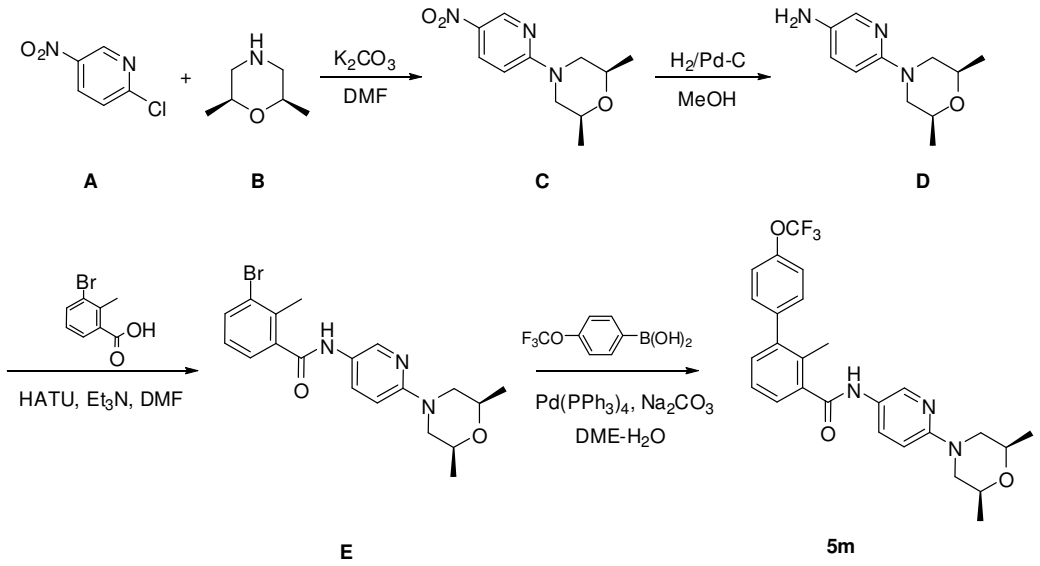
Supporting Information

Discovery of NVP-LDE225, a Potent and Selective Biphenyl-3-carboxamide Smoothened Antagonist

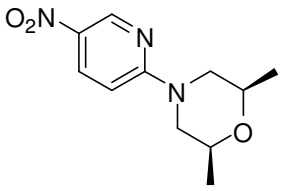
Shifeng Pan,* Xu Wu, Jiqing Jiang, Wenqi Gao, Yongqin Wan, Dai Cheng, Dong Han, Jun Liu, Nathan P. Englund, Yan Wang, Stefan Peukert, Karen Miller-Moslin, , Jing Yuan, Ribo Guo, Melissa Matsumoto, Anthony Vattay, Yun Jiang, Jeffrey Tsao, Fangxiang Sun, AnneMarie C. Pferdekamper, Stephanie Dodd, Tove Tuntland, Wieslawa Maniara, Joseph F. Kelleher, III, Yung-mae Yao, Markus Warmuth, Juliet Williams and Marion Dorsch

Contents include synthetic procedures for compound **5m**, analytical data for final compounds in Table 1, experimental details for in vitro assays presented in Table 1 and in vivo animal models.

Synthetic scheme for 5m



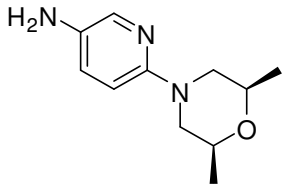
(2S,6R)-2,6-dimethyl-4-(5-nitropyridin-2-yl)morpholine (**C**)



To a solution of 2-chloro-5-nitro-pyridine **A** (5.58 g, 35.2 mmoL) and *cis*-2,6-dimethylmorpholine (4.05 g, 35.2 mmoL) in anhydrous DMF (30 mL) was added K_2CO_3 (9.71 g, 70.4 mmoL). The mixture was heated at 50^0C overnight. After concentration, the residue is partitioned between EtOAc and water. The EtOAc layer is dried over anhydrous Na_2SO_4 and

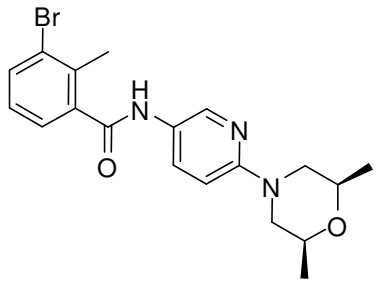
concentrated to give crude product **C** as a yellow solid, after purification by silica gel chromatography, obtained pure product (7.80 g, 93.2%). LC-MS *m/z*: 238.2 (M+1).

