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Article

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Discovery of Novel Macrocyclic Hedgehog Pathway Inhibitors Acting by Suppressing the Gli-mediated Transcription

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Abstract.

A systemic medicinal chemistry campaign was conducted based on a literature hit compound **5** bearing the 4,5-dihydro-2*H*-benzo[*b*][1,5]oxazocin-6(3*H*)-one core through cyclization of two side substituents of the bicyclic skeleton combined with *N*-atom walking or ring-walking and the central ring expansion or extraction approaches, leading to several series of structurally unique tricyclic compounds. Among these, compound **29a** was identified as the most potent against the hedgehog (Hh) signaling pathway showing an IC_{50} value of 23 nM. Mechanism studies indicated that compound **29a** inhibited the Hh signaling pathway by suppressing the expression of the transcriptional factors Gli, rather than by interrupting the binding of Gli with DNA. We further observed that **29a** was equally potent against both Smo wild type and the two major resistant mutants (Smo D473H and Smo W535L). It potently inhibited the proliferation of medulloblastoma cells and showed significant tumor growth inhibition in the ptch+/-;p53-/- medulloblastoma allograft mice model. Though more studies are needed to clarify the precise interaction pattern of **29a** with Gli, its promising *in vitro* and *in vivo* properties encourage further profiling as a new-generation Hh signaling inhibitor to treat tumors primarily or secondarily resistant to current Smo inhibitors.

INTRODUCTION

The Hedgehog (Hh) signaling pathway is a major regulator governing cell proliferation and differentiation, tissue polarity and patterning. It is initiated by binding of its 12-pass transmembrane receptor, patched (Ptch) with one of the three ligands, sonic hedgehog (Shh), indian hedgehog (Ihh), and desert hedgehog (Dhh). The membrane protein Smoothened (Smo), structurally similar to G-protein coupled receptors (GPCRs), is then released and accumulated in cilia. The ciliary accumulated Smo can promote activation of transcriptional factor Gli (Gli1, Gli2, and Gli3) by inhibition of suppressor of fused (Sufu) and protein kinase A (PKA), ultimately leading to target genes transcription.[1,2] It has been long recognized that aberrant activation of the Hh pathway after birth drives initiation and maintenance of numerous types of cancers, such as basal cellular carcinoma (BCC) and medulloblastoma.[2,3] Molecular mechanisms behind the aberrant activation of the Hh pathway in cancers include irregular elevation of its ligands in stromal cells or tumor cells, Ptch-inactivating mutations, Smo-activating mutations, Sufu-inactivating mutations, as well as genetic changes in Gli1 or Gli2.[2,4]

Addiction of various types of tumors to the hyperactive Hh pathway activity has attracted intensive effort to develop targeted Hh pathway inhibitors for therapeutic use.[5-7] Smo, a central effector of the Hh pathway, has emerged as a predominant drug target in the development of small molecular Hh pathway inhibitors.[8-10] All the current drug candidates in clinical trials for combating Hh-driven cancers act directly on Smo,[10] among which, vismodegib[11,12] (**2**, GDC-0449) from Genentech, and sonidegib[13,14] (**3**, NVP-LDE225) from Novartis were successively approved for clinical use of locally advanced or metastatic BCC in 2012 and 2015, respectively (Figure 1). However, tumors harboring aberrant Hh activity by genetic alterations downstream from Ptch as mentioned above, are conceivably insensitive to these Smo inhibitors, a phenomenon proved by both

bench and bedside investigations.[15-18] Moreover, like other molecular targeted anti-cancer drugs, acquired resistance is rapidly developed in approximately 20% patients after initial dramatic response to **2**.[19,20] This subpopulation of patients was further demonstrated to be refractory to **3** as well.[18] Studies in mice and humans have delineated the molecular basis underlying how tumors evolved resistance to Smo inhibitors, including additional mutations in Smo, loss-of-function mutation of Sufu, and amplification of Gli2.[21-25] Hence, the primary and acquired resistance to current Smo inhibitors highlights the critical need to develop novel next-generation Hh inhibitors, either with distinct chemical structures or with alternative mechanisms of action, particularly those acting downstream of Smo.[26]

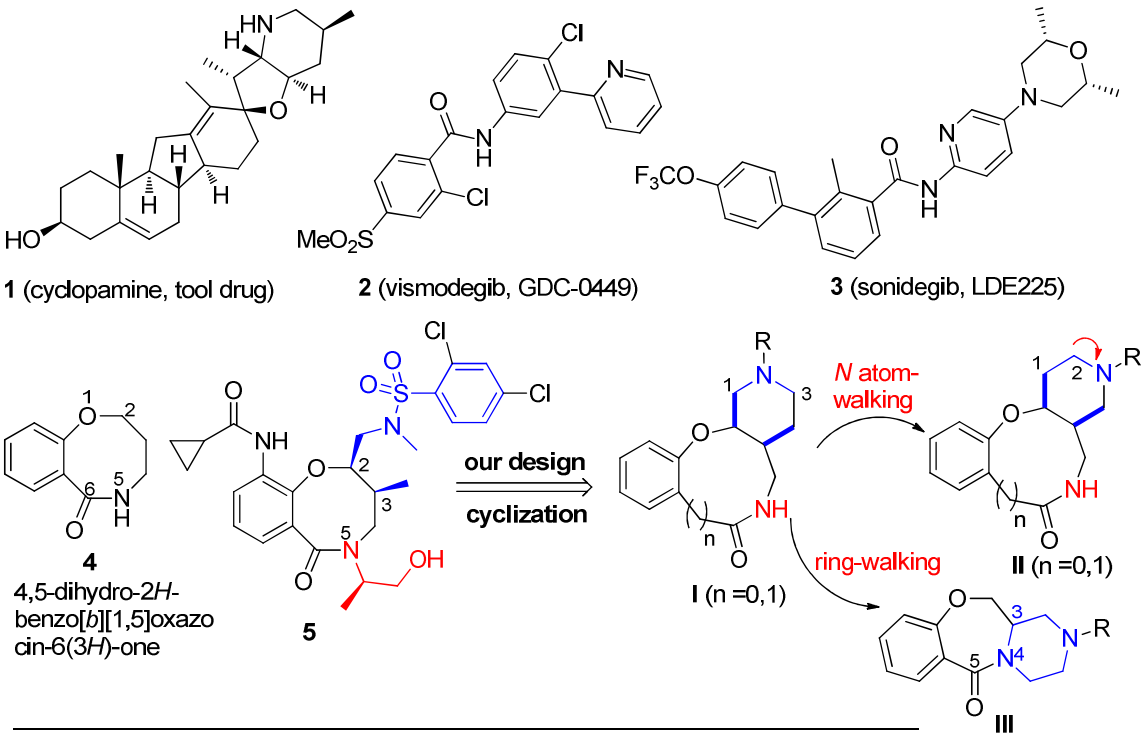


Figure 1. Reported Hedgehog pathway inhibitors and our designed series I-III.

Recently, emerging evidence has showed that targeting Hh pathway at the level of its final effector Gli represents one promising strategy to combat primary and acquired resistance to current

Smo inhibitors.[23,27-31] Several types of small molecule Gli inhibitors have been developed, such as the Gli antagonist - 2,2'-((2-(pyridin-4-yl)dihydropyrimidine-1,3(2*H*,4*H*)-diyl)bis(methylene) bis(*N,N*- dimethylaniline) (GANT61),[32] arsenic trioxide (As_2O_3),[28,33] the bromodomain-containing protein 4 (BRD4) inhibitor - (*6S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4] triazolo[4,3-*a*] [1,4]diazepine-6-acetic acid 1,1-dimethylethyl ester ((+)-JQ1),[27] and among others[34,35]. Unfortunately, these Gli inhibitors generally exhibited low efficacy against the Hh pathway activity and need further structural optimization. Recently, Schreiber and co-workers[36,37] performed a cell-based high-throughput screening and identified compound **5** (BRD9526)[37] bearing the 4,5-dihydro- 2*H*-benzo[*b*][1,5]oxazocin-6(3*H*)-one bicyclic core **4** that showed an EC_{50} value of 60 nM in the Hh-induced differentiation of C3H10T1/2 cells and partially lowered the Gli1 expression at concentrations of 10 μM. Although the cellular potency is weak and its mechanism whether acting at the level of Gli or downstream of SuFu is not clear, the structural novelty, distinctly different from the well-known Hh inhibitors **1-3**, encourages us to conduct a systemic structural modification of **5** to generate more potent new-generation Hh pathway inhibitors. Since the Schreiber group has demonstrated that, among the eight stereoisomers relevant to the three stereogenic carbons, only the two C2,C3-*cis* isomers showed high potency,[37] we decide to design several series of compounds (formula **I**-**III**, Figure 1) by cyclization of C2 and C3 of **5** to form an additional piperidinyl ring sitting on the eight-membered 4,5-dihydro-2*H*-benzo[*b*][1,5]oxazocin-6(3*H*)-one center (**I**, n = 0, Figure 1) or on the nine-membered 2,3,4,5-tetrahydrobenzo[*h*][1,5]oxazonin-6(7*H*)-one center (**I**, n = 1) in *cis*-configuration. Compound series **II** can be viewed from series **I** through an *N*-atom walking process (from 2- to 3-position on the ring as shown in red arrow), whereas series **III** can be viewed from **I** through a piperidinyl ring-walking process combined with a center-ring contraction from the

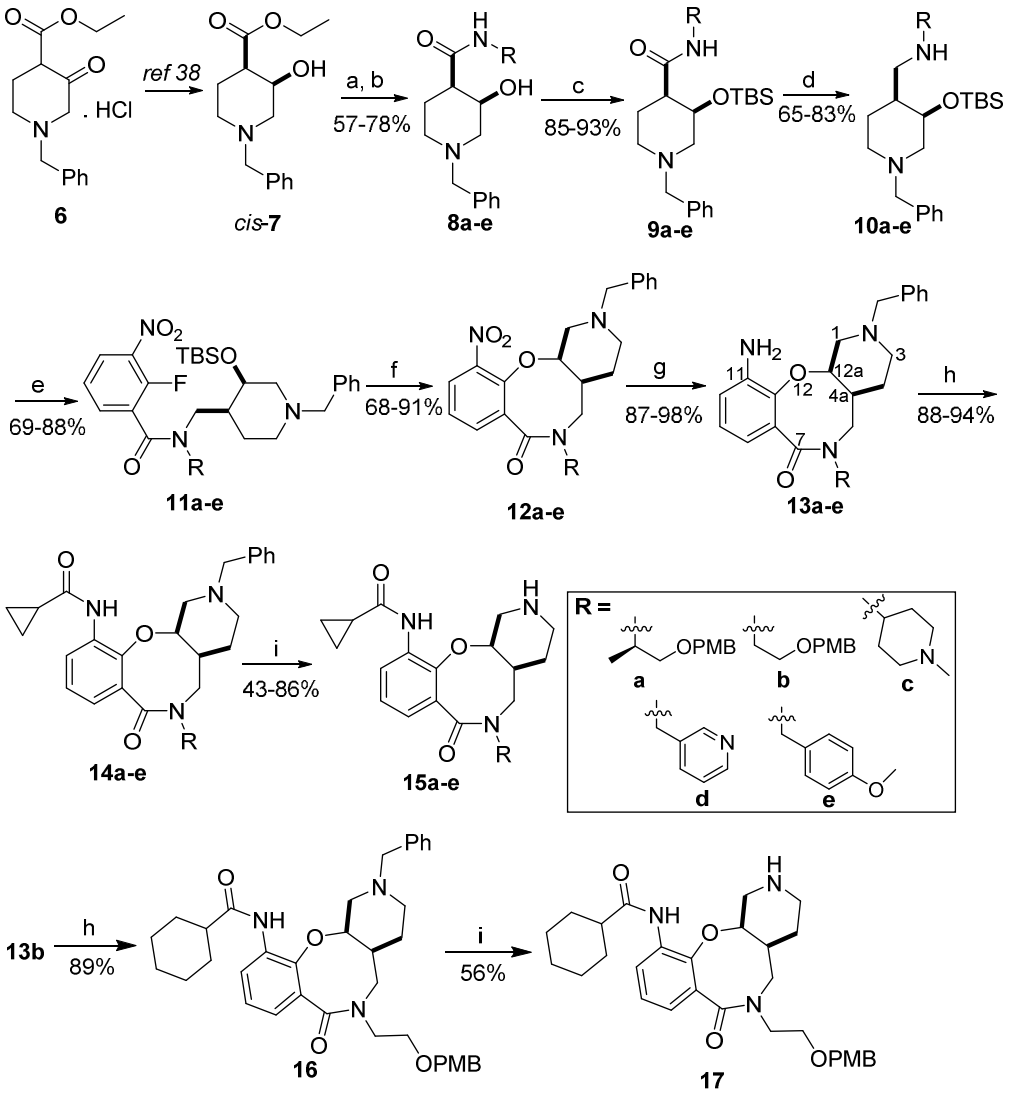
eight-membered 1,5-oxazocan-6-one to the seven-membered 3,4-dihydrobenzo[*f*][1,4] oxazepin-5(2*H*)-one center. Compared to the prototypic bicyclic compound **5**, all the new compounds contain a unique fused tricyclic framework with reduced stereogenic carbons. Herein, we report our synthesis and pharmacological evaluation of these new compounds as Hh pathway inhibitors.

RESULTS AND DISCUSSION

Chemistry. The synthesis of tricyclic benzo[*b*]pyrido[4,3-*g*][1,5]oxazocin-7(12a*H*)-ones is shown in Scheme 1. Starting from 1-benzyl-3-oxopiperidine-4-carboxylate **6**, the *cis*-configured hydroxypiperidine **7** was prepared by following the literature procedure.[38] Hydrolysis of **7** followed by amidation with various amines in the presence of 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU), 1-hydroxy-7-azabenzotriazole (HOAT) and diisopropyl ethylamine (DIPEA) provided amides **8a-e** in 57-78% overall yields. Without purification, protection[39] of **8a-e** via treatment with *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) afforded the TBS-ethers **9a-e** in 85-93% yields. Reduction[37] of amides **9a-e** with borane dimethylsulfide (BH_3.DMS) in THF followed by coupling with 2-fluoro-3-nitrobenzoic acid under the standard condensation conditions (HATU, HOAT, DIPEA) delivered amides **11a-e**, which were subsequently cyclized[37] by treating with CsF to furnish the key tricyclic intermediates - (4a,12a)-*cis*-2,3,4,4a,5,6-hexahydro-1*H*-benzo[*b*]pyrido [4,3-*g*][1,5]oxazocin-7(12a*H*)-ones **12a-e** in 68-91% yields. Reduction of **12a-e** with Fe powder in ethyl alcohol and subsequent acylation of the resulting aniline intermediate with cyclopropanecarbonyl chloride produced the cyclopropyl-substituted amides **14a-e** in 76-92% overall yields. Debenzylation of **14a-e** with $Pd(OH)_2$/C under hydrogen atmosphere gave the key intermediates **15a-e** in 43-86% yields. Meanwhile, the *N*-cyclohexanecarbonyl-substituted analog **17**

was obtained from **13b** in 50% overall yield by following similar reaction procedures as that for preparation of **15a-e**.

Scheme 1. Synthesis of intermediates 15a-e and 17.[a]

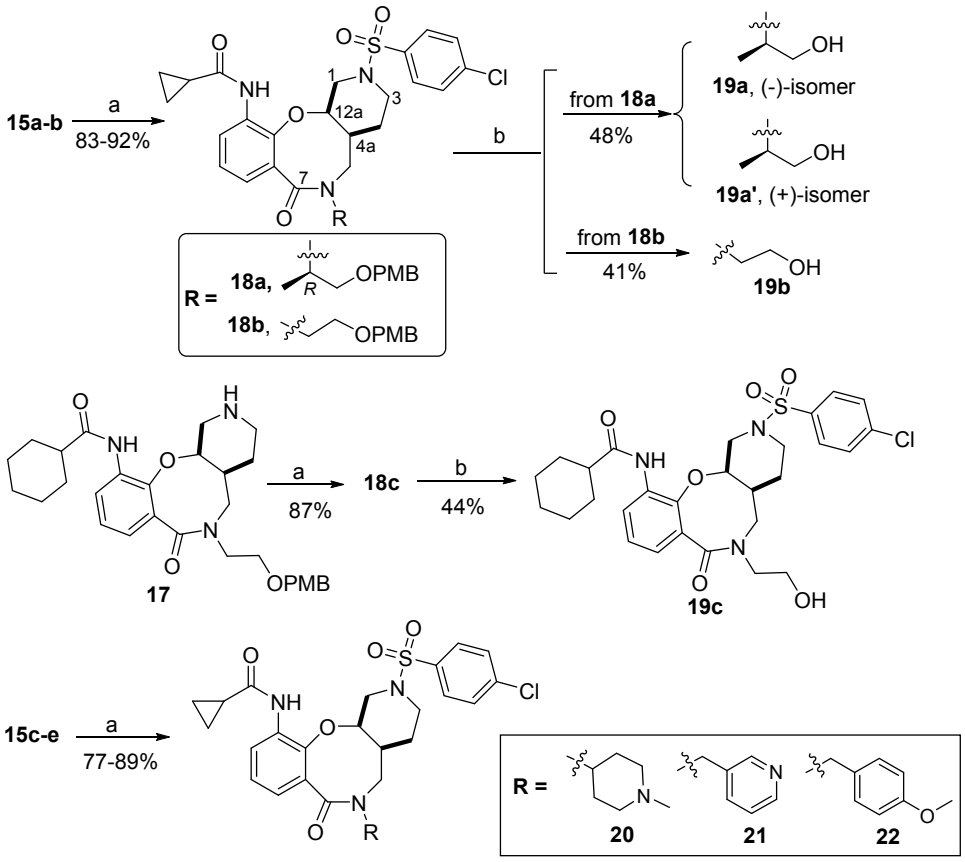


[a]Reagents and conditions: (a) $LiOH.H_2O$, EtOH-H_2O (1:1), rt, 2 h; (b) RNH_2, HATU, HOAT, DIPEA, CH_2Cl_2, rt, 12 h; (c) TBSOTf, Et_3N, CH_2Cl_2, 0 °C, 1 h; (d) BH_3.DMS, THF, 65 °C, 6 h, then 10% potassium sodium tartrate and MeOH (3:2), reflux, 18 h; (e) 2-fluoro-3-nitrobenzoic acid, HOAT, HATU, DIPEA, CH_2Cl_2, rt, 6 h; (f) CsF, DMF, 85 °C, 4 h; (g) Fe, NH_4Cl, EtOH-H_2O (5:2), reflux, 1 h; (h) for **14a-e**, cyclopropanecarbonyl chloride, 2,6-lutidine, CH_2Cl_2, 0 °C, 4 h; for **16**, cyclohexanecarbonyl chloride, 2,6-lutidine, CH_2Cl_2, 0 °C, 3 h; (i) $Pd(OH)_2/C$, EtOH, H_2, 40 °C, 8 h;

2,6,11-Trisubstituted benzo[*b*]pyrido[4,3-*g*][1,5]oxazocin-7(12a*H*)-ones **19-22** were synthesized as described in Scheme 2. Treatment of **15a-b** with 4-chlorobenzene-1-sulfonyl chloride led to

arylsulfonamides **18a-b** in 83-92% yields, which were further converted to final products **19a,b** and **19a'**, respectively, by removal of the *O*-PMB (PMB = *para*-methoxybenzyl) group with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ).[37] Compounds **19a** and **19a'** were generated as a pair of C4a,C12a-*cis* diastereomeric isomers with opposite optical rotations in about 1:1 ratio, due to the existence of the *R*-configurated *N6*-substituent. Both isomers were isolated by HPLC and their absolute configurations were not determined. Similarly, the intermediate **17** was converted to arylsulfonamides **19c** through a similar procedure as that for **19a,b**. In the meantime, *N6*-substituted analogues **20-22** were prepared as well in 77-89% yields by treating intermediates **15c-e** with 4-chlorobenzene-1-sulfonyl chloride under the aforementioned conditions.

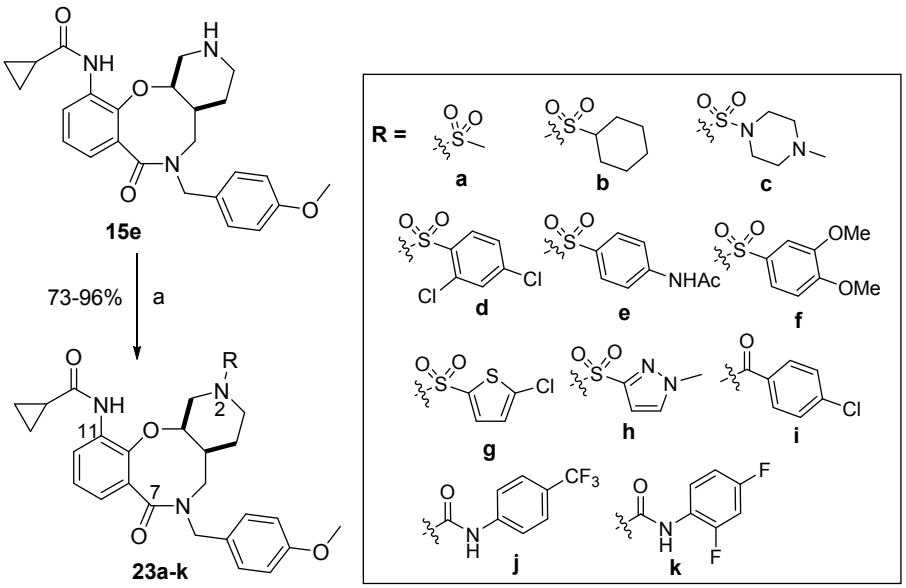
Scheme 2. Synthesis of compounds 19-22.[a]



[a]Reagents and conditions: (a) 4-chlorobenzene-1-sulfonyl chloride, 2,6-lutidine, CH_2Cl_2, 0 °C, 3 h; (b) DDQ, pH = 7, buffer/CH_2Cl_2, rt, 12 h.

As shown in Scheme 3, treatment of compound **15e** with diverse alkyl- or aryl-sulfonyl chlorides provided sulfonamides **23a-h** in 73-96% yields. Meanwhile, treating **15e** with acyl chlorides or isocyanates afforded substituted benzamides **23i-k** in 74-93% yields.

Scheme 3. Synthesis of compounds 23a-k.[a]

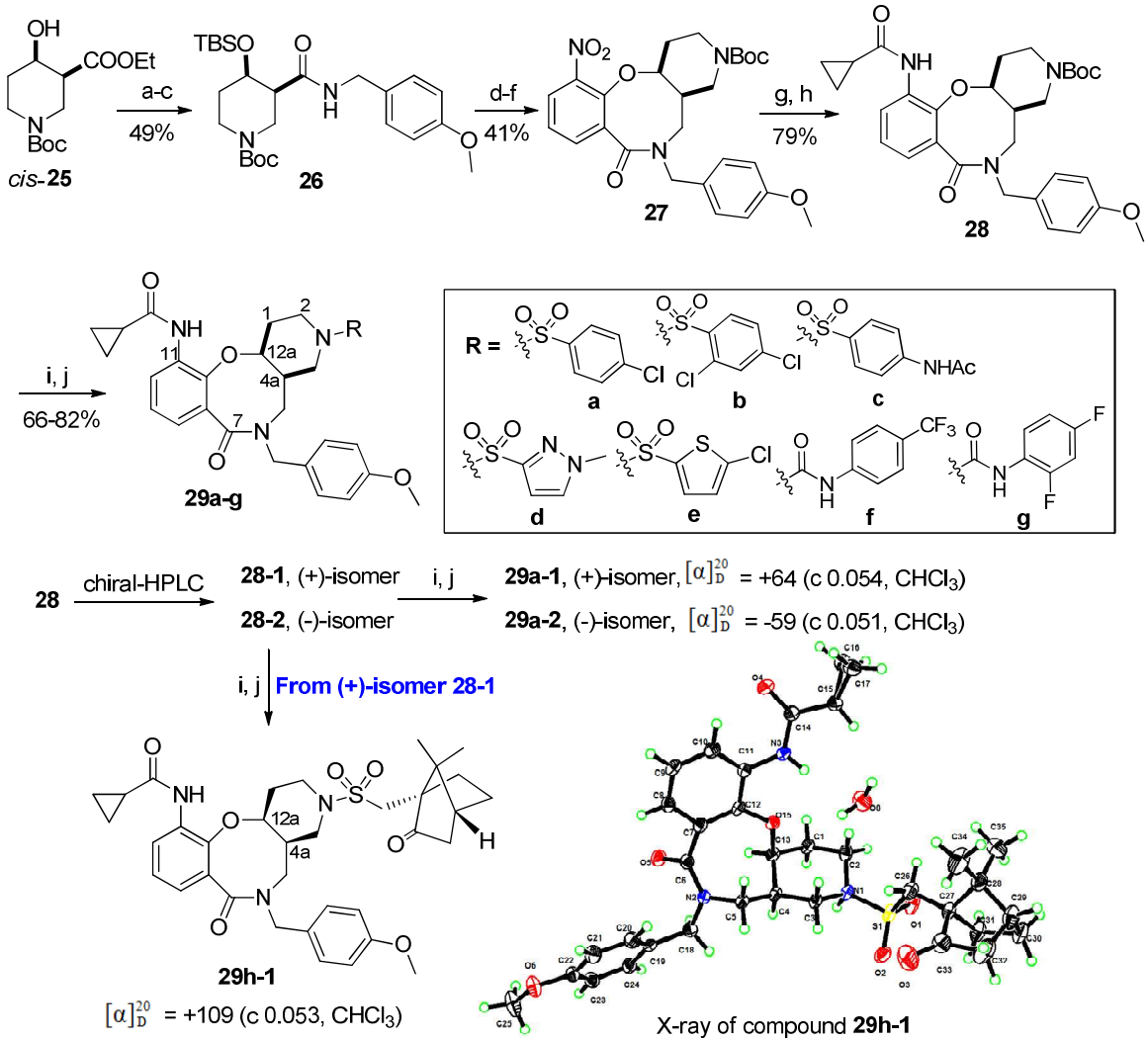


[a]Reagents and conditions: (a) for **23a-h**, sulfonyl chlorides, 2,6-lutidine, CH_2Cl_2, 0 °C, 3 h, 73-96%; for **23i**, acyl chlorides, 2,6-lutidine, CH_2Cl_2, 0 °C, 3 h, 93%; for **23j-k**, isocynates, CH_2Cl_2, rt, 3 h, 74-75%.

