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Optimization of tetrahydronaphthalene inhibitors of Raf with selectivity over hERG

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ABSTRACT

Investigations of a biaryl ether scaffold identified tetrahydronaphthalene Raf inhibitors with good in vivo activity; however these compounds had affinity toward the hERG potassium channel. Herein we describe our work to eliminate this hERG activity via alteration of the substituents on the benzoic amide functionality. The resulting compounds have improved selectivity against the hERG channel, good pharmacokinetic properties and potently inhibit the Raf pathway in vivo.

Raf, a serine/threonine protein kinase, is an integral part of the MAP kinase signaling pathway and is a component of the Raf-MEK-ERK signaling cascade.[1] Understanding the complex and intricate role of Raf pathway signaling in cancer is the continuing aim of intensive research.[2] One component of this pathway, the Raf isoform B-Raf, has a high rate of activating mutation in melanoma (50–70%) and other cancers, including papillary thyroid (49%), colorectal (~15%), and ovarian (~30%).[3,4] The V600E activating mutation is most common and significantly increases basal level activity of the enzyme.[5] Suppression of B-Raf (V600E) activity in human melanoma cells leads to a down-regulation of the MAP kinase signaling pathway and apoptosis,[2,6] and recently, selective B-Raf inhibitors have shown significant antitumor activity in patients with B-Raf-mutant melanoma.[7] Thus, inhibition of mutated B-Raf could prove to be a useful therapeutic strategy against melanoma as well as a number of other cancers.

We recently reported the discovery and evolution of a novel series of tetrahydronaphthalenes as potent inhibitors for Raf, typified by compound **1** (Fig. 1).[8] However, this compound inhibits hERG ion channel activity (IC_{50} = 6.3 μM)[9] at concentrations near those required for effects on the Raf pathway. The blockade of hERG channels has been cited as a major factor in the drug-induced alteration of cardiac ventricular repolarization that results in prolongation of QT interval.[10] Since QT prolongation is believed to increase the risk of cardiac arrhythmia, including torsades de points (TdP), possibly resulting in sudden death, further optimization of this series was focused on reducing the hERG channel activity while retaining the desirable Raf activity.

There have been several ligand-based quantitative structure–activity relationships (QSAR) and pharmacophore models built using structurally diverse compounds known to bind and block the hERG channels.[14] These models clearly suggest that lipophilic amines are often hERG binders. As we focused on reducing the hERG activity of our molecules, we concentrated our efforts on modification of the benzylic amine moiety present in **1**. Compound **1** has excellent pharmacokinetic (PK) properties and inhibits the growth of B-Raf mutant tumor xenografts in mice.[8] While we were interested in modifying the hERG activity of this compound, we

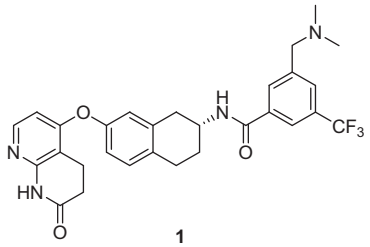

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Figure 1. Structure of lead compound **1**.

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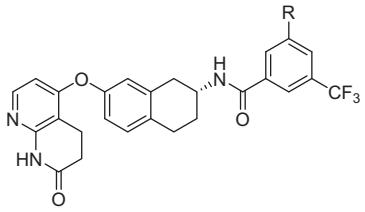
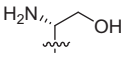
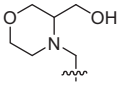
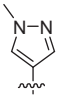
Table 1
Biological activity of tetrahydronaphthalene analogs



