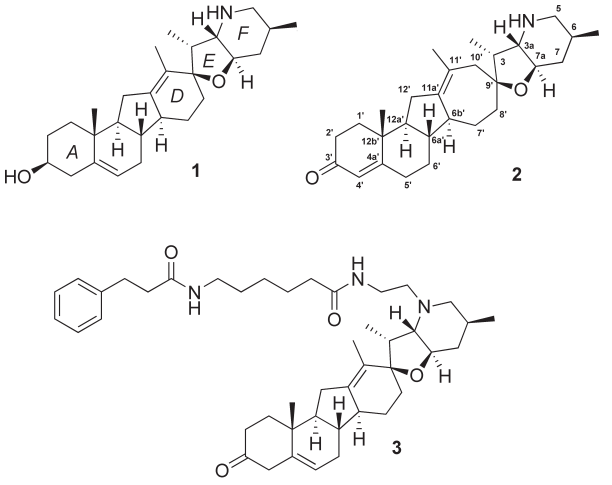


Figure 2. Structures of cyclopamine (**1**), IPI-269609 (**2**), and **3**.

equipotent analogues of cyclopamine with fair-to-good solubility in aqueous media.[38] Another approach conjugated cyclopamine to prostate specific, antigen activated peptides to produce prostate cancer targeted prodrugs.[39] We have also previously reported the development of a prototype analogue **2** (Figure 2) that exhibited improved chemical stability and aqueous solubility relative to cyclopamine.[40] Compound **2** demonstrated in vivo efficacy in several mouse xenograft models.[41,42] However, development of compound **2** as a drug candidate was limited by potency and metabolic stability.

We now report the further optimization of compound **2**, which has led to the discovery of the more potent *cis*-decalone analogue **6** from which three series of A-ring modified analogues of reduced D-homocyclopamine were identified. Among them, compound **28** emerged as the most promising clinical development candidate with improved potency, pharmacokinetic, and pharmaceutical properties relative to cyclopamine and compound **2**.

Chemistry

The α/β-unsaturated ketone system found in compound **2** is a very common and structurally important functionality in endogenous steroid hormones. However, this functionality in compound **2** is readily metabolized to the corresponding saturated alcohol and glucuronide conjugate after oral administration in cynomolgus monkeys.[43] We chose to explore the importance of the half-chair conformation of the A-ring system of compound **2** on the potency. Stereoselective reduction of the enone of **2** with Li/NH_3 provided *trans*-decalone **4** (Scheme 1),[44] whereas hydrogenation catalyzed with Pd/C in presence of pyridine[45] yielded the *cis*-decalone as the major product (ratio of ~9:1). Protection of the piperidine nitrogen of *cis*-decalone as a benzyl carbamate provided stereochemically pure material (**5**) after silica gel chromatography, and removal of protecting group provided **6**. Hedgehog inhibitory activity of these new compounds was measured by inhibition of oxysterol-dependent differentiation of C3H10T1/2 cells.[46] In this assay, *cis*-decalone **6** was found to be distinctly more potent (30-fold) than *trans*-decalone **4** and, more importantly, enone **2** (Table 1). Utilizing the 3-ketone as a synthetic handle, three series of *cis*-decalone analogues were pursued: 3-substituted analogues, fused heterocycles, and A-ring lactams.

The scalable intermediate **5** served as a useful starting point for additional elaboration of the A-ring. Schemes 2 and 3 show the synthesis of compounds that have been modified at the C3′-position (Table 1, compounds **6**−**29**). The A-ring ketone was reductively removed from **6** by a Wolff−Kishner reaction to give cyclohexane analogue **7**. Oxime **10** and oxime methyl ether **11** were prepared by standard procedures from compound **5**. Third, the ketone of compound **5** was reduced in the presence of bulky potassium tri-*sec*-butyl borohydride to the C3′(*S*)-alcohol **12**, whereas Luche reduction ($CeCl_3$, $NaBH_4$) gave the C3′(*R*)-alcohol **13**. Hydrogenation of these protected derivatives provided the desired analogues **14** and **15**. Conversion of the protected alcohols **12** and **13** into methyl ethers was performed using standard procedures to give **16** and **17**, after removal of the benzylcarbamate. As described in Scheme 3, epimeric alcohols **12** and **13** were readily transformed to the corresponding azides **18** and **19**. These azides were key intermediates in the formation of triazoles (**20**, **21**), amines (**24**, **25**), acetamides (**26**, **27**), and sulfonamides (**28**, **29**).

The synthetic approaches to fused-ring analogues (C2′/C3′ and C3′/C4′ fused ring systems) and their derivatives are depicted in Schemes 4−6 (Table 1, compounds **32**−**50**). As

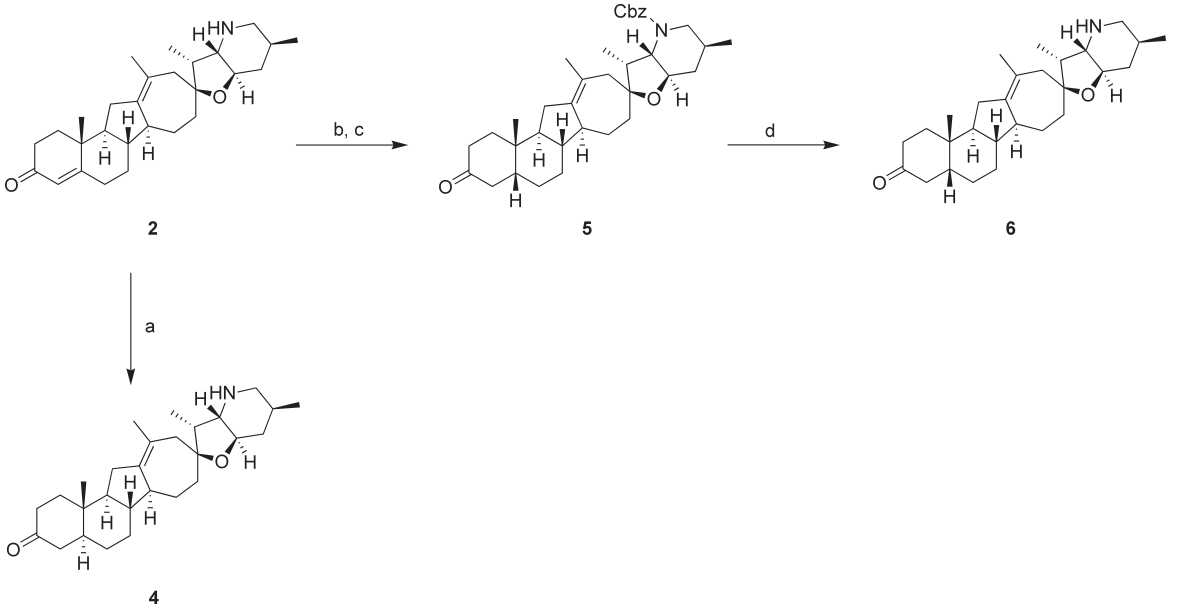
described in Scheme 4, formylation of compound **5** under thermodynamic conditions[47] afforded β-ketoaldehyde **30**. Condensation of the latter with hydrazine followed by ring closure provided, after benzylcarbamate removal, pyrazole **32**. The other regioisomer of **32** was prepared by formylation of the kinetic TES enol ether **33** to give the rather unstable β-hydroxyketone **34**. Subsequent oxidation with Dess–Martin periodinane, followed by condensation with hydrazine, and deprotection gave pyrazole **36**. Evaluation of the relative potency between the pyrazole regioisomers revealed that the C2′/C3′ fused ring system (**32**) was clearly superior to the C3′/C4′ analogue **36** (Table 1). Additional C2′/C3′ fused heterocycles were synthesized from intermediate **30** (Schemes 5). Thiazole **38** was prepared by the condensation of thioacetamide with C2′-bromoketone **37**.[48] The β-ketoaldehyde **30** was transformed to β-ketooxime **39**,[49] which was then converted to oxadiazole **40** using conditions adapted from Ohta and co-workers.[50] On the other hand, β-ketoaldehyde **30** was converted in a regioselective manner to [3,2-*c*] isoxazole (**41**) under basic conditions.[51] Substitutions around the C2′/C3′ fused pyrazole were also explored, and the details of their synthesis are described in Scheme 6. The C3″ position of the pyrazole was substituted with trifluoromethyl (**42**), which was prepared by Claisen condensation from ketone **5** and ethyl trifluoroacetate, followed by ring-closing condensation to give, after deprotection, trifluoromethylpyrazole **44**. Pyrazolone **47** was prepared by condensation of hydrazine on unsaturated β-ketoester **46**, which was synthesized from key intermediate **30**. Condensation of β-ketoaldehyde **30** with N-methylhydrazine gave methylpyrazole **48** as the major regioisomer. Pyrazole **31** was reacted with the appropriate sulfonyl chloride to give sulfamylpyrazoles **49** and **50** as the major regioisomers.

A synthetic sequence for the conversion of the cyclohexanone A-ring of compound **6** to lactams **55** and **56** is shown in Scheme 7. The use of nonreducible azasteroids has been very successful in the development of 5α-reductase inhibitors.[52,53] On the basis of literature precedents,[54,55] it was envisioned that a regioselective Beckmann rearrangement could be achieved using unsaturated oxime **52**. The requisite enone **51** was prepared from key intermediate β-ketoaldehyde **45** via deformylation with Wilkinson's catalyst. Ring enlargement of the oxime **52** through Beckmann rearrangement occurred regioselectively to afford $\Delta^{1'\text{-}2'}$-en-lactam **53**. Standard alkylation

Table 1. Cellular Hh Pathway Inhibition and in Vitro Metabolic Stability in Human Liver Microsomes of A-Ring Analogues of D-Homocyclopamine

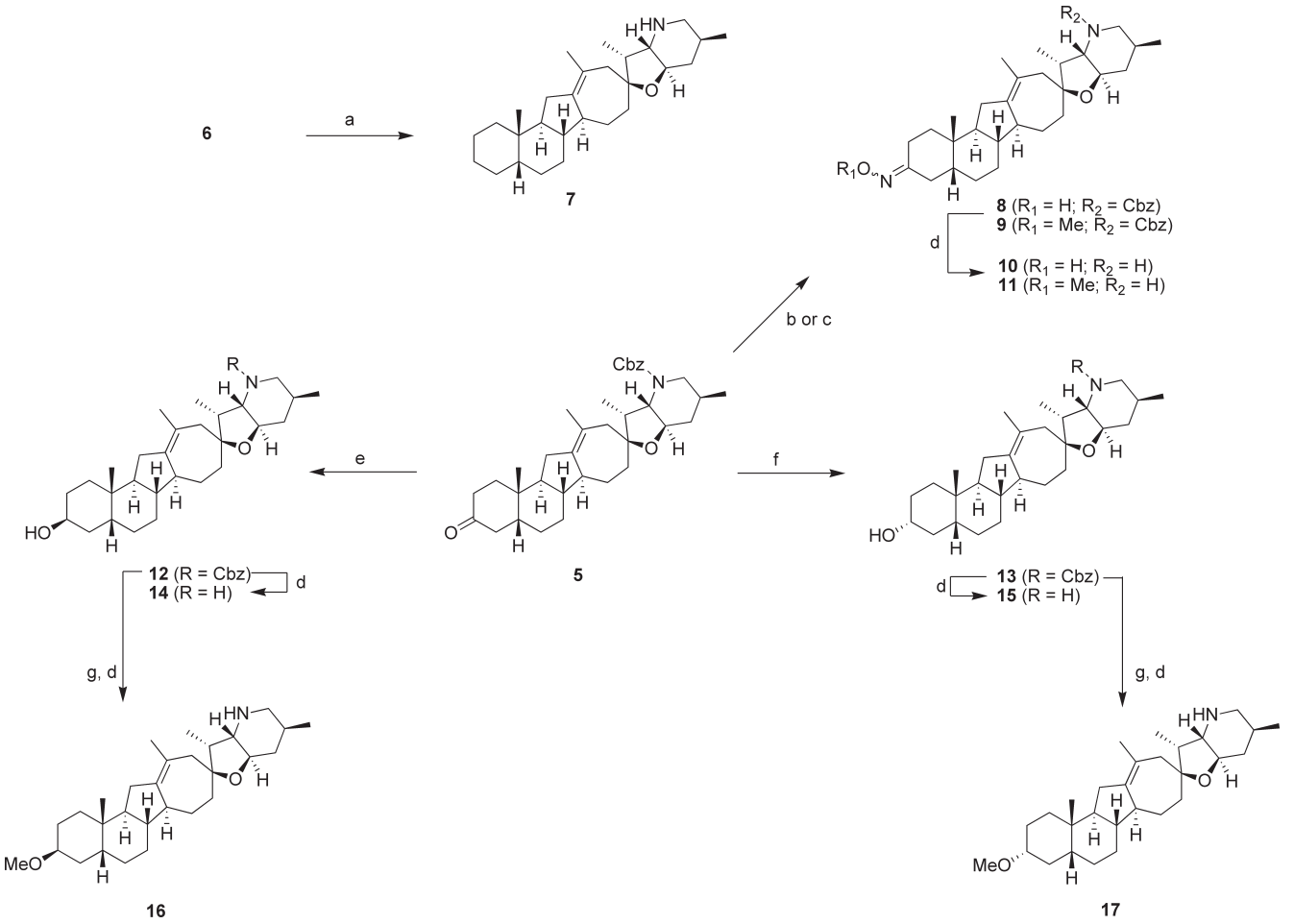
A-ring motif	compd	EC_{50}, μM (*N*)	$t_{1/2}$, min
enone	**2**	0.30 ± 0.05 (9)	75
trans-decalone	**4**	0.4 ± 0.2 (2)	75
cis-decalone	**6**	0.013 ± 0.004 (6)	70
cyclohexyl	**7**	0.76 ± 0.11 (2)	> 200
3′-oxime	**10**	0.015 ± 0.007 (2)	45
3′-methyloxime	**11**	0.16 ± 0.04 (2)	55
3′-(*S*)-hydroxy	**14**	0.023 ± 0.016 (2)	20
3′-(*R*)-hydroxy	**15**	0.009 ± 0.001 (2)	40
3′-(*S*)-methoxy	**16**	0.49 ± 0.04 (2)	> 200
3′-(*R*)-methoxy	**17**	0.18 ± 0.05 (2)	20
3′-(*R*)-triazole	**20**	0.024 ± 0.009 (2)	40
3′-(*S*)-triazole	**21**	0.13 ± 0.03 (2)	70
3′-(*R*)-amino	**24**	> 1 (2)	
3′-(*S*)-amino	**25**	> 1 (2)	
3′-(*R*)-acetamido	**26**	0.017 ± 0.005 (3)	65
3′-(*S*)-acetamido	**27**	0.30 ± 0.08 (3)	60
3′-(*R*)-sulfonamido	**28**	0.007 ± 0.002 (3)	85
3′-(*S*)-sulfonamido	**29**	0.09 ± 0.03 (3)	30
2′,3′-fused pyrazole	**32**	0.013 ± 0.008 (6)	120
3′,4′-fused pyrazole	**36**	0.22	27
2′,3′-fused thiazole	**38**	0.27 ± 0.08 (2)	100
2′,3′-fused oxadiazole	**40**	0.23 ± 0.05 (2)	8
2′,3′-fused isoxazole	**41**	0.062 ± 0.023 (3)	8
2′,3′-fused (CF_3)-pyrazole	**44**	0.17 ± 0.02 (3)	200
2′,3′-fused (OH)-pyrazole	**47**	0.17 ± 0.01 (2)	100
2′,3′-fused N-Me-pyrazole	**48**	0.054 ± 0.007 (4)	35
2′,3′-fused N-(Ms)-pyrazole	**49**	0.14 ± 0.01 (2)	180
2′,3′-fused N-(Ts)-pyrazole	**50**	0.9 ± 0.3 (2)	65
lactam	**55**	0.025 ± 0.005 (3)	65
N-Me-lactam	**56**	0.040 ± 0.003 (2)	46

Scheme 1[a]



[a]Reagents and conditions: (a) Li, NH_3, THF, *t*-BuOH, −78 °C; (b) H_2, Pd/C, pyridine, 25 °C; (c) CbzCl, Et_3N, THF, 25 °C; (d) H_2, Pd/C, EtOAc, toluene, 25 °C.

Scheme 2[a]



[a]Reagents and conditions: (a) $N_2H_4-H_2O$, K_2CO_3, triethylene glycol, 150 °C; (b) $HONH_2-HCl$, NaOAc, wet EtOH, 25 °C; (c) CH_3ONH_2-HCl, NaOAc, wet EtOH, 25 °C; (d) H_2, Pd/C, 25 °C; (e) K-Selectride, THF, −78 °C; (f) $CeCl_3 \cdot 7H_2O$, $NaBH_4$, −78 °C; (g) Me_2SO_4, aq KOH, benzyltriethylammonium chloride, CH_2Cl_2, 25 °C.

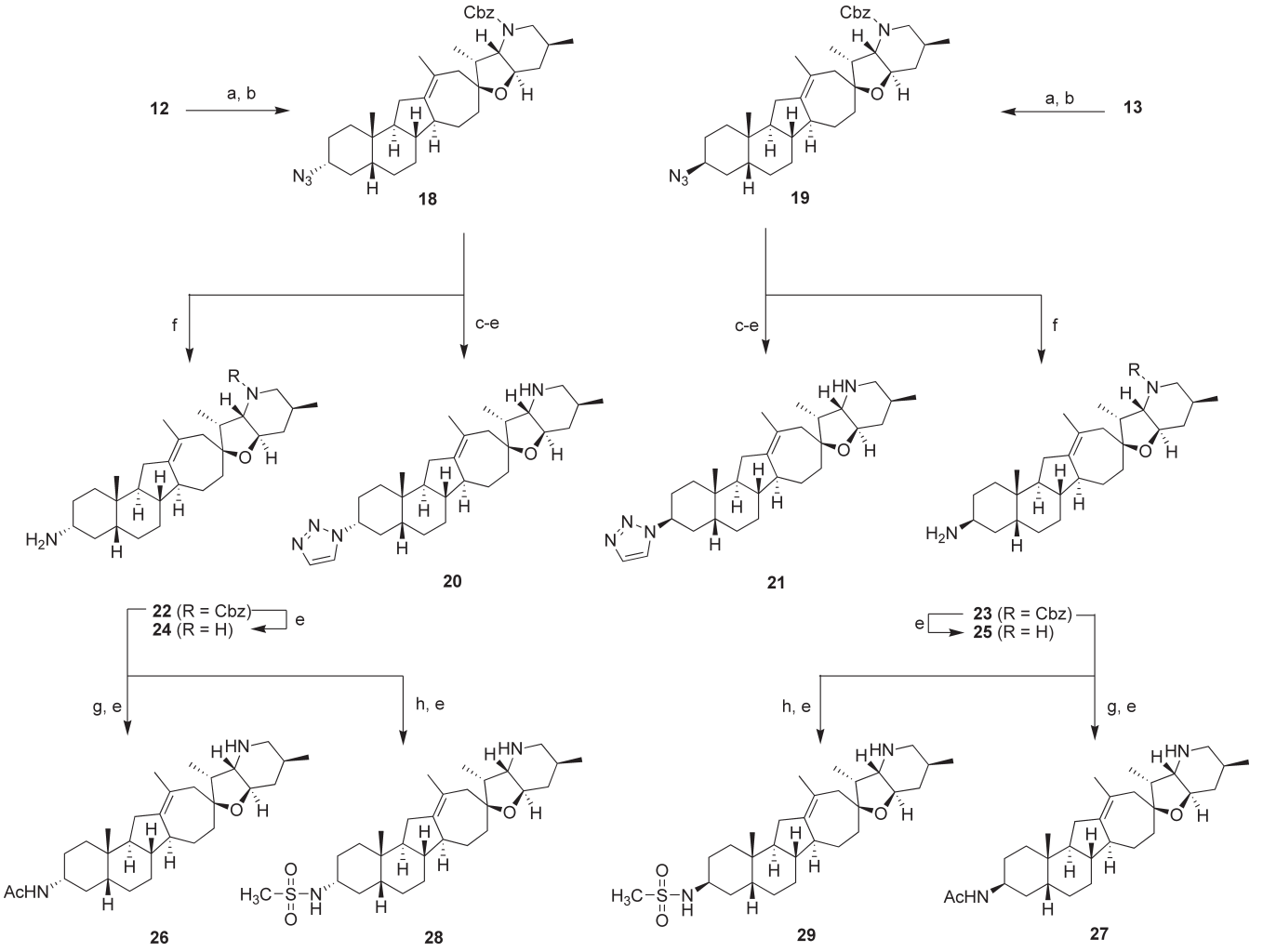
gave the *N*-methyllactam **54**. Hydrogenolysis removes the benzyl carbamate and reduces the olefin of $\Delta^{1'-2'}$-en-lactams **53** and **54** to give saturated the desired saturated analogues **55** and **56**.