6-((2S,6R)-2,6-dimethylmorpholino)pyridin-3-amine (**D**)



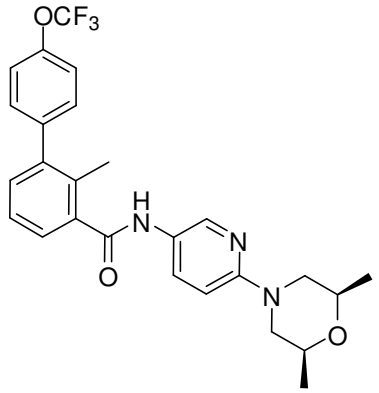
Intermediate **C** (7.30g, 30.8 mmoL) was hydrogenated in the presence of 10% Pd-C (1.0 g) in MeOH (120 mL) under hydrogen overnight. The suspension was filtered through celite and the filtrate was concentrated to give the crude product **4** (5.92 g) as a dark brown oil which was used directly in the next step without further purification. LC-MS *m/z*: 208.2 (M+1).

3-bromo-N-(6-((2S,6R)-2,6-dimethylmorpholino)pyridin-3-yl)-2-methylbenzamide (**E**)



To a solution of 3-bromo-2-methyl benzoic acid (2.71 g, 12.6 mmoL), 6-((2S,6R)-2,6-dimethylmorpholino)pyridin-3-amine **D** (2.61 g, 12.6 mmoL), and HATU (4.80 g, 12.6 mmoL) in anhydrous DMF (30 mL) was added diisopropylethylamine (6.58 mL, 37.8 mmoL) dropwise. The resulting mixture was stirred overnight at room temperature. The reaction mixture was diluted with water (50 mL), and then extracted with EtOAc (3x120 mL). The organic layer was dried and concentrated to give the crude product. This crude product was then purified by flash column chromatography using 30% EtOAc in hexane as eluent to give **E** as a white solid (4.23 g, 83.0%). LC-MS *m/z*: 404.1 (M+1).

N-(6-((2S,6R)-2,6-dimethylmorpholino)pyridin-3-yl)-2-methyl-4'-(trifluoromethoxy)biphenyl-3-carboxamide (**5m**)



A mixture of 4-(trifluoromethoxy)phenylboronic acid (254 mg, 1.24 mmol), 3-bromo-N-[6-(2,6-dimethyl-morpholin-4-yl)-pyridin-3-yl]-4-methyl-benzamide **E** (250 mg, 0.62mmol), $Pd(PPh_3)_4$ (36 mg, 0.03 mmol), Na_2CO_3 (2.0M aqueous solution, 1.23 mL, 2.4 mmol) and DME (4.5 mL) in a sealed tube was heated at 130^0C overnight. The reaction mixture was diluted with EtOAc and water. The aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine and concentrated to give the crude product which was then purified by preparative mass triggered HPLC (C_{18} column, eluted with CH_3CN-H_2O containing 0.05% TFA) to give N-(6-((2S,6R)-2,6-dimethylmorpholino)pyridin-3-yl)-2-methyl-4'-(trifluoromethoxy)biphenyl-3-carboxamide (**5m**, 183.5 mg, 61.1% yield). LC-MS *m/z*: 486.2 (M+1).

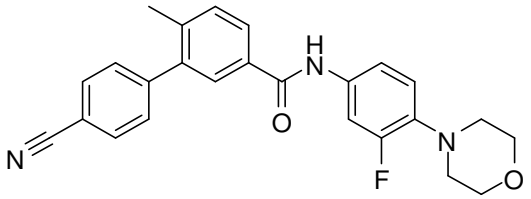
HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{27}N_3O_3F_3$ 486.2005; found 486.1986,

^{1}H-NMR (500 MHz, DMSO-d_6): δ (ppm) 10.15 (s, 1H), 8.43 (d, 1H), 7.94 (dd, 1H), 7.52-7.43 (m, 5H), 7.38 (m, 1H), 7.33 (m, 1H), 6.86 (d, 1H), 4.06 (d, 2H), 3.62 (m, 2H), 2,34 (m, 2H), 2.22 (s, 3H), 1.16 (d, 6H).