	R[a]	V600E B-Raf IC_{50}[b,c] (nM)	pERK IC_{50}[b,d] (nM)	hERG IC_{50}[b,e] (μM)
1		2.7 ± 0.4	48 ± 26	6.3 ± 1.1
2		3.1	487	>11.1
3		2.2 ± 0.5	45 ± 12	10 ± 7.1
4		1.9 ± 0.2	56 ± 25	5.6 ± 3.0
5		2.9	274	6.6 ± 3.1
6		2.1 ± 0.5	56 ± 30	7.4 ± 2.0
7		1.8 ± 0.2	67 ± 19	3.1 ± 1.2
8		1.7	207 ± 59	13.8 ± 3.6
9		2.2	854	4.9 ± 1.1
10		4.0	254	>100
11		2.0 ± 0.5	38 ± 12	>33
12		9.5	121 ± 39	>33
13		2.9 ± 0.6	55 ± 64	>11.1
14		2.1 ± 0.2	123 ± 53	>100
15		1.5	74 ± 38	>11.1
16		2.1 ± 0.8	34 ± 17	>100

[a] All compounds were isolated as either the HCl or bis-HCl salt except compound **10**, which was isolated as free base.[11]
[b] Standard deviations are reported for $N \geqslant 3$. Otherwise IC_{50} values are mean values of two determinations.
[c] Raf enzyme activity was determined using a Flash Plate Assay.[12]
[d] Cellular Raf pathway activity was determined with a whole cell ELISA assay utilizing a human melanoma cell line (A375) possessing the mutation BRaf V600E.[13]
[e] hERG activity was determined with the Predictor™ hERG assay (Invitrogen).[9]

were also keen to maintain its excellent physicochemical and PK properties and its in vivo activity. Earlier work established that substitution at the 5 position of the benzoic amide was important

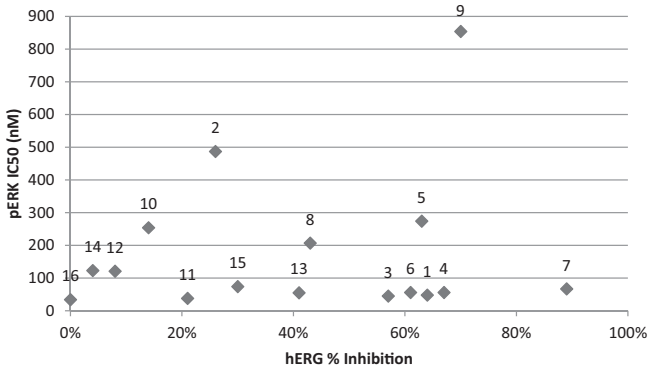


Figure 2. hERG percent inhibition at 11.1 μM and Raf activity.[9]

for cellular potency and the unsubstituted compound **2** (Table 1) is significantly less active in our cell-based pathway assay. To measure the cellular activity of our compounds, we monitored the inhibition of the downstream marker, pERK, rather than the direct marker of Raf activity, pMEK. We found that inhibition of pERK and pMEK correlated well for a small handful of compounds (data not shown) and that the pERK assay was more robust for screening large numbers of compounds. We also noted that while our compounds in general potently inhibited the enzymatic Raf activity, inhibition of the cellular activity was more variable. Although we have not rigorously confirmed this, our observations indicate poor compound solubility and permeability[15] as possible explanations for the lack of cellular activity.

Efforts to alter hERG activity in bioactive molecules have been well documented and we took a cue from the literature in our initial efforts. Attempts to modify the pK_a of our compounds by modifying the amine's alkyl substituents had no effect on either hERG activity or B-Raf enzyme potency (compounds **3**–**5**). Attempts to alter the electron density of the benzyl ring itself, by adding electron-withdrawing substituents to the phenyl ring did not alter hERG activity but did decrease Raf enzyme potency (data not shown). Increasing the steric bulk around the basic nitrogen had no effect on hERG activity (compounds **6** and **7**). The addition of additional polar functionality near the basic site has been shown in some cases to reduce hERG activity,[16] however in our case the introduction of an alcohol moiety offered little benefit with respect to hERG and led to a decrease in cellular potency (compounds **8** and **9**), most likely due to decreased cellular permeability.