Potency and Metabolic Stability

The major disadvantages with the prototype compound **2** are its limited potency and metabolic stability. The remarkable improvement in potency demonstrated by *cis*-decalone analogue **6** in the differentiation assay is tempered by the likelihood that it would suffer from the same metabolic fate as compound **2**. Efforts to improve the metabolic stability while maintaining (or improving) the biological activity of the potent *cis*-decalone scaffold led to the synthesis of multiple classes of analogues without the C3′-ketone. New analogues were evaluated for their ability to inhibit the hedgehog pathway using oxysterol-dependent differentiation of C3H10T1/2 cells,[46] as well as for their metabolic stability in human liver microsomes (Table 1).

Polar functionalities on the C3′ position provided analogues with the best biological activity on the hedgehog pathway. For instance, the more hydrophobic deoxygenated compound **7** is almost 3 orders of magnitude less potent than ketone **6** in the differentiation assay. Methyloxime analogue **11** was found to be 10-fold less active than oxime **10**, suggesting the preference for a hydrogen bond donor. Although oxime **10** was found to be as active in the differentiation assay as ketone **6**, it was less metabolically stable. It was found that the sp^2 hybridization at the C3′ position is not critical for biological activity as demonstrated by the potency of hydroxyl and methoxy analogues (**14**−**17**). The *R* configuration and again a hydrogen bond donor at the C3′ position were clearly preferred for improved biological activity (cf. **15** vs **14**, and **17** vs **16**). In vitro and in vivo metabolism studies with compound **2** revealed the formation of metabolite alcohol **15**, which is cleared from the plasma along with its glucuronide conjugate in cynomolgus monkeys.[43] In light of this observation we searched for analogues devoid of this metabolic liability while also maintaining a hydrogen bond donor at the C3′position. Consequently, additional functional groups were introduced at the C3′ position to further probe the SAR. While the primary amine analogues **24** and **25** were found to be very weak inhibitors regardless of the stereochemistry at C3′ position, the amide and sulfonamide analogues proved more interesting. As was the case with the C3′ alcohol analogues, the *R*-analogues (**26** and **28**) were more potent than the corresponding *S*-analogues (**27** and **29**). The sulfonamides were also 2-fold superior to the amides, possibly because of a lower pK_a of the N−H, coupled with a larger volume occupied by the sulfonamide group. Importantly, the C3′-(*R*)-sulfonamide **28** was found to be similar in potency to the C3′-(*R*)-alcohol **15** (EC_{50} = 7 nM vs 9 nM) and yet had the advantage of being more metabolically stable in the human liver microsomes assay ($t_{1/2}$ = 85 min vs 40 min).

Scheme 3[a]



[a]Reagents and conditions: (a) MsCl, Et_3N, CH_2Cl_2, 0 °C; (b) NaN_3, DMPU, 60 °C; (c) TMS−acetylene, toluene, reflux; (d) TBAF, THF, 50 °C; (e) H_2, Pd/C, 25 °C; (f) PPh_3, wet THF, 55 °C; (g) Ac_2O, pyridine, 25 °C; (h) MsCl, *i*-Pr_2EtN, CH_2Cl_2, 0 °C.

We envisioned that the metabolic liability of the C3′ ketone in compound **6** could be reduced by introducing a heterocycle such as pyrazole and isoxazole fused onto the A-ring. The position of the pyrazole on the A ring has a dramatic effect on the potency and metabolic fate, with the C2′−C3′ fused analogue **32** being superior with respect to both, compared to the C3′−C4′ fused analogue **36**. The nature of the heterocyclic ring is also very important for biological activity, favoring pyrazole (**32**) over thiazole (**38**), oxadiazole (**40**), and isoxazole (**41**). Notably, ring systems with a heteroatom at the C3′′ position (**38** and **40**) were clearly disfavored compared to the unsubstituted pyrazole ring (**32**). Likewise, trifluoromethyl **44** and pyrazolone **47** were also significantly less potent than compound **32**. Substitution on the nitrogen of the pyrazole ring also resulted in a loss of potency as exemplified with analogues **48**−**50**, with the least pronounced effect coming from the N2′′-methyl substitution (∼3-fold). The metabolic stability was greatly affected by the nature of the fused heterocycle with pyrazole **32** demonstrating superior biological activity in the differentiation assay (EC_{50} = 13 nM) and superior metabolic stability in human liver microsomes ($t_{1/2}$ = 120 min).

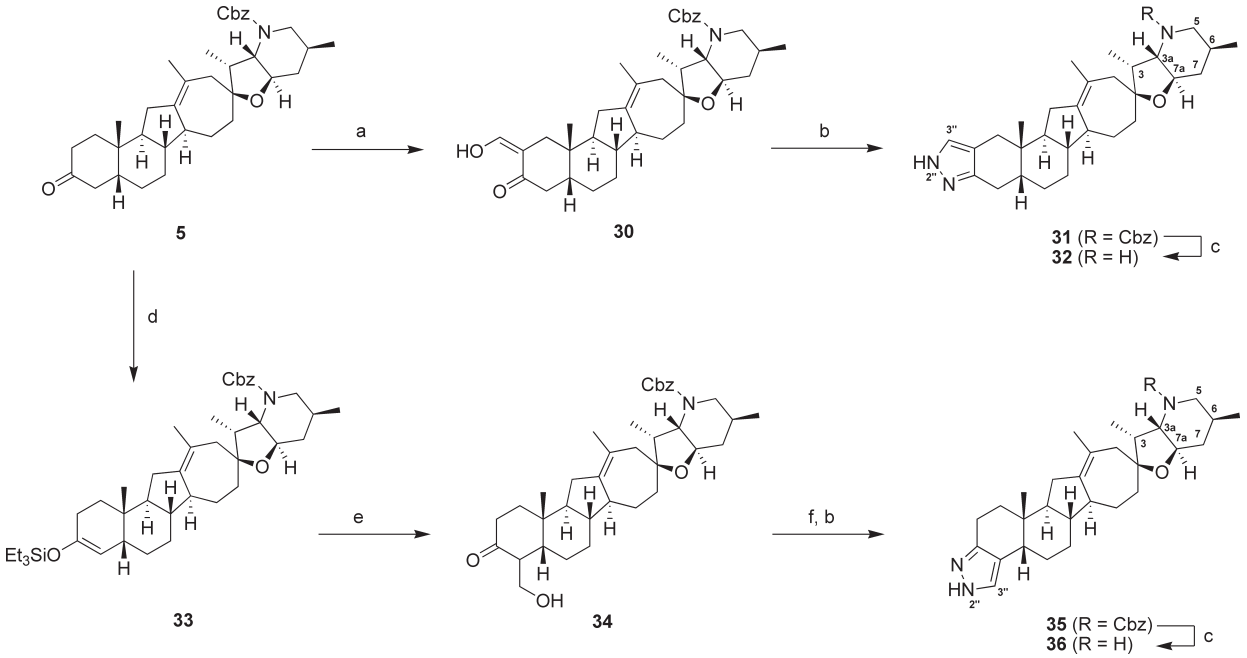
In another approach to modify the A-ring, we prepared seven-membered ring lactam analogue **55** and *N*-methyl lactam analogue **56**. Like pyrazole **32** and sulfonamide **28**, lactam **55** displays a hydrogen bond acceptor/donor pair in the same region of the molecule and was expected to provide similar potency. Lactam **55** was only slightly less potent than the pyrazole **32** and sulfonamide **28**, despite the expanded A-ring (EC_{50} = 25 nM vs 13 nM and 7 nM, respectively). *N*-Methyllactam **56** showed decreased potency compared to lactam **55**, an effect similar to findings with N-methylation of pyrazole (**48** vs **32**) and consistent with the notion that a hydrogen bond donor is preferable. To our surprise, the in vitro metabolic stability of lactam **55** was almost the same as that of the 3-ketone *cis*-decalone **6** ($t_{1/2}$ = 65 min vs 70 min).

After extensive exploration of the SAR around the A-ring of reduced D-homocyclopamine, three lead compounds emerged based on in vitro assay data (C3H10T1/2 differentiation and human liver microsome stability): sulfonamide **28**, pyrazole **32**, and lactam **55**. The pharmacokinetic properties of these three distinct chemotypes were evaluated to help guide selection of a superior development candidate.

Pharmacokinetic Properties in Multiple Mammalian Species

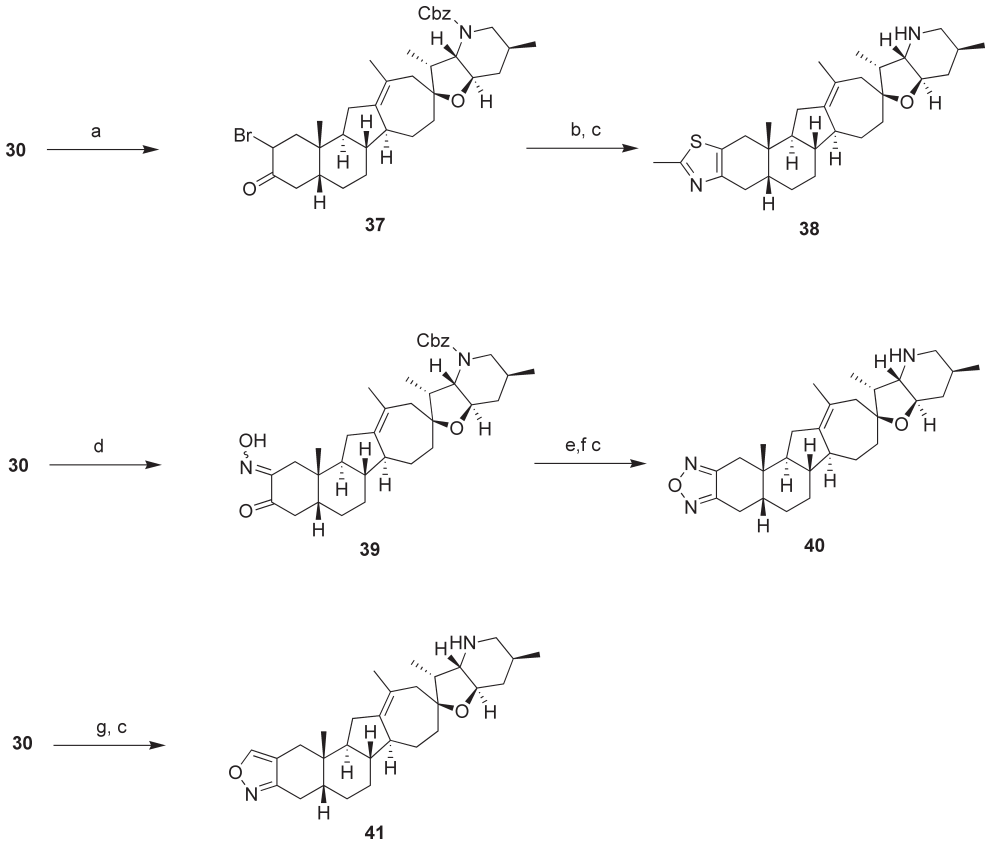
The plasma pharmacokinetic properties of the three lead compounds (**28**, **32**, and **55**) were investigated in mice, rats,

Scheme 4[a]



[a]Reagents and conditions: (a) HCOOEt, *t*-BuOK, *t*-BuOH, 25 °C; (b) $N_2H_4-H_2O$, EtOH, 80 °C; (c) H_2, Pd/C, 25 °C; (d) KHMDS, Et_3SiCl, THF, −78 °C; (e) HCHO, TBAF, CH_2Cl_2, −20 °C; (f) Dess−Martin periodinane, CH_2Cl_2, 0 °C.

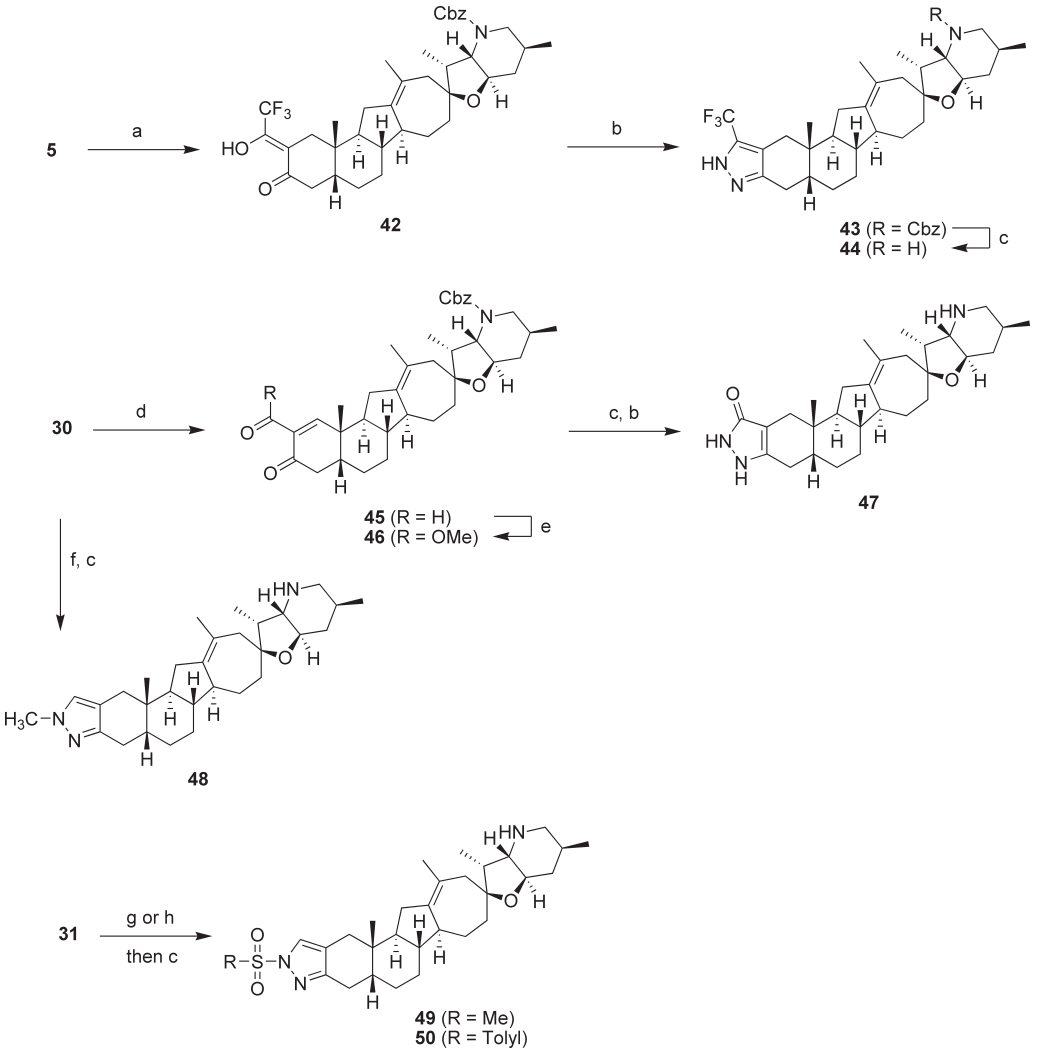
Scheme 5[a]



[a]Reagents and conditions: (a) NBS, HOAc, NaOAc, wet dioxane, 25 °C; (b) $CH_3C(S)NH_2$, EtOH, reflux; (c) H_2, Pd/C, 25 °C; (d) $NaNO_2$, aq AcOH, CH_2Cl_2, 0 °C; (e) $HONH_2-HCl$, NaOAc, EtOH, 25 °C; (f) KOH, dioxane, ethylene glycol, 120 °C; (g) $HONH_2-HCl$, pyridine, 120 °C.

dogs, and cynomolgus monkeys and compared to the prototype compound **2** (Table 2). Compound **2** exhibited a relatively short elimination half-life (1.7−3.5 h), high plasma clearance, and variable oral bioavailability across all species. Of significance, sulfonamide **28** and lactam **55** have much lower clearance compared to enone **2** and have excellent oral bioavailability in rodents and nonrodents. Sulfonamide **28** has a volume of distribution (11−30 L/kg) similar to the

Scheme 6[a]



[a]Reagents and conditions: (a) CF_3COOEt, *t*-BuOK, *t*-BuOH, 25 °C; (b) $N_2H_4-H_2O$, EtOH, 80 °C; (c) H_2, Pd/C, 25°C; (d) DDQ, toluene, 25 °C; (e) PDC, MeOH, DMF, 25 °C; (f) CH_3NHNH_2, EtOH, 80 °C; (g) MsCl, pyridine, 25 °C; (h) TsCl, pyridine, CH_2Cl_2, 25 °C.

enone **2** (13.9−28 L/kg). Overall, the encouraging pharmacokinetic profile of the potent lead compounds **28**, **32**, and **55** provided impetus to evaluate them further in a murine tumor model in vivo.

Pharmacology

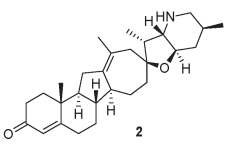
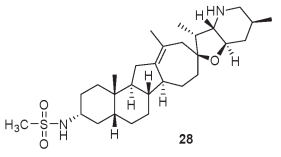
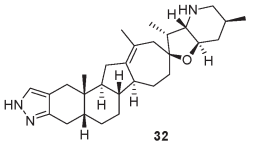
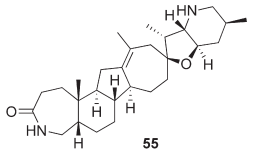
Antitumor activity and pharmacology of the three lead compounds were explored using a genetic model of medulloblastoma derived from mice engineered to be heterozygous for both the *Ptch1* and *Hic1* genes. These animals develop spontaneous, aggressive medulloblastoma with an incidence of 40%.[56] B837Tx is a serially passaged subcutaneous allograft derived from a medulloblastoma that developed in these mice. The B837Tx medulloblastoma tumor is dependent on the Hh pathway for growth and survival.[56]

Multidose tolerability studies performed on tumor-bearing immunocompromised mice demonstrated maximum tolerated oral doses of 40, 80, and 30 mg/kg for compounds **28**, **32**, and **55**, respectively. Daily oral administration of compounds **28**, **32**, and **55** at these tolerated doses led to complete tumor regression during the treatment phase, and median time to unmeasurable tumor volume was 9 days for all three compounds (Table 3). This is in stark contrast to cyclopamine and compound **2**, which showed no activity in this model when orally administered at a dose near the maximum tolerated dose (MTD). Growth inhibition of murine medulloblastoma model ($Ptc1^{+/-}/p53^{+/-}$; $Ptc1^{+/-}/p53^{-/-}$) has been shown with cyclopamine administered subcutaneously[34] (50 mg/kg) or intraperitoneally[35] (10 mg/kg). Our results underscore the superior oral efficacy of these novel analogues relative to cyclopamine. To better ascertain differences among these three compounds, time to tumor regrowth, during a 21-day post-treatment phase, was measured. Tumors in animals treated with compound **28** did not recur during the post-treatment period, whereas 4/10 and 3/10 animals regrew tumors when treated with compounds **32** and **55**, respectively. These findings suggest that compound **28** demonstrates superior antitumor activity relative to compounds **32** and **55**, possibly resulting in the complete elimination of all viable tumor cells.

