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Second generation 2-pyridyl biphenyl amide inhibitors of the hedgehog pathway

Georgette M. Castanedo [a,†], Shumei Wang [a,†], Kirk D. Robarge [a], Elizabeth Blackwood [a], Daniel Burdick [a], Christine Chang [a], Gerrit J. P. Dijkgraaf [a], Stephen Gould [a], Janet Gunzner [a], Oivin Guichert [b], Jason Halladay [a], Cyrus Khojasteh [a], Leslie Lee [a], James C. Marsters Jr. [a], Lesley Murray [a], David Peterson [a], Emile Plise [a], Laurent Salphati [a], Frederic J. de Sauvage [a], Susan Wong [a], Daniel P. Sutherlin [a,*]

[a] Genentech, 1 DNA Way, South San Francisco, CA 94080, United States
[b] Curis Inc., 45 Moulton Street, Cambridge, MA 02138-1118, United States

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ABSTRACT

Potent and efficacious inhibitors of the hedgehog pathway for the treatment of cancer have been prepared using the 2-pyridyl biphenyl amide scaffold common to the clinical lead GDC-0449. Analogs with polar groups in the *para*-position of the aryl amide ring optimized potency, had minimal CYP inhibition, and possessed good exposure in rats. Compounds **9d** and **14f** potently inhibited hedgehog signaling as measured by Gli1 mRNA and were found to be equivalent or more potent than GDC-0449, respectively, when studied in a $Ptch^{+/-}$ medulloblastoma allograft model, that is, highly dependent on hedgehog signaling.

While the hedgehog pathway plays an important role in embryonic tissue development, hedgehog signaling occurs to a lesser extent in the adult where pathway activation contributes to tissue maintenance and repair.[1,2] In addition to its normal functions, aberrant activation of this pathway has also been found to contribute to the growth of certain cancers via mutation of pathway genes in basal cell carcinoma (BCC) and meduloblastoma.[3–8] Aberrant hedgehog expression in colorectal and pancreatic tumors lead to the up regulation of pathway genes in the surrounding tumor stroma.[9–11]

The pathway is activated when a hedgehog ligand (Hh) binds to the transmembrane protein patched (PTCH), relieving suppression of smoothened (SMO) by PTCH and allowing SMO to signal. The result of this pathway initiation is the activation of transcription factors GLI2 and GLI3, which in turn transcribe a number of proteins important for differentiation, growth, and survival. One of these transcribed genes, *Gli1* can also be used to specifically monitor pathway activity either in vitro in cell based assays or in vivo as a PD marker.[12]

The first demonstration that a small molecule could effectively inhibit this pathway came from work done with the natural product cyclopamine. This compound and other small molecules were found to bind SMO, and inhibit pathway activation.[13] With the goal of developing a potentially novel therapeutic for cancer, **2** (GDC-0449) was discovered as a potent and efficacious inhibitor of the pathway that was recently communicated in this journal (Fig. 1).[12] This compound has shown clinical responses in patients with metastatic and inoperable BCC[14] and is currently in clinical

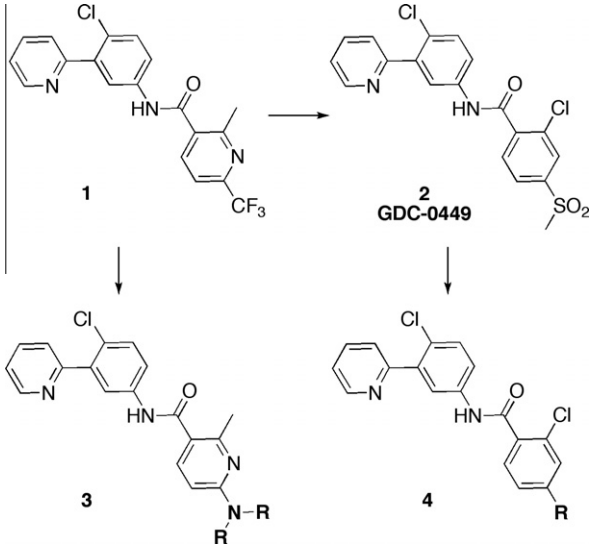

Figure 1. Schematic for the discovery of **2** (GDC-0449) from **1**, and the inspiration for further analogs **3** and **4**.