Preparation of the diphosphate salt

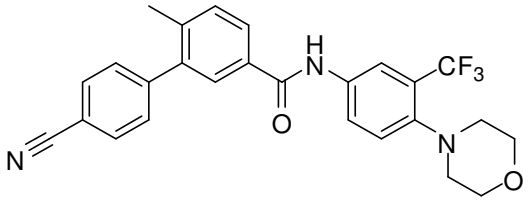
To a 250 mL, three-necked reaction flask 7.0 g (0.0144 mole) of 2-methyl-4'-trifluoromethoxy-biphenyl-3-carboxylic acid [6-(cis-2,6-dimethyl-morpholin-4-yl)-pyridin-3-yl]-amide free base (5m) and acetonitrile (178.5 mL, HPLC grade) was added under nitrogen. The suspension was heated to 58°C under nitrogen over 20 minutes to obtain a clear solution. To the reaction solution 3.403 g of 85% phosphoric acid in water (2 equiv) was added over 18 minutes. Within 5 minutes of the phosphoric acid addition, N-[6-(cis-2,6-dimethylmorpholin-4-yl)pyridine-3-yl]-2-methyl-4'-(trifluoromethoxy)[1,1'-biphenyl]-3-carboxamide di-phosphate precipitates out. The white slurry was stirred and cooled to room temp over 100 minutes. The slurry was then cooled to 0 ± 5°C over 5 minutes and stirred for 1 hour. The mixture was filtered under suction and solid was washed with acetonitrile (3 x 9.4 mL). The drug substance was dried under vacuum at 50°C for 16 hours to obtain 9.63 g of the phosphate salt (yield: 98%).

4'-cyano-N-(3-fluoro-4-morpholinophenyl)-6-methylbiphenyl-3-carboxamide (**5b**)



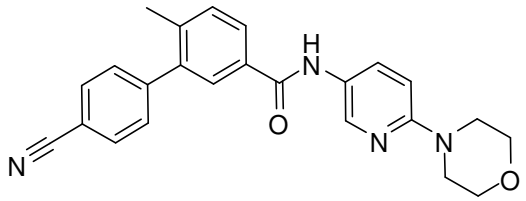
^{1}HNMR (400 MHz, DMSO -d_6): δ (ppm) 10.25 (s, 1H), 7.91-7.98 (m, 3H), 7.84 (s, 1H), 7.65-7.71 (m, 3H), 7.45-7.51 (m, 2H), 7.01 (m, 1H), 3.73 (m, 4H), 2.97 (m, 4H), 2.30 (s, 3H). Exact mass calculated for $C_{25}H_{22}FN_3O_2$: 415.17. Found: LC/MS (ES+) *m/z*=416.2 $[M+H]^+$.

4'-cyano-6-methyl-N-(4-morpholino-3-(trifluoromethyl)phenyl)biphenyl-3-carboxamide (**5c**)



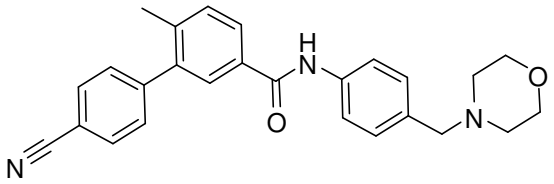
^{1}HNMR (400 MHz, DMSO -d_6): δ (ppm) 10.43 (s, 1H), 8.14 (d, *J*=2.4Hz, 1H), 8.07 (dd, *J*=2.0, 8.8 Hz, 1H), 7.94-7.96 (m, 3H), 7.89 (m, 1H), 7.66 (m, 2H), 7.60 (d, *J*=8.8 Hz, 1H), 7.52 (d, *J*=8.0 Hz, 1H), 3.70 (m, 4H), 2.83 (m, 4H), 2.31 (s, 3H). Exact mass calculated for $C_{26}H_{22}F_3N_3O_2$: 465.17. Found: LC/MS (ES+) m/z=466.2 $[M+H]^+$.