The synthesis of the 2,3,4,4a,5,6-hexahydro-1*H*-benzo[*b*]pyrido[3,4-*g*][1,5]oxazocin-7(12a*H*)-ones **29a-g** is illustrated in Scheme 4. The *cis*-configurated hydroxypiperidine **25** was prepared according to the literature procedure.[38] Hydrolysis of **25** followed by amination with (4-methoxyphenyl)methanamine and protection of the hydroxyl with TBSOTf delivered amide **26** in 49% overall yield. The *N3,N6,C11*-trisubstituted (4a,12a)-*cis* 2,3,4,4a,5,6-hexahydro-1*H*-benzo[*b*]pyrido[3,4-*g*][1,5]oxazocin-7(12a*H*)-one **27** was obtained through BH_3.DMS reduction of the amido moiety, condensation with 2-fluoro-3-nitrobenzoic acid and CsF-assisted cyclization by following similar reaction procedures as that for preparation of **12a-e**. Reduction of **27** with Fe powder and subsequent amidation with cyclopropanecarbonyl chloride produced the intermediate **28** in 79% overall yield. Removal of the *tert*-butoxycarbonyl (Boc) group with TFA followed by treating

with various arylsulfonyl chlorides afforded target compounds **29a-g** as racemates in 66-82% yields. The formation of the tricyclic skeleton was confirmed by NMR correlations between H-12a (δ_H = 4.60 ppm) and C-12a (δ_C = 69.44 ppm) as well as the correlation between H-4a (δ_H = 1.15 – 1.10 ppm) and C-4a (δ_C = 15.98 ppm) in the heteronuclear single quantum coherence spectrum (HSQC) of compound **29a** (See SI).

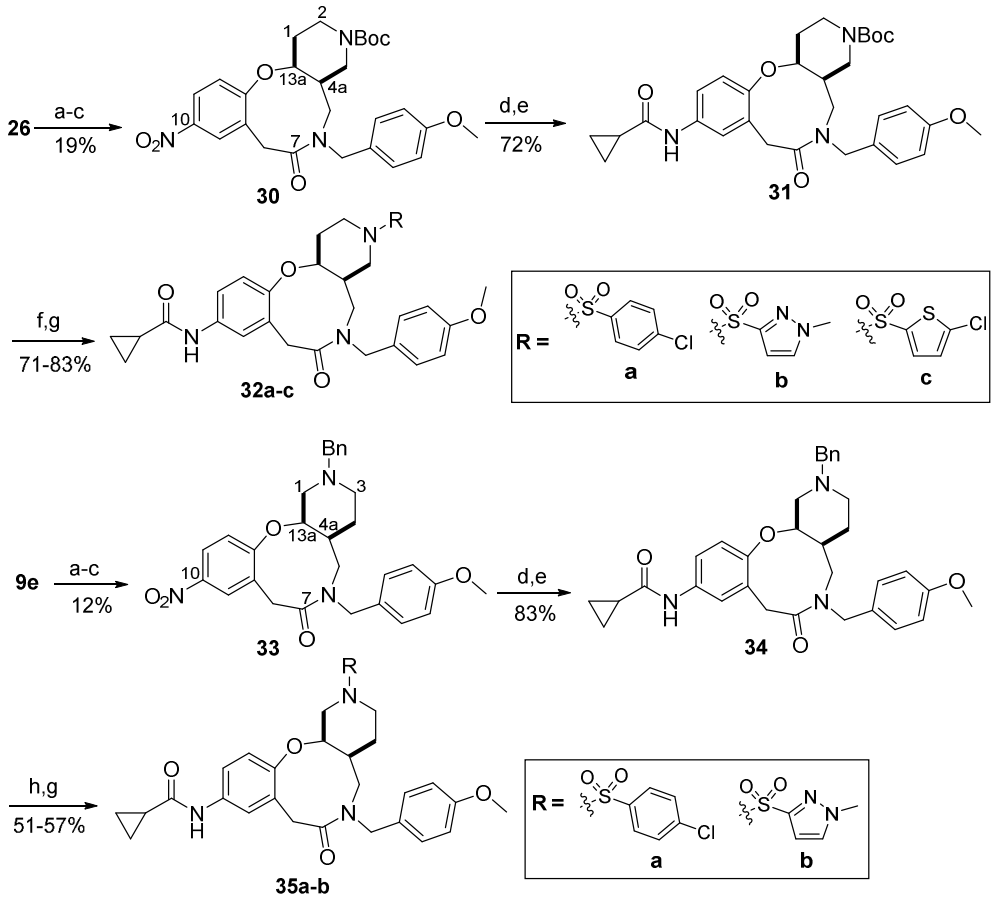
Scheme 4. Synthesis of compounds 29a-g.[a]



[a]Reagents and conditions: (a) $LiOH.H_2O$, $EtOH-H_2O$ (1:1), rt, 2 h; (b) (4-methoxyphenyl)methanamine, HATU, HOAT, DIPEA, CH_2Cl_2, rt, 12 h; (c) TBSOTf, Et_3N, CH_2Cl_2, 0 °C, 1 h; (d) BH_3.DMS, THF, 65 °C, 6 h, then 10% potassium sodium tartrate and MeOH (3:2), reflux, 18 h; (e) 2-fluoro-3-nitrobenzoic acid, HOAT, HATU, DIPEA, CH_2Cl_2, rt, 6 h; (f) CsF, DMF, 85 °C, 4 h, 84%; (g) Fe, NH_4Cl, $EtOH-H_2O$ (5:2), reflux, 1 h; (h) acyl chloride, 2,6-lutidine, CH_2Cl_2, 0 °C, 4 h; (i) TFA, CH_2Cl_2, rt, 4 h; (j) for **29a-e** and **29h**, sulfonyl chlorides, 2,6-lutidine, CH_2Cl_2, 0 °C, 3 h; for **29f-g**, isocynates, 2,6-lutidine, CH_2Cl_2, 0 °C, 3 h.

In addition, the racemic **28** was optically resolved by preparative chiral-HPLC to provide **28-1** and **28-2** as the (+)- and (-)-isomers, respectively. They were subsequently converted to the optically pure compounds **29a-1** ((+)-isomer) and **29a-2** ((-)-isomer), respectively (chiral-HPLC report, see SI). To determine the absolute configuration of **29a-1** and **29a-2**, we prepared camphorsulfonamide **29h-1** by treating (+)-enantiomer **28-1** with D(+)-10-camphorsulfonyl chloride. The absolute configuration of **29h-1** was successfully secured by single-crystal X-ray diffraction analysis as (4a*R*,12a*S*)-isomer (CCDC: 1564315). Accordingly, compounds **29a-1** and **29a-2** were deduced to be (4a*R*,12a*S*)- and (4a*S*,12a*R*)-enantiomer, respectively.

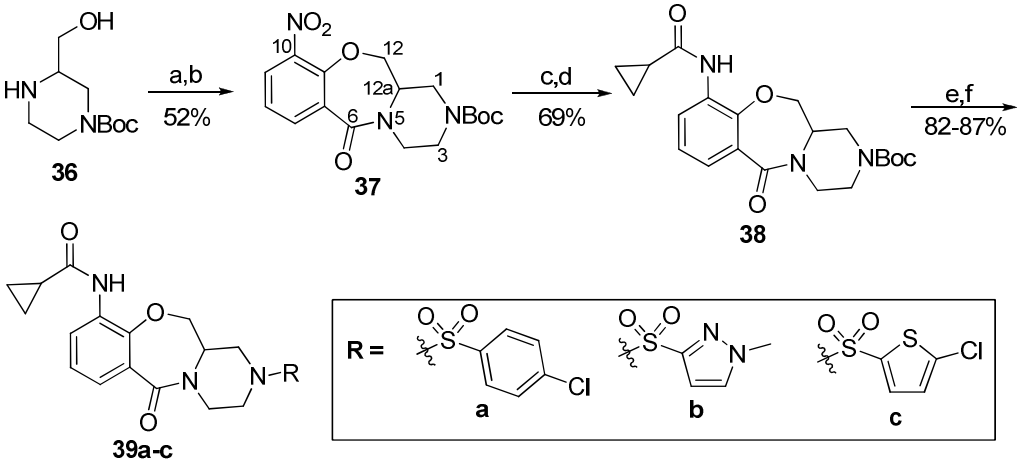
Scheme 5. Synthesis of compounds 32-35.[a]



[a]Reagents and conditions: (a) BH_3.DMS, THF, 65 °C, 6 h, then 10% potassium sodium tartrate and MeOH (3:2), reflux, 18 h; (b) 2-(2-fluoro-5-nitrophenyl)acetic acid, HOAT, HATU, DIPEA, CH_2Cl_2, rt, 6 h; (c) for **30**, NH_4F, TBAF.$3H_2O$, THF, 40 °C, 12 h, 38%; for **33**, NH_4F, TBAF.$3H_2O$, THF, 40 °C, 4 h, then Na_2CO_3, DMF, 80 °C, 3 h, 26%; (d) Fe, NH_4Cl, EtOH-H_2O (5:2), reflux, 1 h; (e) cyclopropanecarbonyl chloride, 2,6-lutidine, CH_2Cl_2, 0 °C, 4 h; (f) TFA, CH_2Cl_2, rt, 4 h; (g) sulfonyl chlorides, 2,6-lutidine, CH_2Cl_2, 0 °C, 3 h; (h) $Pd(OH)_2$/C, EtOH, H_2, 50 °C, 8 h, 63%.

As shown in Scheme 5, compounds **32a-c** bearing the (4a,13a)-*cis*-1,2,3,4,4a,5,6,13a-octahydrobenzo[*h*]pyrido[4,3-*b*][1,5]oxazonin-7(8*H*)-one scaffold were prepared using compound **26** as the starting material. Reduction of **26** with BH_3.DMS[37] and condensation with 2-(2-fluoro-5-nitrophenyl)acetic acid followed by cyclization of the resulting intermediate with the assistance of TBAF furnished 1,2,3,4,4a,5,6,13a-octahydrobenzo[*h*]pyrido[4,3-*b*][1,5] oxazonin-7(8*H*)-one **30** in 19% overall yield. Subsequent reduction of the nitro group with Fe powder followed by acylation of the resulting aniline with cyclopropanecarbonyl chloride gave compound **31** in 72% overall yield. Removal of the *N*-Boc group followed by treating with various arylsulfonyl chlorides yielded target compounds **32a-c** in 71-83% yields. Similarly, the isomeric (4a,13a)-*cis*-1,2,3,4,4a,5,6,13a-octahydrobenzo[*h*]-pyrido[3,4-*b*][1,5]oxazonin-7(8*H*)-ones **35a,b** were prepared by following a set of similar reaction procedures starting from compound **9e** as that for preparation of compounds **32a-c**.

Scheme 6. Synthesis of compounds 39a-c.[a]



[a]Reagents and conditions: (a) 2-fluoro-3-nitrobenzoic acid, HATU, Et_3N, THF, rt, 4 h; (b) NaH, DMF, rt, 4 h; (c) Fe, NH_4Cl, EtOH-H_2O (5:2), 80 °C, 1 h; (d) cyclopropanecarbonyl chloride, 2,6-lutidine, CH_2Cl_2, 0 °C, 4 h; (e) TFA, CH_2Cl_2, rt, 4 h; (j) sulfonyl chlorides, 2,6-lutidine, CH_2Cl_2, 0 °C, 3 h;

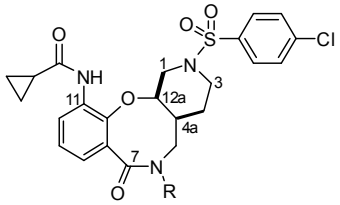
As shown in Scheme 6, compound **37** bearing the 3,4,12,12a-tetrahydro-1*H*-benzo[*f*] pyrazino[2,1-*c*][1,4]oxazepin-6(2*H*)-one scaffold was prepared by condensation[40] of *tert*-butyl 3-(hydroxymethyl)piperazine-1-carboxylate (**36**) with 2-fluoro-3-nitrobenzoic acid under

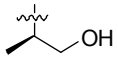
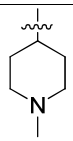
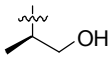
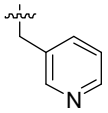
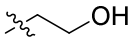
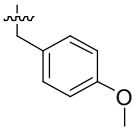
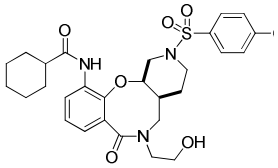
HATU/Et_3N followed by NaH-promoted cyclization[41] in 52% overall yield. Reduction of the nitro group with Fe powder followed by amidation with cyclopropanecarbonyl chloride provided amide **38** in 69% overall yield. Removal of the *N*-Boc group with TFA followed by sulfonamidation with diverse arylsulfonyl chlorides delivered target compounds **39a-c** in 82-87% yields.

Structure-Activity Relationship (SAR) Study. All the new compounds were first evaluated for their ability to inhibit the Hh signaling pathway by dual luciferase reporter assays with light II cells, which were NIH-3T3 cells stably transfected with a Gli-responsive firefly luciferase reporter and Renilla-luciferase expression vector.[42] IC_{50} values were tested only for compounds showing inhibitory rates greater than 50% at the concentration of 1 μM. Compounds **19a**, **19a'**, **29a-1**, and **29a-2** are single stereoisomer, whereas the rest of tested compounds were racemic mixtures.

As shown in Table 1, we first evaluated benzo[*b*]pyrido[4,3-*g*][1,5]oxazocin-7(12a*H*)-ones **19-22** bearing *N*2-(*para*-chlorophenyl) sulfonyl and C11-cyclopropanecarboxamido moieties with diverse *N*6-substituents. Unfortunately, the two diastereoisomers **19a** and **19a'** bearing the same *R*-configured *N*-(1-hydroxypropan-2-yl) moiety as the *N*6-substituent as that in **5** showed rather weak inhibitory effects at the concentration of 1 μM against the Hh signaling pathway. The 2-hydroxyethyl substituted analog **19b** also exhibited negligible activity. Replacement of the C11-substituent with cyclohexanecarboxamido moiety afforded compound **19c** showing low inhibition as well. Again, rather weak inhibitory effect was observed from **21** bearing pyridine-3-methyl as the *N*6-substituent. To our delight, the *N*-methylpiperidin-4-yl substituted analog **20** and *N*6-(*para*-methoxybenzyl) substituted analog **22** showed appreciable inhibition against the Hh signaling pathway with an IC_{50} value of 344 nM and 304 nM, respectively. Both compounds are slightly more potent than the prototypic compound **5** (IC_{50} = 383 nM) in our test system.

Table 1. Hh pathway inhibition of compounds by dual luciferase reporter assays in light II cells.[a]



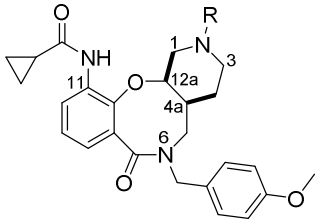
R	Gli-luc reporter		R	Gli-luc reporter	
	1 μM (%)	IC_{50} (nM)		1 μM (%)	IC_{50} (nM)
 **19a**	34 ± 16	>1000	 **20**	61 ± 23	344 ± 6
 **19a'**	40 ± 12	>1000	 **21**	28 ± 8	>1000
 **19b**	25 ± 12	>1000	 **22**	97 ± 8	304 ± 59
 **19c**	32 ± 9	>1000	**5**	67 ± 22	383 ± 74
			2	93 ± 6	39 ± 2.0

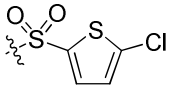
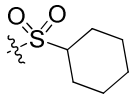
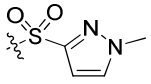
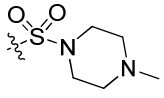
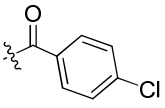
[a]The IC_{50} values are shown as the mean ± SD (nM) from three separate experiments.

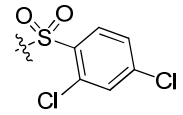
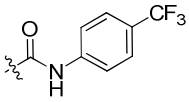
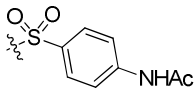
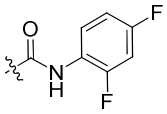
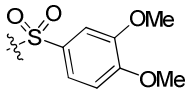
Since compound **22** showed an inhibitory potency compatible to that of **5**, we then retained *para*-methoxybenzyl moiety as the *N*6-substituent together with cyclopropanecarboxiamo moiety as the C11-substituent, and screened various sulfonyl or acyl groups as the *N*2-substituent. As shown in Table 2, methanesulfonyl substituted compound **23a** showed much reduced inhibitory effect, compared to compound **22**. Likewise, both cyclohexanesulfonyl and 4-methylpiperazine-1-sulfonyl

analogs **23b** and **23c** showed inappreciable potency. However, moderate to good potency was found from arylsulfonyl substituted compounds **23d-f**. Compounds **23d** and **23f** bearing two substituents showed lower potency, whereas the (4-acylamino)phenylsulfonyl analog **23e** showed an enhanced potency with an IC_{50} value of 42 nM, which is 7- and 9-fold more potent than **22** and **5**, respectively. Compound **23e** is also nearly equally potent to the clinically prescribed drug **2** (39 nM, Table 1). The result indicated that our tricyclic framework well maintains the interaction with the pathway as that of the bicyclic framework **5**, and an *N*-arylsulfonyl substituent on the piperidine ring seems optimal. Thus analysis is further validated from *N*2-heteroarylsulfonyl substituted **23g** and **23h** that exhibited good potency as well, especially 1-methyl-1*H*-pyrazole-3-sulfonamide **23h** having a high potency of 94 nM, which is 4-fold more potent than both **22** and **5**.

Table 2. Hh pathway inhibition of compounds by dual luciferase reporter assays in light II cells.[a]



R	Gli-luc reporter		R	Gli-luc reporter	
	1 μM (%)	IC_{50} (nM)		1 μM (%)	IC_{50} (nM)
 **23a**	45 ± 16	>1000	 **23g**	113 ± 3	167 ± 88
 **23b**	42 ± 0.9	>1000	 **23h**	74 ± 8	94 ± 25
 **23c**	45 ± 6	>1000	 **23i**	22 ± 13	>1000

 **23d**	102 ± 5	766 ± 134	 **23j**	100 ± 5	292 ± 104
 **23e**	105 ± 5	42 ± 7.0	 **23k**	100 ± 5	26 ± 6.0
 **23f**	54 ± 16	>1000	-	-	-

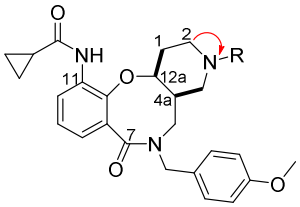
[a]The IC_{50} values are shown as the mean ± SD (nM) from three separate experiments.

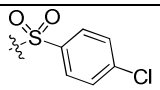
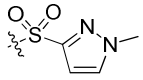
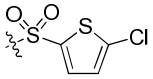
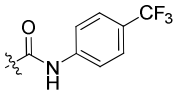
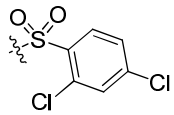
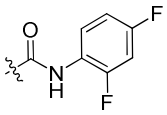
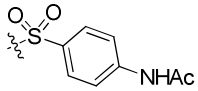
Completely no inhibitory effect was observed from 4-chlorobenzoyl substituted analog **23i**, indicating both the oxygen atoms in the sulfonyl moiety of either **23e** or **23h** other than the only oxygen atom in the acyl **23i** involved in the compound-target interaction model. Further, compounds **23j** and **23k** bearing *N*2-(4-(trifluoromethyl)phenyl)carbamic moiety and *N*2-(2,4-difluorophenyl)carbamic moiety, respectively, displayed good potency, especially compound **23k** showing an IC_{50} value of 26 nM that is more than 10-fold more potent than the prototypic compound **5** and even 1.5-fold more potent than the clinically prescribed Hh inhibitor **2**. This result highlights the critical contribution of the piperidine *N*-substituent in our tricyclic skeleton and both arylsulfonyl and arylacyl bearing appropriate substituents are well tolerant.

Compounds **29a-g** can be viewed as isomers of **22e, 23d-e, 23g-h** and **23j-k**, respectively, with the *N*2-atom walking to *N*3 position. By taking advantage of the result in Table 1 and Table 2, we directly elected the piperidine *N*-substituents showing good potency to the isomeric tricyclic framework. Gratifyingly, this small series of compounds retained good to high potency against the Hh signaling pathway, especially compounds **29a** and **29b** showing IC_{50} values of 23 and 33 nM, respectively (Table 3). Other *N*-substituted analogues (**29c-d**, **29f-g**) showed much less potency, except the 5-chlorothiophene-2sulfonyl **29e** which retained high potency of 46 nM. Since racemic **29a** showed the highest potency, we then evaluated its two enantiomeric isomers. The result shows

that the stereogenic character plays a critical role on the Hh activity. The (+)-enantiomer **29a-1** with *4aR,12aS*-configuration retains the high potency (12 nM), whereas the (-)-enantiomer **29a-2** with *4aS,12aR*-configuration is nearly 17-fold less potent (200 nM). These results may be roughly rationalized based on the X-ray structure of compound **29h-1**, where the phenyl and piperidinyl rings are bended toward each other to form an appropriate dihedral angle, which can be adjusted by both the stereogenic configuration of the piperidine ring (upside or downside of the central ring) and the location of the piperidine *N*-atom, together with its substituent.

Table 3. Hh pathway inhibition of compounds by dual luciferase reporter assays in light II cells.[a]



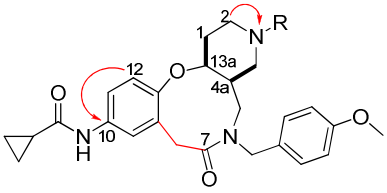
R	Gli-luc reporter		R	Gli-luc reporter	
	1 μM (%)	IC_{50} (nM)		1 μM (%)	IC_{50} (nM)
 **29a**	97 ± 9	23 ± 9	 **29d**	87 ± 1	145 ± 47
29a-1, (+)-isomer	117 ± 15	12 ± 1	 **29e**	108 ± 9	46 ± 4
29a-2, (-)-isomer	92 ± 0.8	200 ± 44	 **29f**	68 ± 9	>1000
 **29b**	107 ± 15	33 ± 17	 **29g**	87 ± 9	540 ± 79
	82 ± 18	773 ± 268	–	–	–

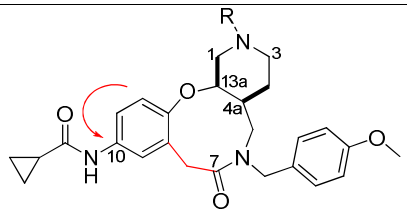
29c	

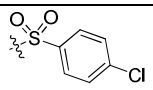
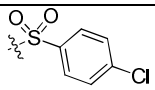
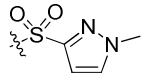
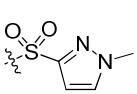
[a]The IC_{50} values are shown as the mean ± SD (nM) from three separate experiments.

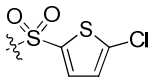
Compounds **32a-c** represent a subseries of ring-expanded analogs bearing a nine-membered 1,5-oxazonan-6-one structural motif along with the cyclopropanecarboxamido moiety moving downside to C10. Although bearing similar substituents at *N*3-, *N*6- and C10 as that in **29a**, **29d** and **29e**, these new compounds showed negligible potency against the Hh pathway. Similarly, compounds **35a,b** are the ring-enlarged analogs of **22** and **23h**, and both showed low inhibitory effects with IC_{50} values of 502 nM and >1μM, respectively (Table 4). We envisioned that in addition to the location of C10-substituent, the larger central ring significantly altered the dihedral angle between the phenyl and piperidnyl of the tricyclic skeleton and subsequently impeded the interaction patterns.

Table 4. Hh pathway inhibition of compounds by dual luciferase reporter assays in light II cells.[a]



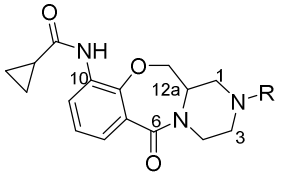


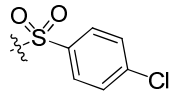
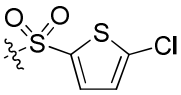
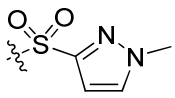
R	Gli-luc reporter		R	Gli-luc reporter	
	1 μM (%)	IC_{50} (nM)		1 μM (%)	IC_{50} (nM)
 **32a**	36 ± 23	>1000	 **35a**	60 ± 11	502 ± 92
 **32b**	-15 ± 21	>1000	 **35b**	16 ± 3	>1000

 **32c**	65 ± 5	1620 ± 43	–	–	–

[a]The IC_{50} values are shown as the mean ± SD (nM) from three separate experiments.

Table 5. Hh pathway inhibition of compounds by dual luciferase reporter assays in light II cells.[a]



R	Gli-luc reporter		R	Gli-luc reporter	
	1 μM (%)	IC_{50} (nM)		1 μM (%)	IC_{50} (nM)
 **39a**	35 ± 26	>1000	 **39c**	80 ± 11	482 ± 27
 **39b**	58 ± 7	>1000	–	–	–

[a]The IC_{50} values are shown as the mean ± SD (nM) from three separate experiments.