These relatively extensive explorations of the benzyl amine moiety led us to conclude that the structure–activity relationships for hERG activity and the Raf activity were closely linked as we were only able to reduce hERG activity in this series when we added functionality that also reduced Raf inhibition in cells. We were fortunate however in that many types of substitution are tolerated at this position on the phenyl ring and therefore we expanded our search for benzyl amine replacements to include

Table 2
Pharmacokinetic properties of compounds **1** and **16**[a]

	Compound **1**	Compound **16**
Clp (L/h/kg)	0.23 ± 0.01	0.08 ± 0.00
V_{ss} (L/kg)	0.75 ± 0.10	0.41 ± 0.05
po AUC (μM h)	98.6 ± 37.5	125.7 ± 3.5
po% F	100	54

[a] Pharmacokinetic experiments were performed by dosing either intravenously (1 mg/kg) or orally (10 mg/kg) in male SD rats.[17] Plasma clearance (Clp); Steady-state volume of distribution (V_{ss}); Area under the curve for oral administration (po AUC); Oral bioavailability (po% F).

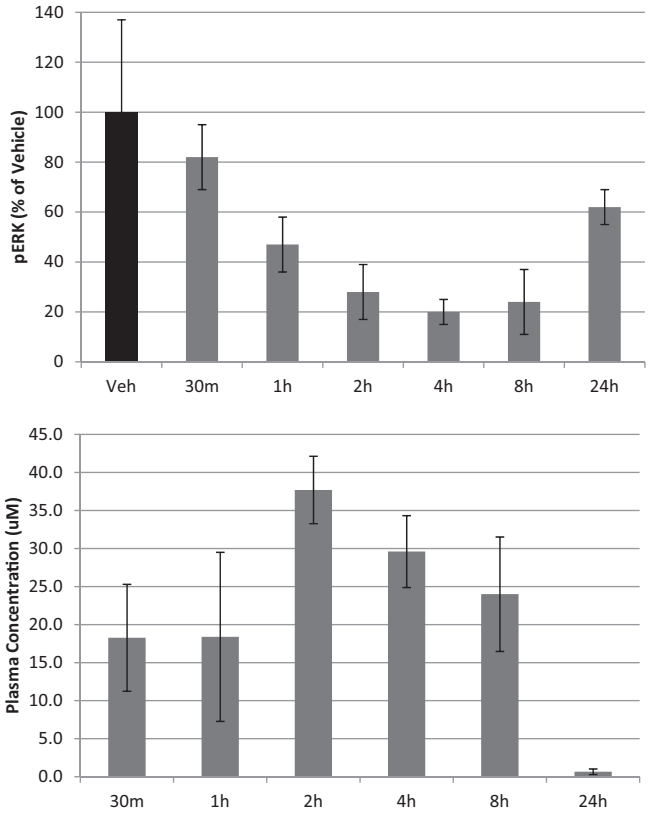


Figure 3. PK/PD study of compound **16** following a 33 mg/kg oral dose.[18]

heterocycles. We initially hoped that we would be able to identify a heterocycle that would also maintain the good physicochemical properties of this series. Saturated heterocycles, such as pyrrolidine and piperidine at the 5 position provided some of our most potent Raf inhibitors, but also were very potent against hERG (data not shown), while the substituted morpholine **10** showed better selectivity versus hERG with poor cellular activity. We were pleased to find that many different 5- and 6-member aromatic heterocycles are well tolerated at this position. Imidazole and pyrazole in particular showed promise with respect to Raf potency (compounds **11**, **12**, and **13**); hERG activity for these compounds decreased significantly. We next explored the effect of alkylation on these heterocycles and found the optimal combination of cellular Raf inhibition with minimal hERG activity in compound **16**. The limited solubility of these compounds affected our ability to determine an exact IC_{50} in the hERG assay however we were able to compare the hERG percent inhibition at the lower concentration as shown in Figure 2.