4'-cyano-6-methyl-N-(6-morpholinopyridin-3-yl)biphenyl-3-carboxamide (**5d**)



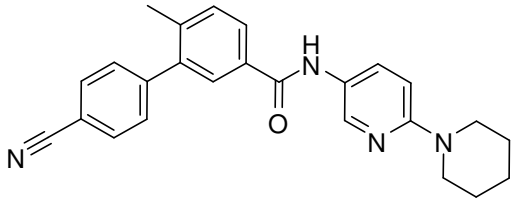
^{1}HNMR (400 MHz, DMSO -d_6): δ (ppm) 10.27 (s, 1H), 8.50 (d, J=2.4 Hz, 1H), 8.01 (dd, *J*=2.4, 9.6 Hz, 1H), 7.98 (m, 2H), 7.90 (dd, *J*=2.0, 8.0 Hz, 1H), 7.87 (d,*J*=1.6 Hz, 1H), 7.66 (m, 2H), 7.52 (m, 1H), 7.05 (d, *J*=9.2 Hz, 1H), 3.72 (t, *J*=4.8 Hz, 4H), 3.45 (t, *J*=4.8 Hz, 4H), 2.31 (s, 3H). Exact mass calculated for $C_{24}H_{22}N_4O_2$: 398.17. Found: LC/MS (ES+) m/z=399.2 $[M+H]^+$.

4'-cyano-6-methyl-N-(4-(morpholinomethyl)phenyl)biphenyl-3-carboxamide HCl salt (**5e**)



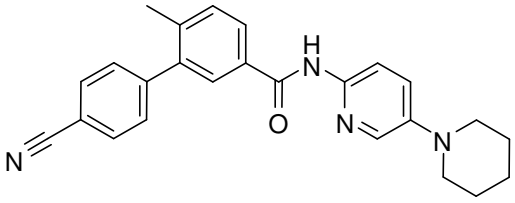
^{1}HNMR (400 MHz, DMSO -d_6): δ (ppm) 10.98 (s, 1H), 10.44 (brs, 1H), 7.96 (d, J = 8.0 Hz, 2H), 7.95 (d, J = 8.4 Hz, 1H), 7.87 (s, 1H), 7.86 (d, J = 8.0 Hz, 2H), 7.66 (d, J = 8.0 Hz, 2H), 7.55 (d, J = 8.4 Hz, 2H), 7.51 (d, J = 8.4 Hz, 1H), 4.29 (s, 2H), 3.88-3.97 (m, 2H), 3.74-3.80 (m, 2H), 3.18-3.26 (m, 2H), 3.02-3.12 (m, 2H), 2.31 (s, 3H). Exact mass calculated for $C_{26}H_{26}N_3O_2$: 411.2. Found: LC/MS (ES+) m/z=412.2 $[M+H]^+$.

4'-cyano-6-methyl-N-(6-(piperidin-1-yl)pyridin-3-yl)biphenyl-3-carboxamide (**5f**)



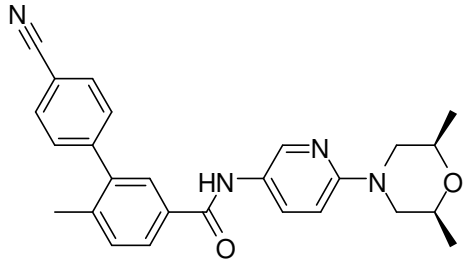
^{1}HNMR (400 MHz, DMSO -d_6): δ (ppm) 10.32 (s, 1H), 8.49 (s, 1H), 8.05-7.93 (m, 4H), 7.87 (s, 1H), 7.66 (d, *J*=8.0 Hz, 2H), 7.52 (d, *J*=8.0Hz, 1H), 7.22 (m, 1H), 3.59 (m, 4H), 2.31 (s, 3H), 1.61 (m, 6H). Exact mass calculated for $C_{25}H_{24}N_4O$: 396.20. Found: LC/MS (ES+) m/z=397.2 $[M+H]^+$.

4'-cyano-6-methyl-N-(5-(piperidin-1-yl)pyridin-2-yl)biphenyl-3-carboxamide (**5g**)



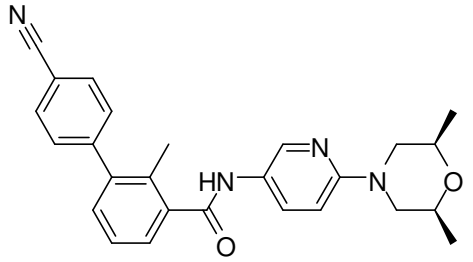
^{1}HNMR (400 MHz, DMSO -d_6): δ (ppm) 10.89 (s, 1H), 8.09 (m, 1H), 7.97-7.93 (m, 5H), 7.70 (m, 2H), 7.59 (m, 1H), 7.50 (d, *J*=8.0 Hz, 1H), 3.21 (t, *J*=4.8 Hz, 4H), 2.31 (s, 3H), 1.66-1.63 (m, 4H), 1.58-1.55 (m, 2H). Exact mass calculated for $C_{25}H_{24}N_4O$: 396.20. Found: LC/MS (ES+) m/z=397.2 $[M+H]^+$.