To further determine the effect of the topology of the tricyclic framework on the Hh pathway activity, compounds **39a-c** bearing the seven-membered 3,4-dihydrobenzo[*f*][1,4]oxazepin-5(2*H*)-one center were designed and tested (Table 5). As mentioned earlier, these compounds can be viewed as the central ring-contracting and the piperidine-ring moving (on the central ring) analogs of **19a-22**. Disappointingly, these compounds showed weak inhibitory effects at the concentration of 1 μM, and only compound **39c** bearing a *N*2-(5-chloro-thiophen-2-yl)-sulfonyl substituent showed a moderate IC_{50} value of 482 nM.

Table 6. Cytotoxicity and hERG Inhibition of Potent Compounds

Compound	IC_{50}		
	Gli-luc reporter assay	squamous carcinoma KB cell	hERG
29a	23 ± 9	> 20 μM	> 20 μM

Overall Safety Profile of Compound 29a. From the SAR analysis above, compound **29a**, bearing the tricyclic center - (4a,12a)-*cis* 1,2,3,4,4a,5,6,12a-octahydro-7*H*-benzo[*b*]pyrido[3,4-*g*][1,5] oxazocin-7-one turns out to be the most potent against the Hh signaling pathway showing an IC_{50} values of 23 nM. It is up to 17-fold more potent than the prototypic compound **5** and 1.7-fold more potent than the clinically prescribed Hh pathway inhibitor drug **2**. Although the (+)-isomer **29a-1** is slightly more potent than both the racemic and the (-)-isomer, it was difficult to prepare in large scale. Therefore, to quickly gain the developmental value of the tricyclic skeleton as the new-generation Hh inhibitor, racemic **29a** was selected for further evaluation. First, to exclude the chemical structure relevant cytotoxicity and cardiac toxicity, we tested the inhibitory effects of **29a** against the proliferation of squamous carcinoma KB cells and against the hERG channel.[42] As shown in Table 6, **29a** showed no significant effects against the proliferation of KB cell (IC_{50} > 20 μM). This compound also has no cardiac toxicity potential (IC_{50} > 20 μM), indicating its overall safety suitable for further profiling.

Compound 29a Inhibited the Hh Pathway Activity. As shown in Figure 2A, compound **29a** was found to inhibit the Gli-luciferase activity stimulated by ShhN conditioned medium (ShhN CM) in a dose-dependent manner as revealed by the normalized Gli-luciferase activity (the relative firely and renilla luciferase values are separately shown in supplementary Figure S2). Further, we examined the effects of **29a** on the mRNA expression of Gli1 and ptch1, two Gli transcriptional target genes which frequently serve as readout of the Hh signaling pathway activity.[31] As shown in

Figures 2B and 2C, exposure of compound **29a** to NIH-3T3 cells or C3H10T1/2 cells was found to significantly inhibit the expression of Gli1 mRNA and ptch1 stimulated by ShhN CM, confirming that **29a** is indeed a potent inhibitor of the Hh signaling pathway. It is of note that compared to the higher potency of **29a** than **2** in the luciferase reporter assay, the inhibitory effect of **29a** in the Gli transcriptional experiment is somewhat lower than that of **2**. The potency discrepancy likely reflected that the inhibitory activity of **29a** in the latter experiment plateaus with about 30% Gli reporter activity remaining, whereas compound **2** can inhibit Hh pathway activity completely.

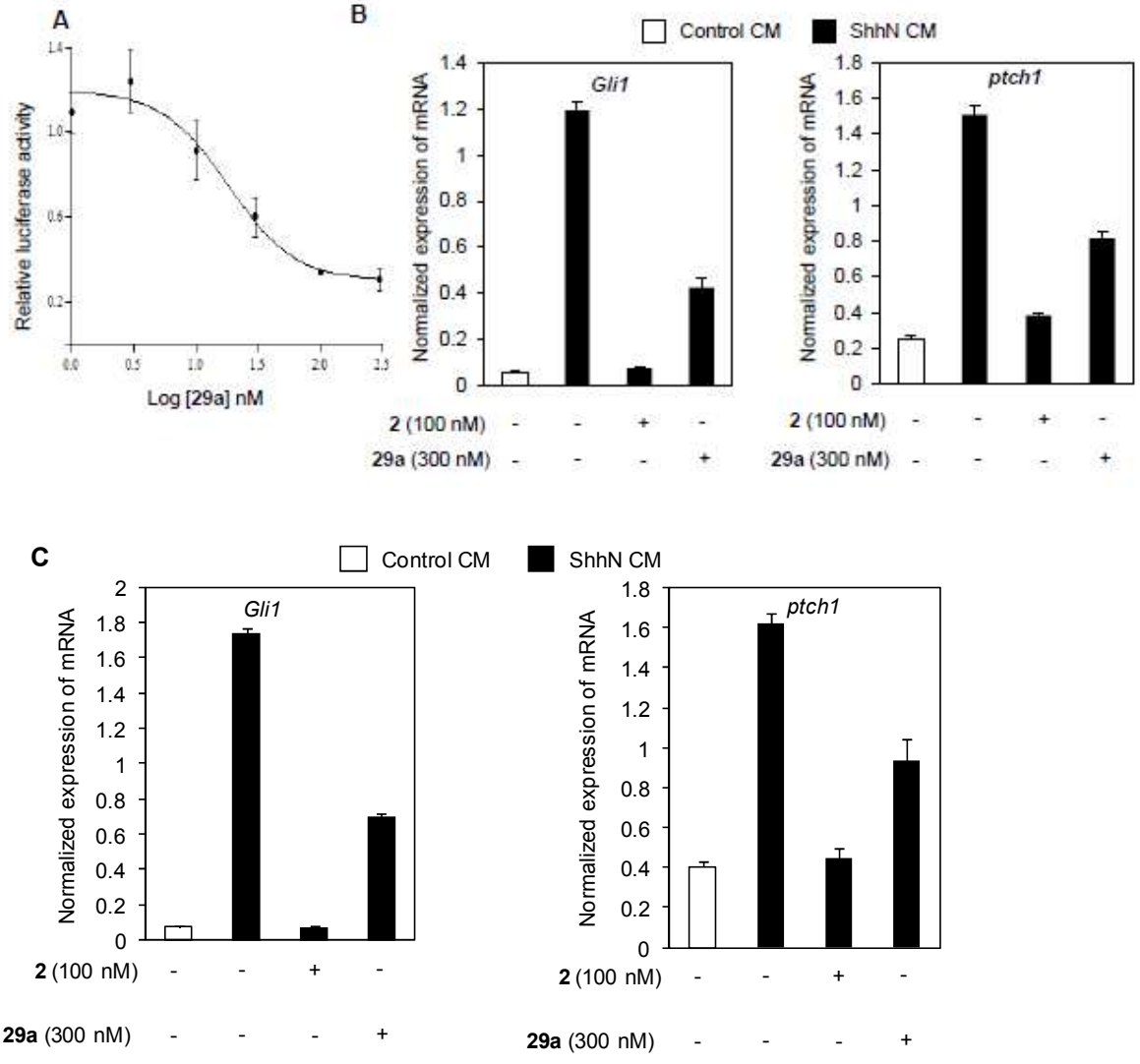


Figure 2. Inhibition of 29a on the Hh activity. A, dose curve of **29a** on the Gli-luciferase activity of light II cells after treated with multiple concentrations of **29a**. **B-C,** representative results of **29a** on the mRNA expression of Gli target

genes Gli1 and ptch1 in NIH-3T3 cells (B) and C3H10T1/2 cells (C). After various treatments as indicated for 36 h, cells were collected for RT-qPCR analysis of the Gli1 and ptch1 mRNA expression. The results were expressed as the mean ± SD.

Compound 29a Selectively Inhibited the Hh Signaling Pathway. To exclude the possibility that **29a** inhibited the Hh pathway activity due to its non-specific off-target effects, we used the dual luciferase reporter assay to examine its effect on two other unrelated transcriptional factors NF-κB in response to TNF-α, and T-cell factor (TCF)/lymphoid enhancer factor (LEF) stimulated by prostaglandin E2 (PGE2).[42,43] As shown in Figure 3A, at the concentration of 1 μM, a concentration completely inhibiting the Hh pathway activity, **29a** had no inhibitory effect on the NF-κB transcriptional activity, whereas its specific inhibitor, (*E*)-3-tosylacrylonitrile (BAY11-7082) significantly suppressed the NF-κB transcriptional activity. Moreover, compound **29a** at the concentration of 1 μM exhibited only minor inhibitory effect on the TCF/LEF activity in response to PGE2 (Figure 3B), whereas its specific inhibitor (*N*-[2-((p-bromocinnamyl)-amino)ethyl]-5-isoquinolinesulfonamide dihydrochloride salt (H89) exhibited significant inhibition. Hence, these data suggested that **29a** selectively inhibited the Hh signaling pathway. The target specificity of **29a** was further investigated by a kinase selectivity profiling against a panel of 468 kinases (including 403 non-mutated kinases) at 1.0 μM using the DiscoveRx screening platform, and **29a** showed no significant inhibition against all these kinases (Figure 3C).

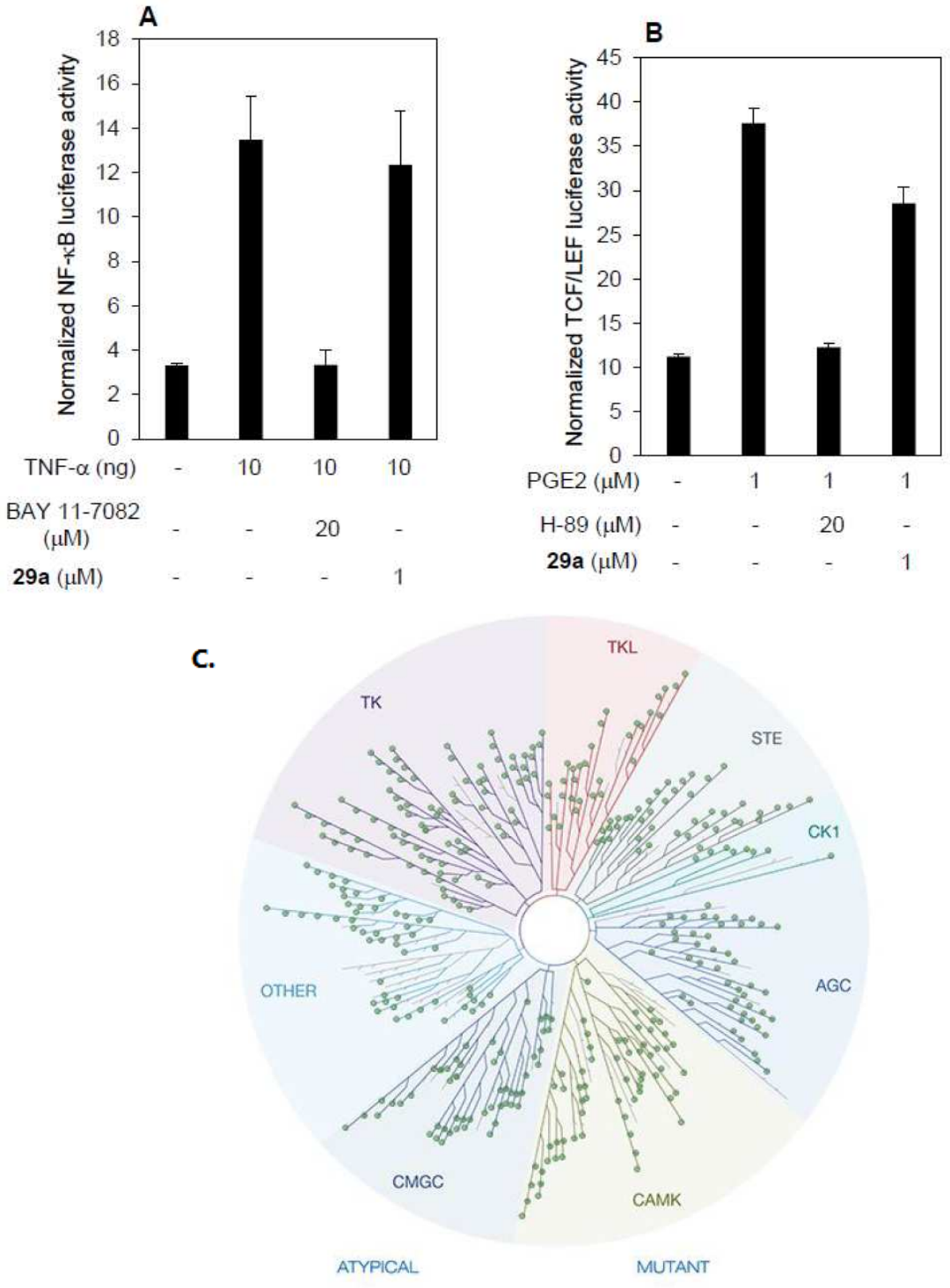
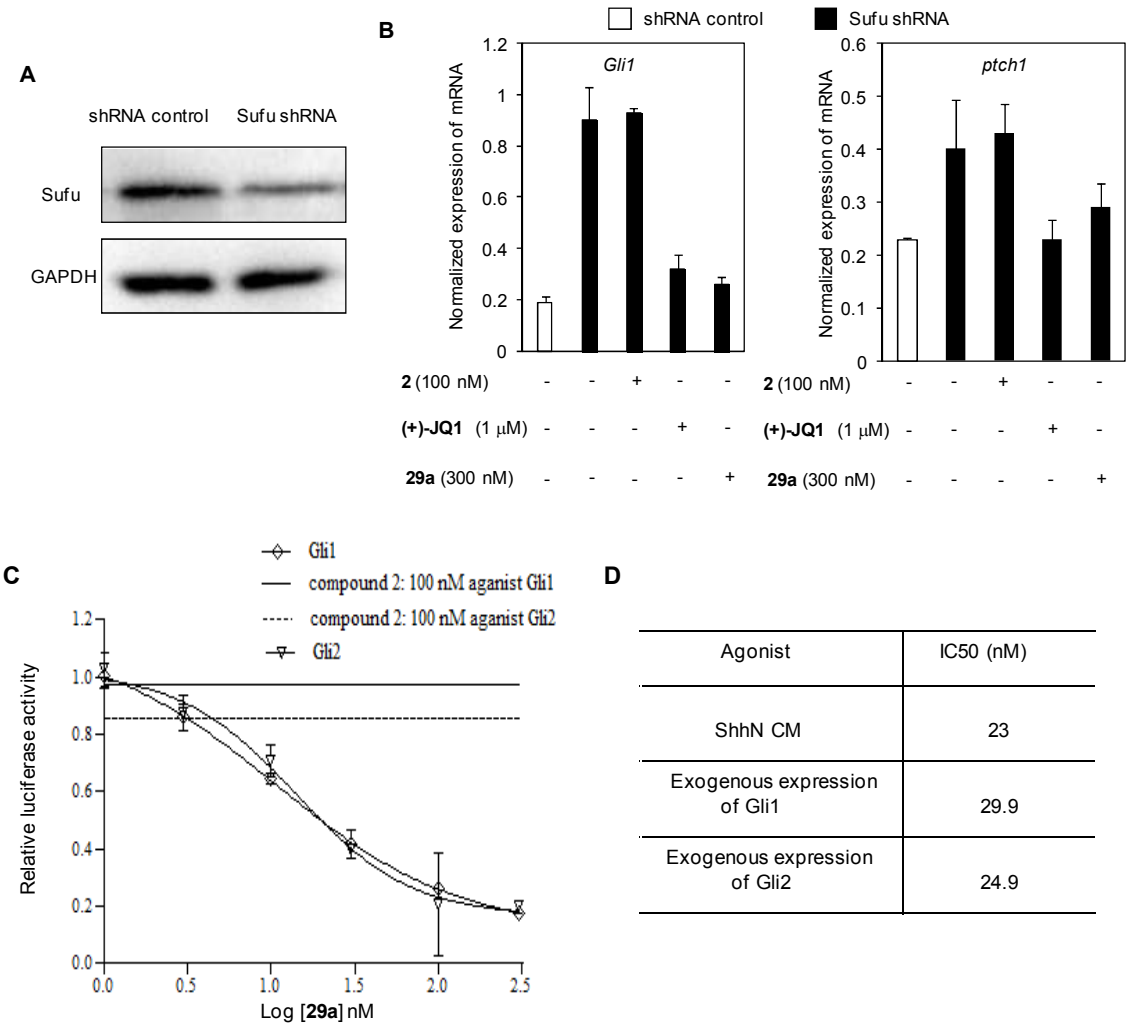


Figure 3. Selective inhibition of 29a on the Hh signaling pathway. A, NF-κB activity in HEK293 cells after various treatments as indicated for 24 h were measured by dual luciferase reporter analysis. **B,** LS174T cells after various treatments as indicated for 6 h were collected for dual luciferase reporter analysis of TCF/LEF activity. The results were

expressed as the mean ± SD. **C,** KinomeScan kinase selectivity profile for **29a** at a concentration of 1.0 μM against a diverse panel of 468 kinases by DiscoveRx.

Compound 29a Inhibited the Hh Signaling Pathway Activity Provoked by Gli1 and Gli2, Respectively. Having well characterized the inhibitory effect of **29a** on Hh signaling pathway, we continued to map its molecular target. Since Sufu negatively controls Hh signaling pathway by binding and sequestering the Gli transcriptional factor in cytoplasm,[44] we then limited the Sufu expression in NIH-3T3 cells by lentivirus harboring Sufu short hairpin RNA (shRNA) (Figure 4A), and determined whether **29a** acts downstream or upstream of Sufu. The reverse transcription and quantitative polymerase chain reaction (RT-qPCR) analysis showed that limiting the Sufu expression abundantly increased the mRNA expression of Gli1 and ptch1 (Figure 4B), whereas treatment with **29a** significantly inhibited the Gli1 and ptch1 mRNA expression (Figure 4B), a phenomenon similar to that of BRD4 inhibitor (+)-JQ1 that inhibits the Hh pathway by epigenetically targeting the transcriptional factor Gli.[27] Meanwhile, the Smo inhibitor **2** failed to reduce the mRNA expression of Gli1 and ptch1 (Figure 4B). To exclude the possible off-target effect, we tested **29a** against BRD4 and no significant inhibition was observed at the concentration of 10 μM (data not shown). These data clearly suggested that **29a** inhibited the Hh signaling pathway by acting downstream of Sufu rather than Smo.



Agonist	IC50 (nM)
ShhN CM	23
Exogenous expression of Gli1	29.9
Exogenous expression of Gli2	24.9

Figure 4. Inhibitory effects of 29a on the Hh signaling pathway. A, western blot analysis of the expression of Sufu in NIH-3T3 cells after infected with lentivirus harboring shRNA control or Sufu shRNA; **B**, effect of **29a** on the expression of Gli target genes Gli1 and ptch1 mRNA expression in the NIH-3T3 cells infected with Sufu shRNA; **C**, effect of **29a** on the Gli luciferase activity initiated by ectopic expression of Gli1 and Gli2 plasmids, respectively. The results were expressed as the mean ± SD; **D**, IC_{50} values of **29a** inhibiting the Gli-luciferase activity provoked by ShhN CM, ectopic expression of Gli1 and Gli2, respectively.

Having characterized **29a** acting downstream of Sufu, we then set out to determine whether **29a** can inhibit the Hh pathway activity initiated by Gli, the last node of the Hh signaling pathway. As Gli1 and Gli2 are mainly responsible for stimulating the Hh pathway activity, we analyzed the effect of **29a** on the Hh pathway activity provoked by forced expression of Gli1 and Gli2 in light II cells,

respectively. As shown in Figure 4C, **29a** significantly suppressed the Gli-luciferase activity stimulated by either ectopic expression of Gli1 or Gli2 in a dose-dependent manner, whereas Smo-targeting inhibitor **2** had no effects (Figure 4C; the relative firely and renilla luciferase values are separately shown in supplementary Figure S3A, B). Meanwhile, the IC_{50} values of **29a** to inhibit the Gli-luciferase activity provoked by artificially expressed Gli1 or Gli2 were similar (23-30 nM) to those of inhibiting the Gli-luciferase activity in response to ShhN CM (Figure 4D). Taken together, these data clearly demonstrated that **29a** potently inhibited the Hh pathway activity provoked by Gli.

Compound 29a had No Influence on the DNA Sequence Binding Ability of Gli, but Decreased the Protein Expression of Gli1 and Gli2. As we have showed that **29a** inhibited the Hh pathway activity provoked by ectopic expression of Gli1 or Gli2, we decide to further clarify its molecular mechanism. First, we tested the effect of **29a** on the binding ability of transcriptional factor Gli1 to the DNA sequence of its transcriptional target genes, one of the most critical steps for initiating the transcriptional activity of Gli. In the electrophoretic mobility shift assay (EMSA) using biotin-labeled Gli-binding sequence in the promoter of Gli target gene twist1,[45] compound **29a** exhibited no inhibitory effect on the DNA binding ability of Gli1, at the concentration of 1 uM that completely suppressed the Hh activity in response to ShhN CM (Figure 5A; line 6 compared to line 2). However, different concentrations of non-labeled Gli binding sequence completely inhibited the Gli1 binding to the DNA sequence (Figure 5A; lanes 4 and 5 compared to lane 2, respectively). These results suggested that the inhibitory effect of **29a** on the Hh pathway activity was not caused by interruption of the binding of Gli to the DNA sequence of its target genes.

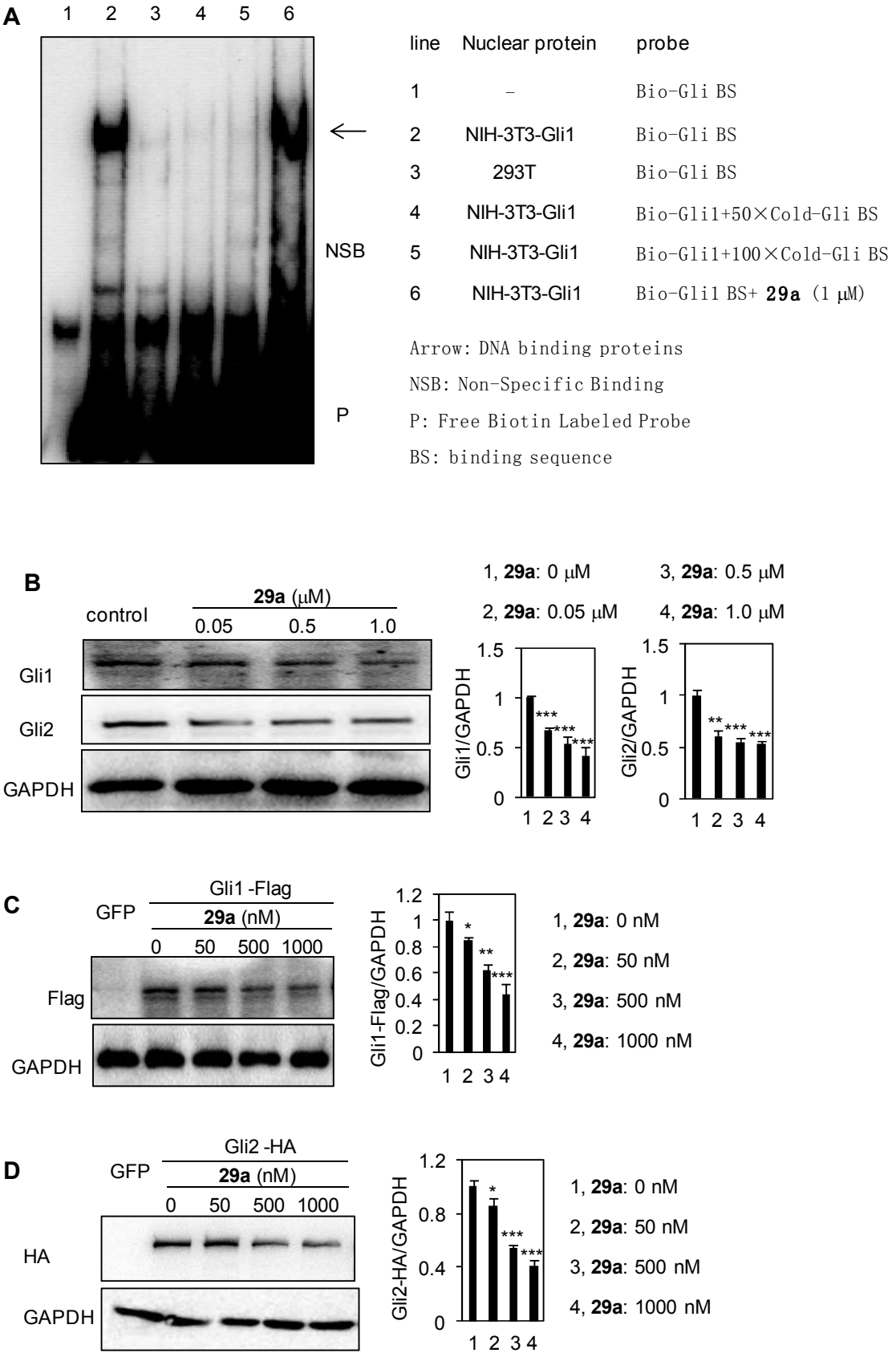

A
1 2 3 4 5 6
NSB
P
line Nuclear protein probe
1 – Bio-Gli BS
2 NIH-3T3-Gli1 Bio-Gli BS
3 293T Bio-Gli BS
4 NIH-3T3-Gli1 Bio-Gli1+50×Cold-Gli BS
5 NIH-3T3-Gli1 Bio-Gli1+100×Cold-Gli BS
6 NIH-3T3-Gli1 Bio-Gli1 BS+ 29a (1 μM)
Arrow: DNA binding proteins
NSB: Non-Specific Binding
P: Free Biotin Labeled Probe
BS: binding sequence
B
29a (μM)
control 0.05 0.5 1.0
Gli1
Gli2
GAPDH
1, 29a: 0 μM
2, 29a: 0.05 μM
3, 29a: 0.5 μM
4, 29a: 1.0 μM
Gli1/GAPDH
Gli2/GAPDH
C
GFP
Gli1 -Flag
29a (nM)
0 50 500 1000
Flag
GAPDH
Gli1-Flag/GAPDH
1, 29a: 0 nM
2, 29a: 50 nM
3, 29a: 500 nM
4, 29a: 1000 nM
D
GFP
Gli2 -HA
29a (nM)
0 50 500 1000
HA
GAPDH
Gli2-HA/GAPDH
1, 29a: 0 nM
2, 29a: 50 nM
3, 29a: 500 nM
4, 29a: 1000 nM

Figure 5. Effects of 29a on the DNA sequence binding ability of Gli, and the protein expression of Gli1 and Gli2. A, effect of **29a** on the DNA binding ability of Gli1 examined by EMSA; **B-D**, Representative immunoblots and semi-quantitative analysis of the effect of **29a** on the expression of endogenous Gli1 and Gli2 proteins (**B**) and artificially expressed Gli1 (**C**) and Gli2 (**D**) in the NIH-3T3 cells. Statistical analysis using Student's T-test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Cells after subjected to **29a** at indicated concentrations for 24 h were collected for western blot analysis.