Discussion

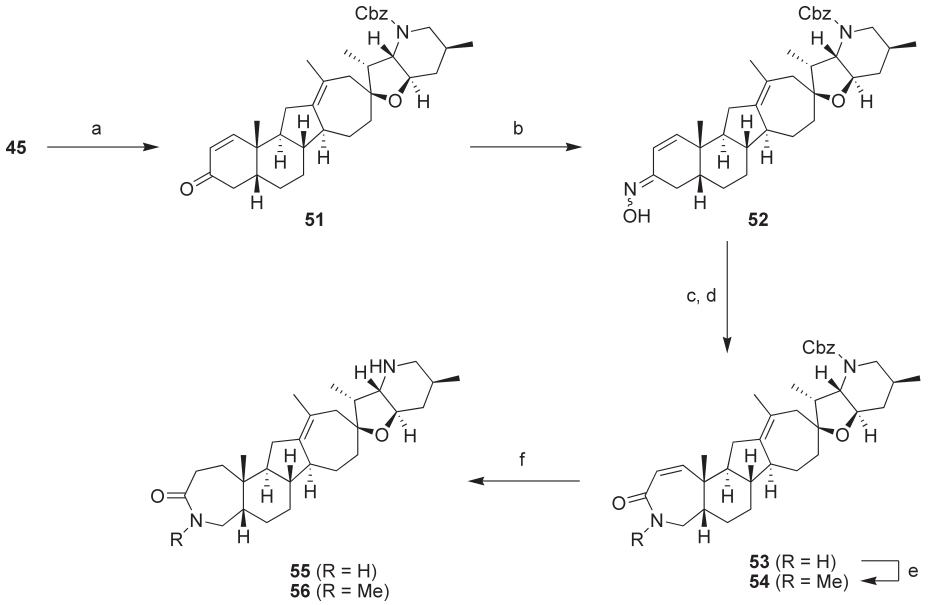
The Hh signaling pathway is an emerging target for the treatment of cancer. The majority of anticancer drugs potently inhibit the growth of cancer cells in culture, which provides information on potential sensitivity of tumor types for these

Table 2. Pharmacokinetic Parameters for Enone **2**, Sulfonamide **28**, Pyrazole **32**, and Lactam **55** in Multiple Mammalian Species

Compounds	Species	% F (PO)	Cl (L/hr/kg)[a]	V_d (L/kg)[a]	$T_{½}$ (hr)[a]
 **2**	CD-1 mouse (5 mg/kg, PO)	79%	3.6	18	3.5
	Sprague Dawley rat (5 mg/kg, PO)	13%	12.4	28	1.7
	Beagle dog (4 mg/kg, PO)	7%	4.7	13.9	2.2
	Cynomolgus monkey (4 mg/kg, PO)	69%	6.2	21.3	2.4
 **28**	CD-1 mouse (5 mg/kg, PO)	~ 100%	0.74	11	10.5
	Sprague Dawley rat (5 mg/kg, PO)	~ 100%	0.21	30	> 24
	Beagle dog (4 mg/kg, PO)	50%	0.66	15.3	~ 15.5
	Cynomolgus monkey (4 mg/kg, PO)	74%	0.9	9.5	8.2
 **32**	CD-1 mouse (5 mg/kg, PO)	52%	2	9	3
	Sprague Dawley rat (5 mg/kg, PO)	34%	7.5	13	1.2
	Beagle dog (4 mg/kg, PO)	73%	0.6	6.0	6.8
	Cynomolgus monkey (4 mg/kg, PO)	84%	1.8	13	5.6
 **55**	CD-1 mouse (5 mg/kg, PO)	~100%	0.5	2	3
	Sprague Dawley rat (5 mg/kg, PO)	~100%	0.63	4.9	5.4
	Beagle dog (4 mg/kg, PO)	97%	0.33	3.0	6.5
	Cynomolgus monkey (4 mg/kg, PO)	~ 100%	0.8	4.3	3.8

[a]Parameters obtained from the concentration−time profile after iv administration (1 mg/kg). T_{last} = 24 h in all studies.

Scheme 7[a]

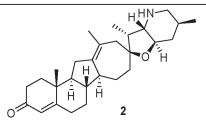
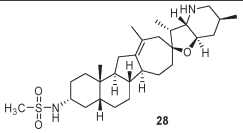
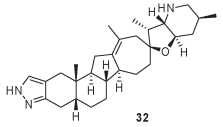
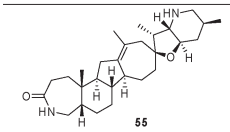


[a]Reagents and conditions: (a) $(PPh_3)_3RhCl$, toluene, 80 °C; (b) $HONH_2$−HCl, NaOAc, EtOH, 25 °C; (c) MsCl, pyridine, 5 °C; (d) HCl, MeOH, toluene, 60 °C; (e) KHMDS, CH_3I, THF, −78 to 25 °C; (f) H_2, Pd/C, alcohol, 25 °C.

drugs. However, there are significant limitations in using conventional in vitro growth inhibition assays to predict the in vivo efficacy of Hh pathway antagonists. Cancer cells can lose their dependency on Hh signaling pathway for proliferation and growth when grown in culture.[57−59] The antitumor activity of Hh antagonists is thus best assessed in vivo, but this poses significant challenges for lead identification and the lead optimization process. The natural product cyclopamine has shown in vivo efficacy in several pharmacological models and served as an excellent starting point for our medicinal chemistry effort.

Alterations to the cyclopamine core structure have produced very potent Hh antagonists. Homologation of the D-ring of cyclopamine and change in the oxidation state of the A−B ring system provided first generation compounds such as compound **2**. These modifications were shown to increase chemical stability and solubility relative to the natural product. Despite favorable pharmaceutical properties,

Table 3. In Vivo Antitumor Activity of Cyclopamine, Enone **2**, Sulfonamide **28**, Pyrazole **32**, and Lactam **55** in the $Ptc1^{+/-}/Hic1^{+/-}$ B837Tx Allograft Mouse Model

Compounds	Dosing regimen near MTD[a]	Median time to unmeasurable tumor volume	Tumor-free interval 21 days post-treatment	Recurrence rate post-treatment
cyclopamine	40 mg/kg (qd PO)	No regression	No efficacy	No efficacy
 **2**	25 mg/kg (qd PO)	No regression	No efficacy	No efficacy
 **28**	40 mg/kg (qd PO)	9 days	21 days	0/10
 **32**	80 mg/kg (qd PO)	9 days	19 days	4/10
 **55**	30 mg/kg (qd PO)	9 days	10 days	3/10

[a] The maximum tolerated dose (MTD) is the dose tolerated by all animals ($N = 3$) after repeat oral dose administration (5 days).

compound **2** was extensively metabolized in multiple species after oral administration[43] (Table 2). Consequently, compound **2** has shown modest efficacy in certain tumor xenograft models[41,42] and demonstrated no efficacy in the B837Tx medulloblastoma allograft model when administered orally at doses approaching the MTD.

The conversion of the half-chair A-ring system of compound **2** to a *cis*-ring fusion system provided a significant improvement in potency of these D-homocyclopamine derivatives. Furthermore, the presence of a pair of H-bond acceptor/donor on the A-ring was a common feature of the most active analogues such as sulfonamide **28**, pyrazole **32**, and lactam **55**. It is noteworthy that all three compounds displayed better activity than compound **2** in Hh pathway-mediated differentiation in vitro and Hh-dependent tumor growth in vivo. The clear correlation between these two activities provides strong evidence that these molecules are acting on-mechanism in vivo. The A-rings of the three lead compounds (**28**, **32**, and **55**) possess functional moieties that are metabolically more stable than the enone on first generation compound **2** and ketone of the compound **6**. Sulfonamide **28**, pyrazole **32**, and lactam **55** were all significantly more potent and less susceptible to metabolism than the first generation compound **2**.

One key discriminating factor between the three leads is their pharmacokinetic profiles. Although all three leads displayed good exposure when administered orally to multiple species, the sulfonamide **28** showed a significant increase in plasma half-life due to low clearance and high tissue distribution. These properties translated into greater tumor-free intervals following treatment and more robust efficacy than the two other lead compounds when studied in a Hh-dependent B837Tx tumor model. Compound **28** (IPI-926) has also shown in vivo efficacy in lung[60] and pancreatic[61] cancer xenograft models. Compound **28** is a very potent SMO antagonist, as shown by its ability to inhibit the association of BODIPY-cyclopamine (5 nM) on C3H10T1/2 transiently expressing recombinant human SMO with an IC_{50} value of 1.4 nM compared to 114 nM for cyclopamine (data not shown).[33] Further preclinical investigation corroborated the decision to pursue compound **28** as a clinical development candidate. Compound **28** is currently undergoing clinical evaluation in a phase 1 clinical trial in cancer patients.

In summary, structural modifications on the first generation antagonist compound **2** led to the discovery of compound **28**, a novel Hh antagonist with improved pharmaceutical properties and potency, as well as a superior plasma pharmacokinetic profile relative to cyclopamine and compound **2**. Clinical studies are ongoing to fully scope the full potential of this novel drug candidate.[29]

Experimental Section

General Methods. Commercial reagents and solvents were used as received without further purification or drying. All experiments involving water-sensitive compounds were carried out under argon and scrupulously dry conditions, using commercially available anhydrous solvents. Thin-layer chromatography was performed on glass-backed precoated Merck silica gel (60 F254) plates, and compounds were visualized using UV light, ceric ammonium molybdate, or 2% aqueous potassium permanganate solution. Silica gel column chromatography was carried out using Merck silica gel 60. Flash chromatography was run using silica gel (200−400 mesh) from Sorbent Technologies. The purity of tested compounds was determined by analytical liquid chromatography (HPLC) performed on an Agilent 1100 HPLC system equipped with a Waters C18-Symmetry reverse-phase column (4.6 mm × 150 mm, 5 μm). The mobile phases were acetonitrile (0.1% trifluoroacetic acid) and water (0.1% trifluoroacetic acid). After injection the gradient holds at 10% acetonitrile for 2 min followed by a gradient to 60% over 18 min, a 1 min organic flush at 95% acetonitrile, and a 6 min re-equilibration at a flow rate of 1.5 mL/min, column temperature at 40 °C, and detection wavelength of 215 nm. ^{1}H NMR spectra were recorded on a Bruker 400 spectrometer (400 MHz). Chemical shifts are reported in ppm with the solvent resonance as the internal standard ($CHCl_3$: δ 7.26). Data are

reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, dd = doublet of doublet, ddd = doublet of doublet of doublet, t = triplet, q = quartet, br = broad, m = multiplet), and coupling constants (Hz). ^{13}C NMR spectra were recorded on a Bruker 400 spectrometer (100 MHz) with complete proton decoupling. Chemical shifts are reported in ppm with the solvent as the internal reference ($CDCl_3$: δ 77.16). Electrospray positive ionization mass spectrometry was performed on samples using a reversed phase HPLC−MS system. The system was equipped with a diode array UV detector and a single quadrupole mass spectrometer and utilized the above-mentioned reversed-phase HPLC gradient method.

Preparation of Compound Included in Scheme 1. Compound 4. Freshly condensed ammonia (300 mL) at −78 °C was treated with lithium (1.5 g, 215 mmol, 22.7 equiv) and stirred for 10 min. The mixture was then charged with a solution of enone **2**[40,62] (4.01 g, 9.46 mmol, 1 equiv) in anhydrous tetrahydrofuran (40 mL) and *tert*-butanol (2.6 mL) over 15 min. After 2 h, the mixture was treated with saturated aqueous ammonium chloride. The reaction mixture was warmed to room temperature and the ammonia evaporated overnight. The residue was then partitioned between dichloromethane and water. The aqueous layer was separated and extracted with dichloromethane. The combined organic layers were washed with saturated aqueous ammonium chloride. The organic phase was dried over sodium sulfate and concentrated to white foam. The residue was purified using silica gel chromatography (10−20% EtOAc/hexanes) to give the desired ketone (3.46 g, 8.14 mmol, 86%). NMR δ_H (400 MHz, $CDCl_3$) 3.34 (dt, J = 10.6, 3.9 Hz, 1H), 3.03 (dd, J = 12.6, 4.2 Hz, 1H), 2.66 (dd, J = 9.8, 7.3 Hz, 1H), 2.47−0.95 (m, 31H), 0.93 (s, 3H), 0.90 (s, 3H), 0.88 (s, 3H); NMR δ_C (100 MHz, $CDCl_3$) 210.01, 140.75, 124.71, 82.70, 64.18, 54.86, 54.75, 49.89, 49.25, 46.57, 46.52, 44.73, 44.51, 40.30, 39.10, 38.21, 37.30, 35.46, 31.69, 30.77, 30.55, 29.69, 27.88, 23.94, 19.03, 10.68, 10.31; m/z = 426.26 $[M + H]^+$; HPLC 94.5 a/a % @ 215 nm.

Compound 6. A suspension of enone **2** (4.20 g, 9.94 mmol, 1 equiv) and 10% palladium on carbon (0.850 g) in pyridine (40 mL) was placed under hydrogen atmosphere and stirred for 16 h at room temperature. The reaction mixture was filtered on Celite and the filtrate concentrated to dryness. The residue was purified using silica gel chromatography (0−5% DCM/MeOH). A solution of the isolated product (4.23 g, 9.94 mmol, 1 equiv) in tetrahydrofuran (60 mL) was treated with triethylamine (5.03 g, 49.7 mmol, 5.0 equiv) and benzyl chloroformate (1.87 g, 10.9 mmol, 1.1 equiv). The reaction mixture was stirred at room temperature for 2 h and partitioned between saturated aqueous sodium bicarbonate and ethyl acetate. The organic phase was separated, dried over sodium sulfate, and concentrated to dryness. The residue was purified using silica gel chromatography (2−14% EtOAc/hexanes) to afford the desired product as single isomer **5** (4.65 g, 8.3 mmol, 84%). NMR δ_H (400 MHz, $CDCl_3$) 7.39−7.27 (m, 5H), 5.15 (d, J = 12.6 Hz, 1H), 5.11 (d, J = 12.7, 1H), 3.82 (dd, J = 12.7, 4.4 Hz, 1H), 3.63 (dt, J = 10.5, 4.1 Hz, 1H), 3.09 (dd, J = 10.1, 6.0 Hz, 1H), 2.69−2.55 (m, 3H), 2.50−2.38 (m, 2H), 2.26−2.14 (m, 3H), 2.10−1.10 (m, H), 0.97 (d, J = 6.8, 3H), 0.95 (s, 3H), 0.91 (d, J = 7.1, 3H); HPLC 96.6 a/a % at 215 nm.

A suspension of compound **5** (1.35 g, 2.41 mmol, 1 equiv) and 10% palladium on carbon (300 mg) in ethyl acetate (30 mL) was placed under hydrogen atmosphere and stirred for 3 h at room temperature. The reaction mixture was filtered on Celite and the filtrate concentrated to dryness. The oil was purified by silica gel chromatography (5% MeOH/DCM) to give compound **6** (950 mg, 2.23 mmol, 93%). NMR δ_H (400 MHz, $CDCl_3$) 3.37 (dt, J = 9.2, 2.9 Hz, 1H), 3.07 (dd, J = 12.3, 2.8 Hz, 1H), 2.73−2.60 (m, 2H), 2.51−2.40 (m, 2H), 2.37−2.16 (m, 5H), 2.11−1.03 (m, 23H), 0.96 (s, 3H), 0.95−0.90 (m, 6H); NMR δ_C (100 MHz, $CDCl_3$) 213.45, 140.18, 124.94, 82.64, 64.15, 54.81, 49.89, 49.65, 46.69, 44.72, 43.95, 42.51, 42.12, 40.27, 38.29, 37.56, 37.29, 34.86, 31.66, 30.44, 30.40, 27.98, 27.90, 25.50, 24.00, 21.66, 19.01, 10.29; m/z = 426.19 $[M + H]^+$; HPLC 95.4 a/a % at 215 nm.

Preparation of Compound Included in Scheme 2. Compound 7. A solution of ketone **6** (85 mg, 0.199 mmol, 1 equiv) in triethylene glycol (2 mL) was treated with hydrazine monohydrate (500 mg, 10 mmol, 50 equiv) and potassium carbonate (138 mg, 1 mmol, 5 equiv). The reaction mixture was sealed in a tube and heated to 150 °C for 16 h. The reaction mixture was cooled to room temperature and partitioned between water and chloroform. The organic layer was washed with water, dried over sodium sulfate, and concentrated to dryness. The residue was purified using silica gel chromatography (4% MeOH/DCM). The purified fractions were pooled and concentrated to dryness. The resulting oil was dissolved in methyl *tert*-butyl ether, washed with water, 2 N aqueous sodium hydroxide, and saturated aqueous sodium chloride. The combined organic layers were dried over sodium sulfate, filtered, and concentrated to afford the desired compound **7** as a white foam (64 mg, 0.155 mmol, 75%). NMR δ_H (400 MHz, $CDCl_3$) 3.35 (dt, J = 10.1, 3.6 Hz, 1H), 3.21 (s, 1H), 3.05 (dd, J = 12.8, 3.8 Hz, 1H), 2.69 (dd, J = 9.5, 7.6 Hz, 1H), 2.45 (d, J = 14.0 Hz, 1H), 2.31 (t, J = 11.6 Hz, 1H), 2.22 (br d, J = 11.4 Hz, 1H), 2.12 (dd, J = 14.4, 6.0 Hz, 1H), 2.05−1.00 (m, 28H), 0.92 (d, J = 7.3 Hz, 3H), 0.91 (d, J = 6.6 Hz, 3H), 0.86 (s, 3H). NMR δ_C (100 MHz, $CDCl_3$) 141.43, 124.02, 82.84, 64.19, 54.87, 49.93, 49.82, 47.12, 44.69, 42.96, 42.04, 40.32, 38.67, 37.33, 35.25, 31.74, 31.36, 30.40, 28.72, 27.90, 27.36, 27.20, 26.30, 23.95, 23.34, 21.76, 19.03, 10.31; m/z = 412.29 $[M + H]^+$; HPLC 98.2 a/a % at 215 nm.

Compound 10. A solution of ketone **5** (185 mg, 0.3 mmol, 1 equiv) in ethanol (3 mL) and water (0.3 mL) was treated with hydroxylamine hydrochloride (140 mg, 2 mmol, 6 equiv) and sodium acetate (160 mg, 2 mmol, 6 equiv). The reaction mixture was stirred for 1 h at room temperature and partitioned between ethyl acetate and water. The organic layer was washed with saturated aqueous sodium chloride, dried over sodium sulfate, and concentrated to a white residue. The crude product was purified by silica gel chromatography (2:3 to 1:1 Et_2O/hexanes) to give the oxime **8** (193 mg, 0.3 mmol, 100%). A solution of compound **8** (65 mg, 0.113 mmol) and 10% palladium on carbon (20 mg) in ethyl acetate (7 mL) was placed under hydrogen atmosphere and stirred for 3 h at room temperature. The reaction mixture was filtered on Celite and the filtrate concentrated to dryness. The residue was purified using silica gel chromatography (0.5:2:97.5 to 0.5:6:93.5 NH_4OH(aq)/MeOH/DCM) to give compound **10** as a white powder (15 mg, 0.113 mmol, 36%, 1/1 cis and trans oxime isomers). NMR δ_H (400 MHz, $CDCl_3$) 3.36 (dt, J = 10.8, 4.5 Hz, 1H), 3.16−3.10 (m, 1H), 3.06 (dd, J = 12.6, 4.4 Hz, 0.5H), 2.94 (dd, J = 15.2, 5.0 Hz, 0.5H), 2.70 (dd, J = 9.4, 7.6 Hz, 1H), 2.50−2.40 (m, 2H), 2.31 (t, J = 12 Hz, 1H), 2.27−1.14 (m, 28H), 0.95−088 (m, 9H); NMR δ_C (100 MHz, $CDCl_3$) 162.08, 161.88, 140.86, 124.89, 83.02, 77.56, 64.33, 56.98, 50.14, 49.94, 44.88, 43.32, 42.25, 42.20, 42.05, 40.49, 38.52, 37.56, 37.53, 35.66, 32.79, 31.89, 31.57, 30.68, 28.23, 28.18, 27.63, 25.98, 25.94, 25.27, 24.24, 22.43, 22.34, 20.30, 10.33; m/z = 441.50 $[M + H]^+$.