4'-cyano-N-(6-((2S,6R)-2,6-dimethylmorpholino)pyridin-3-yl)-6-methylbiphenyl-3-carboxamide (**5h**)



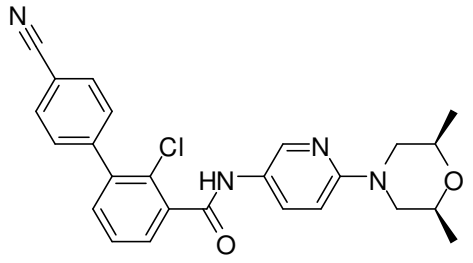
^{1}HNMR (400 MHz, DMSO -d_6): δ (ppm) 10.14 (s, 1H), 8.43 (d, *J*=2.8 Hz, 1H), 7.98-7.90 (m, 4H), 7.87 (d, *J*=2.0 Hz, 1H), 7.68-7.65 (m, 2H), 7.50 (d, *J*=8.0 Hz, 1H), 6.87 (d, *J*=9.2 Hz, 1H), 4.07 (dd, *J*=2.0, 12.8 Hz, 2H), 3.65-3.58 (m, 2H), 2.35 (dd, *J*=10.8, 12.8 Hz, 2H), 2.31 (s, 3H), 1.16 (d, *J*=6.4 Hz, 6H). Exact mass calculated for $C_{26}H_{26}N_4O_2$: 426.21. Found: LC/MS (ES+) m/z=427.2 $[M+H]^+$.

4'-cyano-N-(6-((2S,6R)-2,6-dimethylmorpholino)pyridin-3-yl)-2-methylbiphenyl-3-carboxamide (**5i**)



^{1}HNMR (400 MHz, DMSO -d_6): δ (ppm) 10.33 (s, 1H), 8.49 (d, *J*=2.4 Hz, 1H), 8.01-7.98 (m, 3H), 7.62 (d, *J*=8.0 Hz, 2H), 7.56 (dd, *J*=1.2, 7.6 Hz, 1H), 7.47 (t, *J*=7.6 Hz, 1H), 7.39 (dd, *J*=1.2, 7.6 Hz, 1H), 6.92 (d, *J*=9.2 Hz, 1H), 4.10 (m, 2H), 3.71-3.64 (m, 2H), 2.40 (dd, *J*=10.8, 12.8 Hz, 2H), 2.27 (s, 3H), 1.22 (d, *J*=6.4 Hz, 6H). Exact mass calculated for $C_{26}H_{26}N_4O_2$: 426.21. Found: LC/MS (ES+) m/z=427.2 $[M+H]^+$.

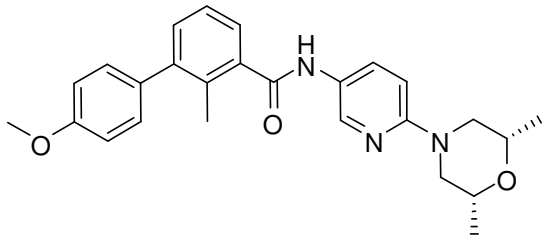
2-chloro-4'-cyano-N-(6-((2S,6R)-2,6-dimethylmorpholino)pyridin-3-yl)biphenyl-3-carboxamide (**5j**)



^{1}HNMR (400 MHz, CD_3OD): δ (ppm) 8.41 (d, J=2.8Hz, 1H), 7.96 (dd, *J*=9.2, 2.8Hz, 1H), 7.82 (d, *J*=8.4Hz, 2H), 7.62 (d, *J*=8.4Hz, 2H), 7.59 (d, *J*=2.0Hz, 1H), 7.54-7.46 (m, 2H), 6.82 (d, *J*=9.2Hz, 1H), 4.02 (dd, *J*=12.8, 1.6Hz, 2H), 3.78-3.70 (m, 2H), 2.50 (dd, *J*=12.4, 10.4Hz, 2H),

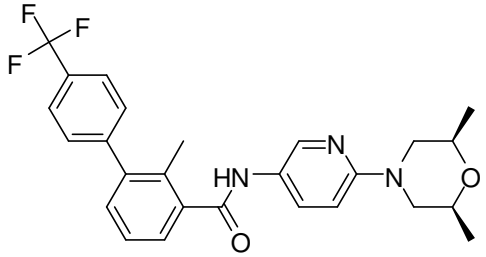
1.26 (d, *J*=6.4Hz, 2H). Exact mass calculated for $C_{25}H_{23}ClN_4O_2$: 446.15. Found: LC/MS (ES+) m/z=447.2 $[M+H]^+$.