* Corresponding author. Tel.: +1 650 225 3171; fax: +1 650 225 2061.
E-mail address: dans@gene.com (D.P. Sutherlin).
† These authors contributed equally to this work.

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trials targeting this and other indications.[15] Additionally, a number of other compounds are being pursued both preclinically and clinically as modulators of the Hh pathway.[16,17]

During our investigation of **2** (GDC-0449) in xenograft and allograft models, we noted that concentrations in the micromolar range were necessary to achieve sufficient efficacy. Our backup strategy then became centered around the development of compounds with greater in vivo potency through the increase of cell based in vitro potency. Ultimately, compound **2** was able to successfully attain high concentrations in patients that were sufficient to modulate the Hh pathway.

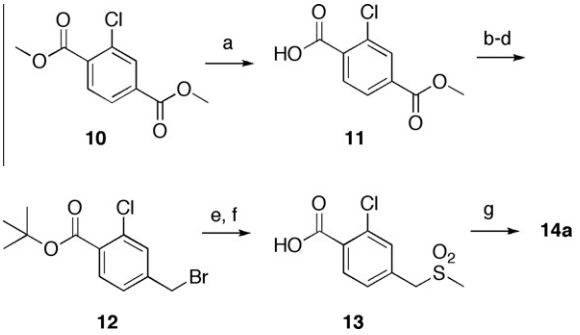
The 2-pyridyl biphenyl amide **2** originated from a lead optimization program centered around analog **1** (Fig. 1). Modification of the trifluoro methyl nicotinic amide found in **1** to a 2-chloro-4-methanesulphonyl benzamide in **2** was effective in improving the solubility of the molecule while maintaining good potency and pharmacokinetics in rats and dogs.[12] We considered both of these molecules to be promising starting points to further explore the SAR around the aryl amide, in that previous SAR demonstrated that substitutions around this ring were well tolerated. Described herein is the discovery of potent compounds of the general structures **3** and **4** that were explored further in PK/PD and efficacy studies.

Starting with the previously described pyridyl biphenyl aniline **5**,[18–20] 6-chloronicotinamides **8** were prepared by amide coupling with chlorinated nicotinic acids **7**,[18] prepared from the ester **6** (Scheme 1). This advanced intermediate was then used to generate a number of N-substituted replacements for the trifluoromethyl group **9a–f**.

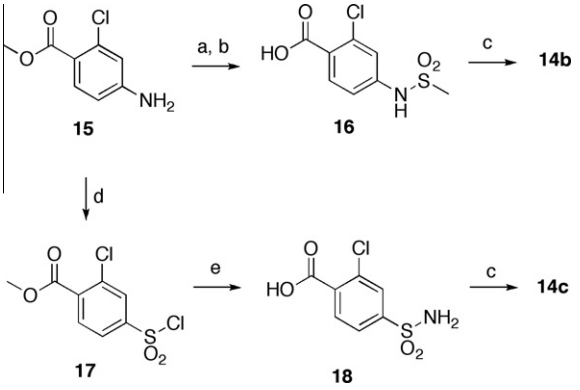
The extended sulfone was prepared by selective demethylation of the bis-methylester **10** with BBr_3 to yield **11** (Scheme 2).[21] This acid **11** was then converted to the *t*-butyl ester prior to reduction of the remaining methyl ester to a benzyl alcohol which was then used to prepare benzyl bromide **22**. The bromide was then treated with methyl sulfinate to make the sulfone. Following the removal of the *t*-butyl group the acid **13** was coupled to aniline **5** to prepare compound **14a**.

Sulfonamides **14b** and **14c** were both made starting from aniline **15**, prepared following an iron reduction of 2-chloro-4-nitrobenzoic acid methyl ester (Scheme 3). Simple manipulations led to **16** that was then coupled to aniline **5** to make methylsulfonamide **14b**. A Sandmeyer reaction was used to convert the aniline **15** to the aryl sulfonyl chloride **17**.[22] Addition of ammonia and then hydrolysis of the methyl ester prepared the acid **18** that was used to prepare the primary aryl sulfonamide **14c**. Finally, amides **14d–f** were made from the advanced intermediate **19**, prepared beginning with the coupling of aniline **5** and the acid **11** from Scheme 1 (Scheme 4).