Compound 11. 11 was prepared as described for compound **10**. The ketone **5** (100 mg, 0.179 mmol) and methoxyamine hydrochloride with all other reagents scaled accordingly were used to generate the desired methyloxime **11** (45 mg, 0.99 mmol, 55% for 2 steps). NMR δ_H (400 MHz, $CDCl_3$) 4.78 (br s, 3H), 3.33 (dt, J = 10.2, 3.8 Hz, 1H), 3.03 (dd, J = 12.3, 3.8 Hz, 1H), 2.82 (dd, J = 14.9, 5.1 Hz, 1H), 2.66 (dd, J = 9.8, 7.2 Hz, 1H), 2.48−2.38 (m, 1H), 2.29 (t, J = 11.5 Hz, 1H), 1.25−1.00 (m, 28H), 0.92−0.86 (m, 9H); NMR δ_C (100 MHz, $CDCl_3$) 161.27, 161.08, 140.59, 140.57, 124.67, 82.74, 64.18, 61.09, 61.07, 54.85, 49.92, 49.69, 46.86, 46.80, 44.72, 43.73, 42.11, 41.99, 41.83, 40.29, 38.36, 37.49, 37.30, 35.41, 35.31, 32.58, 31.70, 31.36, 30.43, 27.97, 27.91, 27.38, 25.86, 25.76, 25.70, 24.02, 24.01,

22.20, 22.09, 20.89, 19.03, 10.13; $m/z = 455.30$ $[M + H]^+$; HPLC 96.5 a/a % at 215 nm.

Compound 14. A solution ketone **5** (300 mg, 0.536 mmol, 1 equiv) in anhydrous tetrahydrofuran (10 mL) was cooled to −78 °C and treated with 1.0 M potassium tri-*sec*-butyl borohydride in tetrahydrofuran (0.536 mL, 0.536 mmol, 1 equiv). The reaction mixture was stirred for 4 h and treated with methanol. The mixture was warmed to −30 °C and treated with 3 N aqueous sodium hydroxide and 15% aqueous sodium peroxide, keeping the temperature below 5 °C. The mixture was warmed to room temperature and stirred for 20 h. The mixture was partitioned between ethyl acetate and 1 N aqueous hydrochloric acid. The organic phase was separated, washed with saturated aqueous sodium chloride, dried over sodium sulfate, and concentrated. The residue was purified using silica gel chromatography (25−40% EtOAc/hexanes) to give the desired alcohol **12** (250 mg, 0.446 mmol, 90%). NMR δ_H (400 MHz, $CDCl_3$) 7.40−7.29 (m, 5H), 5.17 (d, $J = 12.6$ Hz, 1H), 5.13 (d, $J = 12.7$, 1H), 4.08 (br s, 1H), 3.82 (dd, $J = 12.9$, 5.3 Hz, 1H), 3.63 (dt, $J = 10.6$, 4.7 Hz, 1H), 3.08 (dd, $J = 10.3$, 7.3 Hz, 1H), 2.68−2.55 (m, 3H), 2.45 (br d, $J = 14.1$ Hz, 1H), 2.20 (td, $J = 11.2$, 4.1 Hz, 1H), 2.13 (dd, $J = 14.7$, 5.9 Hz, 1H), 2.01−1.00 (m, 25H), 0.97 (d, $J = 7.1$, 3H) 0.94−0.89 (m, 6H).

A suspension of compound **12** (170 mg, 0.303 mmol, 1 equiv) and 10% palladium on carbon (37 mg) in ethyl acetate (5 mL) was placed under hydrogen atmosphere, and the mixture was stirred for 3 h at room temperature. The reaction mixture was filtered on Celite and the filtrate concentrated to dryness. The oil was purified by silica gel flash chromatography (0.1:10:89.9 NH_4OH(aq)/MeOH/DCM) to give compound **14** as a white powder (72 mg, 0.168 mmol, 56%). NMR δ_H (400 MHz, $CDCl_3$) 4.05 (br s, 1H), 3.34 (dt, $J = 10.3$, 3.8 Hz, 1H), 3.04 (dd, $J = 12.9$, 4.1 Hz, 1H), 2.67 (dd, $J = 10.3$, 7.3 Hz, 1H), 2.43 (br d, $J = 14.1$ Hz 1H), 2.29 (t, $J = 12$ Hz, 1H), 2.21 (br td, $J = 11.8$, 3.9 Hz 1H), 2.11 (dd, $J = 14.7$, 6.4 Hz 1H), 2.01−1.00 (m, 28H), 0.92−0.87 (m, 9H); NMR δ_C (100 MHz, $CDCl_3$) 141.11, 124.22, 82.80, 67.05, 64.08, 54.72, 49.87, 49.78, 46.84, 44.62, 41.28, 40.24, 37.30, 35.79, 34.96, 33.65, 31.69, 31.32, 31.16, 30.51, 28.06, 27.90, 27.87, 26.02, 23.94, 22.90, 18.99, 10.27; $m/z = 428.29$ $[M + H]^+$; HPLC 99.2 a/a % at 215 nm.

Compound 15. A solution of cerium trichloride heptahydrate (260 mg, 0.697 mmol, 1.3 equiv) in methanol (10 mL) was cooled to 0 °C and treated with sodium borohydride (24 mg, 0.643 mmol, 1.2 equiv). After 10 min, the mixture was cooled to −78 °C and treated with a solution of ketone **5** (300 mg, 0.536 mmol, 1 equiv) in anhydrous tetrahydrofuran (5 mL). The mixture was stirred for 1 h, treated with water, ethyl acetate, and warmed to room temperature. The mixture was partitioned between water and 1 N aqueous hydrochloric acid. The organic phase was separated, washed with saturated aqueous sodium chloride, dried over sodium sulfate, and concentrated. The residue was purified using silica gel chromatography (30−50% Et_2O/hexanes) to give the desired alcohol **13** (235 mg, 0.418 mmol, 78%). NMR δ_H (400 MHz, $CDCl_3$) 7.38−7.29 (m, 5H), 5.15 (d, $J = 12.6$ Hz, 1H), 5.11 (d, $J = 12.7$, 1H), 3.81 (dd, $J = 13.7$, 3.7 Hz 1H), 3.67−3.54 (m, 1H), 3.08 (dd, $J = 10.0$, 6.7 Hz, 1H), 2.63 (dd, $J = 12.9$, 10.0 Hz, 1H), 2.59 (br t, $J = 6.7$ Hz, 1H), 2.45 (br d, $J = 14.1$ Hz, 1H), 2.19 (br td, $J = 12.1$, 3.5 Hz, 1H), 2.13 (dd, $J = 15.0$, 5.9 Hz, 1H), 2.06−1.10 (m, H), 0.97 (d, $J = 6.7$ Hz, 3H), 0.90 (d, $J = 7.0$ Hz, 3H), 0.86 (s, 3H).

A suspension of compound **13** (235 mg, 0.418 mmol, 1 equiv) and 10% palladium on carbon (51 mg) in ethyl acetate (5 mL) was placed under hydrogen atmosphere and stirred for 3 h at room temperature. The reaction mixture was filtered on Celite and the filtrate concentrated to dryness. The oil was purified by silica gel chromatography (0.1:6:93.9 NH_4OH(aq)/MeOH/DCM) to give compound **15** as a white foam (130 mg, 0.304 mmol, 73%). NMR δ_H (400 MHz, $CDCl_3$) 3.52 (br s, 1H), 3.33 (dt, $J = 10.3$, 3.5 Hz, 1H), 3.02 (dd, $J = 13.0$, 4.1 Hz, 1H), 2.66 (dd, $J = 9.7$, 7.1 Hz, 1H), 2.40 (br d, $J = 13.8$ Hz, 1H), 2.27 (t, $J = 12.1$ Hz, 1H), 2.21 (br td, $J = 12.1$, 3.5 Hz, 1H), 2.12 (dd, $J = 15.0$, 5.9 Hz, 1H), 2.01−1.00 (m, 28H), 0.91 (d, $J = 8.0$ Hz, 3H), 0.89 (d, $J = 6.4$ Hz, 3H), 0.85 (s, 3H); NMR δ_C (100 MHz, $CDCl_3$) 140.87, 124.26, 82.74, 71.64, 63.90, 54.55, 49.99, 49.87, 47.09, 44.63, 42.05, 41.63, 40.22, 37.41, 36.97, 36.76, 34.53, 31.77, 31.32, 31.27, 30.39, 28.52, 27.87, 26.21, 24.03, 22.50, 18.96, 10.21; $m/z = 428.30$ $[M + H]^+$; HPLC 98.9 a/a % at 215 nm.

Compound 16. A solution of alcohol **12** (110 mg, 0.196 mmol, 1 equiv) in dichloromethane (4 mL) was treated with benzyltriethylammonium chloride (9 mg, 0.039 mmol, 0.2 equiv), dimethyl sulfate (99 mg, 0.783 mmol, 4 equiv), and 50% (w/w) aqueous potassium hydroxide (0.300 mL). The mixture was stirred for 18 h at room temperature and partitioned between ethyl acetate and water. The organic phase was separated, washed with aqueous sodium chloride, dried over sodium sulfate, and concentrated to dryness. The residue was purified using silica gel chromatography (10% Et_2O/hexanes). A suspension of isolated product and 10% palladium on carbon (6 mg) in ethyl acetate (3 mL) was placed under hydrogen atmosphere and stirred for 3 h at room temperature. The reaction mixture was filtered on Celite and the filtrate concentrated to dryness. The oil was purified by silica gel chromatography (0.1:2:97.9 to 0.1/12/87.9 NH_4OH(aq)/MeOH/DCM) to give compound **16** as a white foam (9 mg, 0.020 mmol, 43%). NMR δ_H (400 MHz, $CDCl_3$) 3.47 (br s, 1H), 3.37 (t, $J = 10.3$ Hz, 1H), 3.28 (s, 3H), 3.07 (br d, $J = 12.2$ Hz, 1H), 2.70 (t, $J = 8.1$ Hz, 1H), 2.45 (br d, $J = 14.1$ Hz 1H), 2.32 (t, $J = 12$ Hz, 1H), 2.24 (br d, $J = 11.8$ Hz 1H), 2.14 (dd, $J = 14.7$, 6.4 Hz 1H), 2.01−1.00 (m, 27H), 0.95−0.86 (m, 9H); NMR δ_C (100 MHz, $CDCl_3$) 141.28, 124.23, 82.85, 76.12, 64.20, 55.67, 54.85, 49.96, 49.88, 46.95, 44.71, 41.59, 40.32, 37.37, 36.24, 34.86, 31.70, 31.57, 31.37, 30.65, 30.60, 28.08, 27.94, 26.11, 24.49, 24.00, 22.87, 19.05, 10.36; $m/z = 442.30$ $[M + H]^+$; HPLC 98.0 a/a % at 215 nm.

Compound 17. 13 was prepared as described for compound **16**. The alcohol **13** (100 mg, 0.178 mmol) with all other reagents scaled accordingly were used to generate the desired compound **17** (22 mg, 0.050 mmol, 32% for 2 steps). NMR δ_H (400 MHz, $CDCl_3$) 3.40−3.32 (m, 1H), 3.33 (s, 3H), 3.18−3.09 (m, 1H), 3.06 (dd, $J = 13.6$, 4.1 Hz, 1H), 2.69 (dd, $J = 10.2$, 7.3 Hz, 1H), 2.45 (br d, $J = 14.1$ Hz 1H), 2.31 (t, $J = 11.8$ Hz, 1H), 2.26−2.18 (m, 1H), 2.12 (dd, $J = 14.7$, 6.4 Hz 1H), 2.01−1.00 (m, 28H), 0.92−0.87 (m, 9H); NMR δ_C (100 MHz, $CDCl_3$) 140.96, 124.21, 82.96, 80.43, 64.21, 55.62, 54.86, 49.94, 49.74, 47.06, 44.67, 41.85, 41.51, 40.33, 37.27, 36.59, 34.87, 33.08, 31.70, 31.37, 30.36, 28.62, 27.88, 27.15, 26.12, 23.99, 22.54, 19.05, 10.36; $m/z = 442.31$ $[M + H]^+$; HPLC 99.7 a/a % at 215 nm.

Preparation of Compound Included in Scheme 3. Compound 20. A solution of alcohol **12** (7.60 g, 13.53 mmol, 1 equiv) in dichloromethane (115 mL) was treated with triethylamine (8.21 g, 81 mmol, 6.0 equiv), cooled to 0 °C, and treated with methanesulfonyl chloride (1.86 g, 16.2 mmol, 1.2 equiv). After 30 min, the reaction mixture was partitioned between saturated aqueous sodium bicarbonate and ethyl acetate. The organic layer was separated, dried over sodium sulfate, and concentrated to dryness. The residue was purified using silica gel chromatography (10−25% EtOAc/hexanes). A solution of the isolated product in DMPU (50 mL) was treated with sodium azide (4.17 g, 64.3 mmol, 5.0 equiv), heated to 60 °C, and stirred for 17 h. The reaction mixture was cooled to room temperature and partitioned between methyl *tert*-butyl ether and 5% aqueous sodium chloride. The organic layer was separated, washed with 5% aqueous sodium chloride, dried over sodium sulfate, and concentrated to dryness. The residue was purified using silica gel chromatography (5−15% EtOAc/hexanes) to give the desired azide **18** (7.16 g, 12.2 mmol, 90% for two steps). NMR δ_H (400 MHz, $CDCl_3$) 7.40−7.30 (m, 5H), 5.17 (d, $J = 12.6$ Hz, 1H), 5.13 (d, $J = 12.7$, 1H), 3.83 (dd, $J = 12.8$, 3.9, 1H), 3.64 (dt, $J = 10.9$, 3.8 Hz, 1H), 3.32−3.21 (m, 1H), 3.10 (dd, $J = 13.1$, 6.3 Hz, 1H), 2.70−2.56 (m, 2H), 2.47 (br d, $J = 14.4$ Hz, 1H),

2.33 (br td, J = 11.6, 4.3 Hz, 1H), 2.14 (dd, J = 14.8, 5.9 Hz, 1H), 2.06−1.12 (m, 25H), 0.98 (d, J = 6.6 Hz, 3H), 0.92 (d, J = 7.4 Hz, 3H), 0.89 (s, 3H).

A solution of azide **18** (49 mg, 0.084 mmol, 1 equiv) in toluene (4 mL) was treated with trimethylsilylacetylene (164 mg, 1.67 mmol, 20 equiv). The reaction mixture was sealed and heated to 115 °C for 16 h. The reaction mixture was cooled to room temperature and the solvent removed under vacuum. The residue was purified using silica gel chromatography (2−20% EtOAc/hexanes). A solution of isolated product in anhydrous tetrahydrofuran (3 mL) was treated with 1.0 M tetrabutylammonium fluoride in tetrahydrofuran (0.131 mL, 0.131 mmol, 2 equiv) and heated to 55 °C. The mixture was stirred for 4 h, cooled to room temperature, and partitioned between saturated aqueous bicarbonate and methyl *tert*-butyl ether. The organic phase was separated, dried over sodium sulfate, and concentrated. The residue was purified by silica gel chromatography (EtOAc/hexanes). A suspension of isolated product and 10% palladium on carbon (10 mg) in ethanol (5 mL) was placed under hydrogen atmosphere and stirred for 1.5 h at room temperature. The reaction mixture was filtered on Celite and the filtrate concentrated to dryness. The residue was purified using silica gel chromatography (0−10% MeOH/DCM) to give compound **20** (8 mg, 0.017 mmol, 26% for three steps). NMR δ_H (400 MHz, $CDCl_3$) 7.69 (s, 1H), 7.58 (s, 1H), 4.50 (m, 1H), 3.36 (dt, J = 10.2, 4.2 Hz, 1H), 3.06 (dd, J = 12.7, 5.1 Hz, 1H), 2.70 (dd, J = 9.3, 6.7 Hz, 1H), 2.47 (d, J = 13.5 Hz, 1H), 2.32 (t, J = 11.9 Hz, 1H), 2.24 (br d, J = 12.7 Hz, 1H), 2.19 (dd, J = 15.2, 6.8 Hz, 2H), 2.10−1.00 (m, 26H), 0.96 (s, 3H), 0.93 (d, J = 7.6 Hz, 3H), 0.91 (d, J = 6 Hz, 3H); NMR δ_C (100 MHz, $CDCl_3$) 140.52, 133.47, 124.80, 121.30, 82.75, 64.22, 60.89, 54.90, 49.85, 49.70, 46.99, 44.78, 42.09, 42.07, 40.35, 37.40, 37.14, 34.75, 34.44, 31.75, 31.18 30.33, 28.46, 28.25, 27.95, 26.10, 24.02, 22.52, 19.07, 10.35; m/z = 479.31 $[M + H]^+$; HPLC 95.5 a/a % at 215 nm.

Compound 21. 19 was prepared as described for compound **18**. The alcohol **13** (174 mg, 0.310 mmol) and all other reagents scaled accordingly were used to generate the desired azide **19** (174 mg, 0.298 mmol, 96% for two steps). NMR δ_H (400 MHz, $CDCl_3$) 7.38−7.30 (m, 5H), 5.16 (d, J = 12.3 Hz, 1H), 5.12 (d, J = 12.5 Hz, 1H), 3.93 (br s, 1H), 3.82 (dd, J = 12.3, 3.6, 1H), 3.63 (dt, J = 10.7, 4.0 Hz, 1H), 3.09 (dd, J = 13.3, 6.0 Hz, 1H), 2.68−2.56 (m, 2H), 2.44 (br d, J = 14.0 Hz, 1H), 2.20 (br td, J = 11.6, 4.3 Hz, 1H), 2.13 (dd, J = 14.8, 5.9 Hz, 1H), 2.06−1.07 (m, 25H), 0.98 (d, J = 6.3 Hz, 3H), 0.91 (d, J = 7.1 Hz, 3H), 0.90 (s, 3H).

21 was prepared as described for compound **20**. The azide **19** (60 mg, 0.102 mmol) and all other reagents scaled accordingly were used to generate the desired triazole **21** (8 mg, 0.018 mmol, 12% for three steps). NMR δ_H (400 MHz, $CDCl_3$) 7.69 (br d, J = 13.1 Hz, 2H), 4.69 (br s, 1H), 3.37 (dt, J = 10.2, 3.4 Hz, 1H), 3.07 (dd, J = 12.5, 4.2 Hz, 1H), 2.70 (dd, J = 10.7, 7.6 Hz, 1H), 2.46 (br d, J = 13.6 Hz, 1H), 2.39−1.13 (m, 28H), 1.07 (t, J = 11.4 Hz, 2H) 0.98−0.87 (m, 6H), 0.85 (s, 3H); NMR δ_C (100 MHz, $CDCl_3$) 140.71, 133.55, 124.68, 122.61, 82.77, 64.18, 56.62, 54.82, 49.96, 49.83, 46.85, 44.73, 42.04, 40.30, 37.38, 36.54, 34.72, 31.87, 31.67, 31.38, 30.48, 30.20, 27.92, 27.29, 25.98, 25.33, 24.04, 22.87, 19.04, 10.36; m/z = 479.30 $[M + H]^+$; HPLC 98.0 a/a % at 215 nm.