N-(6-((2S,6R)-2,6-dimethylmorpholino)pyridin-3-yl)-4'-methoxy-2-methylbiphenyl-3-carboxamide (**5k**)



^{1}HNMR (400 MHz, DMSO -d_6): δ (ppm) 10.16 (s, 1H), 8.37 (d, *J*=2.8Hz, 1H), 7.87 (dd, *J*=2.4, 9.2 Hz, 1H), 7.34-7.17 (m, 5H), 6.97 (m, 2H), 6.80 (d, *J*=9.2 Hz, 1H), 3.98 (m, 2H), 3.74 (s, 3H), 3.59-3.51 (m, 2H), 2.28 (dd, *J*=2.0, 7.2 Hz, 2H), 2.16 (s, 3H), 1.09 (d, *J*=6.0 Hz, 6H). Exact mass calculated for $C_{26}H_{29}N_3O_3$: 431.22. Found: LC/MS (ES+) m/z=432.2 $[M+H]^+$.

N-(6-((2S,6R)-2,6-dimethylmorpholino)pyridin-3-yl)-2-methyl-4'-(trifluoromethyl)biphenyl-3-carboxamide (**5l**)



^{1}HNMR (400 MHz, DMSO -d_6): δ (ppm) 10.33 (s, 1H), 8.49 (d, *J*=2.8 Hz, 1H), 8.00 (dd, *J*=2.8, 9.2 Hz, 1H), 7.90 (d, *J*=8.0 Hz, 2H), 7.64 (d, *J*=8.0 Hz, 2H), 7.56 (m, 1H), 7.46-7.40 (m, 2H), 6.92 (d, *J*=9.2 Hz, 1H), 4.10 (m, 2H), 3.71-3.64 (m, 2H), 2.40 (dd, *J*=10.4, 12.4 Hz, 2H), 2.28 (s, 3H), 1.22 (d, *J*=6.4 Hz, 6H). Exact mass calculated for $C_{26}H_{26}F_3N_3O_2$: 469.20. Found: LC/MS (ES+) m/z=470.2 $[M+H]^+$.

TM3-Gli-Luc reporter gene assay

Test compounds were prepared for assay by serial dilution in DMSO and then added to empty assay plates. TM3Hh12 cells (TM3 cells containing Hh-responsive reporter gene construct pTA-8xGli-Luc) were cultured in F12 Ham's/DMEM (1:1) containing 5% horse serum, 2.5% fetal bovine serum (FBS), and 15 mM HEPES, pH 7.3. Cells were harvested by trypsin treatment, resuspended in F12 Ham's/DMEM (1:1) containing 5% horse serum and 15 mM HEPES, pH

7.3, added to assay plates, and incubated with test compounds for approximately 30 min at 37 °C in 5% CO_2. Then 1 or 25 nM Ag1.5 was added to assay plates and incubated at 37 °C in the presence of 5% CO_2. After 48 h, either Bright-Glo (Promega E2650) or MTS reagent (Promega G258B) was added to the assay plates and luminescence or absorbance at 492 nm was determined. IC_{50} values, defined as the inflection point of the logistic curve, were determined by nonlinear regression of the Gli-driven luciferase luminescence or absorbance signal from MTS assay vs log10 (concentration) of test compounds using the R statistical software package.

Fluorescence binding assays using BODIPY-cyclopamine

Fluorescence binding assays using BODIPY FL or BODIPY® 558/568 labeled binding assays were conducted as described. Briefly, binding assays were conducted in 384-well plates using fixed CHO cells stably expressing mouse or human Smo. Cells were fixed with 4% paraformaldehyde for 15 min at room temperature, washed, covered in PBS buffer containing 0.5% fetal bovine serum, and incubated with fluorescence labeled BODIPY-cyclopamine (20 nM) and the test compounds for 4 h at 37 °C. The treated cells then were washed with PBS, stained with Hoechst 33258, and analyzed by ImageXpress® Ultra imaging system (Molecular Devices).