Further, we examined whether **29a** influences the expression of Gli proteins. As shown in Figure 5B, exposure of NIH-3T3 cells to different concentrations of **29a** dose-dependently suppressed the endogenous expression of Gli1 and Gli2. It should be noted that the endogenous Gli1 expression in NIH-3T3 cells might be caused by basal activity of both canonical and non-canonical Hh pathway activity as the cells were cultured[5,46] with 10% FBS (fetal bovine serum) when exposed to **29a**. Moreover, using the antibodies targeting the Tag of respective plasmids, we found that **29a** also reduced the ectopic expression of Gli1 (Figure 5C) and Gli2 (Figure 5D) in NIH-3T3 cells. Collectively, these observations indicated that **29a** inhibited the Hh pathway activity by a mechanism not involving disruption of DNA binding, but likely involving reduction of the Gli proteins expression, including Gli1 and Gli2.

Compound 29a Significantly Suppressed the Hh Pathway Activity Provoked by Smo Mutants Which Cause Resistance to Current Smo Inhibitors. Smo mutations in its ligand binding pocket or in pivotal residues that ensuring Smo receptor autoinhibition are the predominant molecular mechanisms, responsible for the resistance to current Smo inhibitors.[23] Having profiled that compound **29a** inhibited the Hh signaling pathway by reducing the expression of its final effectors Glis, we envisioned that compound **29a** may inhibit the Hh pathway activity provoked by Smo mutants, especially Smo D473H and Smo W535L, two key Smo resistant mutations observed clinically.[23] To this aim, we separately transfected three distinct Smo plasmids, including Smo wild type and the two Smo mutants D473H and W535L into light II cells and analyzed the effect of

compound **29a** on the Hh pathway activity provoked by these ectopic plasmids. As shown in Figure 6A-C (the relative firely and renilla luciferase values are separately shown in supplementary Figure S4A-C), compound **29a** robustly inhibited the Gli-luciferase activity provoked by all these three distinct Smo plasmids in a dose-dependent manner. Meanwhile, the IC_{50} values revealed that compound **29a** exhibited statistically similar potency in inhibiting the Gli-luciferase activity provoked by Smo wild type (35.72 nM), Smo D473H (37.46 nM), and Smo W535L (39.81 nM), respectively (Figure 6D). Hence, these data supported the potential value of compound **29a** to combat the resistance to current Smo inhibitors caused by distinct Smo mutations.

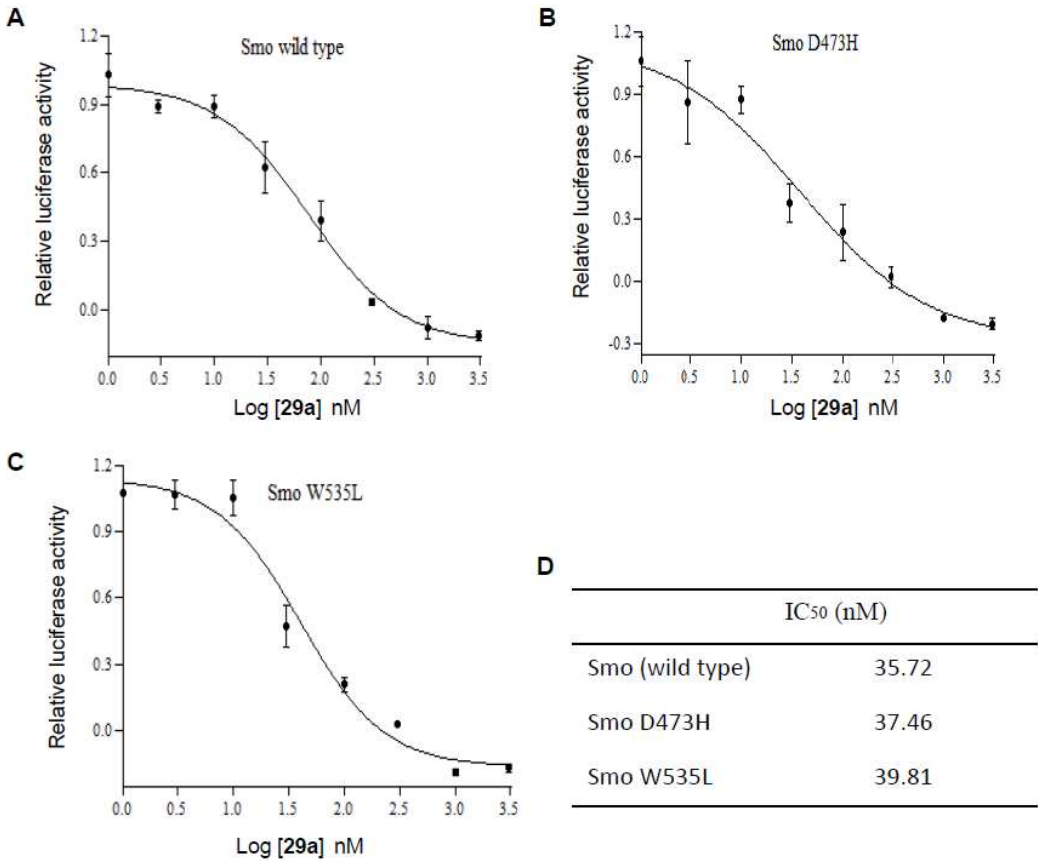


Figure 6. Suppression of 29a on the Hh pathway activity provoked by Smo wild type and mutants. A, effect of **29a** on the Gli luciferase activity initiated by ectopic expression of wild type Smo plasmid. **B**, effect of **29a** on the Gli luciferase activity initiated by ectopic expression of Smo D473H plasmid. **C**, effect of **29a** on the Gli luciferase activity initiated by ectopic expression of Smo W535L plasmid. **D**, IC_{50} values of **29a** inhibiting the Gli-luciferase activity provoked by Smo wild type, Smo D473H, and Smo W535L plasmids, respectively. Light II cells transfected with Smo wild type, SmoW539L or SmoD473H plasmids were exposed to **29a** for 36 h, and then were harvested for dual luciferase

reporter assays. The results were expressed as the mean ± SD.

Compound 29a Inhibited the Proliferation of Medulloblastoma Cells Isolated from ptch+/-;p53-/- Mice. Since the growth of medulloblastoma from ptch+/-;p53-/- mice is driven by the constitutive Hh activity caused by loss of one allele of ptch,[47] we isolated medulloblastoma cells from ptch+/-;p53-/- mice and determined the inhibitory effect of **29a** on the proliferation of these tumor cells. As shown in Figure 7A, **29a** dose-dependently inhibited the proliferation of ptch+/-;p53-/-medulloblastoma cells in a fashion similar to that of **2**. Meanwhile, we observed that the proliferative inhibition of medulloblastoma cells by **29a** was accompanied by suppression of Hh pathway activity, as judged by the reduction of Gli1 and ptch1 mRNA expression (Figure 7B). It is of note that both compounds exhibited weaker effects on the proliferation and Hh pathway activity of ptch+/-;p53-/- medulloblastoma cells (Figure 7A, B) compared to their potencies against the Hh pathway in light II cells and NIH-3T3 cells (Figure 2A, B). This discrepancy is likely due to the partial loss of Hh activity in medulloblastoma cells when cultured *in vitro*.[48] In addition, we also compared compound **29a** with the prototypic compound **5**, which was reported to have little inhibitory effect on the Hh activity in ptch+/- cells. Consistent with the literature report,[37] we found that **5** lacked significant effects against the Hh pathway of ptch+/-;p53 medulloblastoma cells, as revealed by no influence on both Gli1 and ptch1 mRNA expressions (Figure 7C). This observation implicated that our tricyclic compounds are not only more potent than the "parental" bicyclic inhibitor, but also act through a different mechanism of action.

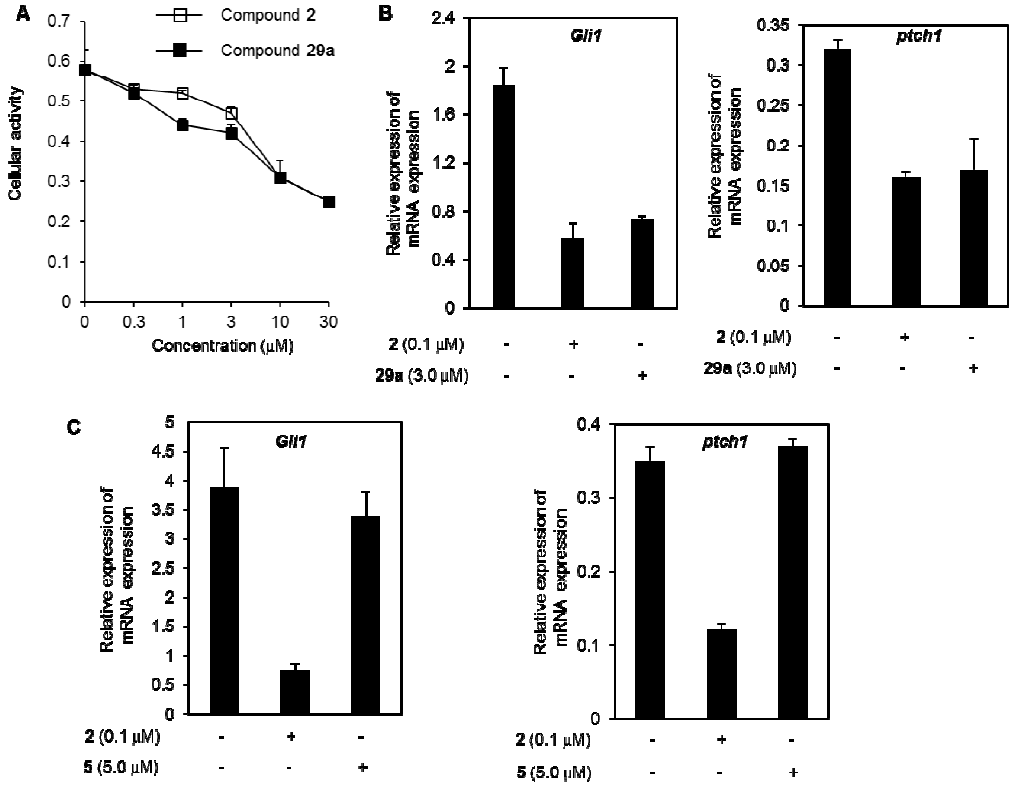


Figure 7. Effects of 29a on proliferation of ptch+/-;p53-/- medulloblastoma cells. A, effect of **29a** on the proliferation of ptch+/-;p53-/- medulloblastoma cells. **B**, effect of **29a** on the expression of Gli1 and ptch1 at mRNA level in ptch+/-;p53-/- medulloblastoma cells. **C**, effect of **5** on on the expression of Gli1 and ptch1 at mRNA level in ptch+/-;p53-/- medulloblastoma cells. The results were expressed as the mean ± SD.

Compound 29a Significantly Inhibited the Tumor Growth in the ptch+/-;p53-/- Medulloblastoma Allograft Mice Model. To test whether the good *in vitro* potency of the new Gli inhibitor **29a** can be translated into *in vivo* antitumor efficacy, we subcutaneously allografted a primary medulloblastoma from a ptch+/-;p53-/- mouse into nude mice and examined the tumor growth inhibition (TGI) of **29a** in this model. Compound **29a** was administered via intraperitoneal injection (ip) at a dose of 25 or 50 mg/kg twice a day for 15 consecutive days. Compared to the vehicle control, compound **29a** was found to significantly inhibit the growth of ptch+/−;p53−/− mededulloblastoma at either dose (Figure 8A) with TGI of 57.5% and 73.4%, respectively. No substantial body weight loss was observed at both doses (Figure 8B). Meanwhile, the growth

inhibition of medulloblastoma by compound **29a** paralleled the repression of Hh pathway activity, as reflected by the similar reduction of the mRNA expression of Gli1 (Figure 8C) and ptch1 (Figure 8D). Given that Hh signaling pathway can prevent cellular apoptosis via transcriptionally controlling the expression of Bcl-2,[48] we further determined whether administration of **29a** induces apoptosis of medulloblasotma cells using terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling (TUNEL) assay. As illustrated in Figure 8E, administration of **29a** robustly induced apoptosis of medulloblastoma cells *in vivo*, similar to the effect of compound **2**.

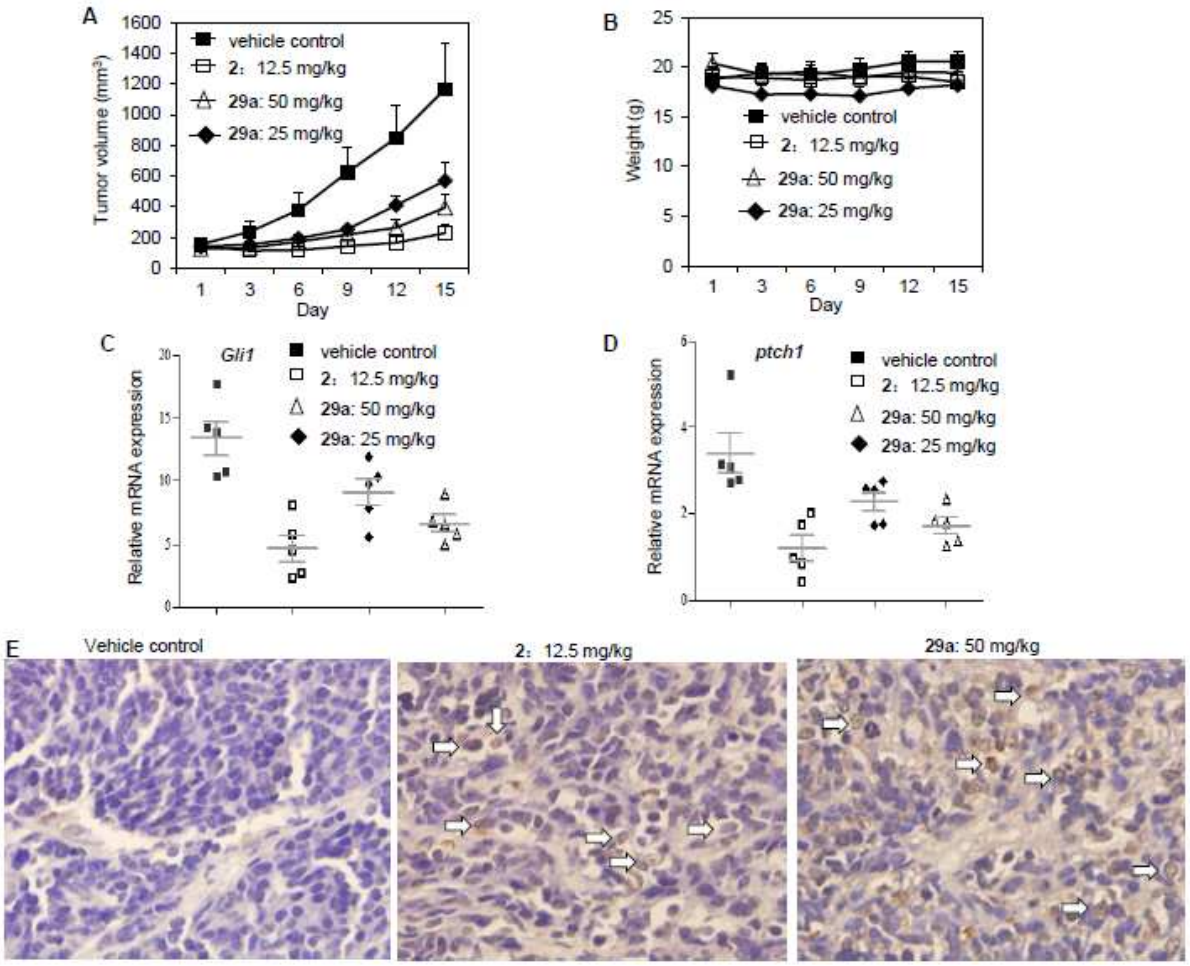


Figure 8. Inhibitory effects of 29a on the tumor growth in the ptch+/-;p53-/- medulloblastoma allograft mice model. Mice (n = 5) allografted with medulloblastoma were administered **2** or **29a** twice a day for 15 days. **A,** effect of **29a** on the growth of ptch+/-;p53-/- medulloblastoma. The results were expressed as the mean ± SEM. **B,** Effect of **29a**

on the body weight of mice. The results were expressed as the mean ± SD. **C-D**, effect of **29a** on the Gli1 and ptch1 mRNA expression of medulloblastoma. After 4 h of last dosage, tumor samples were collected for examining the expression of Gli1and ptch1 mRNA. The results were expressed as the mean ± SD. **E**, effect of **29a** on apoptosis of medulloblastoma cells (white arrows: apoptotic cells; 60×)

CONCLUSIONS

In summary, the membrane protein Smo, a central effector of the Hh signaling pathway, has been well studied as a drug target in recent years and two first-generation of Smo-targeting Hh pathway inhibitors have been approved for clinical use to treat locally advanced or metastatic BCC. However, the primary and acquired resistance to these inhibitors limited their broad application and thereby highlighted the emergent need to develop novel next-generation Hh inhibitors either with distinct chemical structures or with alternative mechanisms of action, particularly those acting downstream of Smo. Based on a hit compound 4,5-dihydro-2*H*-benzo[*b*][1,5]oxazocin-6(3*H*)-one **5** reported by the Schreiber group recently, we conducted a systemic structural optimization by cyclization of two side substituents of the bicyclic skeleton combined with *N*-atom walking or ring-walking and the central ring expansion or extraction approaches. Five series of structurally unique tricyclic compounds were prepared and evaluated. Compound **29a** was identified as the most potent compound against the Hh signaling pathway with an IC_{50} value of 23 nM, which is up to 17-fold more potent than the prototypic compound **5** and nearly 2-fold more potent than the marketed first-generation Hh inhibitor **2**. Mechanism study indicated that **29a** selectively inhibited Hh signaling pathway and was inactive against a panel of 468 kinases and other transcriptional factors. This compound was effective against both Smo wild type and the two major mutants (Smo D473H and Smo W535L). Further, **29a** potently inhibited the proliferation of medulloblastoma cells isolated

from ptch+/-;p53-/- mice and showed significant tumor growth inhibition in the ptch+/-;p53-/- medulloblastoma allograft mice model. Based on the mode of action, compound **29a** might be classified as an indirect Gli antagonist functioning by suppressing its transcriptional activity, rather than by interrupting with its binding to DNA.[29,30] The precise mechanism of how compound **29a** down-regulates Gli expression is currently being further investigated.

EXPERIMENTAL SECTION

Chemistry. All reactions were performed in glassware containing a Tefloncoated stir bar. Solvents and chemical reagents were obtained from commercial sources and used without further purification. ^{1}H NMR spectral data was recorded in $CDCl_3$ or $CDCl_3$ + CD_3OD on Varian Mercury 300, 400 or 500 NMR spectrometer and ^{13}C NMR was recorded in $CDCl_3$ or $CDCl_3$ + CD_3OD on Varian Mercury 500 or 600 NMR spectrometer. Chemical shifts (δ) were reported in ppm downfield from an internal TMS standard. Low and high-resolution mass spectra were obtained in the ESI mode. Flash column chromatography on silica gel (200-300 mesh) was used for the routine purification of reaction products. The column output was monitored by TLC on silica gel (200-300 mesh) precoated on glass plates (15 x 50 mm), and spots were visualized by UV light at 254 or 365 nM. HSQC were used in the structural assignment. Compounds **7**[38] and **25**[38] were prepared according to corresponding literature procedures. HPLC analysis was conducted for all bioassayed compounds on an Agilent Technologies 1260 series LC system (Agilent ChemStation Rev.A.10.02; ZORBAX-C18, 4.6 mm × 150 mm, 5 μM, MeOH (0.1% DEA)/H_2O, rt) with two ultraviolet wavelengths (uv 254 and 210 nM). All the assayed compounds displayed a chemical purity of 95%-99% in both wavelengths.

*General Procedure for Synthesis of Intermediates **15a-e** and **17**.* To a solution of compound **7** (263 mg, 1.0 mmol) in EtOH (5 mL) and H_2O (3 mL) was added lithium hydroxide monohydrate (84 mg, 2.0 mmol). The reaction was stirred for 2 h at rt. The mixture was adjusted to pH 5 by adding

hydrochloride acid (0.5 M) and then concentrated to give the acid intermediate as pale oil. Without further purification, the acid intermediate was dissolved in DMF (10 mL), HATU (760 mg, 2.0 mmol) and HOAT (136 mg, 1.0 mmol) were added. The mixture was stirred at rt for 20 min and then the appropriate amine (1.2 mmol) and DIPEA (387 mg, 3.0 mmol) were added. The reaction was stirred at rt for 12 h and then treated with saturated $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layer was washed with water, dried over Na_2SO_4, filtered, and concentrated in vacuo to give **8a-e** as colorless oil. The crude products were used without further purification.

To a solution of **8a-e** (0.5 mmol) in CH_2Cl_2 (5 mL) at 0 °C was slowly added TBSOTf (264 mg, 1.0 mmol). The reaction was stirred for another 1 h at 0 °C and then treated with saturated $NaHCO_3$ (10 mL). The mixture was extracted with CH_2Cl_2 (3 × 10 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica column chromatography (petroleum ether/EtOAc) to give corresponding compound **9a-e** as pale oil.

*(±)-cis-1-Benzyl-3-(tert-butyldimethylsilyloxy)-N-((R)-1-(4-methoxybenzyloxy)propan-2-yl)piperidine-4-carboxamide (**9a**)*. Yield 244 mg, 93%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.19 (m, 7H), 6.92 – 6.83 (m, 2H), 4.53 – 4.40 (m, 2H), 4.34 – 4.20 (m, 1H), 4.12 (dt, J = 8.4, 4.2 Hz, 1H), 3.82 and 3.81 (s, 3H), 3.56 – 3.31 (m, 4H), 2.63 – 2.04 (m, 6H), 1.67 – 1.53 (m, 1H), 1.20 and 1.18 (s, 3H), 0.90 and 0.88 (s, 9H), 0.08 – 0.03 (m, 6H).

*(±)-cis-1-Benzyl-3-(tert-butyldimethylsilyloxy)-N-(2-(4-methoxybenzyloxy)ethyl)piperidine-4-carboxamide (**9b**)*. Yield 221 mg, 86%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 – 7.09 (m, 7H), 6.87 (d, J = 8.4 Hz, 2H), 4.44 (d, J = 2.7 Hz, 2H), 4.14 – 4.04 (m, 1H), 3.80 (s, 3H), 3.60 – 3.46 (m, 4H), 3.41 – 3.35 (m, 1H), 2.49 – 2.29 (m, 6H), 1.87 – 1.85 (m, 1H), 1.62 – 1.55 (m, 1H), 0.86 (s, 9H), 0.02 (d, J = 10.3 Hz, 6H).

*(±)-cis-1-Benzyl-3-(tert-butyldimethylsilyloxy)-N-(1-methylpiperidin-4-yl)piperidine-4-carboxamide (**9c**)*. Yield 202 mg, 91%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 – 7.23 (m, 5H), 4.08 (dt, J = 8.7,

4.2 Hz, 1H), 3.85 – 3.75 (m, 1H), 3.47 (d, *J* = 2.4 Hz, 2H), 2.77 (d, *J* = 11.7 Hz, 2H), 2.61 – 2.47 (m, 3H), 2.39 – 2.33 (m, 1H), 2.27 – 2.17 (m, 4H), 2.12 – 2.05 (m, 2H), 1.92 – 1.84 (m, 3H), 1.62 – 1.37 (m, 3H), 0.88 (s, 9H), 0.05 (d, *J* = 10.6 Hz, 6H).

(±)-cis-1-Benzyl-3-(tert-butyldimethylsilyloxy)-N-(pyridin-3-ylmethyl)piperidine-4-carboxamide ***(9d)***. Yield 187 mg, 85%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.59 – 8.56 (m, 2H), 7.72 – 7.64 (m, 2H), 7.38 – 7.27 (m, 6H), 4.57 (dd, *J* = 14.7, 5.7 Hz, 1H), 4.43 (dd, *J* = 14.7, 5.7 Hz, 1H), 4.15 (dt, *J* = 8.5, 4.2 Hz, 1H), 3.53 (d, *J* = 2.4 Hz, 2H), 2.65 – 2.31 (m, 6H), 1.73 – 1.63 (m, 1H), 0.82 (s, 9H), 0.03 (d, *J* = 10.4 Hz, 6H).