Compound 24. A solution of azide **18** (7.16 g, 12.2 mmol, 1 equiv) in tetrahydrofuran (120 mL) was treated with triphenylphosphine (9.60 g, 36.6 mmol, 3.0 equiv) and water (2.19 g, 122.3 mmol, 10 equiv). The mixture was heated to 55 °C, stirred for 20 h, cooled to room temperature, and concentrated to dryness. The residue was purified using silica gel chromatography (10−20% MeOH/DCM) to afford the desired amine **22** (5.83 g, 10.4 mmol, 85%). NMR δ_H (400 MHz, $CDCl_3$) 8.38−8.34 (m, 1H), 7.39−7.30 (m, 5H), 5.16 (d, J = 12.2 Hz, 1H), 5.12 (d, J = 12.3 Hz, 1H), 3.83 (dd, J = 12.0, 3.9 Hz, 1H), 3.63 (dt, J = 10.8, 4.0 Hz, 1H), 3.10 (dd, J = 13.3, 6.0 Hz, 1H), 2.70−2.57 (m, 4H), 2.46 (br d, J = 14.0 Hz, 1H), 2.20 (br td, J = 11.2, 4.3 Hz, 1H), 2.13 (dd, J = 14.8, 5.9 Hz, 1H), 2.06−1.07 (m, 25H), 0.98 (d, J = 6.3 Hz, 3H), 0.91 (d, J = 6.8 Hz, 3H), 0.87 (s, 3H).

A solution of compound **22** (206 mg, 0.368 mmol, 1 equiv) and 10% palladium on carbon (42 mg) in 2-propanol (5 mL) was placed under hydrogen atmosphere and stirred for 18 h at room temperature. The reaction mixture was filtered on Celite and the filtrate concentrated to dryness. The residue was purified using silica gel chromatography (0−10% MeOH/DCM) to give compound **24** (110 mg, 0.258 mmol, 70%). NMR δ_H (400 MHz, $CDCl_3$) 3.35 (dt, J = 10.0, 3.2 Hz, 1H), 3.05 (dd, J = 12.6, 3.5 Hz, 1H), 2.74−2.60 (m, 2H), 2.44 (br d, J = 13.8 Hz, 1H), 2.30 (t, J = 11.9 Hz, 1H), 2.22 (br d, J = 11.2 Hz, 1H), 2.11 (dd, J = 14.7, 5.6 Hz 1H), 2.01−1.00 (m, 29H), 0.95−0.88 (m, 6H), 0.86 (s, 3H); NMR δ_C (100 MHz, $CDCl_3$) 140.93, 124.14, 82.78, 76.77, 64.11, 54.76, 51.41, 49.86, 49.68, 46.98, 44.61, 41.87, 40.24, 37.49, 37.28, 37.23, 34.46, 31.64, 31.59, 30.27, 28.42, 27.82, 26.11, 25.47, 23.90, 22.59, 18.97, 10.27; m/z = 427.30 $[M + H]^+$; HPLC 95.1 a/a % at 215 nm.

Compound 25. 23 was prepared as described for compound **22**. The azide **19** (126 mg, 0.215 mmol) and all other reagents scaled accordingly were used to generate the desired amine **23** (107 mg, 0.191 mmol, 85%). NMR δ_H (400 MHz, $CDCl_3$) 7.37−7.29 (m, 5H), 5.15 (d, J = 12.2 Hz, 1H), 5.11 (d, J = 12.3 Hz, 1H), 3.81 (dd, J = 13.2, 3.8 Hz, 1H), 3.62 (dt, J = 10.6, 4.0 Hz, 1H), 3.21 (br s, 1H), 3.08 (dd, J = 9.8, 5.4 Hz, 1H), 2.67−2.55 (m, 2H), 2.44 (br d, J = 14.0 Hz, 1H), 2.19 (br td, J = 11.5, 4.2 Hz, 1H), 2.12 (dd, J = 14.2, 5.9 Hz, 1H), 2.04−1.11 (m, 25H), 0.96 (d, J = 6.3 Hz, 3H), 0.90 (d, J = 6.8 Hz, 3H), 0.89 (s, 3H).

25 was prepared as described for compound **24**. The amine **23** (107 mg, 0.191 mmol) and all other reagents scaled accordingly were used to generate the desired amine **21** (73 mg, 0.171 mmol, 90%). NMR δ_H (400 MHz, $CDCl_3$) 3.36 (dt, J = 10.5, 3.3 Hz, 1H), 3.23 (br s, 1H), 3.06 (dd, J = 11.9, 3.3 Hz, 1H), 2.69 (t, J = 8.1 Hz, 1H), 2.46 (br d, J = 13.4 Hz, 1H), 2.32 (t, J = 11.9 Hz, 1H), 2.24 (br d, J = 11.4 Hz, 1H), 2.14 (dd, J = 14.4, 6.2 Hz, 1H), 2.04−1.03 (m, 29H), 0.95−0.89 (m, 9H); NMR δ_C (100 MHz, $CDCl_3$) 141.16, 124.26, 82.84, 64.20, 54.88, 49.95, 49.85, 46.91, 46.44, 44.73, 41.31, 40.34, 37.37, 35.75, 35.23, 33.99, 31.77, 31.37, 31.18, 30.52, 28.39, 28.18, 27.93, 26.14, 23.99, 23.15, 19.05, 10.34; m/z = 427.31 $[M + H]^+$; HPLC 92.7 a/a % at 215 nm.

Compound 26. A solution of amine **22** (510 mg, 0.933 mmol, 1 equiv) in pyridine (5 mL) was treated with acetic anhydride (95 mg, 0.933 mmol, 1 equiv) and stirred for 1 h. The reaction mixture was partitioned between saturated aqueous sodium bicarbonate and ethyl acetate. The organic phase was separated, washed with saturated sodium chloride, dried over sodium sulfate, and concentrated to an oil. A solution of the residue (245 mg, 0.406 mmol, 1 equiv) and 10% palladium on carbon (46 mg) in 2-propanol (5 mL) was placed under hydrogen atmosphere and stirred for 16 h at room temperature. The reaction mixture was filtered on Celite and the filtrate concentrated to dryness. The residue was purified using silica gel chromatography (0−10% MeOH/DCM) to give the acetamide **26** (185 mg, 0.406 mmol, 100% for two steps). NMR δ_H (400 MHz, $CDCl_3$) 5.44 (br d, J = 7.8 Hz, 1H), 3.72 (br s, 1H), 3.33 (dt, J = 10.4, 3.5 Hz, 1H), 3.03 (dd, J = 12.6, 3.9 Hz, 1H), 2.66 (t, J = 7.3 Hz, 1H), 2.41 (br d, J = 13.9 Hz, 1H), 2.29 (t, J = 11.7 Hz, 1H), 2.21 (br d, J = 11.7 Hz, 1H), 2.10 (dd, J = 14.7, 6.0 Hz, 1H), 1.91 (s, 3H), 1.99−1.03 (m, 27H), 0.90 (d, J = 3.0 Hz, 3H), 0.88 (d, J = 2.2 Hz, 3H), 0.85 (s, 3H); NMR δ_C (100 MHz, $CDCl_3$) 169.27, 140.87, 124.41, 82.73, 64.12, 54.77, 49.89, 49.80, 49.40, 46.97, 44.66, 42.02, 41.63, 40.27, 37.33, 37.05, 34.47, 33.92, 31.72, 31.33, 30.31, 28.27, 28.23, 27.88, 26.16, 23.97, 23.67, 22.55, 19.00, 10.30; m/z = 469.32 $[M + H]^+$; HPLC 95.8 a/a % at 215 nm.

Compound 27. 27 was prepared as described for compound **26**. The amine **23** (50 mg, 0.089 mmol) and all other reagents scaled accordingly were used to generate the desired acetamide **27** (34 mg, 0.070 mmol, 78% for two steps). NMR δ_H (400 MHz, $CDCl_3$) 5.69 (br d, J = 7.1 Hz, 1H), 4.18 (br s, 1H), 3.38 (dt, J = 10.5, 3.9 Hz, 1H), 3.08 (dd, J = 13.1, 4.5 Hz, 1H),

2.71(dd, J = 9.7, 7.9 Hz, 1H), 2.46 (br d, J = 14.3 Hz, 1H), 2.33 (t, J = 11.8 Hz, 1H), 2.24 (br d, J = 11.7 Hz, 1H), 2.14 (dd, J = 15.0, 5.8 Hz, 1H), 2.00 (s, 3H), 1.99−1.03 (m, 27H), 0.96−0.90 (m, 9H); NMR δ_C (100 MHz, $CDCl_3$) 169.40, 140.79, 124.49, 82.86, 64.10, 54.79, 49.92, 49.70, 46.86, 45.34, 44.64, 41.30, 40.23, 37.30, 37.25, 34.97, 32.49, 31.50, 31.36, 30.76, 30.47, 27.99, 27.90, 25.93, 25.09, 23.99, 23.85, 23.18, 19.00, 10.36; m/z = 469.32 $[M + H]^+$; HPLC 98.8 a/a % at 215 nm.

Compound 28. A solution of amine **22** (5.10 g, 9.09 mmol, 1 equiv) in dichloromethane (60 mL) was treated with diisopropylethylamine (5.88 g, 45.5 mmol, 5.0 equiv), cooled to 0 °C, and treated with methanesulfonyl chloride (2.08 g, 18.2 mmol, 2.0 equiv). The reaction mixture was stirred for 30 min and partitioned between saturated aqueous sodium bicarbonate and ethyl acetate. The organic layer was separated, dried over sodium sulfate, and concentrated to dryness. The residue was purified using silica gel chromatography (10−30% EtOAc/hexanes). A suspension of the isolated product and 10% palladium on carbon (1.0 g) in 2-propanol (50 mL) was placed under hydrogen atmosphere and stirred for 4 h at room temperature. The reaction mixture was then filtered on Celite and the filtrate concentrated to dryness. The residue was then purified using silica gel chromatography (0−5% DCM/MeOH) to give the desired compound **28** (4.06 g, 8.05 mmol, 95% for two steps). NMR δ_H (400 MHz, $CDCl_3$) 6.90 (br s, 1H), 3.31 (dt, J = 10.6, 3.8 Hz, 1H), 3.20 (br s, 1H), 3.10 (dd, J = 13.7, 4.5 Hz, 1H), 2.91 (s, 3H), 2.62 (dd, J = 9.9, 7.6 Hz, 1H), 2.33 (br d, J = 14.5 Hz, 1H), 2.27−2.15 (m, 1H), 2.10 (dd, J = 14.5, 6.9 Hz, 1H), 1.99−1.17 (m, 28H), 1.05 (q, J = 11.6 Hz, 1H), 0.93 (d, J = 7.4 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H), 0.86 (s, 3H); NMR δ_C (100 MHz, $CDCl_3$) 140.47, 124.53, 82.48, 76.97, 63.73, 54.08, 53.87, 50.12, 49.98, 47.19, 44.73, 42.27, 42.10, 40.24, 37.55, 37.44, 36.04, 34.44, 31.87, 31.33, 30.46, 29.79, 28.37, 27.94, 26.26, 24.19, 22.70, 18.92, 10.19; m/z = 505.29 $[M + H]^+$; HPLC 99.1 a/a % at 215 nm.

Compound 29. 29 was prepared as described for compound **28**. The amine **23** (50 mg, 0.089 mmol) and all other reagents scaled accordingly were used to generate the desired compound **29** (35 mg, 0.072 mmol, 90% for two steps). NMR δ_H (400 MHz, $CDCl_3$) 4.47 (d, J = 7.5 Hz, 1H), 3.81−3.74 (m, 1H), 3.36 (dt, J = 10.0, 3.5 Hz, 1H), 3.07 (dd, J = 12.7, 3.6 Hz, 1H), 2.96 (s, 3H), 2.69 (dd, J = 9.6, 7.7 Hz, 1H), 2.44 (d, J = 13.6 Hz, 1H), 2.32 (t, d, J = 13.4 Hz, 1H), 2.26−2.19 (m, 1H) 2.13(dd, J = 14.3, 6.1 Hz, 1H), 2.08−1.02 (m, 28H) 0.96−0.88 (m, 9H); NMR δ_C (100 MHz, $CDCl_3$) 140.66, 124.61, 82.77, 64.11, 54.73, 50.14, 49.91, 49.70, 46.80, 44.66, 41.66, 41.48, 40.24, 37.31, 36.59, 34.87, 32.21, 31.71, 31.58, 31.36, 30.47, 27.95, 27.88, 26.53, 25.98, 24.01, 23.01, 19.01, 10.35; m/z = 505.38 $[M + H]^+$; HPLC 98.6 a/a % at 215 nm.

Preparation of Compound Included in Scheme 4. Compound 32. A solution of ketone **5** (6.3 g, 11.25 mmol, 1 equiv) in *tert*-butanol (65 mL) was treated with potassium *tert*-butoxide (8.84 g, 79 mmol, 7 equiv) and stirred for 10 min. The mixture was treated with ethyl formate (5.00 g, 67.5 mmol, 6 equiv) and stirred for 48 h at room temperature. The mixture was partitioned between diethyl ether and 1 N aqueous sodium hydroxide. The aqueous layer was separated and acidified to pH 1−2 with concentrated aqueous hydrochloric acid and extracted with dichloromethane. The combined organic extracts were dried with sodium sulfate and concentrated to give the desired compound **30** (6.0 g, 10.21 mmol, 91%, 9/1 C2/C4 epimer). NMR δ_H (400 MHz, $CDCl_3$) 8.75 (d, J = 4 Hz, 1H C4 epimer), 8.45 (d, J = 3.4 Hz, 1H C2 epimer), 7.39−7.29 (m, 5H), 5.16 (d, J = 12.6 Hz, 1H), 5.12 (d, J = 12.7, 1H), 3.82 (dd, J = 12.6, 4.0 Hz, 1H), 3.63 (dt, J = 10.8, 3.8 Hz, 1H), 3.08 (dd, J = 9.7, 6.2 Hz, 1H), 2.64 (dd, J = 13.1, 9.4 Hz, 1H), 2.59 (t, J = 6.2 Hz, 1H), 2.53−2.39 (m, 2H), 2.33−1.15 (m, 27H), 1.00 (s, 3H), 0.97 (d, J = 6.2 Hz, 3H), 0.90 (d, J = 6.8 Hz, 3H).

A solution of ketone **30** (6.0 g, 10.21 mmol, 1 equiv) in ethanol (100 mL) was treated with hydrazine hydrate (1.022 g, 20.42 mmol, 2 equiv), heated to 80 °C, stirred for 30 min, and concentrated to dryness. The residue was purified by silica gel chromatography (20−55% EtOAc/hexanes) to give the desired pyrazole **31** (4.5 g, 7.71 mmol, 76%). NMR δ_H (400 MHz, $CDCl_3$) 7.37−7.30 (m, 5H), 7.29 (s, 1H), 5.14 (d, J = 12.5 Hz, 1H), 5.10 (d, J = 12.4, 1H), 3.81 (dd, J = 12.7, 4.1 Hz, 1H), 3.62 (dt, J = 10.6, 3.8 Hz, 1H), 3.06 (dd, J = 9.5, 6.3 Hz, 1H), 2.80 (dd, J = 17.4, 10.8 Hz, 1H), 2.68−2.52 (m, 4H), 2.37 (br d, J = 13.8 Hz, 1H), 2.28 (br d, J = 16.1 Hz, 1H), 2.19 (dt, J = 11.8, 4.5 Hz, 1H), 2.10−1.18 (m, 22H), 1.05 (s, 3H), 0.97 (d, J = 6.8 Hz, 3H), 0.89 (d, J = 7.1 Hz, 3H).

A suspension of pyrazole **31** (4.5 g, 7.71 mmol, 1 equiv) and 10% palladium on carbon (350 mg) in ethanol (50 mL) and ethyl acetate (50 mL) was placed under hydrogen atmosphere and stirred for 1.5 h at room temperature. The reaction mixture was filtered on Celite and the filtrate concentrated to dryness. The residue was purified using silica gel chromatography (0.5/2/97.5 to 0.5/6/93.5% NH_4OH/DCM/MeOH) to afford the desired product **32** (3.0 g, 6.67 mmol, 87%). NMR δ_H (400 MHz, $CDCl_3$) 3.32 (dt, J = 10.2, 3.5 Hz, 1H), 3.02 (dd, J = 12.5, 4.1 Hz, 1H), 2.75 (dd, J = 17.1, 11.5 Hz, 1H), 2.63 (t, J = 8.6 Hz, 1H), 2.58−2.49 (m, 2H), 2.37−2.15 (m, 4H), 2.07−1.18 (m, 24H), 1.00 (s, 3H), 0.9−0.85 (m, 6H); NMR δ_C (100 MHz, $CDCl_3$) 140.64, 124.38, 113.97, 82.86, 64.14, 54.76, 49.99, 49.89, 46.55, 44.68, 42.30, 40.27, 39.07, 37.29, 35.62, 32.73, 31.65, 31.37, 30.85, 27.84, 27.20, 25.42, 23.97, 23.03, 22.23, 19.03, 10.35; m/z = 450.19 $[M + H]^+$; HPLC 99.8 a/a % at 215 nm.

Compound 36. A solution of potassium hexamethyldisilazane (310 mg, 1.55 mmol, 1.5 equiv) in anhydrous THF (10 mL) was cooled to −78 °C and treated with a solution of ketone **5** (580 mg, 1.036 mmol, 1 equiv) in anhydrous tetrahydrofuran (2 mL) followed by triethylsilyl chloride (234 mg, 1.55 mmol, 1.5 equiv). The reaction mixture was stirred for 15 min, partitioned between water and ethyl acetate, and warmed to room temperature. The organic phase was separated, dried over sodium sulfate, and concentrated to a colorless oil. The residue was purified by silica gel chromatography (2−6% EtOAc/hexanes) to give the desired compound **33** (546 mg, 0.810 mmol, 78%). NMR δ_H (400 MHz, $CDCl_3$) 7.39−7.31 (m, 5H), 5.14 (d, J = 12.5 Hz, 1H), 5.10 (d, J = 12.4, 1H), 4.73 (br d, 1H minor C2 isomer), 4.50 (s, 1H major C4 isomer), 3.82 (dd, J = 13.1, 4.3 Hz, 1H), 3.63 (dt, J = 10.7, 4.9 Hz, 1H), 3.19 (dd, J = 10.7, 6.3 Hz, 1H), 2.66 (dd, J = 13.1, 10.1 Hz, 1H), 2.59 (t, J = 6.3 Hz, 1H), 2.43 (br d, J = 14.1 Hz, 1H), 2.24−1.03 (m, 25H), 1.00−0.95 (m, 12H), 0.93−0.87 (s, 6H), 0.69−0.61 (m 6H).

A 37% aqueous formaldehyde solution (0.302 mL, 4.05 mmol, 5 equiv) was diluted in tetrahydrofuran (10 mL), stirred for 10 min, and cooled to −20 °C. The reaction mixture was treated with a solution of the compound **33** (546 mg, 0.810 mmol, 1 equiv) and 1.0 M of tetrabutylammonium fluoride in tetrahydrofuran (4.05 mL, 4.05 mmol, 5 equiv), keeping the internal temperature below −20 °C. The mixture was stirred for 30 min and quenched with saturated aqueous sodium chloride. The mixture was warmed to room temperature and extracted with ethyl acetate. The combined extracts were dried over sodium sulfate and concentrated to dryness. The residue was purified by silica gel chromatography (10−20% EtOAc/hexanes) to give the desired alcohol **34** (330 mg, 0.560 mmol, 69%). NMR δ_H (400 MHz, $CDCl_3$) 7.37−7.30 (m, 5H), 5.14 (d, J = 12.5 Hz, 1H), 5.10 (d, J = 12.4 Hz, 1H), 3.90−3.78 (m, 2H), 3.70−3.58 (m, 2H), 3.08 (dd, J = 13.1, 4.3 Hz, 1H), 2.77−2.41 (m, 6H), 2.26−2.16 (m, 3H), 2.08−1.11 (m, 23H), 0.99−0.88 (m, 9H).