Our attempts to modify the hERG potency of our Raf inhibitors led us a few conclusions. First, the basic amine substituent of the benzamide moiety in compound **1** is responsible for the hERG activity of this compound and attempts to modify this substituent while maintaining its basic and solubilizing nature did not lead to improved hERG selectivity. The SAR for the Raf and hERG activities track closely for the basic amine substituted analogs. Substitution of benzyl amine with a heterocycle led to more selective compounds and further substitution of these heterocycles provided an optimal combination of Raf activity with hERG selectivity indicating that both reduction of basicity and addition of steric bulk were required to achieve the desired selective compound.

Compound **16** has good cellular potency without any hERG activity at 11.1 μM. We were pleased also to find that despite its poor solubility, it maintains good PK properties in rodents as shown in Table 2.

Compound **16** was found to have pharmacodynamic (PD) and antitumor activity in vivo. As shown in Figure 3, strong inhibition of the Raf pathway is observed following a 33 mg/kg oral dose. Furthermore, twice daily dosing for 21 days at 33 mg/kg induces partial regressions of an A375M tumor xenograft with significant tumor growth reduction at lower daily doses. These doses are well tolerated by the mice, and no indication of toxicity or weight loss as compared to vehicle treated animals was observed (Fig. 4).

Our synthetic exploration of this series required the preparation of a number of benzoic acid derivatives which were then coupled to amino tetralin **17**[8] as illustrated in Scheme 1.[11]

The syntheses of the required benzoic acid derivatives are summarized in Schemes 2 and 3. Scheme 2 details the preparation of benzoic acids derived from aldehyde **20**, which can be prepared in two steps from benzoic acid **18**. One-step reductive amination followed by a deprotection/protection sequence allowed access to a number of benzylamines including ethylamine **22**, and the substituted morpholino-benzoate used to prepare compound **10**. The primary benzylamine **24** was accessible through Raney®-Ni-mediated reduction of the aldoxime derived from **20**. Aryl

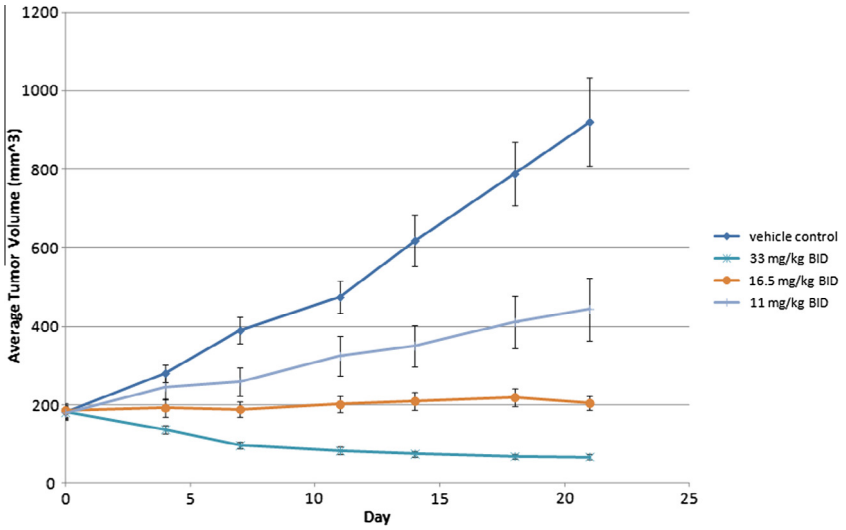
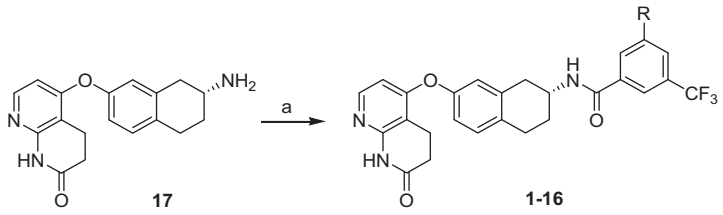
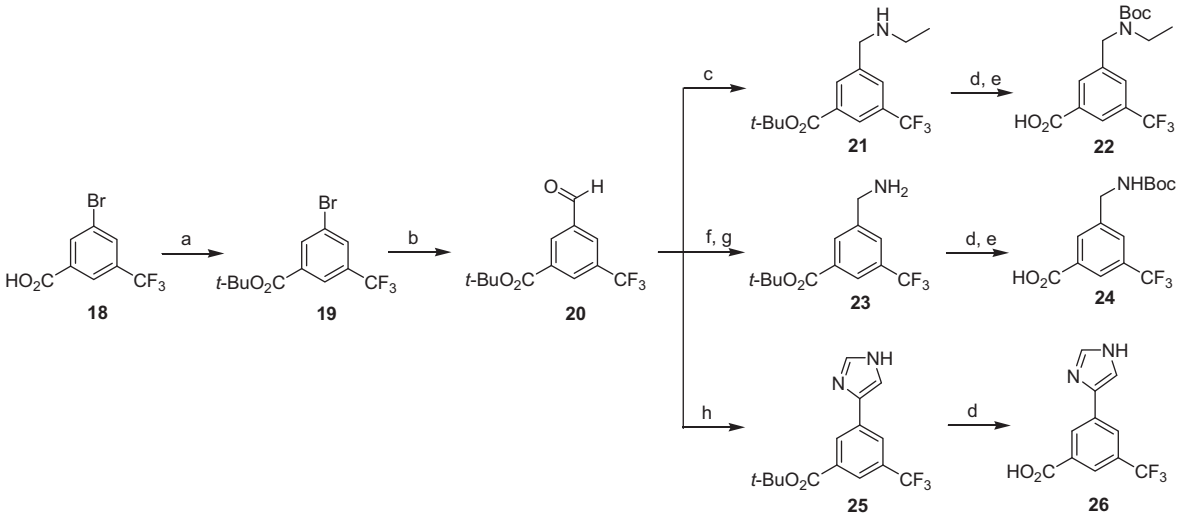