(±)-cis-1-Benzyl-3-(tert-butyldimethylsilyloxy)-N-(4-methoxybenzyl)piperidine-4-carboxamide ***(9e)***. Yield 211 mg, 90%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 – 7.22 (m, 8H), 6.89 (d, *J* = 8.4 Hz, 2H).4.48 (dd, *J* = 14.4, 5.7 Hz, 1H), 4.34 (dd, *J* = 14.4, 5.4 Hz, 1H), 4.17 – 4.11 (m, 1H), 3.84 (s, 3H), 3.52 (s, 2H), 2.61 – 2.37 (m, 6H), 1.70 – 1.57 (m, 1H), 0.80 (s, 9H), 0.02 (d, *J* = 10.4 Hz, 6H).

Compounds **9a-e** (0.5 mmol) was dissolved in THF (5 mL) under N_2 and borane dimethylsulfide complex (BH_3.DMS) (1.25 mL, 2.5 mmol) was added dropwise. The reaction was stirred at 65 °C for 6 h and then cooled to room temperature. The mixture was quenched by carefully adding MeOH and concentrated under reduced pressure to remove excess $B(OMe)_3$, and then co-evaporated with MeOH three times. The resulting oil was re-dissolved in a solution of MeOH (4 mL) and 10% aqueous potassium sodium tartrate (6 mL) and heated at reflux for 18 h. The product was concentrated in vacuo and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give **10a-e** as colorless oil. The crude products were used without further purification.

To a solution of 2-fluoro-3-nitrobenzoic acid (111 mg, 0.6 mmol) in CH_2Cl_2 (5 mL) was added HATU (380 mg, 1.0 mmol) and HOAT (78 mg, 0.5 mmol). The mixture was stirred at rt for 20 min, then the crude amines **10a-e** (0.5 mmol) and DIPEA (194 mg, 1.5 mmol) was added. The reaction was stirred at rt for 6 h and then treated with saturated $NaHCO_3$ (10 mL) and was extracted with

CH_2Cl_2 (3 × 10 mL). The combined organic layer was washed with water, dried over Na_2SO_4, filtered, and concentrated in vacuo to give corresponding amides **11a-e** as pale oil.

Without purification, the crude products **11a-e** was dissolved in DMF (5 mL) and CsF (228 mg, 1.5 mmol) was added. The reaction was stirred at 85 °C for 4 h. After complete consumption of starting material, the reaction was diluted with water (20 mL) and extracted with EtOAc (3 × 10 mL), the organic layer was washed with water three times, dried with Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica column chromatography (petroleum ether/EtOAc) to give the corresponding compounds **12a-e**.

(±)-cis-2-Benzyl-6-((R)-1-(4-methoxybenzyloxy)propan-2-yl)-11-nitro-2,3,4,4a,5,6-hexahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-7(12aH)-one ***(12a)***. White foam (121 mg, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 – 7.66 (m, 1H), 7.59 – 7.56 (m, 1H), 7.37 – 7.30 (m, 4H), 7.23 – 7.18 (m, 3H), 6.97 – 6.92 (m, 1H), 6.88 – 6.81 (m, 2H), 4.88 (q, J = 6.8 Hz, 0.5H), 4.56 (s, 1H), 4.50 (d, J = 11.2 Hz, 0.5H), 4.42 (q, J = 5.6 Hz, 1H), 4.36 (d, J = 11.2 Hz, 0.5H), 4.13 – 4.07 (m, 1H), 3.79 (s, 3H), 3.63 – 3.57 (m, 2H), 3.48 (d, J = 5.2 Hz, 0.5H), 3.35 (d, J = 13.2 Hz, 0.5H), 3.30 – 3.22 (m, 2H), 3.09 (dd, J = 15.2, 4.8 Hz, 0.5H), 3.00 (dd, J = 12.6, 2.8 Hz, 0.5H), 2.95 – 2.88 (m, 1.5H), 2.03 – 1.91 (m, 2.5H), 1.82 (d, J = 13.2 Hz, 0.5H), 1.77 – 1.66 (m, 1H), 1.52 (d, J = 12.4 Hz, 0.5H), 1.38 – 1.34 (m, 2H), 1.23 (d, J = 7.2 Hz, 1.5H).

(±)-cis-2-Benzyl-6-(2-(4-methoxybenzyloxy)ethyl)-11-nitro-2,3,4,4a,5,6-hexahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-7(12aH)-one ***(12b)***. White foam (124 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (dd, J = 8.0, 2.0 Hz, 1H), 7.61 (dd, J = 8.0, 2.0 Hz, 1H), 7.40 – 7.31 (m, 4H), 7.24 – 7.19 (m, 3H), 6.98 (t, J = 8.0 Hz, 1H), 6.85 (d, J = 8.8 Hz, 2H), 4.48 – 4.40 (m, 3H), 4.01 – 3.95 (m, 1H), 3.80 – 3.76 (m, 4H), 3.69 – 3.63 (m, 2H), 3.45 – 3.36 (m, 3H), 3.28 (dd, J = 15.2, 4.8 Hz, 1H), 3.11 (d, J = 12.4 Hz, 1H), 2.97 (d, J = 11.2 Hz, 1H), 2.08 – 1.93 (m, 3H), 1.83 – 1.73 (m, 1H), 1.41 (d, J = 13.2 Hz, 1H).

(±)-cis-2-Benzyl-6-(1-methylpiperidin-4-yl)-11-nitro-2,3,4,4a,5,6-hexahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-7(12aH)-one ***(12c)***. White foam (106 mg, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (dd, J = 8.0, 1.8 Hz, 1H), 7.62 (dd, J = 8.0, 1.8 Hz, 1H), 7.42 – 7.33 (m, 4H), 7.28 – 7.24 (m, 1H), 6.99 (t, J = 8.0 Hz, 1H), 4.60 – 4.49 (m, 2H), 3.70 (d, J = 13.6 Hz, 1H), 3.45 (d, J = 13.6 Hz, 1H), 3.36 – 3.27 (m, 2H), 3.21 – 3.17 (m, 1H), 3.03 – 3.00 (m, 3H), 2.37 (s, 3H), 2.29 – 2.00 (m, 5H), 1.98 – 1.76 (m, 4H), 1.72 – 1.68 (m, 1H), 1.47 – 1.44 (m, 1H).

(±)-cis-2-Benzyl-11-nitro-6-(pyridin-3-ylmethyl)-2,3,4,4a,5,6-hexahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-7(12aH)-one ***(12d)***. White foam (109 mg, 68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.58 – 8.56 (m, 2H), 7.79 (dd, J = 8.1, 1.8 Hz, 1H), 7.71 – 7.63 (m, 2H), 7.37 – 7.18 (m, 6H), 7.02 (t, J = 8.1 Hz, 1H), 5.34 (d, J = 14.7 Hz, 1H), 4.45 (s, 1H), 4.19 (d, J = 14.7 Hz, 1H), 3.68 (d, J = 13.5 Hz, 1H), 3.46 – 3.38 (m, 2H), 3.21 – 2.97 (m, 3H), 2.14 – 2.04 (m, 2H), 1.84 – 1.77 (m, 2H), 1.43 – 1.40 (m, 1H).

(±)-cis-2-Benzyl-6-(4-methoxybenzyl)-11-nitro-2,3,4,4a,5,6-hexahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-7(12aH)-one ***(12e)***. White foam (132 mg, 91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (dd, J = 7.8, 1.8 Hz, 1H), 7.57 (dd, J = 7.8, 1.8 Hz, 1H), 7.31 – 7.25 (m, 3H), 7.20 – 7.15 (m, 4H), 6.95 (t, J = 7.8 Hz, 1H), 6.81 (d, J = 8.4 Hz, 2H), 5.12 (d, J = 14.4 Hz, 1H), 4.39 (s, 1H), 4.16 (d, J = 14.4 Hz, 1H), 3.75 (s, 3H), 3.60 (d, J = 13.5 Hz, 1H), 3.38 – 3.22 (m, 2H), 3.13 – 3.00 (m, 2H), 2.90 (d, J = 11.1 Hz, 1H), 2.04 – 1.94 (m, 2H), 1.74 – 1.67 (m, 2H), 1.37 – 1.23 (m, 1H).

To a solution of **12a-e** (0.5 mmol) in EtOH (5 mL) and H_2O (2 mL) was added Fe powder (196 mg, 3.5 mmol) and NH_4Cl (259 mg, 5.0 mmol). The reaction mixture was heated under reflux for 1 h and then filtered over a pad of celite and concentrated in vacuo. The residue was diluted with water (20 mL) and extracted with CH_2Cl_2 (3 × 10 mL), and the combined organic layer was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give compounds **13a-e** as pale-yellow solid.

To a solution of **13a-e** (0.5 mmol) and 2,6-lutidine (107 mg, 1.0 mmol) in CH_2Cl_2 (5 mL) at

0 °C was slowly added cyclopropanecarbonyl chloride or cyclohexanecarbonyl chloride (0.6 mmol). The reaction was stirred for 4 h at 0 °C and then treated with saturated $NaHCO_3$ (15 mL). The mixture was extracted with CH_2Cl_2 (3 × 10 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo to give the corresponding compounds **14a-e** or **16**. Compound **14a** was used for the next step without purification. **14b-e** and **16** were purified by silica column chromatography (CH_2Cl_2/EtOAc).

(±)-N-(cis-2-Benzyl-6-(2-(4-methoxybenzyloxy)ethyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide ***(14b)***. White solid (264 mg, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (s, 1H), 8.49 (d, J = 8.0 Hz, 1H), 7.38 – 7.26 (m, 5H), 7.25 – 7.21 (m, 3H), 6.95 (t, J = 8.0 Hz, 1H), 6.88 (d, J = 8.4 Hz, 2H), 4.49 – 4.43 (m, 3H), 4.02 – 3.97 (m, 1H), 3.83 – 3.81 (m, 4H), 3.71 – 3.66 (m, 1H), 3.60 (d, J = 13.2 Hz, 1H), 3.53 – 3.38 (m, 3H), 3.29 – 3.18 (m, 2H), 3.04 (d, J = 11.2 Hz, 1H), 2.08 – 1.99 (m, 3H), 1.77 – 1.66 (m, 2H), 1.47 (d, J = 15.2 Hz, 1H), 1.16 – 1.12 (m, 2H), 0.92 – 0.86 (m, 2H).

(±)-N-(cis-2-Benzyl-6-(1-methylpiperidin-4-yl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide ***(14c)***. White solid (220 mg, 88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.75 (s, 1H), 8.45 (d, J = 8.1 Hz, 1H), 7.43 – 7.25 (m, 5H), 7.14 (d, J = 8.1 Hz, 1H), 6.92 (t, J = 8.1 Hz, 1H), 4.64 – 4.55 (m, 2H), 3.68 – 3.53 (m, 2H), 3.31 – 3.25 (m, 3H), 3.07 – 3.03 (m, 3H), 2.39 (s, 3H), 2.32 – 2.20 (m, 3H), 2.13 – 1.81 (m, 5H), 1.73 – 1.66 (m, 3H), 1.52 – 1.42 (m, 1H), 1.13 – 1.09 (m, 2H), 0.90 – 0.79 (m, 2H).

(±)-N-(cis-2-Benzyl-7-oxo-6-(pyridin-3-ylmethyl)-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide ***(14d)***. White solid (233 mg, 94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.65 (s, 1H), 8.56 – 8.49 (m, 3H), 7.70 (d, J = 7.8 Hz, 1H), 7.38 – 7.18 (m, 7H), 6.96 (t, J = 7.8 Hz, 1H), 5.36 (d, J = 14.7 Hz, 1H), 4.48 (s, 1H), 4.17 (d, J = 14.7 Hz, 1H), 3.66 – 3.51 (m, 2H), 3.40 (dd, J = 14.7, 11.7 Hz, 1H), 3.31 – 3.25 (m, 1H), 3.09 – 3.01 (m, 2H), 2.19 – 2.15 (m, 1H), 2.10 – 2.03 (m, 1H), 1.85 – 1.63 (m, 3H), 1.50 – 1.43 (m, 1H), 1.15 – 1.10 (m, 2H), 0.90 – 0.83 (m, 2H).

*(±)-N-(cis-2-Benzyl-6-(4-methoxybenzyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide **(14e)***. White solid (239 mg, 91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.60 (s, 1H), 8.49 (d, J = 8.1 Hz, 1H), 7.38 – 7.20 (m, 8H), 6.95 (t, J = 8.1 Hz, 1H), 6.86 (d, J = 8.4 Hz, 2H), 5.21 (d, J = 14.5 Hz, 1H), 4.49 (s, 1H), 4.19 (d, J = 14.5 Hz, 1H), 3.80 (s, 3H), 3.62 – 3.50 (m, 2H), 3.35 – 3.23 (m, 2H), 3.07 – 3.00 (m, 2H), 2.13 (d, J = 12.3 Hz, 1H), 2.06 – 1.98 (m, 1H), 1.77 – 1.59 (m, 3H), 1.42 – 1.38 (m, 1H), 1.14 – 1.11 (m, 2H), 0.89 – 0.84 (m, 2H).

*(±)-N-(cis-2-Benzyl-6-(2-(4-methoxybenzyloxy)ethyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclohexanecarboxamide (**16**)*. White solid (272 mg, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (dd, J = 8.0, 1.8 Hz, 1H), 8.39 (s, 1H), 7.39 – 7.30 (m, 5H), 7.25 – 7.20 (m, 3H), 6.97 (t, J = 8.0 Hz, 1H), 6.87 (d, J = 8.4 Hz, 2H), 4.52 – 4.39 (m, 3H), 4.06 – 3.96 (m, 1H), 3.83 – 3.79 (m, 4H), 3.73 – 3.65 (m, 1H), 3.63 (d, J = 13.2 Hz, 1H), 3.56 (d, J = 13.2 Hz, 1H), 3.48 – 3.38 (m, 2H), 3.32 – 3.14 (m, 2H), 3.00 (d, J = 11.2 Hz, 1H), 2.37 – 2.34 (m, 1H), 2.14 – 2.08 (m, 1H), 2.04 – 1.91 (m, 3H), 1.93 (d, J = 10.6 Hz, 1H), 1.87 – 1.83 (m, 2H), 1.72 – 1.58 (m, 4H), 1.44 (d, J = 9.2 Hz, 1H), 1.37 – 1.28 (m, 3H).

A solution of **14a-e** or **16** (0.2 mmol) in EtOH (5 mL) containing 10% $Pd(OH)_2/C$ (0.5 eq) was hydrogenated for 8 h at 40 °C. The catalyst was filtered and the solvent was evaporated to give **15a-e** or **17** as white solid. The crude products were used for the next step without further purification.

*General Procedure for Synthesis of Compounds **19a, 19a', 19b** and **19c**.* To a solution of **15a, 15b** or **17** (0.2 mmol) and 2,6-lutidine (43 mg, 0.4 mmol) in CH_2Cl_2 (2 mL) at 0 °C was added 4-chlorobenzene-1-sulfonyl chloride (51 mg, 0.24 mmol). The reaction was stirred for 3 h at 0 °C and then treated with saturated $NaHCO_3$ (10 mL). The mixture was extracted with CH_2Cl_2 (3 × 5 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo to give **18a-c**. **18a** was used for the next step without purification. **18b-c** were purified by silica column chromatography (CH_2Cl_2/EtOAc) to give the corresponding pure compound as white solid.

*(±)-N-(cis-2-(4-Chlorophenylsulfonyl)-6-(2-(4-methoxybenzyloxy)ethyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide (**18b**)*. Yield 120 mg, 92%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.63 (s, 1H), 8.49 (dd, J = 8.0, 1.8 Hz, 1H), 7.62 (d, J = 8.4 Hz, 2H), 7.46 (d, J = 8.4 Hz, 2H), 7.18 – 7.07 (m, 3H), 6.91 (t, J = 8.0 Hz, 1H), 6.74 (d, J = 8.6 Hz, 2H), 4.4 – 4.30 (m, 3H), 4.05 (d, J = 13.2 Hz, 1H), 3.91 – 3.71 (m, 6H), 3.62 – 3.37 (m, 3H), 3.20 (dd, J = 14.7, 4.8 Hz, 1H), 2.47 – 2.36 (m, 2H), 2.05 – 1.70 (m, 3H), 1.47 – 1.43 (m, 1H), 1.01 – 0.98 (m, 2H), 0.80 – 0.75 (m, 2H).

*(±)-N-(cis-2-(4-Chlorophenylsulfonyl)-6-(2-(4-methoxybenzyloxy)ethyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclohexanecarboxamide (**18c**)*. Yield 121 mg, 87%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, J = 7.8 Hz, 1H), 8.37 (s, 1H), 7.68 (d, J = 8.4 Hz, 2H), 7.54 (d, J = 8.4 Hz, 2H), 7.24 – 7.15 (m, 3H), 6.98 (t, J = 7.8 Hz, 1H), 6.79 (d, J = 8.4 Hz, 2H), 4.49 – 4.36 (m, 3H), 4.09 (d, J = 12.7 Hz, 1H), 3.96 – 3.78 (m, 6H), 3.67 – 3.60 (m, 1H), 3.52 – 3.41 (m, 2H), 3.23 (dd, J = 15.0, 4.5 Hz, 1H), 2.57 – 2.52 (m, 1H), 2.42 – 2.37 (m, 2H), 2.04 – 1.96 (m, 3H), 1.83 – 1.67 (m, 4H), 1.57 – 1.49 (m, 3H), 1.43 – 1.31 (m, 3H).

Compound **18a-c** (107 mg, 0.16 mmol) was then dissolved in CH_2Cl_2 (4 mL) and pH 7 buffer solution (1 mL). The mixture was cooled to 0 °C and DDQ (55 mg, 0.24 mmol) was added. The reaction was recovered to room temperature and stirred for 12 h, quenched with water, and then extracted with CH_2Cl_2. The combined organic extracts were washed with saturated $NaHCO_3$ solution, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica column chromatography (CH_2Cl_2/MeOH). **19a** and **19a'** were obtained as a pair of separable isomers from **18a**.

*(-)-N-(2-(4-Chlorophenylsulfonyl)-6-((R)-1-hydroxypropan-2-yl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclopropane carboxamide (**19a**)*. White solid (21 mg, 24%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (s, 1H), 8.57 (dd, J = 8.0, 1.6 Hz 1H), 7.71 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.4 Hz, 2H), 7.14 (dd, J = 8.0, 1.6 Hz, 1H), 6.96 (t, J = 8.0 Hz, 1H), 4.93 –

4.87 (m, 1H), 4.78 (s, 1H), 4.12 (d, *J* = 13.2 Hz, 1H), 4.02 (d, *J* = 12.8 Hz, 1H), 3.88 (dd, *J* = 11.6, 2.4 Hz, 1H), 3.51 (q, *J* = 10.4 Hz, 1H), 3.39 – 3.32 (m, 1H), 3.26 – 3.16 (m, 2H), 2.64 – 2.56 (m, 2H), 2.05 – 1.96 (m, 2H), 1.89 – 1.80 (m, 1H), 1.59 – 1.56 (m, 1H), 1.19 (d, *J* = 6.8 Hz, 3H), 1.07 – 1.05 (m, 2H), 0.88 – 0.84 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.27, 171.67, 143.10, 139.43, 136.91, 129.62, 128.72, 128.34, 125.79, 121.97, 121.43, 120.40, 69.82, 66.25, 52.99, 50.08, 45.85, 45.10, 38.88, 24.70, 15.63, 14.23, 8.22. MS (ESI, $[M + H]^+$) m/z 548.2. HRMS (ESI) calcd for $C_{26}H_{30}ClN_3NaO_6S$, 570.1436; found, 570.1430. $[\alpha]_D^{20}$ = -29 (c 0.055, $CHCl_3$).

(+)-N-(2-(4-Chlorophenylsulfonyl)-6-((R)-1-hydroxypropan-2-yl)-7-oxo-2,3,4,4a,5,6,7,12a-octa hydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide ***(19a')***. White solid (21 mg, 24%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (s, 1H), 8.55 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.72 (d, *J* = 8.4 Hz, 2H), 7.52 (d, *J* = 8.4 Hz, 2H), 7.19 (dd, *J* = 8.0, 1.6 Hz, 1H), 6.95 (t, *J* = 8.0 Hz, 1H), 4.71 (s, 1H), 4.20 – 4.10 (m, 2H), 3.99 (d, *J* = 12.8 Hz, 1H), 3.85 – 3.81 (m, 1H), 3.69 – 3.64 (m, 1H), 3.49 – 3.40 (m, 2H), 3.09 (dd, *J* = 15.2, 4.8 Hz, 1H), 2.67 – 2.58 (m, 2H), 2.09 – 1.96 (m, 2H), 1.90 – 1.80 (m, 1H), 1.57 – 1.53 (m, 1H), 1.32 (d, *J* = 6.8 Hz, 3H), 1.06 – 1.04 (m, 2H), 0.88 – 0.82 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.28, 169.99, 143.30, 139.44, 137.15, 129.65, 128.74, 128.35, 126.23, 122.20, 121.41, 120.84, 69.78, 64.25, 58.66, 50.52, 49.98, 45.04, 38.14, 24.44, 15.58, 14.27, 8.22, 8.19. MS (ESI, $[M + H]^+$) m/z 548.2. HRMS (ESI) calcd for $C_{26}H_{30}ClN_3NaO_6S$, 570.1436; found, 570.1441. $[\alpha]_D^{20}$ = +29 (c 0.052, $CHCl_3$).

(±)-N-(cis-2-(4-Chlorophenylsulfonyl)-6-(2-hydroxyethyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1 H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide ***(19b)***. White solid (35 mg, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 1H), 8.55 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.73 (d, *J* = 8.4 Hz, 2H), 7.52 (d, *J* = 8.4 Hz, 2H), 7.23 (dd, *J* = 8.0, 1.6 Hz, 1H), 6.97 (t, *J* = 8.0 Hz, 1H), 4.57 (s, 1H), 4.25 (d, *J* = 13.2 Hz, 1H), 4.03 (d, *J* = 12.8 Hz, 1H), 3.92 – 3.82 (m, 3H), 3.58 – 3.44 (m, 2H), 3.22 (dd, *J* = 15.2, 4.8 Hz, 1H), 3.06 – 3.04 (m, 1H), 2.77 (d, *J* = 13.2 Hz, 1H), 2.68 – 2.62 (m, 1H), 2.07 – 1.85 (m, 3H), 1.62 – 1.57 (m, 1H), 1.07 – 1.04 (m, 2H), 0.90 – 0.84 (m, 2H). ^{13}C NMR (151 MHz,

$CDCl_3$) δ 173.21, 170.95, 143.55, 139.51, 137.00, 129.66, 128.67, 128.33, 126.46, 122.52, 121.55, 119.57, 69.81, 61.94, 51.42, 50.89, 50.10, 45.00, 37.45, 24.40, 15.54, 8.21. MS (ESI, $[M - H]^-$) m/z 532.2. HRMS (ESI) calcd for $C_{25}H_{27}ClN_3O_6S$, 532.1315; found, 532.1309.

*(±)-N-(cis-2-(4-Chlorophenylsulfonyl)-6-(2-hydroxyethyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclohexanecarboxamide (**19c**)*. White solid (52 mg, 56%). ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$) δ 8.56 (d, J = 8.1 Hz, 1H), 8.39 (s, 1H), 7.72 (d, J = 8.4 Hz, 2H), 7.54 (d, J = 8.4 Hz, 2H), 7.21 (d, J = 8.1 Hz, 1H), 6.97 (t, J = 8.1 Hz, 1H), 4.58 (s, 1H), 4.20 (d, J = 13.2 Hz, 1H), 4.00 (d, J = 12.1 Hz, 1H), 3.93 – 3.78 (m, 3H), 3.57 – 3.43 (m, 2H), 3.21 (dd, J = 15.0, 4.5 Hz, 1H), 2.67 (d, J = 13.2 Hz, 1H), 2.59 – 2.47 (m, 2H), 2.04 – 1.97 (m, 3H), 1.89 – 1.78 (m, 3H), 1.67 – 1.48 (m, 4H), 1.44 – 1.23 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3/CD_3OD$) δ 176.26, 170.67, 143.86, 139.58, 136.03, 129.65, 128.48, 128.11, 126.54, 122.51, 121.37, 119.51, 69.80, 60.36, 51.10, 50.35, 50.19, 46.17, 45.16, 36.98, 29.83, 29.51, 25.66, 25.62, 25.55, 24.17. MS (ESI, $[M + H]^+$) m/z 576.2. HRMS (ESI) calcd for $C_{28}H_{33}ClN_3O_6S$, 574.1784; found, 574.1787.

*General Procedure for Synthesis of Compounds **20-22***. To a solution of **15c-e** (0.2 mmol) and 2,6-lutidine (43 mg, 0.4 mmol) in CH_2Cl_2 (2 mL) at 0 °C was added 4-chlorobenzene-1-sulfonyl chloride (51 mg, 0.24 mmol). The reaction was stirred for 3 h at 0 °C and then treated with saturated $NaHCO_3$ (15 mL). The mixture was extracted with CH_2Cl_2 (3 × 10 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica column chromatography (CH_2Cl_2/EtOAc) to give corresponding target compounds **20-22**.