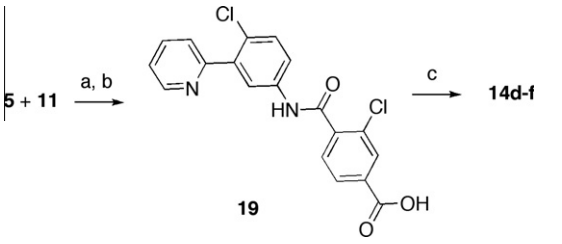
Nicotinic amide **1** was considered to be a good starting point for new analog design in that the compound had good potency, no CYP inhibition, and low clearance (CL) in vitro and in vivo (Table 1). Despite these promising properties, this compound failed to show a strong modulation of downstream *Gli1* transcript when dosed at



Scheme 2. Reagents and conditions: (a) BBr_3, CH_2Cl_2, 0 °C to rt, 2.5 h, 55%; (b) isobutylene, H_2SO_4, CH_2Cl_2, −78 °C, 48 h, 73%; (c) $NaBH_4$, EtOH, 0–40 °C, 0.5 h, 97%; (d) NBS, PPh_3, DCM, 0 °C, 2 h, 87%; (e) $NaSO_2Me$, DMF, 1.5 h, 60 °C, 98%; (f) 4 N HCl, CH_2Cl_2, 1.5 h, 45 °C, 93%; (g) HATU, HoAT, DIPEA, **5**, DMF, rt, 18 h, 79%.

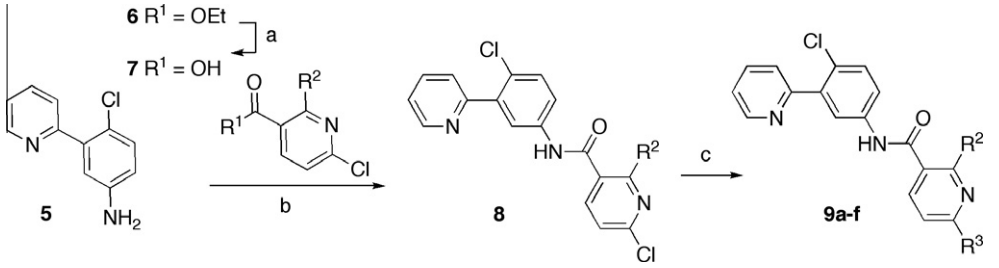


Scheme 3. Reagents and conditions: (a) $MeSO_2Cl$, py, DCM, 0 °C to rt, 18 h, 100%; (b) LiOH, THF/H_2O, 5 h, 50 °C, 80%; (c) HATU, DIPEA, **5**, DMF, 18 h, rt (46–48%); (d) $NaNO_2$, HCl, $CuCl_2$, SO_2, AcOH, H_2O, 2 h, 0 °C, 75%; (e) 2 M NH_3 in MeOH, DIPEA, 5 min, rt, 84%.



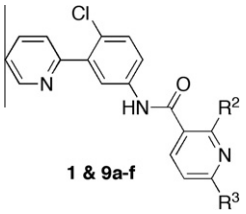
Scheme 4. Reagents and conditions: (a) HATU, DIPEA, DMF, 2 h, rt; (b) LiOH, THF/H_2O; (c) HATU, DIPEA, amine, DMF (49–82%).

75 mg/kg in a Calu-6 PK/PD model.[12] This poor in vivo activity correlated well with a lower than expected C_{max} possibly due to

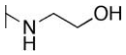
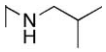
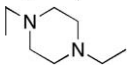
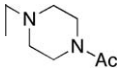
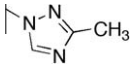


Scheme 1. Reagents and conditions: (a) LiOH, THF/H_2O, 45%; (b) HATU, HOAT, DIPEA, DMF; rt, R_2 = H, 5 h, 76%; R_2 = CH_3, 18 h, 95%; (c) for amines: *n*-butanol or ethylene glycol, 175–220 °C, 1 h, microwave, 34–71%; for triazoles: NaH, DMF, 140 °C, 20 h, 68%.