Subcutaneous $Ptch^{+/-}p53^{-/-}$ medulloblastoma allograft model.

Mouse Ptch+/-p53-/- medulloblastoma cells ((1.0-5.0) × 10^6), dissociated directly from tumor fragments, were inoculated subcutaneously into the right flank of Harlan nu/nu mice. Treatment was initiated approximately 7 days after implantation. Animals were randomized into treatment groups with similar mean tumor volumes of 271 mm^3 with individual tumor sizes ranging from approximately 200 to 340 mm^3. Tumor volumes (mm^3) and body weights (g) were recorded two or three times per week from all groups for analysis. Dose was body weight adjusted at time of dosing. Comparisons between treatment groups was performed using ANOVA (Tukey) rank sum test.

Gli1 mRNA expression analysis.

Gli1 mRNA levels in tumors were analyzed by real-time PCR. Briefly, total RNA from approximately 100 mg of flash frozen tumor tissue was purified using the Aurum total RNA fatty and fibrous tissue kit (Biorad, Hercules CA). cDNA was synthesized by reverse transcription using qScript cDNA Supermix (Quanta Bioscience Gaithersburg, MD) using 2-5 μg of total RNA. Relative quantitation of mRNA expression was performed by real-time quantitative PCR using TaqMan gene expession reagents (Applied Biosystems, Foster City, CA). An amount of 2 μL of the cDNA reaction was used in a 25 μL reaction with 2× master mix containing Amplitaq gold DNA polymerase, Amperase UNG, dNTPs, UTP, and probes for mouse _-actin (Mm 4352341E) and Gli1 (Mm494645). The reaction was run on an ABI 7900HT fast real-time PCR system.

Bioanalytical method for plasma and tumor PK.

Plasma and samples were processed using protein precipitation with an acetonitrile/methanol mixture. Samples were centrifuged and an aliquot of the supernatant was injected via

autosampler on to an HPLC. Tumor samples were homogenized after dilution with water and then processed using the same procedure as the plasma samples. Separation and analysis was performed by LC-MS/MS using electrospray ionization as an interface.

Orthotopic $Ptch^{+/-}p53^{-/-}$ medulloblastoma allograft model.

Twenty four athymic nude mice (age 6 week, body weight 21.31 ± 1.52 g) were implanted with 100,000 tumor cells 17 days prior to the intiation of dosing. Tumor cells were stereotactically implanted subcortically at a depth of 3 mm and at 1.5 mm posterior to and 2.5 mm right of bregma. MRI was performed on day 4 prior to initiation of treatment for randomization into treatment group (baseline measurement). Nine animals were excluded from the study based on tumor size. The remaining 16 mice were sorted into a vehicle-treated group and a 5m-treated group so that the mean and SEM were similar. One animal in the5m -treatment group was subsequently excluded from the analysis because the tumor volume did not change over the observation period, and the finding was confirmed by histological evaluation. The mean (± SEM) tumor volume of the 5m-treated group was 3.39 ± 0.26 mm^3, and the mean (± SEM) tumor volume of the vehicle-treated group was 3.19 ± 0.39 mm^3. Treatment (vehicle or 5m at 40 mg/kg/day p.o. b.i.d) was initiated on day 0 (17 days following tumor implantation). Doses are provided as free base equivalents started on day 0. MRI scans were performed on days -4, 0 and +4 In reference to initiation of dosing) Mice were euthanized when they exhibited signs of morbidity.

	Tumor volume (mm^3)	
Days on treatment	Vehicle (n=8)	NVP-LDE225 (n=7)
baseline	3.39 ± 0.39 mm^3	3.39 ± 0.26 mm^3
4	31.08 ± 9.44 mm^3	7.31 ± 1.09 mm^3

Values = mean ± SEM

Demonstration of an intact blood-brain barrier in the orthotopic $Ptch^{+/-}p53^{-/-}$ medulloblastoma allograft model.