*(±)-N-(cis-2-(4-Chlorophenylsulfonyl)-6-(1-methylpiperidin-4-yl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide (**20**)*. White solid (90 mg, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H), 8.54 (d, J = 8.0 Hz, 1H), 7.72 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.4 Hz, 2H), 7.17 (d, J = 8.0 Hz, 1H), 6.97 (t, J = 8.0 Hz, 1H), 4.62 – 4.55 (m, 2H), 4.25 (d, J = 13.2 Hz, 1H), 4.03 (d, J = 12.8 Hz, 1H), 3.34 – 3.30 (m, 2H), 3.00 – 2.92 (m, 2H), 2.71 (d, J = 13.2 Hz, 1H), 2.60 (t, J = 12.0 Hz, 1H), 2.33 (s, 3H), 2.21 – 2.14 (m, 2H), 1.97 – 1.83 (m,

4H), 1.70 – 1.54 (m, 4H), 1.08 – 1.04 (m, 2H), 0.88 – 0.83 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.12, 169.72, 142.90, 139.51, 136.92, 129.66, 128.59, 128.34, 126.17, 122.00, 121.54, 120.84, 69.24, 55.18, 55.09, 51.93, 50.25, 45.89, 45.15, 45.04, 40.27, 24.55, 15.59, 8.18. MS (ESI, $[M + H]^+$) m/z 587.4. HRMS (ESI) calcd for $C_{29}H_{36}ClN_4O_5S$, 587.2089; found, 587.2106.

(±)-N-(cis-2-(4-Chlorophenylsulfonyl)-7-oxo-6-(pyridin-3-ylmethyl)-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclopropanecarboxamid ***(21)****.* White solid (103 mg, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (s, 1H), 8.60 – 8.55 (m, 3H), 7.74 – 7.70 (m, 3H), 7.53 (d, J = 8.4 Hz, 2H), 7.30 – 7.28 (m, 2H), 7.01 (t, J = 8.0 Hz, 1H), 5.29 (d, J = 14.8 Hz, 1H), 4.49 (d, J = 2.4 Hz, 1H), 4.29 – 4.20 (m, 2H), 4.01 (d, J = 12.4 Hz, 1H), 3.48 (dd, J = 14.8, 11.2 Hz, 1H), 3.09 (dd, J = 15.2, 3.6 Hz, 1H), 2.71 – 2.59 (m, 2H), 2.00 – 1.94 (m, 1H), 1.91 – 1.86 (m, 2H), 1.56 – 1.53 (m, 1H), 1.08 – 1.04 (m, 2H), 0.87 – 0.83 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.18, 169.89, 149.46, 149.28, 143.46, 139.53, 137.14, 135.85, 132.62, 129.68, 128.74, 128.31, 126.39, 123.91, 122.58, 121.74, 119.53, 69.74, 49.96, 49.05, 47.46, 44.90, 37.15, 24.34, 15.52, 8.20. MS (ESI, $[M + Na]^+$) m/z 603.3. HRMS (ESI) calcd for $C_{29}H_{29}ClN_4NaO_5S$, 603.1439; found, 603.1458.

(±)-N-(cis-2-(4-Chlorophenylsulfonyl)-6-(4-methoxybenzyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide ***(22)****.* White solid (102 mg, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (s, 1H), 8.56 (dd, J = 8.0, 1.6 Hz, 1H), 7.71 (d, J = 8.4 Hz, 2H), 7.51 (d, J = 8.4 Hz, 2H), 7.29 (dd, J = 8.0, 1.6 Hz, 1H), 7.23 (d, J = 8.4 Hz, 2H), 6.99 (t, J = 8.0 Hz, 1H), 6.85 (d, J = 8.4 Hz, 2H), 5.09 (d, J = 14.4 Hz, 1H), 4.48 (s, 1H), 4.28 (d, J = 14.4 Hz, 1H), 4.24 – 4.20 (m, 1H), 3.97 (d, J = 12.6 Hz, 1H), 3.82 (s, 3H), 3.42 – 3.36 (m, 1H), 3.08 (dd, J = 15.2, 4.0Hz, 1H), 2.65 – 2.54 (m, 2H), 2.00 – 1.94 (m, 1H), 1.88 – 1.72 (m, 2H), 1.50 – 1.47 (m, 1H), 1.06 – 1.04 (m, 2H), 0.88 – 0.82 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.17, 169.64, 159.28, 143.39, 139.47, 137.22, 129.67, 129.47, 128.91, 128.64, 128.32, 126.35, 122.20, 121.57, 120.13, 114.22,

69.73, 55.34, 50.00, 49.23, 48.69, 44.98, 37.07, 24.38, 15.54, 8.19. MS (ESI, $[M + H]^+$) m/z 610.2. HRMS (ESI) calcd for $C_{31}H_{31}ClN_3O_6S$, 608.1628; found, 608.1625.

*General Procedure for Synthesis of Compounds **23a-i**.* To a solution of **15e** (52 mg, 0.1 mmol) and 2,6-lutidine (22 mg, 0.2 mmol) in CH_2Cl_2 (1 mL) at 0 °C was added an appropriate sulfochloride (0.12 mmol). The reaction was stirred for 3 h at 0 °C and then treated with saturated $NaHCO_3$ (15 mL). The mixture was extracted with CH_2Cl_2 (3 × 10 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica column chromatography (CH_2Cl_2/EtOAc) to give corresponding compounds **23a-h**.

*(±)-N-(cis-6-(4-Methoxybenzyl)-2-(methylsulfonyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide (**23a**).* White solid (43 mg, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (s, 1H), 8.54 (dd, J = 8.0, 1.6 Hz, 1H), 7.29 – 7.25 (m, 3H), 6.97 (t, J = 8.0 Hz, 1H), 6.87 (d, J = 8.4 Hz, 2H), 5.10 (d, J = 14.4 Hz, 1H), 4.45 (s, 1H), 4.34 (d, J = 14.4 Hz, 1H), 4.23 (d, J = 14.0 Hz, 1H), 3.96 (d, J = 12.8 Hz, 1H), 3.81 (s, 3H), 3.38 (dd, J = 14.8, 11.2 Hz, 1H), 3.07 (dd, J = 14.8, 4.4 Hz, 1H), 2.92 – 2.80 (m, 5H), 1.99 – 1.92 (m, 1H), 1.86 – 1.71 (m, 2H), 1.48 – 1.44 (m, 1H), 1.06 – 0.99 (m, 2H), 0.85 – 0.80 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.24, 169.77, 159.30, 143.5, 129.49, 129.00, 128.61, 126.32, 122.28, 121.42, 120.13, 114.21, 69.50, 55.34, 49.54, 49.25, 48.73, 44.76, 39.26, 37.30, 24.39, 15.28, 8.11, 8.06. MS (ESI, $[M + H]^+$) m/z 514.3. HRMS (ESI) calcd for $C_{26}H_{30}N_3O_6S$, 512.1861; found, 512.1869.

*(±)-N-(cis-2-(Cyclohexylsulfonyl)-6-(4-methoxybenzyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide (**23b**).* White solid (51 mg, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.03 (s, 1H), 8.57 (dd, J = 8.0, 1.6 Hz, 1H), 7.30 – 7.28 (m, 3H), 6.99 (t, J = 8.0 Hz, 1H), 6.89 (d, J = 8.4 Hz, 2H), 5.12 (d, J = 14.4 Hz, 1H), 4.40 – 4.33 (m, 2H), 4.21 (d, J = 14.4 Hz, 1H), 4.01 – 3.97 (m, 1H), 3.83 (s, 3H), 3.40 (dd, J = 14.8, 11.6 Hz, 1H), 3.09 – 3.00 (m, 2H), 2.96 – 2.89 (m, 2H), 2.14 – 2.04 (m, 3H), 1.93 – 1.73 (m, 5H), 1.47 – 1.41 (m, 3H), 1.34 – 1.19 (m, 3H), 1.07 – 1.03 (m, 2H), 0.84 – 0.79 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ

173.51, 169.99, 159.27, 143.83, 129.49, 129.09, 128.75, 126.29, 122.50, 121.31, 120.07, 114.22, 69.42, 62.59, 55.37, 50.30, 49.26, 48.77, 45.89, 37.49, 26.65, 26.53, 25.15, 24.94, 15.12, 8.13, 8.02. MS (ESI, $[M + H]^+$) m/z 582.3. HRMS (ESI) calcd for $C_{31}H_{38}N_3O_6S$, 580.2487; found, 580.2491.

*(±)-N-(cis-6-(4-Methoxybenzyl)-2-(4-methylpiperazin-1-ylsulfonyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide (**23c**).* White solid (44 mg, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (s, 1H), 8.56 (dd, J = 8.0, 1.6 Hz, 1H), 7.28 – 7.24 (m, 3H), 6.97 (t, J = 8.0 Hz, 1H), 6.87 (d, J = 8.4 Hz, 2H), 5.12 (d, J = 14.4 Hz, 1H), 4.40 (s, 1H), 4.31 (d, J = 14.4 Hz, 1H), 4.22 (d, J = 14.8 Hz, 1H), 3.96 (d, J = 13.6 Hz, 1H), 3.81 (s, 3H), 3.37 (dd, J = 14.8, 10.8 Hz, 1H), 3.19 (t, J = 4.8 Hz, 4H), 3.07 – 2.99 (m, 2H), 2.91 (t, J = 12.4 Hz, 1H), 2.48 (t, J = 4.8 Hz, 4H), 2.32 (s, 3H), 2.05 – 1.99 (m, 1H), 1.81 – 1.78 (m, 2H), 1.41 – 1.36 (m, 1H), 1.04 – 0.99 (m, 2H), 0.82 – 0.78 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.42, 169.96, 159.30, 143.78, 129.48, 129.03, 128.62, 126.36, 122.45, 121.37, 120.08, 114.21, 69.71, 55.36, 54.15, 50.94, 49.21, 48.67, 46.22, 46.16, 45.88, 37.49, 24.01, 15.12, 8.13, 8.09. MS (ESI, $[M + H]^+$) m/z 598.4. HRMS (ESI) calcd for $C_{30}H_{40}N_5O_6S$, 598.2694; found, 598.2697.

*(±)-N-(cis-2-(2,4-Dichlorophenylsulfonyl)-6-(4-methoxybenzyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide (**23d**).* White solid (51 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.86 (s, 1H), 8.57 (dd, J = 8.0, 1.6 Hz, 1H), 8.03 (d, J = 8.4 Hz, 1H), 7.55 (d, J = 2.0 Hz, 1H), 7.39 (dd, J = 8.4, 1.6 Hz, 1H), 7.28 (dd, J = 8.0, 1.6 Hz, 1H), 7.24 (d, J = 8.6 Hz, 2H), 6.99 (t, J = 8.0 Hz, 1H), 6.86 (d, J = 8.6 Hz, 2H), 5.11 (d, J = 14.4 Hz, 1H), 4.47 (s, 1H), 4.31 (d, J = 14.6 Hz, 2H), 3.97 (d, J = 12.6 Hz, 1H), 3.82 (s, 3H), 3.44 – 3.38 (m, 1H), 3.08 (dd, J = 15.2, 3.6 Hz, 1H), 2.95 (dd, J = 14.4, 1.6 Hz, 1H), 2.88 – 2.81 (m, 1H), 2.09 – 2.03 (m, 1H), 1.91 – 1.79 (m, 2H), 1.50 – 1.43 (m, 1H), 1.06 – 1.03 (m, 2H), 0.82 – 0.78 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.42, 169.91, 159.29, 143.67, 139.75, 135.94, 133.05, 132.64, 131.89, 129.47, 128.97, 128.60, 127.45, 126.42, 122.53, 121.48, 120.18, 114.23, 69.62, 55.36, 50.02, 49.23, 48.66,

45.39, 37.53, 24.57, 15.26, 8.16, 8.11. MS (ESI, $[M + Na]^+$) m/z 666.2. HRMS (ESI) calcd for $C_{31}H_{30}Cl_2N_3O_6S$, 642.1238; found, 642.1252.

*(±)-N-(cis-2-(4-Acetamidophenylsulfonyl)-6-(4-methoxybenzyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide (**23e**).* White solid (61 mg, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (s, 1H), 8.54 (dd, J = 8.1, 1.6 Hz, 1H), 8.24 (s, 1H), 7.69 (s, 4H), 7.30 – 7.27 (m, 1H), 7.22 (d, J = 8.4 Hz, 2H), 6.97 (t, J = 8.0 Hz, 1H), 6.85 (d, J = 8.4 Hz, 2H), 5.13 (d, J = 14.4 Hz, 1H), 4.47 (s, 1H), 4.26 – 4.20 (m, 2H), 3.95 (d, J = 12.4 Hz, 1H), 3.78 (s, 3H), 3.41 – 3.35 (m, 1H), 3.11 – 3.07 (m, 1H), 2.63 – 2.52 (m, 2H), 2.19 (s, 3H), 2.03 – 1.98 (m, 1H), 1.84 – 1.76 (m, 2H), 1.52 – 1.46 (m, 1H), 1.05 – 1.02 (m, 2H), 0.89 – 0.83 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$/CD_3OD) δ 173.68, 169.95, 169.84, 159.33, 143.64, 142.90, 132.48, 129.44, 128.64, 128.44, 128.10, 126.45, 122.32, 121.44, 120.04, 119.61, 114.30, 70.04, 55.37, 50.02, 49.26, 48.81, 44.97, 36.91, 24.36, 24.22, 15.58, 8.28, 8.25. MS (ESI, $[M + Na]^+$) m/z 655.3. HRMS (ESI) calcd for $C_{33}H_{35}N_4O_7S$, 631.2232; found, 631.2243.

*(±)-N-(cis-2-(3,4-Dimethoxyphenylsulfonyl)-6-(4-methoxybenzyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide (**23f**).* White solid (56 mg, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (s, 1H), 8.57 (dd, J = 8.1, 1.6 Hz, 1H), 7.40 (dd, J = 8.4, 2.4 Hz, 1H), 7.30 – 7.28 (m, 1H), 7.24 – 7.21 (m, 3H), 7.01 – 6.95 (m, 2H), 6.85 (d, J = 8.4 Hz, 2H), 5.13 (d, J = 14.4 Hz, 1H), 4.49 (s, 1H), 4.27 – 4.21 (m, 2H), 3.99 – 3.94 (m, 7H), 3.78 (s, 3H), 3.42 – 3.35 (m, 1H), 3.08 (dd, J = 15.2, 4.0 Hz, 1H), 2.63 – 2.52 (m, 2H), 2.02 – 1.97 (m, 1H), 1.85 – 1.76 (m, 2H), 1.52 – 1.47 (m, 1H), 1.07 – 1.04 (m, 2H), 0.88 – 0.83 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.15, 169.61, 159.23, 152.73, 149.25, 143.37, 130.04, 129.42, 128.87, 128.68, 126.25, 122.03, 121.48, 120.86, 120.07, 114.17, 110.74, 109.36, 69.90, 56.30, 56.24, 55.31, 50.04, 49.14, 48.65, 44.96, 36.98, 24.34, 15.55, 8.19. MS (ESI, $[M + H]^+$) m/z 636.2. HRMS (ESI) calcd for $C_{33}H_{36}N_3O_8S$, 634.2229; found, 634.2232.

*(±)-N-(cis-2-(5-Chlorothiophen-2-ylsulfonyl)-6-(4-methoxybenzyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide (**23g**).* White solid (50 mg, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 – 8.52 (m, 2H), 7.35 (d, J = 4.0 Hz, 1H), 7.29 (dd, J = 8.0, 1.6 Hz, 1H), 7.24 (d, J = 8.4 Hz, 2H), 7.02 – 6.97 (m, 2H), 6.86 (d, J = 8.4 Hz, 2H), 5.11 (d, J = 14.4 Hz, 1H), 4.52 (s, 1H), 4.29 (d, J = 14.4 Hz, 1H), 4.20 (d, J = 13.6Hz, 1H), 3.99 (d, J = 12.4 Hz, 1H), 3.80 (s, 3H), 3.39 (dd, J = 14.8, 11.2 Hz, 1H), 3.09 (dd, J = 14.8, 4.0 Hz, 1H), 2.70 – 2.59 (m, 2H), 1.91 – 1.75 (m, 3H), 1.55 – 1.52 (m, 1H), 1.09 – 0.99 (m, 2H), 0.87 – 0.83 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.99, 169.53, 159.30, 143.24, 137.53, 136.73, 131.39, 129.46, 128.86, 128.55, 127.02, 126.35, 122.16, 121.62, 120.11, 114.23, 69.77, 55.33, 50.21, 49.23, 48.62, 45.08, 36.96, 24.29, 15.60, 8.16. MS (ESI, $[M + H]^+$) m/z 616.1. HRMS (ESI) calcd for $C_{29}H_{29}ClN_3O_6S_2$, 614.1192; found, 614.1198.

*(±)-N-(cis-6-(4-Methoxybenzyl)-2-(1-methyl-1H-pyrazol-3-ylsulfonyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide (**23h**).* White solid (45 mg, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 1H), 8.55 (dd, J = 8.0, 1.6 Hz, 1H), 7.44 (d, J = 2.4 Hz, 1H), 7.29 (dd, J = 8.0, 1.6 Hz, 1H), 7.24 (d, J = 8.4 Hz, 2H), 6.99 (t, J = 8.0 Hz, 1H), 6.86 (d, J = 8.4 Hz, 2H), 6.65 (d, J = 2.4 Hz, 1H), 5.13 (d, J = 14.6 Hz, 1H), 4.50 (s, 1H), 4.30 – 4.24 (m, 2H), 4.06 (d, J = 12.8 Hz, 1H), 3.98 (s, 3H), 3.80 (s, 3H), 3.39 (dd, J = 15.2, 11.2 Hz, 1H), 3.09 (dd, J = 15.2, 3.6 Hz, 1H), 2.82 – 2.71 (m, 2H), 2.02 – 1.95 (m, 1H), 1.81 – 1.78 (m, 2H), 1.51 (d, J = 8.8 Hz, 1H), 1.05 – 1.02 (m, 2H), 0.86 – 0.82 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.22, 169.66, 159.25, 149.38, 143.44, 131.83, 129.45, 128.95, 128.69, 126.26, 122.09, 121.45, 120.09, 114.20, 107.27, 69.89, 55.33, 50.28, 49.20, 48.75, 45.12, 39.89, 37.06, 24.51, 15.56, 8.18. MS (ESI, $[M + H]^+$) m/z 580.3. HRMS (ESI) calcd for $C_{29}H_{32}N_5O_6S$, 578.2079; found, 578.2087.

*Synthesis of Compound **23i**.* To a solution of **15e** (52 mg, 0.1 mmol) and 2,6-lutidine (22 mg, 0.2 mmol) in CH_2Cl_2 (1 mL) at 0 °C was added 4-chlorobenzoyl chloride (21 mg, 0.12 mmol). The reaction was stirred for 3 h at 0 °C and then treated with saturated $NaHCO_3$ (15 mL). The mixture

was extracted with CH_2Cl_2 (3 × 10 mL), dried over Na_2SO_4, filtered, and then concentrated in vacuo. The residue was purified by silica column chromatography (CH_2Cl_2/EtOAc) to give compound **23i**.

*(±)-N-(cis-2-(4-Chlorobenzoyl)-6-(4-methoxybenzyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide (**23i**).* White solid (53 mg, 93%). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD) δ 8.73 (s, 0.38H), 8.51 (dd, J = 8.0, 1.6 Hz, 1H), 7.42 – 7.38 (m, 4H), 7.32 (dd, J = 8.0, 1.6 Hz, 1H), 7.26 (d, J = 8.0 Hz, 2H), 7.00 (t, J = 8.0 Hz, 1H), 6.88 (d, J = 8.0 Hz, 2H), 5.19 – 5.15 (m, 2H), 4.61 (s, 1H), 4.29 (d, J = 14.4 Hz, 1H), 3.80 – 3.78 (s, 4H), 3.35 (t, J = 13.2 Hz, 1H), 3.14 (t, J = 13.2 Hz, 1H), 3.04 (dd, J = 14.8, 4.0 Hz, 1H), 2.90 (d, J = 13.6 Hz, 1H), 2.23 – 2.17 (m, 1H), 2.02 – 1.96 (m, 1H), 1.49 – 1.31 (m, 2H), 1.11 – 0.97 (m, 2H), 0.82 – 0.76 (m, 1H), 0.59 – 0.53 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$/CD_3OD) δ 173.60, 171.82, 169.72, 159.28, 144.08, 136.21, 133.84, 129.42, 129.04, 128.74, 128.22, 128.09, 127.00, 122.86, 121.49, 120.30, 114.23, 72.06, 55.30, 49.20, 48.60, 47.44, 47.16, 37.40, 25.37, 15.07, 8.44, 8.27. MS (ESI, $[M + H]^+$) m/z 574.2. HRMS (ESI) calcd for $C_{32}H_{31}ClN_3O_5$, 572.1958; found, 572.1967.

*General Procedure for Synthesis of Compounds **23j-k**.* To a solution of **15e** (52 mg, 0.1 mmol) in CH_2Cl_2 (1 mL) was added an appropriate isocyanate (0.12 mmol). The reaction was stirred for 3 h at rt and then treated with saturated $NaHCO_3$ (15 mL). The mixture was extracted with CH_2Cl_2, dried over Na_2SO_4, filtered, and then concentrated in vacuo. The residue was purified by silica column chromatography (CH_2Cl_2/EtOAc) to give corresponding compounds **23j-k**.

*(±)-cis-11-(Cyclopropanecarboxamido)-6-(4-methoxybenzyl)-7-oxo-N-(4-(trifluoromethyl)phenyl)-3,4,4a,5,6,7-hexahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocine-2(12aH)-carboxamide (**23j**).* White solid (45 mg, 74%). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD) δ 8.46 (dd, J = 8.0, 1.6 Hz, 1H), 7.56 (d, J = 8.4 Hz, 2H), 7.43 (d, J = 8.4 Hz, 2H), 7.28 – 7.22 (m, 3H), 6.96 (t, J = 8.0 Hz, 1H), 6.89 (d, J = 8.0 Hz, 2H), 5.15 (d, J = 14.4 Hz, 1H), 4.79 (d, J = 14.4 Hz, 1H), 4.31 – 4.24 (m, 3H), 3.81 (s, 3H), 3.34 – 3.20 (m, 1H), 3.08 (dd, J = 14.8, 4.4 Hz, 1H), 2.97 (t, J = 13.2 Hz, 1H), 2.83 (d, J = 14.4 Hz, 1H), 1.99 – 1.92 (m, 1H), 1.67 – 1.61 (m, 2H), 1.51 – 1.47 (m, 1H), 0.84 – 0.78 (m, 1H), 0.75 –

0.69 (m, 1H), 0.33 – 0.31 (m, 1H), 0.04 – 0.01 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3/CD_3OD$) δ 173.52, 169.93, 159.31, 155.26, 143.81, 142.56, 129.20, 128.48, 128.31, 126.56, 125.67 (q, J = 3.4 Hz), 124.65 (q, J = 33 Hz), 124.14 (q, J = 272 Hz), 122.36, 121.32, 120.00, 119.75, 114.27, 71.69, 55.29, 49.13, 48.77, 48.37, 44.20, 37.73, 24.60, 15.34, 8.11, 7.68. MS (ESI, $[M + H]^+$) m/z 623.2. HRMS (ESI) calcd for $C_{33}H_{32}F_3N_4O_5$, 621.2330; found, 621.2344.

(±)-cis-11-(Cyclopropanecarboxamido)-N-(2,4-difluorophenyl)-6-(4-methoxybenzyl)-7-oxo-3,4, 4a,5,6,7-hexahydro-1H-benzo[b]pyrido[4,3-g][1,5]oxazocine-2(12aH)-carboxamide ***(23k)***. White solid (44 mg, 75%). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$) δ 8.60 (s, 0.56H), 8.54 (d, J = 8.0 Hz, 1H), 7.89 – 7.80 (m, 1H), 7.31 – 7.24 (m, 3H), 6.96 (t, J = 8.0 Hz, 1H), 6.90 – 6.83 (m, 3H), 6.80 – 6.75 (m, 1H), 6.68 (s, 0.54H), 5.18 (d, J = 14.4 Hz, 1H), 4.83 (d, J = 14.4 Hz, 1H), 4.47 (s, 1H), 4.27 (d, J = 14.4 Hz, 1H), 3.97 (d, J = 14.0 Hz, 1H), 3.81 (s, 3H), 3.38 (dd, J = 14.8, 12.0 Hz, 1H), 3.22 – 3.02 (m, 2H), 2.89 (d, J = 14.0 Hz, 1H), 2.02 – 1.96 (m, 1H), 1.74 – 1.64 (m, 2H), 1.54 – 1.50 (m, 1H), 0.92 – 0.87 (m, 1H), 0.83 – 0.77 (m, 1H), 0.54 – 0.50 (m, 1H), 0.12 – 0.05 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3/CD_3OD$) δ 173.15 and 173.07 (1C), 169.28, 158.77, 158.12 (d, J = 243.2 Hz), 155.02 and 154.94 (1C), 153.48 (d, J = 246.9 Hz), 143.43 and 143.40 (1C), 128.89, 128.27, 128.11 and 128.02 (1C), 126.19, 124.05 (m, 1C), 122.47 (m, 1C), 121.74 and 121.68 (1C), 120.88, 119.70, 113.72, 110.22 (dd, J = 24.7, 3.18 Hz), 103.22 (t, J = 24.7 Hz), 71.34, 54.81, 48.67, 48.10, 47.80, 44.69, 37.25, 24.14, 14.52, 7.72, 7.13. MS (ESI, $[M + H]^+$) m/z 591.3. HRMS (ESI) calcd for $C_{32}H_{33}F_2N_4O_5$, 591.2414; found, 591.2419.