Table 1



1 & 9a-f

Compd	R^2	R^3	Mouse S12 (nM)	Human HEPM (nM)	CYP 3A4/ 2Cp (μm)	Predicated CL in rat[a] (mL/min/kg)	In vivo CL rat[b] (mL/min/kg)	V_{ss} rat (L/kg)	Thermodynamic solubility	
									pH 1 mg/mL	pH 6.5 mg/mL
1	CH_3		42	15	>20/>20	5.9	4.5	0.95	1.0	0.002
9a	CH_3		62	23	>20/>20	34	—	—	—	—
9b	CH_3		53	14	19/0.5	48	—	—	—	—
9c	CH_3		160	5.0	>20/>20	48	—	—	—	—
9d	CH_3		22	5.0	>20/16	12	2.0	0.38	3.2	0.006
9e	H		47	8.0	0.2/0.2	0.6	4.0	1.4	4.0	0.008
9f	CH_3		67	8.0	>20/>20	19	0.7	0.24	0.9	0.006

[a] Based on rat liver microsomes.
[b] Male rats were dosed with the TFA salt as a solution intravenously (1 mg/kg) and orally (5 mg/kg) in a solution of 60–80% PEG.

dose-limited solubility that resulted in lower absorption (this compound had high permeability in MDCK and Caco-2 cell lines with no evidence of efflux). Additionally, as we have reported previously, a small library of aryl amides in place of the nicotinic amide had indicated that polar groups para to the amide bond appeared to maintain potency relative to **1**. Based on these observations, we sought to replace the lipophilic trifluoromethyl group in **1** with more polar substituents.

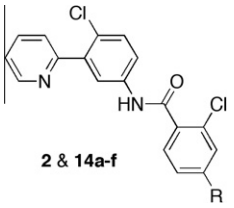
Table 1 describes a subset of representative analogs of **1** that highlights general structure–activity relationship (SAR) trends and our strategy for selecting molecules for further study. The nicotinic amide tolerated a variety of substitutions at the 6 position including primary amines, cyclic amines, and N-linked heterocycles which had cell based potencies near **1**. Nearly all compounds prepared were more potent in the reporter gene assay derived from human cells (HEPM) than in the mouse-derived cell line (S12) and the majority of compounds had the same rank order in both assays.

Moving beyond potency we observed that more hydrophobic substituents began to increase inhibition of CYP 2C9, illustrated in the comparison of **9a** and **9b**. Charged groups, exemplified by methyl piperidine **9c** tended to have less CYP inhibition but had predicted clearances in the moderate range. The acylated piperazine **9d** had seemed to strike a good balance with minimal CYP inhibition and lower predicted hepatic clearance in rat that translated to lower in vivo CL. This compound was twofold more potent, more stable, and showed a similar pH dependent solubility to **1**. The triazole **9e** was also pursued based on its very low predicted clearance in rat despite strong CYP inhibition for isoforms 3A4 and 2C9, potentially contributing to the increased stability. Once low clearance was confirmed in vivo, the introduction of a methyl group adjacent to the ring nitrogen of the nicotinic amide and the triazole resulted in **9f** and significantly reduced CYP inhibition, presumably by sterically blocking the heterocyclic nitrogens. Interestingly, a single methyl group on the triazole or the nicotinic amide alone was sufficient to reduce inhibition of CYPs (analogs not shown). Like **9d**, methyl triazole **9f** also had a low in vivo clearance in rats and was considered a promising lead.

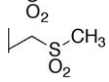
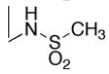
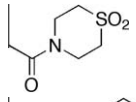
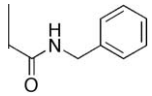
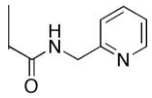
Additionally, GDC-0449 (**2**) served as an excellent starting point from which to discover new benzamide analogs. Again we focused our attention on the para substituent and chose to retain the *ortho*-chloro group that we had previously determined to be critical to for acceptable solubility. Table 2 describes some of the SAR that was discovered and exemplifies many of the molecules that were pursued further into PK/PD and efficacy studies. As we saw with the nicotinamides (Table 1) a variety of polar functional groups are tolerated in the *para*-position. The extended sulfone **14a** had excellent activity in both mouse and human assays, low Cl in rats, but a surprisingly >10-fold lower solubility at pH 1 when compared to most analogs. Sulfonamide **14b** was favored over analog **14c** due to better potency. Simple amides (Me, Et, etc.) lost potency relative to **2** and additional functionality had to be incorporated to recover better than average potencies, resulting in **14d–f**. Similar trends observed in ADME and PK for the nicotinic amide series were also seen in the benzamide series including CYP inhibition with more hydrophobic groups, exemplified by **14e**, and high in vitro and in vivo clearance for charged substituents. Encouraged by the potency of benzyl amide **14e** we prepared three pyridyl analogs, in hopes to add some additional solubility to the molecule and to reduce the CYP inhibition. Compound **14f** was by far the most potent of the three pyridyl regioisomers and had acceptable solubility, a slight improvement in CYP inhibition, and improved microsomal stability relative to **14e**. Although this compound had microsomal clearance that was higher than most analogs, the improved potency relative to **2** prompted us to move forward into rat PK where the clearance was found to be low.