A solution of alcohol **34** (100 mg, 0.170 mmol, 1 equiv) in dichloromethane (5 mL) was cooled to 5 °C and treated with Dess−Martin periodinane (144 mg, 0.339 mmol, 2 equiv). The reaction mixture was stirred for 30 min and partitioned between saturated aqueous bicarbonate and dichloromethane. The organic phase was separated, dried over sodium sulfate, and concentrated to dryness. The residue was purified by silica gel

chromatography (5−10% EtOAc/hexanes). A solution of the isolated product in ethanol (5 mL) was treated with hydrazine (27 mg, 0.85 mmol, 10 equiv) and heated to 80 °C. The reaction mixture was stirred for 15 min and concentrated to dryness. The residue was purified by silica gel chromatography (50% EtOAc/hexanes) to give the desired pyrazole **35** (43 mg, 0.073 mmol, 65% for two steps). NMR δ_H (400 MHz, $CDCl_3$) 7.38−7.31 (m, 5H), 7.30 (br s, 1H), 5.15 (d, J = 12.5 Hz, 1H), 5.11 (d, J = 12.7 Hz, 1H), 3.81 (dd, J = 13.1, 4.3 Hz, 1H), 3.62 (dt, J = 10.7, 4.9 Hz, 1H), 3.08 (dd, J = 10.1, 6.2 Hz, 1H), 2.70−2.53 (m, 5H), 2.37 (br d, J = 13.9 Hz, 1H), 2.24−2.13 (m, 2H), 2.10−1.03 (m, 22H), 1.02 (s, 3H), 0.98 (d, J = 6.9 Hz, 3H), 0.89 (d, J = 7 Hz, 3H).

A suspension of pyrazole **35** (43 mg, 0.073 mmol, 1 equiv) and 10% palladium on carbon (8 mg) in ethyl acetate (3 mL) was placed under hydrogen atmosphere and stirred for 16 h at room temperature. The reaction mixture was filtered on Celite and the filtrate concentrated to dryness. The residue was purified using silica gel chromatography (5% DCM/MeOH) to afford the desired product **36** (30 mg, 0.067 mmol, 91%). NMR δ_H (400 MHz, $CDCl_3$) 7.30 (br s, 1H), 3.35 (dt, J = 10.6, 3.8 Hz, 1H), 3.06 (dd, J = 12.6, 4.2 Hz, 1H), 2.90−2.61 (m, 4H), 2.39−2.27 (m, 2H), 2.23 (td, J = 11.8, 3.4, Hz, 1H), 2.16 (dd, J = 14.8, 5.4 Hz, 1H), 2.10−1.03 (m, 23H), 1.00 (s, 3H), 0.93−0.86 (s, 6H); NMR δ_C (100 MHz, $CDCl_3$) 140.77, 124.17, 119.01, 82.88, 76.62, 64.02, 54.55, 49.89, 49.43, 46.63, 44.57, 42.23, 40.22, 39.29, 37.29, 35.39, 35.35, 31.50, 31.37, 30.84, 27.90, 26.78, 26.35, 24.05, 21.22, 19.00, 18.82, 10.33; m/z = 450.28 $[M + H]^+$; HPLC 97.3 a/a % at 215 nm.

Preparation of Compound Included in Scheme 5. Compound 38. A solution of hydroxymethylene **30** (300 mg, 0.510 mmol, 1 equiv) in dioxane (5 mL) and water (0.5 mL) was treated with a solution of sodium acetate (42 mg, 0.510 mmol, 1 equiv) in acetic acid (31 mg, 0.510 mmol, 1 equiv) and *N*-bromosuccinimide (95 mg, 0.536 mmol, 1.05 equiv). The mixture was stirred at room temperature for 16 h and partitioned between ethyl acetate and aqueous sodium sulfite. The organic phase was separated, washed with water, washed with saturated aqueous sodium chloride, dried over sodium sulfate, and concentrated to dryness. A solution of the crude residue in ethanol (5 mL) and sodium methoxide (0.05 mL) was stirred for 40 min and partitioned between ethyl acetate and water. The organic phase was separated, washed with water, washed with saturated aqueous sodium chloride, dried over sodium sulfate, and concentrated to dryness. The residue was purified by silica gel chromatography (5−30% EtOAc/hexanes) to give the desired bromoketone **37** (159 mg, 0.249 mmol, 49%). NMR δ_H (400 MHz, $CDCl_3$) 7.39−7.30 (m, 5H), 5.15 (d, J = 12.6 Hz, 1H), 5.11 (d, J = 12.7, 1H), 4.82 (dd, J = 13.8, 6.4 Hz, 1H), 3.82 (dd, J = 12.8, 4.0 Hz, 1H), 3.63 (dt, J = 11.1, 3.7 Hz, 1H), 3.10 (dd, J = 10.2, 6.4 Hz, 1H), 2.81 (t, J = 13.8, 1H), 2.69−2.16 (m, 7H), 2.14−1.08 (m, 20H), 1.00−0.82 (m, 9H).

A solution of bromoketone **37** (97 mg, 0.152 mmol, 1 equiv) in absolute ethanol (1 mL) was treated with thioacetamide (53 mg, 0.70 mmol, 4.6 equiv) and refluxed for 3 h. The reaction mixture was cooled to room temperature and partitioned between water and ethyl acetate. The organic phase was separated, washed with saturated aqueous sodium chloride, dried over sodium sulfate, and concentrated to dryness. The residue was purified by silica gel chromatography (5−35% EtOAc/hexanes). A suspension of the isolated product and 10% palladium on carbon (8 mg) in ethanol (6 mL) was placed under hydrogen atmosphere and stirred for 3 h at room temperature. The reaction mixture was filtered on Celite and the filtrate concentrated to dryness. The residue was purified using silica gel flash chromatography (5% DCM/MeOH) to afford the desired product (16 mg, 0.034 mmol, 22% for two steps). NMR δ_H (400 MHz, $CDCl_3$) 3.34 (dt, J = 10.6, 4.0 Hz, 1H), 3.04 (dd, J = 12.8, 4.0 Hz, 1H), 2.84 (br dd, J = 17.7, 10.6 Hz, 1H), 2.71 (t, J = 16.8, 1H), 2.68−2.62 (m, 1H), 2.61 (s, 3H), 2.48 (br d, J = 16.8 Hz, 1H), 2.37 (br d, J = 14.0 Hz, 1H), 2.29 (t, J = 11.8 Hz, 1H), 2.22 (td, J = 11.5, 3.8 Hz, 1H), 2.12−1.00 (m, 24H), 1.04 (s, 3H), 0.91−0.89 (m, 6H); NMR δ_C (100 MHz, $CDCl_3$) 162.66, 148.34, 140.53, 126.43, 124.59, 82.86, 64.09, 54.68, 49.95, 49.88, 46.65, 44.60, 42.52, 40.24, 39.01, 37.24, 36.19, 35.20, 31.48, 30.95, 29.85, 28.16, 27.83, 26.96, 25.48, 24.01, 21.94, 19.26, 19.02, 10.39; m/z = 481.23 $[M + H]^+$; HPLC 97.7 a/a % at 215 nm.

Compound 40. A solution of ketone **30** (500 mg, 0.851 mmol, 1 equiv) in dichloromethane (5 mL), acetic acid (20 mL), and water (2.5 mL) was cooled to 0 °C. The mixture was treated with sodium nitrite (59 mg, 0.851 mmol, 1 equiv) and stirred for 30 min. The reaction mixture was concentrated to dryness and purified by silica gel chromatography (50% EtOAc/hexanes) to give the desired oxime **39** (120 mg, 0.204 mmol, 24%).

A solution of compound **39** (120 mg, 0.204 mmol, 1 equiv) in ethanol (5 mL) and water (0.25 mL) was treated with sodium acetate (50 mg, 0.611 mmol, 3 equiv) and hydroxylamine hydrochloride (43 mg, 0.611 mmol, 3 equiv). The mixture was stirred for 3 h at room temperature and partitioned between ethyl acetate and water. The organic layer was separated, dried over sodium sulfate, and concentrated to dryness. The crude product was purified by silica gel chromatography (20−50% EtOAc/hexanes). A solution of the isolated product in dioxane (2 mL) and ethylene glycol (3 mL) was placed in a sealed tube and treated with potassium hydroxide (1 pellet). The mixture was heated to 120 °C for 90 min and partitioned between ethyl acetate and water. The organic layer was separated, dried over sodium sulfate, and concentrated to dryness. The crude product was purified by silica gel chromatography (0.5/1/98.5 to 0.5/10/89.5 NH_4OH/DCM/MeOH). A suspension of the isolated product and 10% palladium on carbon (30 mg) in ethanol (2.5 mL) and ethyl acetate (2.5 mL) was placed under hydrogen atmosphere and stirred for 2 h at room temperature. The reaction mixture was filtered on Celite and washed with ethanol and the filtrate concentrated to dryness. The residue was purified by silica gel chromatography (0.5−6% MeOH/DCM) to give the desired compound **40** (11 mg, 0.024 mmol, 12% for three steps). NMR δ_H (400 MHz, $CDCl_3$) 3.36 (dt, J = 10.1, 4.0 Hz, 1H), 3.11−2.89 (m, 3H), 2.68 (dd, J = 9.8, 7.4 Hz, 1H), 2.45 (d, J = 17.2 Hz, 1H), 2.38 (br d, J = 14.0 Hz, 1H), 2.32 (t, J = 11.7 Hz, 1H), 2.22 (td, J = 11.3, 3.2 Hz, 1H), 2.12 (dd, J = 14.5, 6.2 Hz, 1H), 2.07−1.16 (m, 20H), 1.14 (s, 3H), 1.11−0.97 (m, 2H), 0.94−0.89 (m, 6H); NMR δ_C (100 MHz, $CDCl_3$) 51.36, 151.30, 139.31, 125.42, 82.66, 64.14, 54.82, 49.87, 49.66, 46.55, 44.74, 43.02, 40.27, 38.24, 37.27, 36.02, 31.66, 31.63, 31.39, 30.52, 27.86, 26.76, 24.82, 24.01, 22.00, 20.68, 19.04, 10.35; m/z = 452.29 $[M + H]^+$; HPLC 100.0 a/a % at 215 nm.

Compound 41. A solution of hydroxymethylene **30** (200 mg, 0.340 mmol, 1 equiv) in pyridine (4.5 mL) was heated to 110 °C. After 5 min, the mixture was treated with a solution of hydroxylamine hydrochloride (71 mg, 1.02 mmol, 3 equiv) in water (1.2 mL) and stirred for 4 h, cooled to room temperature, and partitioned between water and dichloromethane. The organic phase was separated, washed with 2 N aqueous hydrochloric acid, washed with saturated aqueous sodium chloride, dried with sodium sulfate, and concentrated to an oil. The residue was purified by silica gel chromatography (10−40% Et_2O/hexanes). A suspension of the isolated product and 10% palladium on carbon (25 mg) in ethyl acetate (3 mL) was placed under hydrogen atmosphere and stirred for 2 h at room temperature. The reaction mixture was then filtered on Celite and the filtrate concentrated to dryness. The residue was then purified using silica gel chromatography (0.5/1/98.5 to 0.5/10/89.5 NH_4-OH/DCM/MeOH) to afford the desired isoxazole **41** (37 mg, 0.082 mmol, 24% for two steps). NMR δ_H (400 MHz, $CDCl_3$) 8.06 (br s, 1H), 3.34 (dt, J = 10.2, 3.5 Hz, 1H), 3.04 (dd, J = 12.6, 4.3 Hz, 1H), 2.85 (dd, J = 18.2, 11.4 Hz, 1H), 2.90−2.61 (m, 4H), 2.38 (br d, J = 14.6 Hz, 1H), 2.30 (t, J = 12.2 Hz, 1H), 2.26−2.18 (m, 1H), 2.10−1.00 (m, 25H), 1.05 (s, 3H), 0.97 (d, J = 6.6 Hz, 3H), 0.89 (d, J = 7.3 Hz, 3H); NMR δ_C (100 MHz, $CDCl_3$) 159.95, 153.06, 140.03, 124.81, 113.75, 82.74, 64.13, 54.79, 49.82,

49.75, 46.50, 44.67, 42.33, 40.27, 38.40, 37.22, 35.27, 31.64, 31.35, 30.66, 30.57, 27.83, 27.04, 24.96, 23.96, 22.00, 21.65, 19.01, 10.30; $m/z = 451.27$ $[M + H]^+$; HPLC 98.4 a/a % at215 nm.

Preparation of Compound Included in Scheme 6. Compound 44. A solution of ketone **5** (500 mg, 0.893 mmol, 1 equiv) in *tert*-butanol (5 mL) and potassium *tert*-butoxide (702 mg, 6.25 mmol, 7 equiv) was treated with ethyl trifluoracetate (761 mg, 5.36 mmol, 6 equiv) dropwise. The mixture was stirred at room temperature for 48 h and partitioned between diethyl ether and 1 N aqueous sodium hydroxide. The organic phase was separated, washed with 1 N aqueous sodium hydroxide, dried with sodium sulfate, and concentrated to desired compound **42** (550 mg, 0.839 mmol, 94%) as a pale-yellow foam. The residue was used without further purification.

A solution of crude compound **42** (210 mg, 0.320 mmol, 1 equiv) in ethanol (6 mL) was treated with hydrazine hydrate (32 mg, 0.640 mmol, 2 equiv) and heated to 80 °C for 3 h. The mixture was cooled to room temperature and concentrated to a yellow oil. The residue was purified by silica gel chromatography (20−55% EtOAc/hexanes) to give the desired pyrazole **43** (95 mg, 0.146 mmol, 46%). NMR δ_H (400 MHz, $CDCl_3$) 7.37−7.29 (m, 5H), 5.15 (d, $J = 12.6$ Hz, 1H), 5.11 (d, $J = 12.7$ Hz, 1H), 3.82 (dd, $J = 13.2$, 4.3 Hz, 1H), 3.62 (dt, $J = 10.8$, 4.6 Hz, 1H), 3.08 (dd, $J = 10.2$, 6.2 Hz, 1H), 2.86−2.54 (m, 4H), 2.38 (br d, $J = 13.8$ Hz, 1H), 2.32 (br d, $J = 16.1$ Hz, 1H), 2.19 (td, $J = 11.9$, 4.2 Hz, 1H), 2.12−1.13 (m, 22H), 1.08 (s, 3H), 0.97 (d, $J = 6.9$ Hz, 3H), 0.90 (d, $J = 7.3$ Hz, 3H).

A suspension of pyrazole **43** (95 mg, 0.146 mmol, 1 equiv) and 10% palladium on carbon (30 mg) in ethyl acetate (6 mL) was placed under hydrogen atmosphere and stirred for 2 h at room temperature. The reaction mixture was then filtered on Celite and the filtrate concentrated to dryness. The residue was then purified using silica gel chromatography (0.5/1/98.5 to 0.5/14/85.5 NH_4OH/DCM/MeOH) to afford the desired trifluoromethylpyrazole **44** (42 mg, 0.081 mmol, 56%). NMR δ_H (400 MHz, $CDCl_3$) 3.36 (dt, $J = 10.2$, 3.8 Hz, 1H), 3.05 (dd, $J = 12.9$, 3.7 Hz, 1H), 2.81−2.63 (m, 3H), 2.55 (dd, $J = 17.2$, 7.1 Hz, 1H), 2.36 (br d, $J = 14.7$ Hz, 1H), 2.30 (t, $J = 8.9$ Hz, 1H), 2.25−2.17 (m, 2H), 2.10−1.00 (m, 23H), 0.91 (s, 3H), 0.89 (s, 3H); NMR δ_C (100 MHz, $CDCl_3$) 140.35, 140.07, 124.72, 112.87, 82.79, 76.68, 63.70, 54.52, 49.96, 49.92, 46.48, 44.63, 42.43, 40.17, 38.33, 37.26, 35.35, 31.79, 31.53, 31.33, 30.77, 27.79, 26.93, 25.39, 24.00, 22.00, 21.96, 18.97, 10.37; $m/z = 518.28$ $[M + H]^+$; HPLC 97.8 a/a % at215 nm.

Compound 47. A solution of compound **30** (360 mg, 0.612 mmol, 1 equiv) in toluene (10 mL) was treated with DDQ (153 mg, 0.674 mmol, 1.1 equiv) and stirred at room temperature for 5 min. The mixture was concentrated to dryness and purified by silica gel chromatography (10−20% EtOAc/hexanes) to give the desired compound **45** (244 mg, 0.417 mmol, 68%). NMR δ_H (400 MHz, $CDCl_3$) 10.08 (s, 1H), 7.54 (s, 1H), 7.38−7.29 (m, 5H), 5.15 (d, $J = 12.6$ Hz, 1H), 5.11 (d, $J = 12.7$ Hz, 1H), 3.82 (dd, $J = 13.3$, 4.4 Hz, 1H), 3.62 (dt, $J = 10.8$, 3.8 Hz, 1H), 3.08 (dd, $J = 10.3$, 6.8 Hz, 1H), 2.81 (dd, $J = 16.6$, 15.1 Hz, 1H), 2.64 (dd, $J = 11.8$, 10.2 Hz, 1H), 2.59 (t, $J = 6.2$ Hz, 1H), 2.42 (br d, $J = 14.2$ Hz, 1H), 2.27−2.10 (m, 5H), 2.05−1.22 (m, 17H), 1.21 (s, 3H), 0.98 (d, $J = 6.8$ Hz, 3H), 0.91 (d, $J = 6.8$ Hz, 3H); $m/z = 558.48$ $[M + H]^+$.

A solution of compound **45** (123 mg, 0.210 mmol, 1 equiv) in *N*,*N*-dimethylformamide (6 mL) was treated with methanol (67 mg, 2.10 mmol, 10 equiv) and PDC (474 mg, 1.26 mmol, 6 equiv). The mixture was stirred for 4 days at room temperature (∼50% conversion). The reaction mixture was partitioned between water and ethyl acetate, filtered on Celite, and washed with ethyl acetate. The filtrate was separated and the aqueous layer back-extracted with ethyl acetate. The combined organics were washed with 1 N aqueous hydrochloric acid, dried with sodium sulfate, and concentrated to dryness. The residue was purified by silica gel chromatography (80% EtOAc/hexanes) to give the desired ester **46** (36 mg, 0.059 mmol, 28%). NMR δ_H (400 MHz, $CDCl_3$) 7.43 (s, 1H), 7.38−7.30 (m, 5H), 5.15 (d, $J = 12.6$ Hz, 1H), 5.11 (d, $J = 12.7$ Hz, 1H), 3.83 (dd, $J = 12.9$, 4.3 Hz, 1H), 3.81 (s, 3H), 3.63 (dt, $J = 10.8$, 3.8 Hz, 1H), 3.09 (dd, $J = 9.4$, 6.2 Hz, 1H), 2.80 (t, $J = 15.6$ Hz, 1H), 2.64 (dd, $J = 12.8$, 10.3 Hz, 1H), 2.60 (t, $J = 6.2$ Hz, 1H), 2.43 (br d, $J = 14.2$ Hz, 1H), 2.30−2.10 (m, 5H), 2.06−1.22 (m, 17H), 1.18 (s, 3H), 0.98 (d, $J = 6.8$ Hz, 3H), 0.91 (d, $J = 6.8$ Hz, 3H); $m/z = 638.41$ $[M + H]^+$.

A suspension of compound **46** (35 mg, 0.057 mmol, 1 equiv) and 10% palladium on carbon (7 mg) in ethanol (3 mL) was placed under hydrogen atmosphere and was stirred for 16 h at room temperature. The mixture was treated with hydrazine hydrate (12 mg, 0.223 mmol, 4 equiv), heated to 80 °C, and stirred for 4 h. The mixture was cooled to room temperature and concentrated to a yellow oil. The residue was purified by silica gel chromatography (10% MeOH/DCM) to give the desired pyrazolone **47** (14 mg, 0.023 mmol, 40%). NMR δ_H (400 MHz, $CDCl_3$) 8.15 (br s, 1H), 3.36 (dt, $J = 10.0$, 4.0 Hz, 1H), 3.10−3.00 (m, 2H), 2.73−2.60 (m, 2H), 2.51−1.20 (m, 28H), 0.96 (s, 3H), 0.94−0.89 (m, 6H); $m/z = 466.30$ $[M + H]^+$; HPLC 89.2 a/a % at 215 nm.