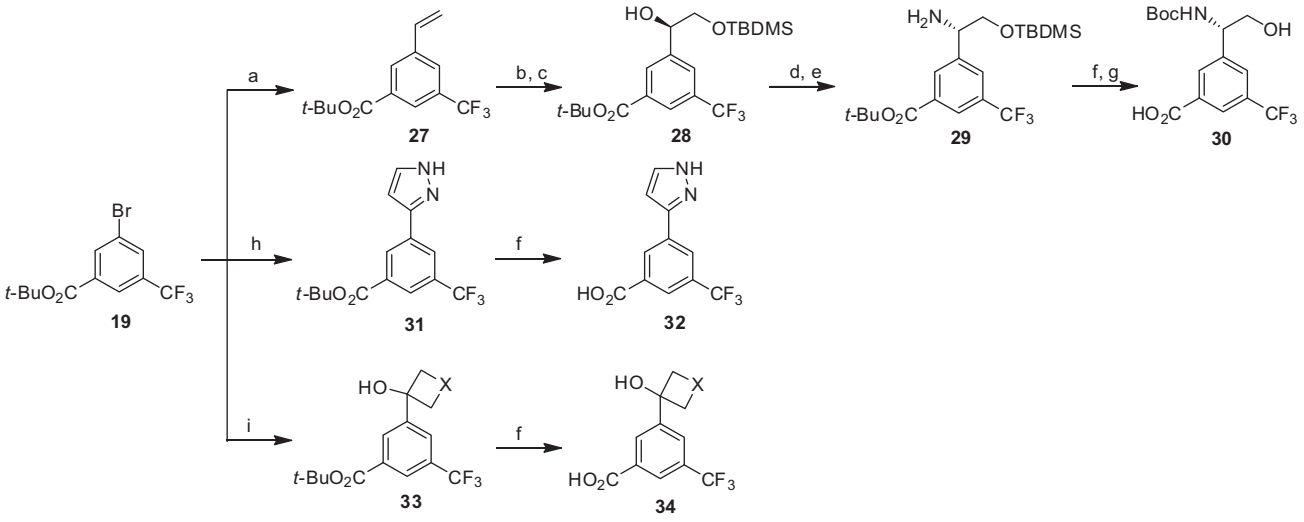
Figure 4. Inhibition of subcutaneous A375M xenografts in immunocompromised mice by **16**.[19]



Scheme 1. Synthesis of amide-based Raf inhibitors. Reagents and conditions: (a) benzoic acid, EDCI, pyridine, rt then HCl, DCM, rt.