To further differentiate the most promising compounds from Tables 1 and 2 we studied their ability to reduce *Gli1* expression in a PK/PD xenograft model derived from the Calu-6 non-small cell lung carcinoma (NSCLC) cell line that recruits a large stromal component when grown as xenografts in nude mice. Evidence strongly suggests that in colon and pancreatic cancers where the Hh path-

Table 2



2 & 14a-f

Compd	R	Mouse S12 (nM)	human HEPM (nM)	CYP 3A4/ 2Cp (μm)	Predicated CL in rat[a] (mL/min/kg)	In vivo CL rat[b] (mL/min/kg)	Thermodynamic solubility	
							pH 1 mg/mL	pH 6.5 mg/mL
2		13	3.0	>20/>20	3.7	3.7	3.5	0.010
14a		3.0	1.0	>20/16	10	5.5	0.11	0.003
14b		17	2.0	>20/8.0	13	2.4	0.91	0.001
14c		35	5.0	>20/>20	18	—	—	—
14d		33	5.0	>20/9.0	18	35	4.3	0.060
14e		7.0	1.0	>20/1.2	42	—	—	—
14f		4.0	0.4	>20/4.5	32	6.7	4.3	0.003

[a] Based on rat liver microsomes.
[b] Male rats were dosed with the TFA salt as a solution intravenously (1 mg/kg) in either 80% PEG or a solution of 5% DMSO/5% cremophor and orally (5 mg/kg) as a solution in 60–80% PEG.

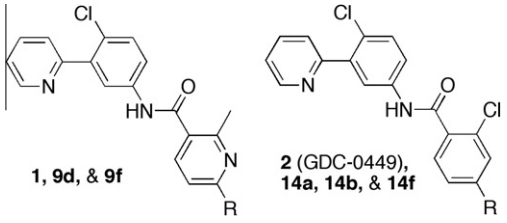
way is active, the tumor produces Hh ligand that then activates PTCH on the surrounding stromal cells.[9] Once the pathway is activated, the stromal respond by producing proteins that promote tumor growth and survival. We felt that this model, governed by this paracrine signaling pathway, would best mimic expectations in the clinic[3] and provide a convenient way to assess the ability of these compounds to block hedgehog signaling in vivo.

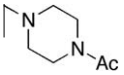
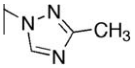
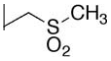
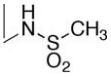
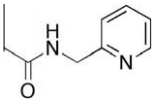
Once Calu-6 tumors were established, mice were dosed by oral gavage at 75 mg/kg twice a day (BID) for five doses and tumors harvested 4 h after the last dose for analysis of stromal (murine) *Gli1* mRNA levels and plotted relative to vehicle treated tumors. We also determined plasma concentrations at this same time point to establish a PK/PD relationship (Table 3). For the nicotinic amide series, the acylated piperazine **9d** showed twofold improvement in *Gli1* suppression relative to **1**, due to more exposure and increased potency for the target. Triazole **9f** did not perform well despite high plasma concentrations, an observation that was ultimately attributed to a very low volume of distribution in mouse of 0.15 L/kg, close to the total blood volume of that animal (~0.08 L/kg)[23] combined with a lack of potency in the mouse S12 assay. In contrast most analogs had volumes closer to 1 L/kg. In the benzamide series, the potent extended sulfone **14a** had very low plasma concentrations and thus did not lead to target modulation. The low exposure for **14a** was thought to occur from solubility-limited absorption, supported by the solubility measurements described in Table 2. Sulfonamide **14b** also had poor exposure which resulted in less impressive PD responses. Finally, the very potent pyridyl amide **14f** demonstrated equivalent *Gli1* suppression to **2** at doses as low as 25 mg/kg.[24]