General Procedure for Synthesis of Compounds ***29a-g***. To a solution of compound **25** (273 mg, 1.0 mmol) in EtOH (5 mL) and H_2O (3 mL) was added lithium hydroxide monohydrate (84 mg, 2.0 mmol). The reaction was stirred for 2 h at rt. The mixture was adjusted to pH 5 with hydrochloride acid (0.5 M) and then concentrated to give the acid intermediate as pale oil. Without further purification, the acid intermediate was dissolved in DMF (10 mL), HATU (760 mg, 2.0 mmol) and HOAT (136 mg, 1.0 mmol) were added. The mixture was stirred at rt for 20 min, then

(4-methoxyphenyl)methanamine (164 mg, 1.2 mmol) and DIPEA (387 mg, 3.0 mmol) were added. The reaction was stirred at rt for 12 h and then treated with saturated $NaHCO_3$ (10 mL). The mixture was extracted with CH_2Cl_2 (3 × 10 mL) and the combined organic layer was washed with water, dried over Na_2SO_4, filtered, and then concentrated in vacuo to give the crude amides.

To a solution of the crude amide products in CH_2Cl_2 (5 mL) at 0 °C was slowly added TBSOTf (528 mg, 2.0 mmol). The reaction was stirred for another 1 h at 0 °C and then treated with saturated $NaHCO_3$ (10 mL). The mixture was extracted with CH_2Cl_2 (3 × 10 mL), dried over Na_2SO_4, filtered, and then concentrated in vacuo. The residue was purified by silica column chromatography (petroleum ether/EtOAc 3:1) to give compound **26** as colorless oil (235 mg, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (d, J = 8.8 Hz, 2H), 6.85 (d, J = 8.8 Hz, 2H), 4.44 – 4.28 (m, 3H), 3.81 – 3.38 (m, 7H), 2.46 (dt, J = 9.6, 3.2 Hz, 1H), 1.65 – 1.59 (m, 2H), 1.46 (s, 9H), 0.83 (s, 9H), 0.04 (d, J = 16.9 Hz, 6H).

Compound **26** (235 mg, 0.5 mmol) was dissolved in THF (5 mL) under N_2 and borane dimethylsulfide complex (BH_3.DMS) (1.25 mL, 2.5 mmol) was added dropwise. The reaction was stirred at 65 °C for 6 h and then cooled to room temperature. The mixture was quenched by carefully adding MeOH and concentrated under reduced pressure to remove excess $B(OMe)_3$, and then co-evaporated with MeOH three times. The resulting oil was re-dissolved in MeOH (4 mL) and 10% aqueous potassium sodium tartrate (6 mL) and the mixture was heated at reflux for 18 h. The mixture was concentrated in vacuo and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layer was washed with water and brine, dried over Na_2SO_4, filtered, and then concentrated under reduced pressure to give the crude amine as colorless oil.

To a solution of 2-fluoro-3-nitrobenzoic acid (111 mg, 0.6 mmol) in CH_2Cl_2 (5 mL) was added HATU (380 mg, 1.0 mmol) and HOAT (78 mg, 0.5 mmol). The mixture was stirred at rt for 20 min, then the crude amine (232 mg, 0.5 mmol) prepared above together with DIPEA (194 mg, 1.5 mmol) were added. The reaction was stirred at rt for 6 h and then treated with saturated $NaHCO_3$ (10 mL).

The mixture was extracted with CH_2Cl_2 (3 × 10 mL) and the combined organic layer was washed with water, dried over Na_2SO_4, filtered, and then concentrated in vacuo to give the crude product as pale foam.

Without purification, the crude product was dissolved in DMF (5 mL) and CsF (228 mg, 1.5 mmol) was added. The reaction was stirred at 85 °C for 4 h. After complete consumption of starting material, the reaction was diluted with water (20 mL) and extracted with EtOAc (3 × 10 mL), the organic layer was washed with water three times, dried with Na_2SO_4, filtered, and then concentrated in vacuo. The residue was purified by silica column chromatography (petroleum ether/EtOAc 3:1) to give compound **27** as white foam (235 mg, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (dd, J = 8.0, 1.6 Hz, 1H), 7.73 (dd, J = 8.0, 1.6 Hz, 1H), 7.27 (d, J = 8.4 Hz, 2H), 7.07 (t, J = 8.0 Hz, 1H), 6.89 (d, J = 8.4 Hz, 2H), 5.14 (d, J = 14.4 Hz, 1H), 4.72 (s, 1H), 4.28 (d, J = 14.4 Hz, 1H), 4.01 – 3.91 (m, 2H), 3.81 (s, 3H), 3.26 (dd, J = 15.2, 12.4 Hz, 1H), 3.17 – 3.07 (m, 2H), 2.80 (t, J = 12.4 Hz, 1H), 2.04 – 1.88 (m, 2H), 1.64 – 1.55 (m, 1H), 1.48 (s, 9H).

To a solution of compound **27** (249 mg, 0.5 mmol) in EtOH (5 mL) and H_2O (2 mL) was added Fe powder (196 mg, 3.5 mmol) and NH_4Cl (259 mg, 5.0 mmol). The reaction mixture was heated under reflux for 1 h and then filtered over celite and concentrated in vacuo. The residue was diluted with water (20 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layer was washed with water and brine, dried over Na_2SO_4, filtered, and then concentrated in vacuo to give the aniline intermediate as pale-yellow solid. Without purification, the aniline (233 mg, 0.5 mmol) was dissolved in CH_2Cl_2 (5 mL) at 0 °C, then 2,6-lutidine (107 mg, 1.0 mmol) and cyclopropanecarbonyl chloride (63 mg, 0.6 mmol) were added. The reaction was stirred for 4 h at 0 °C and then treated with saturated $NaHCO_3$ (15 mL). The mixture was extracted with CH_2Cl_2 (3 × 10 mL), dried over Na_2SO_4, filtered, and then concentrated in vacuo. The residue was purified by silica column chromatography (CH_2Cl_2/EtOAc 3:1) to give compound **28** as white solid (211 mg, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (d, J = 8.0 Hz, 1H), 7.95 (s, 1H), 7.34 (d, J = 8.0 Hz, 1H), 7.29 – 7.21 (d, J

= 8.4 Hz, 2H), 6.99 (t, *J* = 8.0 Hz, 1H), 6.87 (d, *J* = 8.4 Hz, 2H), 5.21 (d, *J* = 14.4 Hz, 1H), 4.69 (s, 1H), 4.17 – 3.89 (m, 3H), 3.80 (s, 3H), 3.31 – 3.24 (m, 1H), 3.09 – 3.04 (m, 2H), 2.81 – 2.71 (m, 1H), 2.10 – 1.93 (m, 2H), 1.72 – 1.68 (m, 1H), 1.51 – 1.44 (m, 10H), 1.11 – 1.09 (m, 2H), 0.90 – 0.86 (m, 2H).

To a solution of **28** (267 mg, 0.5 mmol) in CH_2Cl_2 (10 mL) was added trifluoracetic acid (1.14 g, 10 mmol). The reaction was stirred for 4 h at rt and alkalified with $NaHCO_3$ to pH > 9. The mixture was extracted with CH_2Cl_2 (3 × 10 mL), dried over Na_2SO_4, filtered, and then concentrated in vacuo to give the crude product as white solid.

To a solution of the crude product (43 mg, 0.1 mmol) and 2,6-lutidine (22 mg, 0.2 mmol) in CH_2Cl_2 (1 mL) at 0 °C was added an appropriate sulfonylchloride or isocyanate (0.12 mmol). The reaction was stirred for 3 h at 0 °C and then treated with saturated $NaHCO_3$ (15 mL). The mixture was extracted with CH_2Cl_2 (3 × 10 mL), dried over Na_2SO_4, filtered, and then concentrated in vacuo. The residue was purified by silica column chromatography (CH_2Cl_2/EtOAc) to give corresponding compounds **29a-g**.

*(±)-N-(cis-3-(4-Chlorophenylsulfonyl)-6-(4-methoxybenzyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro -1H-benzo[b]pyrido[3,4-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide (**29a**).* White solid (47 mg, 77%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (s, 1H), 7.69 (d, *J* = 8.5 Hz, 2H), 7.53 – 7.48 (m, 3H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.24 (d, *J* = 8.5 Hz, 2H), 6.95 (t, *J* = 8.0 Hz, 1H), 6.88 (d, *J* = 8.5 Hz, 2H), 5.13 (d, *J* = 14.5 Hz, 1H), 4.60 (s, 1H), 4.26 (d, *J* = 14.5 Hz, 1H), 3.84 – 3.79 (m, 4H), 3.56 (dd, *J* = 11.5, 4.0 Hz, 1H), 3.20 (dd, *J* = 15.0, 12.5 Hz, 1H), 3.06 (dd, *J* = 15.0, 5.0 Hz, 1H), 2.60 (t, *J* = 12.0 Hz, 1H), 2.33 (t, *J* = 11.5 Hz, 1H), 2.21 – 2.08 (m, 2H), 1.92 – 1.85 (m, 1H), 1.15 – 1.10 (m, 1H), 0.98 – 0.92 (m, 2H), 0.81 – 0.76 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.33, 169.51, 159.40, 143.61, 139.84, 134.35, 129.57, 129.56, 128.97, 128.52, 127.23, 122.59, 121.57, 120.30, 114.32, 69.44, 55.34, 49.14, 45.99, 43.77, 41.22, 37.91, 30.82, 15.98, 8.06, 7.90. MS (ESI, $[M + H]^+$) m/z 610.2. HRMS (ESI) calcd for $C_{31}H_{31}ClN_3O_6S$, 608.1628; found, 608.1633.

*(4aR,12aS)-N-(3-(4-Chlorophenylsulfonyl)-6-(4-methoxybenzyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[3,4-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide (**29a-1**).* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.69 (d, J = 8.4 Hz, 2H), 7.53 – 7.48 (m, 3H), 7.30 (d, J = 8.0 Hz, 1H), 7.26 (dd, J = 8.0 Hz, 2H), 6.96 (t, J = 8.0 Hz, 1H), 6.88 (d, J = 8.4 Hz, 2H), 5.13 (d, J = 14.4 Hz, 1H), 4.61 (s, 1H), 4.27 (d, J = 14.4 Hz, 1H), 3.84 – 3.80 (m, 4H), 3.57 (d, J = 11.4 Hz, 1H), 3.24 – 3.18 (m, 1H), 3.06 (dd, J = 15.0, 4.8 Hz, 1H), 2.60 (t, J = 12.4 Hz, 1H), 2.33 (t, J = 11.5 Hz, 1H), 2.16 – 2.12 (m, 2H), 1.93 – 1.87 (m, 1H), 1.13 – 1.08 (m, 1H), 0.99 – 0.88 (m, 2H), 0.82 – 0.80 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.29, 169.48, 159.40, 143.56, 139.84, 134.35, 129.58, 129.56, 128.97, 128.53, 127.23, 122.53, 121.60, 120.30, 114.31, 69.46, 55.33, 49.17, 46.00, 43.77, 41.22, 37.91, 30.83, 16.01, 8.08, 7.91. $[\alpha]_D^{20}$ = +64 (c 0.054, $CHCl_3$).

*(4aS,12aR)-N-(3-(4-Chlorophenylsulfonyl)-6-(4-methoxybenzyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[3,4-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide (**29a-2**).* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.69 (d, J = 8.4 Hz, 2H), 7.53 – 7.48 (m, 3H), 7.31 – 7.24 (m, 3H), 6.96 (t, J = 8.0 Hz, 1H), 6.88 (d, J = 8.4 Hz, 2H), 5.14 (d, J = 14.4 Hz, 1H), 4.61 (s, 1H), 4.27 (d, J = 14.4 Hz, 1H), 3.82 – 3.79 (m, 4H), 3.62 – 3.55 (m, 1H), 3.24 – 3.17 (m, 1H), 3.06 (dd, J = 15.0, 4.8 Hz, 1H), 2.60 (t, J = 12.3 Hz, 1H), 2.33 (t, J = 11.5 Hz, 1H), 2.18 – 2.12 (m, 2H), 1.93 – 1.86 (m, 1H), 1.14 – 1.09 (m, 1H), 1.00 – 0.94 (m, 2H), 0.83 – 0.78 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.30, 169.48, 159.40, 143.60, 139.83, 134.34, 129.57, 128.97, 128.82, 128.53, 127.23, 122.55, 121.58, 120.30, 114.31, 69.47, 55.33, 49.16, 46.00, 43.78, 41.23, 37.91, 30.83, 15.99, 8.08, 7.91. $[\alpha]_D^{20}$ = -59 (c 0.051, $CHCl_3$).

*(±)-N-(3-(2,4-dichlorophenylsulfonyl)-6-(4-methoxybenzyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[3,4-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide (**29b**).* White solid (43 mg, 67%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (s, 1H), 8.01 (d, J = 8.4 Hz, 1H), 7.78 (s, 1H), 7.55 (d, J = 2.0 Hz, 1H), 7.40 (dd, J = 8.4, 2.0 Hz, 1H), 7.32 (d, J = 8.0 Hz, 1H), 7.24 (d, J = 8.0 Hz, 2H), 6.97 (t, J = 8.4 Hz, 1H), 6.87 (d, J = 8.0 Hz, 2H), 5.06 (d, J = 14.4 Hz, 1H), 4.66 (s, 1H), 4.34 (d, J = 14.4

Hz, 1H), 3.87 – 3.81 (m, 4H), 3.57 (d, *J* = 12.4 Hz, 1H), 3.30 – 3.13 (m, 2H), 3.05 (dd, *J* = 14.8, 4.8 Hz, 1H), 2.84 (t, *J* = 12.4 Hz, 1H), 2.15 – 2.04 (m, 2H), 1.82 (t, *J* = 13.6 Hz, 1H), 1.43 – 1.37 (m, 1H), 1.07 – 1.03 (m, 2H), 0.90 – 0.85 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.15, 170.10, 159.96, 144.54, 140.43, 135.47, 133.66, 133.54, 132.55, 130.10, 129.14, 128.04, 127.92, 123.48, 122.08, 120.95, 114.87, 70.58, 55.90, 49.81, 46.64, 43.84, 41.45, 38.61, 31.85, 16.73, 8.74, 8.69. MS (ESI, $[M + H]^+$) m/z 644.2. HRMS (ESI) calcd for $C_{31}H_{32}Cl_2N_3O_6S$, 644.1383; found, 644.1385.

*(±)-N-(-3-(4-acetamidophenylsulfonyl)-6-(4-methoxybenzyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[3,4-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide (**29c**).* White solid (45 mg, 71%). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD) δ 8.23 (s, 1H), 7.93 (s, 1H), 7.71 – 7.64 (m, 4H), 7.56 (s, 1H), 7.29 – 7.23 (m, 3H), 6.94 (t, *J* = 8.0 Hz, 1H), 6.88 (d, *J* = 8.8 Hz, 2H), 5.15 (d, *J* = 14.4 Hz, 1H), 4.58 (s, 1H), 4.24 (d, *J* = 14.4 Hz, 1H), 3.84 – 3.80 (m, 4H), 3.54 (d, *J* = 10.8 Hz, 1H), 3.19 (t, *J* = 13.6 Hz, 1H), 3.06 (dd, *J* = 14.8, 4.8 Hz, 1H), 2.62 (t, *J* = 12.4 Hz, 1H), 2.29 (t, *J* = 11.6 Hz, 1H), 2.19 – 2.11 (m, 5H), 1.88 (t, *J* = 13.6 Hz, 1H), 1.17 – 1.13 (m, 1H), 0.94 – 0.75 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.62, 169.50, 168.77, 159.38, 143.63, 142.61, 130.17, 129.51, 128.81, 128.44, 127.23, 122.57, 121.48, 120.29, 119.18, 114.32, 69.67, 55.34, 49.17, 46.12, 43.79, 41.22, 37.78, 30.82, 24.68, 16.04, 8.15, 7.73. MS (ESI, $[M + H]^+$) m/z 633.3. HRMS (ESI) calcd for $C_{33}H_{37}N_4O_7S$, 633.2377; found, 633.2396.

*(±)-N-(cis-6-(4-Methoxybenzyl)-3-(1-methyl-1H-pyrazol-3-ylsulfonyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[3,4-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide (**29d**).* White solid (44 mg, 77%). ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 8.27 (s, 1H), 7.79 (s, 1H), 7.44 (d, *J* = 2.4 Hz, 1H), 7.31 – 7.21 (m, 3H), 6.96 (t, *J* = 8.1 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 2H), 6.66 (d, *J* = 2.4 Hz, 1H), 5.17 (d, *J* = 14.4 Hz, 1H), 4.63 (s, 1H), 4.23 (d, *J* = 14.4 Hz, 1H), 3.97 – 3.89 (m, 4H), 3.82 (s, 3H), 3.61 – 3.56 (m, 1H), 3.27 – 3.18 (m, 1H), 3.09 – 2.94 (m, 2H), 2.57 (t, *J* = 11.7 Hz, 1H), 2.21 – 2.13 (m, 2H), 1.88 – 1.80 (m, 1H), 1.39 – 1.34 (m, 1H), 1.06 – 1.04 (m, 2H), 0.88 – 0.83 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) *δ* 171.38, 169.59, 159.39, 147.79, 143.62, 131.79, 129.61, 129.32, 128.48,

127.36, 122.45, 121.45, 120.37, 114.31, 108.17, 69.74, 55.33, 49.06, 46.09, 43.86, 41.32, 39.98, 37.71, 30.89, 16.05, 8.24, 7.75. MS (ESI, $[M + H]^+$) m/z 580.1. HRMS (ESI) calcd for $C_{29}H_{32}N_5O_6S$, 578.2079; found, 578.2082.

*(±)-N-(cis-3-(5-Chlorothiophen-2-ylsulfonyl)-6-(4-methoxybenzyl)-7-oxo-2,3,4,4a,5,6,7,12a-octahydro-1H-benzo[b]pyrido[3,4-g][1,5]oxazocin-11-yl)cyclopropanecarboxamide (**29e**).* White solid (56 mg, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (d, J = 7.6 Hz, 1H), 7.64 (s, 1H), 7.33 – 7.25 (m, 4H), 6.99 – 6.94 (m, 2H), 6.88 (d, J = 8.4 Hz, 2H), 5.13 (d, J = 14.4 Hz, 1H), 4.62 (s, 1H), 4.28 (d, J = 14.4 Hz, 1H), 3.84 – 3.79 (m, 4H), 3.55 – 3.51 (m, 1H), 3.24 (dd, J = 14.8, 12.4 Hz, 1H), 3.07 (dd, J = 14.8, 4.8 Hz, 1H), 2.76 (t, J = 12.4 Hz, 1H), 2.42 (t, J = 11.6 Hz, 1H), 2.21 – 2.13 (m, 2H), 1.92 – 1.85 (m, 1H). 1.28 – 1.23 (m, 1H), 1.05 – 0.98 (m, 2H), 0.87 – 0.81 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.40, 169.54, 159.41, 143.58, 137.84, 134.09, 132.15, 129.58, 128.48, 127.28, 127.18, 122.62, 121.59, 120.27, 114.32, 69.30, 55.33, 49.17, 46.01, 43.78, 41.33, 37.82, 30.73, 16.04, 8.09, 7.93. MS (ESI, $[M + H]^+$) m/z 616.1. HRMS (ESI) calcd for $C_{29}H_{29}ClN_3O_6S_2$, 614.1192; found, 614.1196.

*(±)-11-(Cyclopropanecarboxamido)-6-(4-methoxybenzyl)-7-oxo-N-(4-(trifluoromethyl)phenyl)-4,4a,5,6,7,12a-hexahydro-1H-benzo[b]pyrido[3,4-g][1,5]oxazocine-3(2H)-carboxamide (**29f**).* White solid (45 mg, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.93 (s, 1H), 7.50 – 7.46 (m, 4H), 7.32 (d, J = 7.6 Hz, 1H), 7.21 (d, J = 8.0 Hz, 2H), 7.09 – 6.95 (m, 2H), 6.85 (d, J = 8.0 Hz, 2H), 5.15 (d, J = 14.4 Hz, 1H), 4.64 (s, 1H), 4.19 (d, J = 14.4 Hz, 1H), 4.08 (d, J = 12.8 Hz, 1H), 3.89 (d, J = 13.2 Hz, 1H), 3.78 (s, 3H), 3.31 – 3.19 (m, 2H), 3.03 (d, J = 14.8 Hz, 1H), 2.90 – 2.87 (m, 1H), 2.08 – 1.99 (m, 2H), 1.78 – 1.70 (m, 1H), 1.52 – 1.46 (m, 1H), 1.09 – 1.05 (m, 2H), 0.89 – 0.84 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.71, 169.61, 159.37, 154.25, 144.07, 142.01, 129.43, 128.55, 127.41, 126.20 (q, J = 2.9 Hz), 125.05 (q, J = 33 Hz), 124.19 (q, J = 272 Hz), 122.90, 121.51, 120.43, 119.17, 114.29, 70.57, 55.31, 49.15, 46.22, 42.15, 39.92, 37.78, 31.28, 16.30, 8.31, 8.24. MS (ESI, $[M + H]^+$) m/z 623.2. HRMS (ESI) calcd for $C_{33}H_{34}F_3N_4O_5$, 623.2476; found, 623.2473.

*(±)-11-(cyclopropanecarboxamido)-N-(2,4-difluorophenyl)-6-(4-methoxybenzyl)-7-oxo-4,4a,5,6,7,12a-hexahydro-1H-benzo[b]pyrido[3,4-g][1,5]oxazocine-3(2H)-carboxamide (**29g**).* White solid (41 mg, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, *J* = 7.6 Hz, 1H), 7.95 – 7.86 (m, 2H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.24 (d, *J* = 8.0 Hz, 2H), 6.98 (t, *J* = 8.0 Hz, 1H), 6.88 – 6.83 (m, 4H), 6.58 (s, 1H), 5.17 (d, *J* = 14.4 Hz, 1H), 4.71 (s, 1H), 4.23 (d, *J* = 14.4 Hz, 1H), 4.04 (d, *J* = 12.8 Hz, 1H), 3.90 (d, *J* = 12.8 Hz, 1H), 3.79 (s, 3H), 3.39 – 3.27 (m, 2H), 3.13 – 3.08 (m, 1H), 2.93 (t, *J* = 12.4 Hz, 1H), 2.18 – 2.05 (m, 2H), 1.85 – 1.78 (m, 1H), 1.52 – 1.47 (m, 1H), 1.10 – 1.07 (m, 2H), 0.90 – 0.85 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.68, 169.61, 159.34, 158.22 (dd, *J* = 246, 12 Hz), 154.32, 152.94 (dd, *J* = 245, 12 Hz), 144.02, 129.46, 128.61, 127.48, 127.31, 123.34 (d, *J* = 9 Hz), 123.25, 122.77, 121.46, 120.39, 114.27, 111.23 (dd, *J* = 21, 3 Hz), 103.46 (dd, *J* = 26, 24 Hz), 70.58, 55.30, 49.11, 46.22, 42.08, 39.94, 37.70, 31.24, 16.29, 8.26, 8.21. MS (ESI, $[M + H]^+$) m/z 591.2. HRMS (ESI) calcd for $C_{32}H_{33}F_2N_4O_5$, 591.2414; found, 591.2419.

*General Procedure for Synthesis of Compounds **32a-c**.* Reduction of compound **26** to the crude amine was conducted under aforementioned standard conditions.

To a solution of 2-(2-fluoro-5-nitrophenyl)acetic acid (120 mg, 0.6 mmol) in CH_2Cl_2 (5 mL) was added HATU (380 mg, 1.0 mmol) and HOAT (78 mg, 0.5 mmol). The mixture was stirred at rt for 20 min, then the crude amine (232 mg, 0.5 mmol) prepared above and DIPEA (194 mg, 1.5 mmol) were added. The reaction was stirred at rt for 6 h and then treated with saturated $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layer was washed with water, dried over Na_2SO_4, filtered, and then concentrated in vacuo to give the crude product as pale foam.

Without purification, the crude product was dissolved in THF (5 mL), NH_4F (93 mg, 2.5 mmol) and TBAF.$3H_2O$ (788 mg, 2.5 mmol) were added. The reaction was stirred at 40 °C for 12 h, then diluted with water (20 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was washed with water three times, dried with $MgSO_4$, filtered, and then concentrated in vacuo. The residue was purified by silica column chromatography (petroleum ether/EtOAc 3:1) to give compound **30** as

white solid (61 mg, 38%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (d, J = 2.8 Hz, 1H), 8.16 (dd, J = 8.8, 2.8 Hz, 1H), 7.25 (d, J = 8.4 Hz, 2H), 7.12 (d, J = 8.8 Hz, 1H), 6.86 (d, J = 8.4 Hz, 2H), 5.04 (d, J = 14.4 Hz, 1H), 4.54 (d, J = 12.4 Hz, 1H), 4.11 (dd, J = 15.2, 10.4 Hz, 1H), 4.02 – 3.94 (m, 2H), 3.82 – 3.78 (m, 5H), 3.41 (t, J = 12.4 Hz, 1H), 3.28 (d, J = 12.4 Hz, 1H), 3.21 (dd, J = 14.8, 4.8 Hz, 1H), 3.10 (t, J = 12.0 Hz, 1H), 2.14 – 2.09 (m, 1H), 1.97 – 1.94 (m, 1H), 1.63 – 1.58 (m, 1H), 1.51 (s, 9H).

Reduction of **30** and acylation of the resulting aniline were conducted by following procedures similar to that of preparation of compound **28** from **27**. The target compound **31** was obtained as white solid (34 mg, 72%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, J = 8.4 Hz, 1H), 7.33 (s, 1H), 7.21 (d, J = 8.1 Hz, 2H), 6.93 (d, J = 8.4 Hz, 1H), 6.82 (d, J = 8.1 Hz, 2H), 5.04 (d, J = 14.4 Hz, 1H), 4.47 (d, J = 12.3 Hz, 1H), 4.16 – 4.07 (m, 1H), 4.02 – 3.60 (m, 7H), 3.45 – 3.37 (m, 1H), 3.14 – 3.08 (m, 3H), 2.08 – 1.90 (m, 3H), 1.54 – 1.47 (m, 10H), 1.08 – 1.00 (m, 2H), 0.88 – 0.78 (m, 2H).