Scheme 2. Synthesis of benzoic acids. Reagents and conditions: (a) oxalyl chloride, DMF/DCM, rt, 18 h then KO*t*Bu, THF, 0 °C, 1 h, 91%. (b) *i*PrMgCl·LiCl, THF, −20 °C, 1 h then DMF, −20 °C, 30 min, 69%. (c) Ethylamine, $NaB(OAc)_3H$, DCM, rt, overnight, 90%. (d) TFA, DCM, rt, 2 h then HCl, DCM, 90–100%. (e) $(BOC)_2O$, NaOH, dioxane/H_2O, rt, 2 h, 70–95%. (f) NH_4OH·HCl, pyridine, EtOH, rt, 5 h, quant. (g) Raney®-Nickel, NH_3, MeOH, H_2, rt, 18 h, 66%. (h) TosMIC, NaCN, EtOH, rt, 30 min then NH_3, MeOH, 110 °C, 20 min, 17%.



Scheme 3. Synthesis of benzoic acids. Reagents and conditions: (a) potassiumvinyltrifluoroborate, $PdCl_2$, PPh_3, Cs_2CO_3, THF/H_2O, 85 °C, overnight, 65%. (b) AD-mix-alpha, *t*BuOH/H_2O, rt, overnight, 78%. (c) TBDMS-Cl, imidazole, DMF, rt, 4 h, 86%. (d) PPh_3, DPPA, DIAD, THF, 0 °C, 2 h, 83%. (e) PPh_3, THF, H_2O, 60 °C, 3 h, 72%. (f) TFA, DCM, rt then HCl, DCM, 95–100%. (g) $(BOC)_2O$, NaOH, dioxane/H_2O, rt, 3 h, 46%. (h) 5-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole, $Pd(Ph_3P)_4$, Na_2CO_3, dioxane, 100 °C, 10 min, 69%. (i) X = $NCHPh_2$ *i*PrMgCl·LiCl, THF, −20 °C, 90 min then 1-benzhydrylazetidin-3-one, −20 °C to rt, 30 min, 31%.

imidazoles can be accessed through aldehyde **20** by a Van Leusen reaction.[20]

Scheme 3 illustrates the elaboration of bromide **19** into additional benzoates. A sequence involving a modified Suzuki reaction,[21] Sharpless dihydroxylation and Mitsunobu reaction allowed access to amino-alcohol **30**. Suzuki coupling provided pyrazole **31** and similar conditions provided access to additional heteroaryl benzoates required for the synthesis of compounds **13**, **15** and **16** (see Table 1). Grignard reaction of **19** and benzhydrylazetidin-3-one led to azetidine **33**.

In summary, our focus in this study was the improvement of hERG selectivity in the series characterized by lead compound **1**.

Modification of the benzyl amine-containing portion of the molecule provided analogs with reduced hERG channel activity, leading to optimized derivative **16**. Furthermore, compound **16** was found to exhibit good pharmacokinetic properties to display strong inhibition of the Raf pathway in vivo and to potently inhibit tumor growth in mice.