Compound 48. A solution of compound **30** (100 mg, 0.170 mmol, 1 equiv) in ethanol (2 mL) was treated with methylhydrazine (16 mg, 0.340 mmol, 2 equiv), heated to 80 °C, and stirred for 2 h. The mixture was cooled to room temperature and concentrated to a yellow oil. The residue was purified by silica gel chromatography (20−50% EtOAc/hexanes). A suspension of the isolated product and 10% palladium on carbon (10 mg) in ethyl acetate (3 mL) was placed under hydrogen atmosphere and stirred for 2 h at room temperature. The reaction mixture was filtered on Celite and the filtrate concentrated to dryness. The residue was purified using silica gel flash chromatography (0.5/1/98.5 to 0.5/14/85.5 NH_4OH/DCM/MeOH) to afford a mixture of methylpyrazole (34 mg, 0.073 mmol, 43% for two steps, 7:3 mixture of regioisomer). NMR δ_H (400 MHz, $CDCl_3$) 7.00 (br s, 1H) 3.82 (s, 3H minor isomer), 3.80 (s, 3H major isomer), 3.35 (dt, $J = 10.2$, 3.9 Hz, 1H), 3.05 (dd, $J = 12.7$, 4.1 Hz, 1H), 2.84−2.73 (m, 1H), 2.71−2.61 (m, 1H), 2.61−2.50 (m, 2H), 2.48−2.11 (m, 4H), 2.09−1.22 (m, 21H) 1.11−0.97 (m, 1H), 1.02 (s, 3H) 0.90 (d, $J = 11.1$ Hz, 6H); NMR δ_C (100 MHz, $CDCl_3$) 148.17, 140.55, 127.19, 124.21, 114.70, 82.88, 76.72, 64.12, 54.71, 49.95, 49.87, 46.59, 44.62, 42.25, 40.27, 39.21, 38.66, 37.25, 35.42, 32.78, 31.58, 30.83, 27.82, 27.27, 25.32, 23.98, 23.94, 22.24, 19.02, 10.34; $m/z = 464.27$ $[M + H]^+$; HPLC 100 a/a % at 215 nm (71.9/28.1 regioisomers mixture).

Compound 49. A solution of pyrazole **31** (200 mg, 0.346 mmol, 1 equiv) in pyridine (4 mL) was treated with methanesulfonyl chloride (117 mg, 1.03 mmol, 3 equiv) and stirred at room temperature for 30 min. The reaction mixture was partitioned between water and ethyl acetate. The organic layer was separated, washed with saturated aqueous sodium chloride, dried over sodium sulfate, and concentrated to dryness. The residue was purified by silica gel chromatography (10−20% EtOAc/hexanes). A suspension of the isolated product and 10% palladium on carbon (81 mg) in ethyl acetate (15 mL) was placed under hydrogen atmosphere and stirred for 1.5 h at room temperature. The reaction mixture was then filtered on Celite and the filtrate concentrated to dryness. The residue was purified using silica gel chromatography (0.5/1/98.5 to 0.5/10/89.5 NH_4OH/DCM/MeOH) to afford the desired methanesulfonylpyrazole **49** (78 mg, 0.148 mmol, 51% for two steps). NMR δ_H (400 MHz, $CDCl_3$) 7.64 (s, 1H), 3.32 (dt, $J = 10.0$, 3.8 Hz, 1H), 3.25 (s, 3H), 3.02 (dd, $J = 12.7$, 3.9 Hz, 1H), 2.82 (dd, $J = 18.6$, 11.0 Hz, 1H), 2.69−2.58 (m, 3H), 2.36 (br d, $J = 14.2$ Hz, 1H), 2.27 (t, $J = 11.9$ Hz, 1H), 2.24−2.15 (m, 2H), 2.10−0.98 (m, 25H), 0.89 (s, 3H), 0.87 (s, 3H); NMR δ_C (100 MHz, $CDCl_3$) 156.04, 140.20, 127.24, 124.83, 118.67, 82.73, 64.11, 54.76, 49.82, 49.77, 46.57, 44.64, 42.41, 41.40, 40.25, 38.65, 37.23, 35.27, 32.43, 31.60, 31.34, 30.63, 27.83, 27.06, 25.04, 24.07, 23.98, 22.06, 19.01, 10.30; $m/z = 528.30$ $[M + H]^+$; HPLC 100.0 a/a % at 215 nm.

Compound 50. A solution of pyrazole **30** (140 mg, 0.240 mmol, 1 equiv) in dichloromethane (3 mL) was treated with pyridine (95 mg, 1.2 mmol, 5 equiv) and *p*-toluenesulfonyl chloride (55 mg, 0.288 mmol, 1.2 equiv). The mixture was stirred at room temperature for 2 h and partitioned between water and ethyl acetate. The organic layer was washed with saturated aqueous sodium chloride, dried with sodium sulfate, and concentrated to dryness. The residue was purified by silica gel chromatography (5−30% EtOAc/hexanes). A suspension of the isolated product and 10% palladium on carbon (14 mg) in ethanol (2 mL) and ethyl acetate (2 mL) was placed under hydrogen atmosphere and stirred for 1.5 h at room temperature. The reaction mixture was then filtered on Celite and the filtrate concentrated to dryness. The residue was then purified using silica gel chromatography (0.5/1/98.5 to 0.5/14/85.5 NH_4OH/DCM/MeOH) to afford the desired *p*-toluenesulfonylpyrazole **50** (64 mg, 0.106 mmol, 45% for two steps, 88/12 regioisomer ratio). NMR δ_H (400 MHz, $CDCl_3$) 7.88 (d, J = 8.1 Hz, 2H), 7.72 (br s, 1H), 7.31 (d, J = 8.4 Hz, 2H), 3.35 (dt, J = 10.5, 3.8 Hz, 1H), 3.05 (dd, J = 12.6, 3.8 Hz, 1H), 2.77 (dd, J = 17.9, 11.3 Hz, 1H), 2.67 (dd, J = 9.6, 7.7 Hz, 1H), 2.63−2.53 (m, 2H), 2.41 (br s, 3H), 2.40−2.18 (m, 3H), 2.07−1.03 (m, 23H), 1.01 (s, 3H), 0.93−0.88 (m, 6H); NMR δ_C (100 MHz, $CDCl_3$) 156.04, 140.20, 127.24, 124.83, 118.67, 82.73, 64.11, 54.76, 49.82, 49.77, 46.57, 44.64, 42.41, 41.41, 40.25, 38.65, 37.23, 35.27, 32.43, 31.60, 31.34, 30.63, 27.83, 27.06, 25.04, 24.07, 23.98, 22.06, 19.01, 10.30; m/z = 604.43 $[M + H]^+$; HPLC 99.4 a/a % at 215 nm (82/18 epimer ratio).

Preparation of Compound Included in Scheme 7. Compound 55. A solution of compound **45** (500 mg, 0.854 mmol, 1 equiv) in toluene (25 mL) was treated with Wilkinson's catalyst (806 mg, 0.871 mmol, 1.02 equiv). The mixture was stirred at 80 °C for 30 min and cooled to room temperature. The solvent was removed under vacuum and the residue was purified by silica gel chromatography (10−15% EtOAc/hexanes) to give the desired enone **51** (306 mg, 0.549 mmol, 64%). NMR δ_H (400 MHz, $CDCl_3$) 7.37−7.30 (m, 5H), 6.73 (d, J = 10.1 Hz, 1H), 5.88 (d, J = 10.1 Hz, 1H), 5.15 (d, J = 12.2 Hz, 1H), 5.11 (d, J = 12.3, 1H), 3.82 (dd, J = 13.0, 4.1 Hz, 1H), 3.62 (dt, J = 10.8, 4.1 Hz, 1H), 3.08 (dd, J = 10.4, 6 Hz, 1H), 2.75−2.54 (m, 3H), 2.43 (d, J = 14.1 Hz, 1H), 2.25−1.14 (m, 22H), 1.10 (s, 3H), 0.97 (d, J = 6.7 Hz, 3H), 0.90 (d, J = 7.1 Hz, 3H); m/z = 580.46 $[M + Na]^+$.

A solution of enone **51** (42.3 g, 76 mmol, 1 equiv) in ethanol (420 mL was treated with sodium acetate (9.33 g, 114 mmol, 1.5 equiv) and hydroxylamine hydrochloride (7.91 g, 114 mmol, 1.5 equiv). The mixture was stirred at room temperature for 3 h and partitioned between ethyl acetate and water. The organic layer was separated, dried over sodium sulfate, and concentrated to dryness. The residue was purified by silica gel chromatography (20−50% EtOAc/hexanes) to give the desired oxime **52** (43 g, 76 mmol, 100%) as a mixture of regioisomers (2/1 anti/syn). NMR δ_H (400 MHz, $CDCl_3$) 7.37−7.29 (m, 5H), 6.66 (d, J = 10.3 Hz, 1/3H minor), 6.12 (d, J = 10.6 Hz, $^1/_3$H minor), 6.01 (dd, J = 10.6 Hz, $^2/_3$H major), 5.97 (d, J = 10.6 Hz, $^2/_3$H major), 5.15 (d, J = 12.2 Hz, 1H), 5.11 (d, J = 12.3, 1H), 3.82 (dd, J = 13, 4.1 Hz, 1H), 3.62 (dt, J = 10.6, 4.2 Hz, 1H), 3.08 (dd, J = 10.4, 6.3 Hz, 1H), 2.84−2.53 (m, 3H), 2.46−2.36 (m, 2H), 2.23−1.18 (m, 22H), 1.03 (s, 3H), 0.97 (d, J = 6.7 Hz, 3H), 0.90 (d, J = 7.1 Hz, 3H); m/z = 573.56 $[M + H]^+$.

A solution of oxime **52** (5.3 g, 9.25 mmol, 1 equiv) in pyridine (100 mL) was cooled to 5 °C and treated with methanesulfonyl chloride (2.12 g, 18.5 mmol, 2 equiv). The solution was stirred for 1 h, partitioned between water and ethyl acetate, and warmed to room temperature. The organic phase was separated and concentrated under vacuum. The residue was purified by silica gel chromatography (15−20% EtOAc/hexanes) to give the desired intermediate (5.43 g, 8.34 mmol, 90%). A solution of the intermediate (4.26 g, 6.55 mmol, 1 equiv) in methanol (125 mL) and toluene (12.5 mL) was treated with concentrated hydrochloric acid (1.19 g, 32.7 mmol, 5 equiv). The resulting mixture was heated to 60 °C and stirred for 16 h. The mixture was cooled to room temperature and partitioned between saturated aqueous bicarbonate and toluene. The organic phase was separated and concentrated to a brown foam. The residue was crystallized from ethyl acetate to give the desired lactam **53** as a tan solid (14.35 g, 25 mmol, 61%). NMR δ_H (400 MHz, $CDCl_3$) 7.39−7.31 (m, 5H), 6.33 (br s, 1H), 6.10 (d, J = 13.3 Hz, 1H), 5.70 (d, J = 13.6 Hz, 1H), 5.16 (d, J = 12.2 Hz, 1H), 5.12 (d, J = 12.3, 1H), 3.83 (dd, J = 12.6, 4.2 Hz, 1H), 3.71−3.59 (m, 2H), 3.09 (dd, J = 10.6, 6.4 Hz, 1H), 2.85 (dd, J = 14.3, 7.7 Hz, 1H), 2.68−2.57 (m, 2H), 2.44 (br d, J = 15.8 Hz, 1H), 2.39 (dd, J = 14.3, 6 Hz, 1H), 2.23−2.10 (m, 2H), 2.07−1.18 (m, 18H), 1.07 (s, 3H), 0.98 (d, J = 6.8 Hz, 3H), 0.91 (d, J = 7 Hz, 3H); m/z = 573.45 $[M + H]^+$.

A suspension of lactam **53** (12.9 g, 22.5 mmol, 1 equiv) and 10% palladium on carbon (2.5 g) in 2-propanol (195 mL) was placed under hydrogen atmosphere and stirred for 5 h at room temperature. The reaction mixture was treated with ethylenediamine (1.35 g, 22.5 mmol, 1 equiv) and stirred for 20 min. The resulting mixture was filtered on Celite and the cake washed with 2-propanol. The filtrate was concentrated until solid started to separate. Ethyl acetate was added and the mixture further concentrated to 10% of the original reaction volume. The precipitate was collected by filtration and air-dried to yield the desired lactam **55** (8.5 g, 19.2 mmol, 86%) as a white solid. NMR δ_H (400 MHz, $CDCl_3$) 7.04−6.95 (br s, 1H), 3.66 (dd, J = 11.0, 3.7 Hz, 1H), 3.27 (dt, J = 10.7, 3.8 Hz, 1H), 2.97 (dd, J = 12.9, 4.0 Hz, 1H), 2.71−2.51 (m, 3H), 2.37 (br d, J = 13.6 Hz, 1H), 2.23 (t, J = 13.6 Hz, 1H), 2.18−2.02 (br m, 3H), 1.99−1.81 (m, 5H), 1.80−1.14 (m, 18H), 0.87 (s, 3H), 0.85−0.80 (m, 6H); NMR δ_C (100 MHz, $CDCl_3$) 178.89, 139.91, 124.82, 82.46, 64.02, 54.00, 49.75, 49.35, 46.72, 44.73, 44.56, 44.02, 43.03, 40.13, 37.15, 37.11, 34.84, 31.53, 30.22, 30.20, 29.07, 27.69, 26.61, 25.37, 23.90, 22.71, 18.89, 10.16; m/z = 441.29 $[M + H]^+$; HPLC 95.0 a/a % at 215 nm.

Compound 56. A solution of lactam **53** (50 mg, 0.090 mmol, 1 equiv) in anhydrous tetrahydrofuran (5 mL) was cooled to −78 °C and treated with 0.5 M potassium hexamethyldisilazane in toluene (0.215 mL, 0.107 mmol, 1.2 equiv) and methyl iodide (25.4 mg, 0.179 mmol, 2 equiv). The solution was stirred for 30 min, warmed to room temperature, and stirred for 18 h. The reaction mixture was partitioned between ethyl acetate and water. The organic phase was separated, dried with sodium sulfate, and concentrated to dryness. The residue was purified by silica gel chromatography (20−50% EtOAc/hexanes) to give the desired lactam **54** (50 mg, 0.089 mmol, 99%). NMR δ_H (400 MHz, $CDCl_3$) 7.39−7.30 (m, 5H), 6.02 (d, J = 13.2, 1H), 5.84 (d, J = 12.9, 1H), 5.16 (d, J = 12.6 Hz, 1H), 5.12 (d, J = 12.7, 1H), 3.92−3.80 (m, 2H), 3.63 (dd, J = 10.4, 4.0 Hz, 1H), 3.11−3.06 (m, 1H), 3.08 (s, 3H), 2.85 (d, J = 14.7, 1H), 2.68−2.57 (m, 2H), 2.44 (br d, J = 14.7, 1H), 2.39 (dd, J = 14.4, 6.1 Hz, 1H), 2.23−1.17 (m, 20H) 1.03 (s, 3H), 0.98 (d, J = 6.8 Hz, 3H), 0.91 (d, J = 6.9 Hz, 3H); m/z = 587.44 $[M + H]^+$.

A suspension of lactam **54** (50 mg, 0.89 mmol, 1 equiv) and 10% palladium on carbon (10 mg) in ethanol (3 mL) was placed under hydrogen atmosphere and stirred for 4 h at room temperature. The reaction mixture was filtered on Celite and washed with ethanol and the filtrate concentrated to dryness. The residue was purified by silica gel chromatography (8% MeOH/DCM) to give the desired lactam **56** (25 mg, 0.055 mmol, 65%). NMR δ_H (400 MHz, $CDCl_3$) 4.21 (t, J = 7.7 Hz, 1H), 4.05 (dd, J = 7.3, 4.4 Hz, 1H), 3.36 (dt, J = 9.9, 3.9 Hz, 1H), 3.06 (dd, J = 12.0, 4.3 Hz, 1H), 2.94 (s, 3H), 2.74−2.62 (m, 3H), 2.45 (d, J = 13.8 Hz, 1H), 2.31 (t, J = 13.8 Hz, 1H), 2.26−2.10 (br m, 3H), 2.07−1.23 (m, 18H), 1.13−1.00 (m, 1H), 0.99−0.82 (m, 12H); NMR δ_C (100 MHz, $CDCl_3$) 175.79, 140.09, 125.09, 82.72, 64.18, 54.83, 53.17, 49.95, 49.57, 46.89, 44.73, 43.17, 40.30, 38.86, 37.30, 36.87, 35.67, 35.36, 31.68, 31.38, 31.09, 30.51, 29.95, 27.90, 26.77, 24.09, 23.20, 19.04, 10.35; m/z = 455.30 $[M + H]^+$; HPLC 97.0 a/a % at 215 nm.

Hh-Dependent C3H10T1/2 Differentiation Assay. The assay was performed according to the procedure described by Dwyer and co-workers.[46] Briefly, mouse embryonic mesoderm fibroblasts C3H10T1/2 cells (obtained from ATCC catalogue no. CCL-226) were cultured in basal MEM medium (Gibco/Invitrogen) supplemented with 10% heat inactivated FBS (Hyclone), 50 units/mL penicillin, 50 μg/mL streptomycin (Gibco/Invitrogen), and 2 mM glutamine (Gibco/Invitrogen) at 37 °C with 5% CO_2 in air atmosphere. Cells were dissociated with 0.05% trypsin and 0.02% EDTA in PBS for passage and plating. C3H10T1/2 cells were plated in 96 wells with a density of 8×10^3 cells/well. Cells were grown to confluence (72 h). Medium containing 5 μM of 20(*S*)-hydroxycholesterol and 5 μM of 22(*S*)-hydroxycholesterol and/or compound was added at the start of the assay and left for 72 h. The medium was aspirated, and cells were washed once in PBS. Then 100 μL of Tropix CDP-Star with Emerald II (0.4 mM, catalogue no. MS100RY) was added per well and the plate was incubated at room temperature in the dark for 1 h. The plates were read for fluorometric measurement on an Envision plate reader at 405 nm. The percent inhibition with respect to compound concentration was plotted using Prism graphing software on a semilog plot, and EC_{50} values were determined by nonlinear regression analysis with a four-parameter logistic equation.

In Vitro Metabolism Assay. Test compounds and controls (alprenolol and acebutolol) were tested at 500 nM in pooled human liver microsomes (BD Biosciences, Woburn, MA). An incubation solution was made in 85 mM phosphate buffer containing 1 mM EDTA, 3 mM $MgCl_2$, and 0.5 mg/mL human liver microsomes. Compounds were added to a clear flat bottom 96-well plate (Costar, Corning, NY) using a Platemate Plus liquid handler (Matrix). Reaction solution was preincubated at 37 °C for 5 min. Reaction mixture was added to each 96-well plate. To start the reaction, NADPH was added to each plate at a final concentration of 1 mg/mL. Reaction plates were incubated at 37 °C. Reaction was stopped at designated time points (0, 5, 15, 30, 45, and 60 min) by adding 2 volumes of acetonitrile containing internal standard (jervine, AG Scientific). Samples were placed in plate centrifuge (Eppendorf) and spun at 1000 rcf for 10 min. The supernatant was taken and diluted with 1 volume of water in a 96-well autosampler plate with inert glass inserts (MicroLiter). Samples were analyzed by LC-MS. A 2.1 mm $\times$ 20 mm, 3.5 μm Waters C18 Synergy column (Milford, MA) was used, and samples were analyzed using an Applied Biosystems 4000QTrap mass spectrometer. Half-lives were determined by the slope of the line for the percent remaining parent compound over time course.

Dosing Formulation of Cyclopamine and Compounds 28, 32, and 55. In the appropriate size vessel, the Hh antagonists (free bases) were dissolved in a vehicle containing 30% (w/w) of 2-hydroxypropyl-β-cyclodextrin (HPBCD) in 0.1 M citrate phosphate buffer (pH 3) at a nominal concentration of 1.25 mg/mL for pharmacokinetic studies and at concentration of 5−10 mg/mL for efficacy studies.

Pharmacokinetic Studies. Dosing. Aqueous formulations of each compound (vide supra) were administered intravenously or orally via gavage to CD-1 mice, Sprague−Dawley rats, beagle dogs, and cynomolgus monkeys. At 0.083, 0.25, 0.5, 1, 2, 4, 6, and 24 h postdose, blood was collected and processed to plasma by centrifugation and stored at −80 °C until analysis.