Based on their activity in the PK/PD model we determined the efficacy of **9d** and **14f** compared to **2** in a mouse medulloblastoma allograft model. The model uses medulloblastoma tumors developed spontaneously in mice with activating mutations in the Hh pathway ($Ptch^{+/-}$) passaged subcutaneously in nude mice. Mice with established subcutaneous tumors were treated twice daily (BID) with inhibitors for 20 days. Previously, we had determined that 5 mg/kg BID of **2** significantly inhibited the growth of the medulloblastoma tumors but that higher doses of compound were required for tumor regressions. In order to compare the in vivo potency of the new inhibitors to **2** this sub-maximal efficacious dose of 5 mg/kg BID was selected. Compound **9d** performed as well as **2** when dosed at 5 mg/kg BID (Fig. 2A) and only led to partial efficacy at that dose. In a separate experiment, **14f** showed superior potency compared to **2** when both were dosed at 5 mg/kg BID and resulted in complete regressions (Fig. 2B). Additionally, we tested the ability of these two potent SMO antagonists to inhibit pathway signaling using a cell line transiently transfected with SMO-D473H, a point mutant which was recently identified from a medulloblastoma patient who had become resistant to treatment with GDC-0449.[25] Neither **9d** nor **14f** showed significant activity in this assay, resulting in 20% and 2% inhibition, respectively, when tested at 1 μM.

In conclusion, two Hh inhibitors were discovered that demonstrated equal to improved potency relative to **2** (GDC-0449) when compared at a similar dose. These compounds were prepared by modifying the trifluoro nicotinic amide found in **1** or the benzamide ring found in **2**. From both starting points ADME properties (mainly but not limited to in vitro metabolic stability and inhibition of major CYP isoforms) were used as an important selection criteria for further evaluation and a focus for medicinal chemistry optimization. The Calu-6 PK/PD assay was useful in prioritizing compounds for efficacy studies. Compounds that failed in this

Table 3
PK/PD data for advanced Hh pathway inhibitors



Compd	R	Mouse S12 (nM)	4 h plasma[a] concn (μM)	Mouse PPb	Gli sup. versus cont.% at 4 h
1		42	15	95%	40%
9d		22	22	87%	80%
9f		67	150	89%	50%
2		13	23	91%	90%
14a		3.0	0.3	90%	0%
14b		17	2.2	—	25%
14f		4.0	25 mg/kg 1.7 (6 h)	98%	90%

[a] Mice were dosed with the free base or HCl salt as a suspension in a methylcellulose/Tween or Labrafil based formulation.

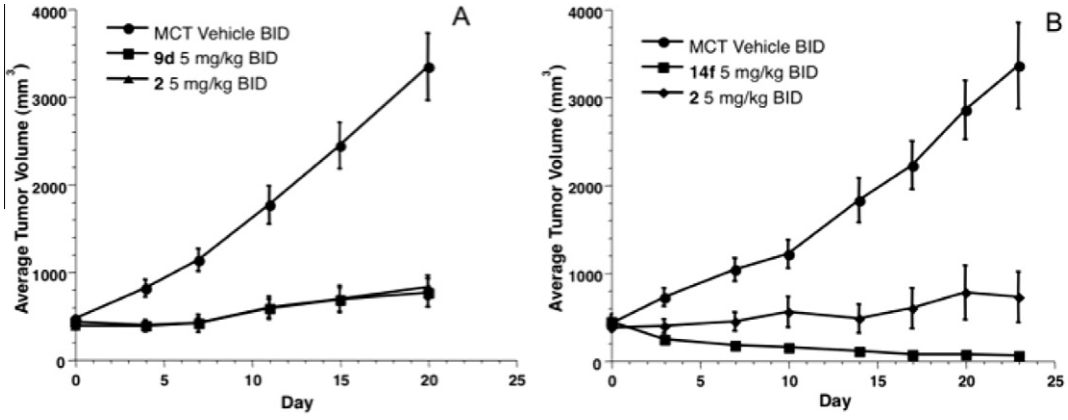


Figure 2. Medulloblastoma allograft models comparing compound two dosed BID in tumor volume over time with BID dosing of compounds **9d** (A) and **14f** (B).

assay were those that lacked sufficient target exposure relative to their potency, likely due to poor solubility exemplified by **9f**, **14a**, and **14b**. Compounds **9d** and **14f** had good potency and high exposure in mice, which translated into excellent in vivo activity in an allograft model that was either similar to or better than **2**, respectively. The improved activity of **14f** in the model was attributed to increased potency of the compound in its ability to inhibit the Hh pathway. Both of these compounds could be considered as potential backups to GDC-0449.

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