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9. hERG activity was determined by the inhibition of Tracer Red fluorescent probe binding to hERG channels expressed in Predictor™ hERG membranes (Invitrogen).
10. In cardiology, the QT interval is a measure of the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle. The QT interval represents electrical depolarization and repolarization of the ventricles. (a) De Ponti, F.; Poluzzi, E.; Cavalli, A.; Recanatini, M.; Montanaro, N. *Drug Safety* **2002**, *25*, 263; (b) Redfern, W. S.; Carlsson, L.; Davis, A. S.; Lynch, W. G.; MacKenzie, I.; Palethorpe, S.; Siegl, P. K.; Strang, I.; Sullivan, A. T.; Wallis, R.; Camm, A. J.; Hammond, T. G. *Cardiovasc. Res.* **2003**, *58*, 32.
11. For synthetic details see U.S. Pat. Appl. Publ. (2010), US 2010/0197924. Compounds **1**, **3** and **6** were prepared as described previously.[8]
12. Enzyme assay details: 15 μL of a solution containing 50 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), pH 7.5, 0.025% Brij 35, 10 mM 1,4-dithiothreitol (DTT), and 10 nM BRafV600E were added to the wells of an assay plate containing compound and the mixture was incubated for 20 min. A substrate solution (15 μL) containing 50 mM HEPES, pH 7.5, 0.025% Brij 35, 5 mM β-glycerol phosphate, 10 mM $MnCl_2$, 2 μM peptide (Biotin-DRGFPRARYRARTTNYNSSRSRFYSGFNSRPRGRVYRGRARATSWYSPY-NH2, New England Peptide), 1 μM ATP, 0.1 mg/mL BSA, and 33P ATP 0.5 μCi/reaction was then added. The reaction mixture was incubated for 3 h and then stopped by the addition of 50 μL of 100 mM ethylenediaminetetraacetic acid (EDTA). The stopped reaction mixture (65 μL) was transferred to a Flash Plate (Perkin-Elmer) and incubated for 2 h. The wells were washed three times with 0.02% Tween-20. Plates were read on a TopCount analyzer.
13. A375 cells seeded overnight were incubated with Raf inhibitors for 3 h. At the end of the incubation, the cells were fixed, permeabilized, blocking buffer added and the plates were incubated overnight. After the blocking buffer was discarded, the plates were incubated with anti-phospho-ERK antibody for 1 h followed by treatment with anti-horse radish peroxidase. Optical density was read at 650 nm for tetramethylbenzidine as substrate.
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15. Permeability was determined with the in vitro PAMPA permeability assay where test compounds are partitioned between two chambers divided by a lipid membrane. Compounds are run in duplicate at one of three pHs: 5.0, 6.2, and 7.4. The donor plate (bottom) contains 50 μM of the test compound at the specified pHs. A filter plate containing a lipid, a negatively charged mixture of 20% phospholipids and dodecane, which has been painted on a porous membrane is then placed on top of the donor plate. The acceptor plate (top) is filled with an acceptor buffer at pH 7.4 containing a chemical scavenger that mimics in vivo carrier proteins. Test compounds are allowed to distribute between the two chambers over the course of four hours. The relative absorbances of the UV traces, 250–490 nm, collected from the blank, reference, donor, and acceptor plates are used to calculate permeability in nm/sec.
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17. Compound **1** was dosed in 10% 2-hydroxypropyl-beta-cyclodextrin (HPBCD) for both routes of administration. Compound **16** was dosed in 10% HPBCD and in ethanol/PEG400/*N*,*N*-dimethylacetamide/water = 1:4:1:4 (by volume) for PO and IV, respectively.
18. The PK/PD study was performed in nude mice bearing A375M xenografts. Compound **16** was dosed as solutions in 10% HPBCD. Xenografts were harvested for analysis at the time listed after a single 33 mg/kg dose. Average of pERK levels determined by Li-Cor quantitative Western blots (3 or 4 animals per group) as compared to vehicle control set at 100%. Average of compound levels in the plasma of mice was determined by LC/MS/MS quantitation (3 or 4 animals per group).
19. 33 mg/kg BID is the maximum daily dose tolerated without body weight loss. Compound **16** was administered PO/BID for 21 days using the formulation described in the PK/PD studies.
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