Animals (8 total; 4 groups of 2 each) were implanted with 50,000 or 100,000 tumor cells, and treated with either with 40 mg/kg/day po bid **5m** or vehicle. MRI was performed at day 9 post implantation. Images were acquired before and after intraperitoneal administration of 0.4 ml/kg of the contrast agent gadopentetate dimeglumine (Gd-DTPA) (Magnevist®, Bayer Healthcare, Wayne, NJ, USA). In 7 out of 8 animals, the brain was unenhanced after contrast injection, while surrounding cranial muscles indicating the integrity of the blood-brain barrier (Figure 1). No difference was observed between the treatment groups. The remaining animal was in the vehicle-treated group implanted with 100,000 cells. In this case, the tumor grew along the great cerebral vein of Galen, and disrupting the blood-brain barrier, resulting in a hyperintense tumor.

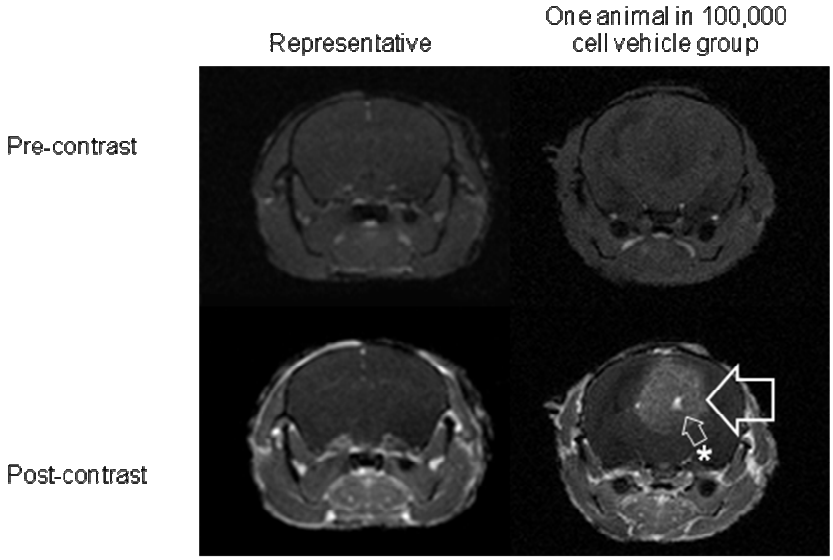


Figure 1. Representative pre- and post- contrast T_1-weighted images. In 7 out of 8 animals, the brain was unenhanced (left column), indicating the integrity of the blood-brain barrier. In the last animal (from vehicle group implanted with 100000 cells), the tumor (wide arrow) was hyperintense after contrast injection since it grew along the great cerebral vein of Galen.

Imaging of orthotopic Ptch$^{+/-}$p53$^{-/-}$ medulloblastoma allograft model. MRI was performed in a Bruker BioSpec 7.0 T scanner (Bruker BioSpin, Ettingen, Germany), using a 35 mm inner-diameter birdcage resonator for transmission and reception. The mice were anaesthetized with 1.2% – 1.5% isoflurane in oxygen. The head of animal was fixed by a tooth bar and a facemask to minimize motion. Respiration rate and body temperature were monitored continuously and temperature maintained between 32 – 35°C by heated airThe T_2-weighted anatomical images were acquired in the coronal view to image the whole mouse brain with a multislice multi-spin-echo sequence. The following parameters including: repetition time of 3000 ms, echo train length of 8, echo spacing of 11.5 ms, effective echo time of 51.75 ms, 160×128 matrix, field of view of 20×20 mm^2, spatial resolution of 0.125×0.156 mm^2/pixel, bandwidth of 50000 Hz, 2×2 oversampling, 2 averages, 30 slices, slice thickness 0.5 mm, and a total scan time of 25 min 36 sec were used. These images were segmented to quantify tumor volume using ITK-SNAP [Yushkevich, P. A., Piven, J., Hazlett, H. C., Smith, R. G., Ho, S., Gee, J. C. and Gerig, G. *Neuroimage* **2006**, *31*, 1116-1128.] For assessment of blood-brain-barrier integrity, T_1-weighted images were acquired with a gradient-echo sequence using the following parameters: repetition time of 200 ms, echo time of 2.7 ms, 128×128 matrix, field of view of 20×20 mm2, spatial resolution of 0.156×0.156 mm2/pixel, 2×1 oversampling, flip angle of 90°, 8 averages, bandwidth of 50505.1 Hz, echo position at 40%, 30 slices, slice thickness 0.5 mm, and a total scan time of 3 min 25 sec.