Bioanalytical Analysis. Plasma samples were thawed at room temperature and processed by protein precipitation with 2 volumes of acetonitrile containing 25 ng/mL jervine (AG Scientific) as an internal standard. After centrifugation, the supernatants of the precipitated plasma samples were diluted 1:1 with water and analyzed by LC/MS/MS. A standard curve was prepared in plasma from 0.5 to 250 ng/mL and processed in the same way with internal standard solution as the samples. Sample analysis was performed on an Agilent 1100 series (Foster City, CA) with an API4000 mass spectrometer from Applied Biosystems (Foster City, CA). Samples were injected on an analytical column (Symmetry IS, 2.1 mm $\times$ 20 mm, C18, 3.5 μm from Waters, Milford, MA) and compounds eluted from the analytical column with a 5 min gradient from 0 to 95% acetonitrile in H_2O, 0.1% (v/v) formic acid. Mass spectrometric detection of the drug as well as jervine as the internal standard was performed by MRM in positive mode. The data were acquired and processed using the software Analyst 1.4 (Applied Biosystems, Foster City, CA). The pharmacokinetics of each compound were analyzed by WinNonlin 5.0.1 (Pharsight, Mountain View, CA) via noncompartmental analysis.

Efficacy Studies in B837Tx-Bearing Nude Mice. The antitumor activity of cyclopamine and the three lead compounds was evaluated in mice bearing $Ptc^{+/-}Hic1^{+/-}$ medulloblastoma B837Tx allografts.[56] The B837Tx tumor line originated as a medulloblastoma in this mouse and is propagated as a mouse-to-mouse allograft. NOD/SCID mice were inoculated in the right hind flank with 1 $\times$ 106 B837Tx cells in a 100 μL injection volume. Once the tumors reached an approximate size of 100−200 mm^3, mice ($N = 10$/group) were dosed orally with vehicle, 30% hydroxypropyl-β-cyclodextrin (HPBCD), or formulated compounds (**28**, 40 mg/kg; **32**, 80 mg/kg; **55**, 30 mg/kg) daily for 21 days. Tumors were measured in millimeters in two perpendicular dimensions with calipers 3 times per week using calipers. In all cases, the tumors regressed with compound treatment. After 21 days of treatment, mice were monitored for regrowth of tumors.

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Supporting Information Available: Physical data (^1H and ^{13}C NMR spectra, mass spectrometry, HPLC) for all tested compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Ruiz i Altaba, A.; Sanchez, M. P.; Dahmane, N. Gli and Hedgehog in cancer: tumours, embryos and stem cells. *Nat. Rev. Cancer* **2002**, *2*, 361–372.
(2) Lum, L.; Beachy, P. A. The Hedgehog response network: sensors, switches, and routers. *Science* **2004**, *304*, 1755–1759.
(3) di Magliano, M. P.; Hebrok, M. Hedgehog signalling in cancer formation and maintenance. *Nat. Rev. Cancer* **2003**, *3*, 903–911.
(4) Beachy, P. A.; Karhadkar, S. S.; Berman, D. M. Tissue repair and stem cell renewal in carcinogenesis. *Nature* **2004**, *432*, 324–331.
(5) Ruiz i Altaba, A. Hedgehog-Gli Signaling in Human Disease; Landes Bioscience/Eurakah.com: Georgetown, TX, 2006; p 226.
(6) Bale, A. E.; Yu, K.-p. The hedgehog pathway and basal cell carcinomas. *Hum. Mol. Genet.* **2001**, *10*, 757–762.
(7) Dahmane, N.; Lee, J.; Robins, P.; Heller, P.; Ruiz i Altaba, A. Activation of the transcription factor Gli1 and the Sonic hedgehog signalling pathway in skin tumours. *Nature* **1997**, *389*, 876–881.
(8) Hutchin, M. E.; Kariapper, M. S. T.; Gratchtchouk, M.; Wang, A.; Wei, L.; Cummings, D.; Liu, J.; Michael, L. R.; Glick, A.; Dlugosz, A. A. Sustained Hedgehog signaling is required for basal cell carcinoma proliferation and survival: conditional skin tumorigenesis recapitulates the hair growth cycle. *Genes Dev.* **2004**, *19*, 214–224.
(9) Kubo, M.; Nakamura, M.; Tasaki, A.; Yamanaka, N.; Nakashima, H.; Nomura, M.; Kuroki, S.; Katano, M. Hedgehog signaling pathway is a new therapeutic target for patients with breast cancer. *Cancer Res.* **2004**, *64*, 6071–6074.

(10) Berman, D. M.; Karhadkar, S. S.; Maitra, A.; Montes de Oca, R.; Gerstenblith, M. R.; Briggs, K.; Parker, A. R.; Shimada, Y.; Eshleman, J. R.; Watkins, D. N.; Beachy, P. A. Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumors. *Nature* **2003**, *425*, 846–851.
(11) Goodrich, L. V.; Scott, M. P. Hedgehog and Patched in neural development and disease. *Neuron* **1998**, *21*, 1243–1257.
(12) Thayer, S. P.; diMagliano, 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. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. *Nature* **2003**, *425*, 851–856.
(13) Karhadkar, S. S.; Bova, G. S.; Abdallah, N.; Dhara, S.; Gardner, D.; Maitra, A.; Isaacs, J. T.; Berman, D. M.; Beachy, P. A. Hedgehog signalling in prostate regeneration, neoplasia and metastasis. *Nature* **2004**, *431*, 707–712.
(14) Watkins, D. N.; Berman, D. M.; Burkholder, S. G.; Wang, B.; Beachy, P. A.; Baylin, S. B. Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. *Nature* **2003**, *422*, 313–317.
(15) Rubin, L. L; de Sauvage, F. J. Target the hedgehog pathway in cancer. *Nat. Rev Cancer* **2006**, *5*, 1026–1033.
(16) Frank-Kamenetsky, M.; Zhang, X. M.; Bottega, S.; Guicherit, O.; Wichterle, H.; Dudek, H.; Bumcrot, D.; Wang, F. Y.; Jones, S.; Shulok, J.; Rubin, L. L.; Porter, J. A. Small-molecule modulators of Hedgehog signaling: identification and characterization of Smoothened agonists and antagonists. *J. Biol.* **2002**, *1*, 10.1–10.19.
(17) Chen, J. K.; Taipale, J.; Young, K. E.; Maiti, T.; Beachy, P. A. Small molecule modulation of Smoothened activity. *Proc. Natl Acad. Sci. U.S.A.* **2002**, *99*, 14071–14076.
(18) Bijlsma, M. F.; Spek, C. A.; Zivkovic, D.; van de Water, S.; Rezaee, F.; Peppelenbosch, M. P. Repression of smoothened by patched-dependent (pro-)vitamin D3 secretion. *PLoS Biol.* **2006**, *4*, 1397–1410.
(19) Remsberg, J. R.; Lou, J.; Tarasov, S. G.; Dean, M.; Tarasova, N. I. Structural analogues of Smoothened intracellular loops as potent inhibitors of hedgehog pathway and cancer cell growth. *J. Med. Chem.* **2007**, *50*, 4534–4538.
(20) Brunton, S. A.; Stibbard, J. H. A.; Rubin, L. L.; Kruse, L. I.; Guicherit, O. M.; Boyd, E. A.; Price, S. Potent inhibitors of the hedgehog signaling pathway. *J. Med. Chem.* **2008**, *51*, 1108–1110.
(21) Peukert, S.; Jain, R.; Geisser, A.; Sun, Y.; Zhang, R.; Bourret, A.; Carlson, A.; DaSilva, J.; Ramamurthy, A.; Kelleher, J. F. Identification and structure−activity relationships of ortho-biphenyl carboxamides as potent Smoothened antagonists inhibiting the Hedgehog signaling pathway. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 328–331.
(22) Lauth, M.; Bergstroem, A.; Shimokawa, T.; Toftgard, R. Inhibition of GLI-mediated transcription and tumor cell growth by small-molecule antagonists. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 8455–8460.
(23) Lee, J.; Wu, X.; diMagliano, M. P.; Peters, E. C.; Wang, Y.; Hong, J.; Hebrok, M.; Ding, S.; Cho, C. Y.; Schultz, P. G. A Small-molecule antagonist of the Hedgehog signaling pathway. *ChemBioChem* **2007**, *8*, 1916–1919.
(24) Arai, M. A.; Tateno, C.; Hosoya, T.; Koyano, T.; Kowithayakorn, T.; Ishibashi, M. Hedgehog/GLI-mediated transcriptional inhibitors from *Zizyphus cambodiana*. *Bioorg. Med. Chem.* **2008**, *16*, 9420–9424.
(25) 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. A small molecule that binds Hedgehog and blocks its signaling in human cells. *Nat. Chem. Biol.* **2009**, *5*, 154–156.
(26) Von Hoff, D. D.; Rudin, C. M.; LoRusso, P. M.; Borad, M. J.; Korn, R. L.; Heath, E. I.; Yauch, R.; Darbonne, W. C.; Kadel, E. E., III; Zerivitz, K. L.; Nelson, L. E.; Mackey, H.; Marsters, J. C., Jr.; de Sauvage, F. J.; Low, J. A. Efficacy Data of GDC-0449, a Systemic Hedgehog Pathway Antagonist, in a First-in-Human, First-in-Class Phase I Study with Locally Advanced, Multifocal or Metastatic Basal Cell Carcinoma Patients. Presented at the AACR Annual Meeting, San Diego, CA, 2008.
(27) A Phase 1 Study of BMS-833923 (XL139) Is Subjects with Advanced of Metastatic Cancer. http://clinicaltrial.gov/ct2/show/NCT00670189.
(28) Dose Finding and Safety of Oral LDE225 in Patients with Advanced Solid Tumors. http://clinicaltrials.gov/ct2/show/NCT00880308?term = LDE-225&rank = 1.
(29) A Phase 1 Study of IPI-926 in Patients with Advanced and/or Metastatic Solid Tumor Malignancies http://clinicaltrial.gov/ct2/show/NCT00761696.
(30) Keeler, R. F. Teratogenic compounds of *Veratrum californicum* (Durand)-IV. First isolation of veratramine and alkaloid Q and a reliable method for isolation of cyclopamine. *Phytochemistry* **1968**, *7*, 303–306.
(31) Keeler, R. F. Teratogenic compounds of *Veratrum californicum* (Durand)-VI. The structure of cyclopamine. *Phytochemistry* **1969**, *8*, 223–225.
(32) Keeler, R. F.; Binns, W. Teratogenic compounds of *Veratrum californicum* (Durand)-V. Comparison of cyclopian effects of steroidal alkaloids from the plant and structurally related compounds from other sources. *Teratology* **1968**, *1*, 5–10.
(33) Chen, J. K.; Taipale, J.; Cooper, M. K.; Beachy, P. A. Inhibition of hedgehog signaling by direct binding of cyclopamine to Smoothened. *Genes Dev.* **2002**, *16*, 2743–2748.
(34) Berman, D. M.; Karhadkar, S. S.; Hallahan, A. R.; Pritchard, J. I.; Eberhart, C. G.; Watkins, D. N.; Chen, J. K.; Cooper, M. K.; Taipale, J.; Olson, J. M.; Beachy, P. A. Medulloblastoma growth inhibition by hedgehog pathway blockade. *Science* **2002**, *297*, 1559–1561.
(35) Sanchez, P.; Ruiz i Altaba, A. In vivo inhibition of endogenous brain tumors through systemic interference of Hedgehog signaling in mice. *Mech. Dev.* **2005**, *122*, 223–230.
(36) Sanchez, P.; Hernandez, A. M.; Stecca, B.; Kahler, A. J.; DeGueme, A. M.; Barrett, A.; Beyna, M.; Datta, M. W.; Datta, S.; Ruiz i Altaba, A. Inhibition of prostate cancer proliferation by interference with SONIC HEDGEHOG-GLI1 signaling. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 12561–12566.
(37) Sheng, T.; Li, C.; Zhang, X.; Chi, S.; He, N.; Chen, K.; McCormick, F.; Gatalica, Z.; Xie, J. Activation of the hedgehog pathway in advanced prostate cancer. *Mol. Cancer* **2004**, *3*, 29–42.
(38) Zhang, J.; Garrossian, M.; Gardner, D.; Garrossian, A.; Chang, Y.-T.; Kim, Y. K.; Chang, C. W. T. Synthesis and anticancer activity studies of cyclopamine derivatives. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1359–1363.
(39) Kumar, S. K.; Roy, I.; Anchoori, R. K.; Fazli, S.; Maitra, A.; Beachy, P. A.; Khan, S. R. Targeted inhibition of Hedgehog signaling by cyclopamine prodrugs for advanced prostate cancer. *Bioorg. Med. Chem.* **2008**, *16*, 2764–2768.
(40) Tremblay, M. R.; Nevalainen, M.; Nair, S. J.; Porter, J. R.; Castro, A. C.; Behnke, M. L.; Yu, L.-C.; Hagel, M.; White, K.; Faia, K.; Grenier, L.; Campbell, M. J.; Cushing, J.; Woodward, C. N.; Hoyt, J.; Foley, M. A.; Read, M. A.; Sydor, J. R.; Tong, J. K.; Palombella, V. J.; McGovern, K.; Adams, J. Semi-synthetic cyclopamine analogues as potent and orally bioavailable Hedgehog pathway antagonists. *J. Med. Chem.* **2008**, *51*, 6646–6649.
(41) Feldmann, G.; Fendrich, V.; McGovern, K.; Bedja, D.; Bisht, S.; Alvarez, H.; Koorstra, J.-B. M.; Habbe, N.; Karikari, C.; Mullendore, M.; Gabrielson, K. L.; Sharma, R.; Matsui, W.; Maitra, A. An orally bioavailable small-molecule inhibitor of Hedgehog signaling inhibits tumor initiation and metastasis in pancreatic cancer. *Mol. Cancer Ther.* **2008**, *7*, 2725–2735.
(42) McGovern, K. J.; Pien, C. S.; Wright, J. L. Dosing Regimen for the Treatment of Cancer. WO2007123511, November 1, 2007.
(43) Manna, J. D.; Alvarez-Diez, T. M.; Grogan, M. J.; Porter, J. R.; Tremblay, M. R.; Castro, A. C.; Sydor, J. R. Metabolite Identification of IPI-609, a Novel and Potent Inhibitor of the Hedgehog Pathway, in Different Species. Presented at the ASMS Conference, Denver, CO, June 1−5, 2008.
(44) Masamune, T.; Sato, N.; Kobayashi, K.; Yamazaki, I.; Mori, Y. Syntheses and NMR spectra of 22,27-imino-17,23-oxidojervane derivatives. *Tetrahedron* **1967**, *23*, 1591–1612.
(45) Tsuji, N.; Suzuki, J.; Shiota, M.; Takahashi, I.; Nishimura, S. Highly stereoselective hydrogenation of 3-oxo-4-ene and -1,4-diene steroids to 5β-compounds with palladium catalyst. *J. Org. Chem.* **1980**, *45*, 2729–2731.
(46) Dwyer, J. R.; Sever, N.; Carlson, M.; Nelson, S. F.; Beachy, P. A.; Parhami, F. Oxysterols are novel activators of the Hedgehog signaling pathway in pluripotent mesenchymal cells. *J. Biol. Chem.* **2007**, *282*, 8959–8968.
(47) Clinton, R. O.; Clarke, R. L.; Stonner, F. W.; Manson, A. J.; Jennings, K. F.; Phillips, D. K. Steroidal heterocycles. VI. Formylation of A/B-*cis* 3-ketosteroids. Preparation of 5β-steroidal [3,2-*c*]pyrazoles. *J. Org. Chem.* **1962**, *27*, 2800–2807.
(48) Holton, P. G.; Necoechea, E. Steroids. CLXXV. Further steroidal anabolic agents. *J. Med. Chem.* **1962**, *5*, 1352–1357.
(49) Mrozik, H.; Buchschacher, P.; Hannah, J.; Fried, J. H. Heterocyclic steroids in the antiinflammatory series. *J. Med. Chem.* **1964**, *7*, 584–589.
(50) Ohta, G.; Takegoshi, T.; Ueno, K.; Shimizu, M. Investigations on steroids. XI. Synthesis of steroidal oxazole, imidazole, and triazole. *Chem. Pharm. Bull.* **1965**, *13*, 1445–1459.

(51) Marchetti, E.; Donini, P. The synthesis of some isoxazole derivatives of androstane. *Chim. Ital.* **1961**, *91*, 1133–1141.
(52) Bakshi, R. K.; Patel, G. F.; Rasmusson, G. H.; Baginsky, W. F.; Cimis, G.; Ellsworth, K.; Chang, B.; Bull, H.; Tolman, R. L.; Harris, G. S. 4,7β-Dimethyl-4-azacholestan-3-one (MK-386) and related 4-azasteroids as selective inhibitors of human type 1 5α-reductase. *J. Med. Chem.* **1994**, *37*, 3871–3874.
(53) Rasmusson, G. H.; Reynolds, G. F.; Utne, T.; Jobson, R. B.; Primka, R. L.; Berman, C.; Brooks, J. R. Azasteroids as inhibitors of rat prostatic 5α-reductase. *J. Med. Chem.* **1984**, *27*, 1690–1701.
(54) Mazur, R. Azasteroids. III. 3-Aza-A-homo androgens. *J. Org. Chem.* **1963**, *28*, 248–250.
(55) Shroff, A. P.; Harper, C. H. 3-Aza-A-homoandrostenes. *J. Med. Chem.* **1969**, *12*, 190–191.
(56) Briggs, K. J.; Corcoran-Schwartz, I. M.; Zhang, W.; Harcke, T.; Devereux, W. L.; Baylin, S. B.; Eberhart, C. G.; Watkins, D. N. Cooperation between the Hic1 and Ptc1 tumor suppressors in medulloblastoma. *Gene Dev.* **2008**, *22*, 770–785.
(57) Sasai, K.; Romer, J. T.; Lee, Y.; Finkelstein, D.; Fuller, C.; McKinnon, P. J.; Curran, T. Shh pathway activity is down-regulated in cultured medulloblastoma cells: implications for preclinical studies. *Cancer Res.* **2006**, *66*, 4215–4222.
(58) Vestergaard, J.; Pedersen Mikkel, W.; Pedersen, N.; Ensinger, C.; Tumer, Z.; Tommerup, N.; Poulsen Hans, S.; Larsen Lars, A. Hedgehog signaling in small-cell lung cancer: frequent in vivo but a rare event in vitro. *Lung Cancer* **2006**, *52*, 281–290.
(59) 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.; DeSauvage, F. J. A paracrine requirement for hedgehog signaling in cancer. *Nature* **2008**, *455*, 406–410.
(60) Travaglione, V.; Peacock, C. D.; MacDougall, J.; McGovern, K.; Cushing, J.; Yu, L.-C.; Trudeau, M.; Palombella, V.; Adams, J.; Hierman, J.; Rhodes, J.; Devereux, W. L.; Watkins, D. N. A Novel Hh Pathway Inhibitor, IPI-926, Delays Recurrence Post-Chemotherapy in a Primary Human SCLC Xenograft Model. Presented at the Annual Meeting of AACR, San Diego, CA, 2008.
(61) Travaglione, V.; Deyneko, I.; Oaks, Z.; Pink, M.; Proctor, J.; Mandley, E.; Gu, L.; Osswalt, M.; Read, M.; Sydor, J.; McGovern, K.; Palombella, V.; MacDougall, J. Activity of IPI-926, a Novel Inhibitor of the HH Pathway, in Subcutaneous and Orthotopically Implanted Xenograft Tumors That Express SHH Ligand. Presented at the EORTC-NCI-AACR Symposium on "Molecular Targets and Cancer Therapeutics", Geneva, Switzerland, 2008.
(62) Austad, B.; Behnke, M. L.; Castro, A. C.; Charette, A. B.; Grogan, M. J.; Janardanannair, S.; Lescarbeau, A.; Peluso, S.; Tremblay, M. Cyclopamine Analogs. US 2009/0012109 A1, January 8, 2009.