Conversion of **31** (45 mg, 0.1 mmol) to the final products **32a-c** was conducted by following procedures similar to that of preparation of compounds **29a-e** from **28**.

*(±)-N-(cis-3-(4-Chlorophenylsulfonyl)-6-(4-methoxybenzyl)-7-oxo-1,2,3,4,4a,5,6,7,8,13a-decah ydrobenzo[h]pyrido[4,3-b][1,5]oxazonin-10-yl)cyclopropanecarboxamid (**32a**).* White solid (51 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD) δ 7.92 (dd, J = 8.8, 2.4 Hz, 1H), 7.69 (d, J = 8.4 Hz, 2H), 7.54 (d, J = 8.4 Hz, 2H), 7.21(d, J = 8.4 Hz, 2H), 7.07 (d, J = 2.4 Hz, 1H), 6.87 (d, J = 8.4 Hz, 2H), 6.82 (d, J = 8.8 Hz, 1H), 4.96 (d, J = 14.4 Hz, 1H), 4.37 (d, J = 8.8 Hz, 1H), 4.11 – 4.05 (m, 2H), 3.82 (s, 3H), 3.55 – 3.49 (m, 2H), 3.27 (d, J = 8.6 Hz, 1H), 3.18 (dd, J = 15.2, 4.8 Hz, 1H), 3.04 – 2.95 (m, 2H), 2.60 – 2.55 (m, 1H), 2.19 – 2.11 (m, 2H), 1.74 – 1.59 (m, 2H), 1.04 – 0.97 (m, 2H), 0.88 – 0.80 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$/CD_3OD) δ 172.95, 172.33, 159.34, 152.30, 139.84, 136.11, 134.25, 129.87, 129.71, 129.35, 129.02, 128.72, 122.93, 122.45, 120.54, 114.22, 76.89, 55.38, 48.51, 46.90, 44.95, 41.76, 38.95, 35.29, 29.44, 15.10, 7.72. MS (ESI, $[M + Na]^+$) m/z 646.2. HRMS (ESI) calcd for $C_{32}H_{33}ClN_3O_6S$, 622.1784; found, 622.1784.

*(±)-N-(cis-6-(4-Methoxybenzyl)-3-(1-methyl-1H-pyrazol-3-ylsulfonyl)-7-oxo-1,2,3,4,4a,5,6,7,8, 13a-decahydrobenzo[h]pyrido[4,3-b][1,5]oxazonin-10-yl)cyclopropanecarboxamide (**32b**).* White solid (47 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.71 (d, J = 8.8 Hz, 1H), 7.45 (d, J = 2.4 Hz, 1H), 7.31 (d, J = 2.4 Hz, 1H), 7.21 (d, J = 8.4 Hz, 2H), 6.88 – 6.83 (m, 3H), 6.66 (d, J = 2.4 Hz, 1H), 5.01 (d, J = 14.4 Hz, 1H), 4.40 (d, J = 12.0 Hz, 1H), 4.08 (dd, J = 14.8, 10.4 Hz, 1H), 4.02 – 3.98 (m, 4H), 3.80 (s, 3H), 3.63 – 3.60 (m, 2H), 3.41 (d, J = 11.2 Hz, 1H), 3.19 – 3.05 (m, 3H), 2.81 (t, J = 11.2 Hz, 1H), 2.19 – 2.11 (m, 2H), 1.73 – 1.66 (m, 1H), 1.53 – 1.47 (m, 1H), 1.04 – 0.97 (m, 2H), 0.79 – 0.73 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.04, 171.34, 159.21, 152.67, 147.22, 135.51, 131.84, 130.15, 129.82, 128.93, 123.44, 122.47, 120.43, 114.11, 108.17, 77.05, 55.31, 48.24, 46.77, 45.01, 41.64, 39.99, 38.94, 35.47, 29.46, 15.54, 7.92, 7.86. MS (ESI, $[M + Na]^+$) m/z 616.3. HRMS (ESI) calcd for $C_{30}H_{35}N_5NaO_6S$, 616.2200; found, 616.2215.

*(±)-N-(cis-3-(5-Chlorothiophen-2-ylsulfonyl)-6-(4-methoxybenzyl)-7-oxo-1,2,3,4,4a,5,6,7,8,13a -decahydrobenzo[h]pyrido[4,3-b][1,5]oxazonin-10-yl)cyclopropanecarboxamide (**32c**).* White solid (58 mg, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (s, 1H), 7.75 (d, J = 8.4 Hz, 1H), 7.34 (d, J = 4.0 Hz, 1H), 7.32 (d, J = 2.8 Hz, 1H), 7.22 (d, J = 8.6 Hz, 2H), 7.01 (d, J = 4.0 Hz, 1H), 6.88 – 6.85 (m, 3H), 4.99 (d, J = 14.4 Hz, 1H), 4.42 (d, J = 12.0 Hz, 1H), 4.16 – 4.07 (m, 2H), 3.82 (s, 3H), 3.64 – 3.57 (m, 2H), 3.33 (d, J = 11.2 Hz, 1H), 3.18 (dd, J = 14.8, 4.8 Hz, 1H), 3.08 – 3.02 (m, 2H), 2.69 (t, J = 10.8 Hz, 1H), 2.24 – 2.14 (m, 2H), 1.75 – 1.62 (m, 1H), 1.54 – 1.49 (m, 1H), 1.07 – 1.04 (m, 2H), 0.84 – 0.76 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.97, 171.32, 159.25, 152.60, 137.70, 135.55, 134.16, 131.83, 130.14, 129.78, 128.92, 127.26, 123.40, 122.49, 120.40, 114.15, 76.69, 55.31, 48.31, 46.81, 44.84, 41.64, 38.95, 35.44, 29.40, 15.58, 7.95, 7.88. MS (ESI, $[M + Na]^+$) m/z 652.2. HRMS (ESI) calcd for $C_{30}H_{31}ClN_3O_6S_2$, 628.1348; found, 628.1341.

*General Procedure for Synthesis of Compounds **35a-b**.* Reduction of compound **9e** to the crude amine was conducted under aforementioned standard conditions.

Condensation of the crude amine (0.5 mmol) and 2-(2-fluoro-5-nitrophenyl)acetic acid (120 mg,

0.6 mmol) was conducted by following a procedure similar to that of preparation of compound **30**.

The crude amide was dissolved in THF (5 mL), NH_4F (93 mg, 2.5 mmol) and TBAF.$3H_2O$ (788 mg, 2.5 mmol) were added. The reaction was stirred at 40 °C for 4 h, and then diluted with water (20 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was washed with water three times, dried with Na_2SO_4, filtered, and concentrated in vacuo. The residue was dissolved in DMF (5 mL) and Na_2CO_3 (2 eq) was added. The reaction was stirred at 80 °C for 3 h, and then diluted with water (20 mL) and extracted with EtOAc (3 × 10 mL). The organic layer was washed with water three times, dried with Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica column chromatography (petroleum ether/EtOAc 3:1) to give the corresponding compound **33** as white solid (30 mg, 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, J = 2.8 Hz, 1H), 7.96 (dd, J = 8.8, 2.8 Hz, 1H), 7.49 – 7.34 (m, 5H), 7.21 (d, J = 8.4 Hz, 2H), 6.86 (d, J = 8.4 Hz, 2H), 6.61 (d, J = 8.8 Hz, 1H), 5.08 (d, J = 14.4 Hz, 1H), 4.55 (d, J = 12.0 Hz, 1H), 4.21 (dd, J = 14.8, 10.8 Hz, 1H), 3.95 (d, J = 14.4 Hz, 1H), 3.89 (d, J = 12.8 Hz, 1H), 3.82 (s, 3H), 3.48 (s, 1H), 3.42 (d, J = 12.8 Hz, 1H), 3.24 – 3.12 (m, 4H), 2.26 – 2.21 (m, 1H), 2.07 – 1.83 (m, 3H), 1.56 – 1.53 (m, 1H).

Reduction of **33** to the corresponding aniline was conducted by following a procedure similar to that of reduction of compound **30**.

The corresponding aniline (233 mg, 0.5 mmol) was dissolved in CH_2Cl_2 (5 mL) at 0 °C, then 2,6-lutidine (107 mg, 1.0 mmol) and cyclopropanecarbonyl chloride (63 mg, 0.6 mmol) were slowly added. The reaction was sitrred for 4 h at 0 °C and white solid was formed. The mixture was filtered and the residue was dried to give compound **34** as white solid (211 mg, 83%). Compound **34** was used for the the next step without further purification.

Conversion of **34** (45 mg, 0.1 mmol) to the final products **35a-b** was conducted by following a procedure similar to that of preparation of compound **22** from **14e**.

*(±)-N-(cis-2-(4-Chlorophenylsulfonyl)-6-(4-methoxybenzyl)-7-oxo-1,2,3,4,4a,5,6,7,8,13a-decah ydrobenzo[h]pyrido[3,4-b][1,5]oxazonin-10-yl)cyclopropanecarboxamid (**35a**).* White solid (45 mg,

73%). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$) δ 7.85 – 7.76 (m, 3H), 7.51 (d, J = 8.4 Hz, 2H), 7.21 – 7.09 (m, 4H), 6.82 (d, J = 8.4 Hz, 2H), 5.01 (d, J = 14.4 Hz, 1H), 4.45 (d, J = 12.0 Hz, 1H), 4.27 – 4.17 (m, 2H), 3.96 – 3.87 (m, 2H), 3.78 (s, 3H), 3.41 (s, 1H), 3.14 – 3.03 (m, 2H), 2.44 (t, J = 12.0 Hz, 1H), 2.27 (d, J = 13.6 Hz, 1H), 1.87 – 1.61 (m, 3H), 1.48 – 1.44 (m, 1H), 1.07 – 1.04 (m, 2H), 0.85 – 0.82 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3/CD_3OD$) δ 172.50, 172.27, 159.23, 152.96, 139.46, 136.11, 135.82, 129.73, 129.58, 129.50, 129.01, 128.83, 123.43, 122.84, 120.70, 114.13, 76.21, 55.34, 49.47, 48.76, 48.02, 45.09, 37.79, 35.38, 23.77, 15.20, 7.70. MS (ESI, $[M + Na]^+$) m/z 646.3. HRMS (ESI) calcd for $C_{32}H_{33}ClN_3O_6S$, 622.1784; found, 622.1773.

(±)-N-(cis-6-(4-Methoxybenzyl)-2-(1-methyl-1H-pyrazol-3-ylsulfonyl)-7-oxo-1,2,3,4,4a,5,6,7,8, 13a-decahydrobenzo[h]pyrido[3,4-b][1,5]oxazonin-10-yl)cyclopropanecarboxamide ***(35b)****.* White solid (48 mg, 81%). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$) δ 7.80 (dd, J = 8.9, 2.4 Hz, 1H), 7.47 (d, J = 2.4 Hz, 1H), 7.22 (d, J = 2.4 Hz, 1H), 7.17 – 7.15 (m, 3H), 6.83 (d, J = 8.4 Hz, 2H), 6.68 (d, J = 2.4 Hz, 1H), 5.05 (d, J = 14.4 Hz, 1H), 4.46 (d, J = 12.4 Hz, 1H), 4.30 (d, J = 13.2 Hz, 1H), 4.28 – 4.18 (m, 1H), 4.01 – 3.97 (m, 4H), 3.92 (d, J = 14.4 Hz, 1H), 3.79 (s, 3H), 3.43 (s, 1H), 3.14 (dd, J = 14.8, 4.8 Hz, 1H), 3.04 (d, J = 12.4 Hz, 1H), 2.64 (t, J = 12.0 Hz, 1H), 2.48 (d, J = 13.2 Hz, 1H), 1.94 – 1.79 (m, 2H), 1.65 – 1.48 (m, 1H), 1.52 – 1.47 (m, 1H), 1.06 – 1.03 (m, 2H), 0.84 – 0.81 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3/CD_3OD$) δ 172.72, 172.33, 159.14, 153.03, 148.33, 135.91, 131.91, 129.64, 129.50, 128.79, 123.33, 122.95, 120.65, 114.06, 107.70, 76.19, 55.26, 49.43 , 48.98, 47.88, 45.22, 39.72, 37.71, 35.34, 23.83, 15.03, 7.56, 7.52. MS (ESI, $[M + Na]^+$) m/z 616.3. HRMS (ESI) calcd for $C_{30}H_{34}N_5O_6S$, 592.2235; found, 592.2238.

General Procedure for Synthesis of Compounds ***39a-c****.* To a solution of 2-fluoro-3-nitrobenzoic acid (111 mg, 0.6 mmol) in THF (5 mL) was added HATU (228 mg, 0.6 mmol). The mixture was stirred at rt for 20 min, then *tert*-butyl 3-(hydroxymethyl)piperazine-1-carboxylate **36** (108 mg, 0.5 mmol) and Et_3N (252 mg, 2.5 mmol) were added. The reaction was stirred at rt for 4 h and then treated with saturated $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined

organic layer was washed with water, dried over Na_2SO_4, filtered, and then concentrated in vacuo to give the amide precursor as white solid.

Without purification, a solution of the amide precursor (383 mg, 1.0 mmol) prepared above in DMF (10 mL) was cooled to 0 ℃, NaH (120 mg, 3.0 mmol) was added. The reaction was stirred at rt for 4 h, quenched with ice-water and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layer was washed with water and brine, dried over Na_2SO_4, filtered, and then concentrated in vacuo to give **37** as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 8.0 Hz, 1H), 7.92 (dd, J = 8.0, 1.6 Hz, 1H), 7.33 (t, J = 8.0 Hz, 1H), 4.49 – 4.43 (m, 2H), 4.08 – 3.90 (m, 3H), 3.74 – 3.64 (m, 4H), 1.49 (s, 9H).

Reduction of **37** and acylation of the resulting aniline were conducted by following procedures similar to that of preparation of compound **28** from **27**. The target compound **38** was obtained as white solid (38 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (d, J = 8.0 Hz, 1H), 8.03 (s, 1H), 7.48 (dd, J = 8.0, 1.6 Hz, 1H), 7.17 (t, J = 8.0 Hz, 1H), 4.40 – 4.35 (m, 1H), 4.27 (dd, J = 10.8, 4.0 Hz, 1H), 4.10 (dt, J = 14.0, 5.2 Hz, 1H), 3.97 – 3.94 (m, 1H), 3.85 – 3.81 (m, 1H), 3.68 – 3.61 (m, 4H), 1.61 – 1.55 (m, 1H), 1.49 (s, 9H), 1.11 – 1.07 (m, 2H), 0.92 – 0.84 (m, 2H).

Conversion of **38** (40 mg, 0.1 mmol) to final products **39a-c** was conducted by following a procedure similar to that of preparation of compounds **29a-e** from **28**.

*N-(2-(4-Chlorophenylsulfonyl)-6-oxo-2,3,4,6,12,12a-hexahydro-1H-benzo[f]pyrazino[2,1-c][1,4]oxazepin-10-yl)cyclopropanecarboxamide (**39a**)*. White solid (43 mg, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (d, J = 8.0 Hz, 1H), 8.04 (s, 1H), 7.69 (d, J = 8.4 Hz, 2H), 7.57 (dd, J = 8.0, 1.6 Hz, 1H), 7.53 (d, J = 8.4 Hz, 2H), 7.13 (t, J = 8.0 Hz, 1H), 4.60 (dd, J = 11.6, 8.8 Hz, 1H), 4.27 (dd, J = 11.8, 3.6 Hz, 1H), 4.23 – 4.17 (m, 1H), 3.98 – 3.93 (m, 1H), 3.81 – 3.75 (m, 1H), 3.27 (dd, J = 12.0, 4.0 Hz, 1H), 3.19 – 3.15 (m, 2H), 3.09 (dd, J = 12.0, 6.0 Hz, 1H), 1.64 – 1.58 (m, 1H), 1.12 – 1.04 (m, 2H), 0.91 – 0.86 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.18, 166.38, 144.13, 140.18, 133.82, 130.41, 129.80, 128.99, 125.76, 125.68, 124.49, 123.81, 73.38, 54.25, 45.77, 45.38, 41.26, 15.98,

8.34, 8.30. MS (ESI, $[M + H]^+$) m/z 476.2. HRMS (ESI) calcd for $C_{22}H_{21}ClN_3O_5S$, 474.0896; found, 474.0899.

*N-(2-(1-Methyl-1H-pyrazol-3-ylsulfonyl)-6-oxo-2,3,4,6,12,12a-hexahydro-1H-benzo[f]pyrazino [2,1-c][1,4]oxazepin-10-yl)cyclopropanecarboxamide (**39b**).* White solid (43 mg, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, J = 8.0 Hz, 1H), 8.12 (s, 1H), 7.59 (dd, J = 8.0, 1.6 Hz, 1H), 7.47 (d, J = 2.4 Hz, 1H), 7.13 (t, J = 8.0 Hz, 1H), 6.67 (d, J = 2.4 Hz, 1H), 4.61 (dd, J = 11.6, 8.8 Hz, 1H), 4.29 (dd, J = 11.8, 3.6 Hz, 1H), 4.23 – 4.15 (m, 1H), 4.02 – 3.96 (m, 4H), 3.83 – 3.77 (m, 1H), 3.47 (dd, J = 12.4, 4.0 Hz, 1H), 3.34 – 3.31 (m, 2H), 3.26 (dd, J = 12.4, 6.0 Hz, 1H), 1.67 – 1.61 (m, 1H), 1.11 – 1.07 (m, 2H), 0.91 – 0.85 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.27, 166.50, 146.87, 144.12, 132.07, 130.52, 125.89, 125.55, 124.35, 123.67, 108.35, 73.69, 54.18, 45.71, 45.43, 41.10, 40.02, 15.95, 8.30, 8.29. MS (ESI, $[M + Na]^+$) m/z 468.2. HRMS (ESI) calcd for $C_{20}H_{22}N_5O_5S$, 444.1347; found, 444.1353.

*N-(2-(5-Chlorothiophen-2-ylsulfonyl)-6-oxo-2,3,4,6,12,12a-hexahydro-1H-benzo[f]pyrazino[2, 1-c][1,4]oxazepin-10-yl)cyclopropanecarboxamide (**39c**).* White solid (45 mg, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, J = 8.0 Hz, 1H), 8.06 (s, 1H), 7.61 (dd, J = 8.0, 1.6 Hz, 1H), 7.35 (d, J = 4.0 Hz, 1H), 7.14 (t, J = 8.0 Hz, 1H), 7.00 (d, J = 4.0 Hz, 1H), 4.60 (dd, J = 11.6, 8.4 Hz, 1H), 4.32 – 4.22 (m, 2H), 4.02 – 3.96 (m, 1H), 3.82 – 3.76 (m, 1H), 3.34 (dd, J = 12.0, 4.0 Hz, 1H), 3.29 – 3.19 (m, 2H), 3.14 (dd, J = 12.0, 6.0 Hz, 1H), 1.66 – 1.59 (m, 1H), 1.11 – 1.08 (m, 2H), 0.91 – 0.87 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.18, 166.31, 144.24, 138.40, 133.32, 132.42, 130.42, 127.45, 125.76, 125.66, 124.49, 123.83, 73.40, 54.31, 45.88, 45.43, 41.23, 15.98, 8.34, 8.30. MS (ESI, $[M + H]^+$) m/z 481.3. HRMS (ESI) calcd for $C_{20}H_{19}ClN_3O_5S_2$, 480.0460; found, 480.0465.

ShhN Conditioned Medium (CM) Preparation. ShhN CM was prepared as previously described.[42] Briefly, after transfection with GFP or plasmid containing the N-terminal signaling domain of the Shh (ShhN), HEK293 cells were changed to be cultured with medium containing 0.1% fetal bovine serum. The CM was then collected for stimulating the Hh pathway activity.

Dual Luciferase Reporter Assay. After various treatments as indicated, cells seeded in 96-well plates were lysed for dual luciferase reporter assay using a kit from Promega according to the manufacturer's instructions in a luminometer (Molecular Devices; Sunnyvale, CA). The firefly luciferase values were normalized to Renilla values.

Reverse Transcription and Quantitative Polymerase Chain Reaction (RT-qPCR). After various indicated treatment, the cells were collected, and total RNA were extracted using an RNAiso Plus Kit (TaKaRa; Dalian, China) according to the manufacturer's instructions. The obtained total RNA was further subjected to reversely transcription to obtain cDNA with a SuperScript III Kit (TaKaRa). The quantitative PCR analyses were conducted in triplicate with a SYBR Green Kit (TaKaRa) in an iCycler iQ system (Bio-Rad; Hercules, CA) using the following primers:

mGUSB: 5'-CTGCCACGGCGATGGA-3';

5'-ACTGCATAATAATGGGCACTGTTG- 3';

mGli1: 5'-GCAGTGGGTAACATGAGTGTCT-3';

5′ -AGGCACTAGAGTTGAGGAATTGT-3'.

mptch1: 5' –GCTACGACTATGTCTCTCACATCAACT-3'

5'-GGCGACACTTTGATGAACCA-3'

The mRNA level of GUSB was used for normalizing the mRNA level of interested genes.

Lentivirus

Lentivirus-mediated Sufu shRNA constructs were obtained from Sunbio. The viral stocks were prepared, and infections were performed according to previous report.[42]

Western blot analysis

Cells were lysed in lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1 % NP-40, 1 mM sodium vanadate, 1 mM PMSF, 1 mM DTT, 10 mg/ml of leupeptin and aprotinin). The protein were then used for immunoblot analysis. Primary antibodies against Sufu and GAPDH (Santa Cruz Biotechnology; Dallas, TX) were used for immunoblot analysis according to the routine procedure.

Densitometric values of immunoreactive bands were semi-quantitative analysis by Image J and were normalized to those of GAPDH.

EMSA

EMSA experiments using whole-cell extracts of Flag-Gli1 overexpressing NIH-3T3 cells were conducted using a commercial kit from Viagene Biotech Co. (Changzhou, China) following the manufacturer's protocol with the on biotin-labeled Gli binding sequence (biotin-twist1-forward: 5'-AGATGAGACATCACCCACTGTGTAG-3'; biotin-twist1-reverse: 5'-CTACACAGTGGGTGATGTCTCATCT-3') in Gli target genes *twist1*.[45]

Medulloblastoma Cells Culture and MTS Assay. Medulloblastoma cells were obtained from Ptch+/-;p53-/- mice as previously reported.[42] Briefly, mechanically minced medulloblastoma allografts were digested by collagenase. The cells were routinely cultured using Neurobasal A medium (Invitrogen) plus B-27 supplement (Invitrogen), EGF 20 ng/ml (Invitrogen), bFGF 20 ng/ml (Invitrogen), nonessential amino acids, N-acetyl cysteine 60 μg/ml. Medulloblastoma cells were seeded into 96-well plates and exposed to various compounds as indicated for 72 h. MTS (3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethonyphenol)-2-(4-sulfonylphenyl)-2H-tetrazolium) assays were performed using the MTS Cell Proliferation Kit (Merck Millipore; Bedford, MA) according to manufacturer's instructions.

Medulloblastoma Allograft Model and TUNEL assay. Mouse medulloblastoma model was established as previously described using Ptch+/-; p53-/- mouse.[42] The primary intracranial medullblastomas spontaneously developed in Ptch+/-; p53-/- mice were harvested and subcutaneously allografted into athymic nude mice (Beijing HFK Bio-Technology; Beijing, China). The tumors in nude mice were further collected, cut into 1 mm^3 fragments and inoculated subcutaneously into the right flank of athymic nude mice. When the tumor volume reached 100–150 mm^3, the mice were administered with vehicle, compound **2**, or compound **29a**. Both compounds were formulated as a suspension in 0.5% methylcellulose, 0.2% Tween-80. The volume of the tumors

was measured twice per week using microcaliper and calculated as 'Volume = [length (mm) ×width^2 (mm^2)]/2. The tumor growth inhibition (TGI) was calculated on the last day of the study by comparing the tumor volume of treated mice with that of the vehicle control mice with the following formula: 100 × {1 − [(tumor volume of treated mice at last day – tumor volume of treated mice at day 0) / (tumor volume of control mice at final day − tumor volume of control mice at day 0)]}. Once the termination of the experiment, the tumor tissues were collected and fixed by formalin for performing TUNEL assays using the kit from Boster (Wuhan, China). All procedures were pre-approved by the Animal Care and Use Committee of Fudan University and performed according to institutional policies.

ASSOCIATED CONTENT

Supporting Information Available. The Supporting information is available free of charge via the Internet at http://pubs.acs.org.

^{1}H and ^{13}C spectra of all new compounds (PDF)

Molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

Hh, hedgehog; Ptch, Patched; Smo, Smoothened; BCC, basal cell carcinoma; MB, medulloblastoma; HSQC, heteronuclear single quantum coherence; TFA, trifluoroacetic acid; DIPEA, diisopropyl ethylamine; HATU, 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate; HOAT, 1-hydroxy-7-azabenzotriazole; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodimide; DMAP, dimethylaminopyridine; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; TBSOTf, *tert*-butyldimethylsilyl trifluoromethanesulfonate; BH_3.DMS, borane dimethylsulfide; TBAF, tetrabutylammonium fluoride; NOE, nuclear overhauser effect; HSQC, heteronuclear single-quantum correlation; PK, pharmacokinetic; SAR, structure-activity relationship; hERG, human Ether-a-go-go Related Gene; Shh CM, the Shh conditioned medium; TNF-α, tumor necrosis factor-α; PGE2, prostaglandin E2; TCF, T-cell factor; LEF, lymphoid enhancer factor; EMSA: electrophoretic mobility shift assay; TGI, tumor growth inhibition; i.p., intraperitoneal; i.v., intravenous; RT-qPCR, Reverse transcription and quantitative polymerase chain reaction; TUNEL, terminal deoxynucleotidyl transferase mediated dUTP nick-end Labeling.

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