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Development of potent B-Raf^{V600E} inhibitors containing an arylsulfonamide headgroup

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ABSTRACT

A potent series of inhibitors against the B-Raf^{V600E} kinase have been developed that show excellent activity in cellular assays and good oral bioavailability in rats. The key structural features of the series are an arylsulfonamide headgroup, a thiazole core, and a fluorine *ortho* to the sulfonamide nitrogen.

B-Raf is a member of the Raf family of protein kinases, and serves as a critical component of the MAPK signal-transduction pathway.[1] Mutations in the regulatory domain of B-Raf, particularly the V600E mutation, cause constitutive activation of the kinase function.[2] This activity is required to maintain the proliferation and oncogenic character of cancers possessing mutated B-Raf.[3] Selective inhibition of mutant B-Raf^{V600E} kinase should therefore provide clinical benefit to patients with cancers that commonly express these mutations, particularly melanoma and colon cancer.[4]

Compound **1** (Fig. 1), a B-Raf lead identified from our oncology-directed kinase programs, features an imidazopyridine core and a large hydrophobic benzamide headgroup. Although this compound displayed good activity in the B-Raf^{V600E} enzyme assay, it had little activity in either mechanistic (pERK) or anti-proliferative cell-based assays using the B-Raf^{V600E} mutant SKMEL28 cell line[5] (Table 1a).

In an effort to improve the cellular activity of the series, SAR in the headgroup, core and tail regions of compound **1** were explored in parallel (Fig. 1).

Headgroup SAR was explored by preparing a set of analogs in which the amide moiety linking the aryl headgroup to the core was replaced with a series of other linkers. The general synthetic route to these analogs is shown in Scheme 1.[6] After esterification, the anilines were reacted with either a benzoyl chloride, an aryl isocyanate, or an arylsulfonyl chloride to form the amide, urea, and sulfonamide linker-containing compounds, respectively. The

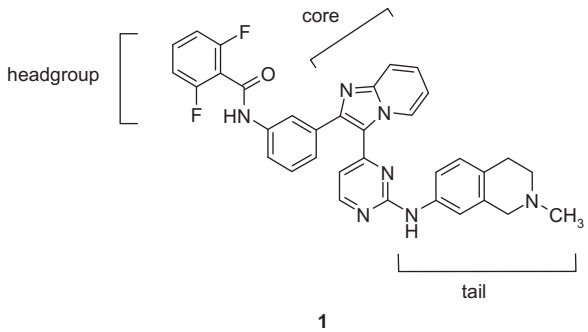

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Figure 1. Early benzamide B-Raf lead.

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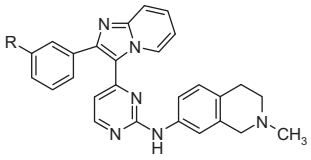
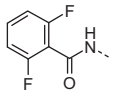
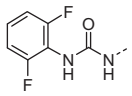
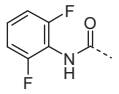
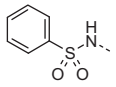
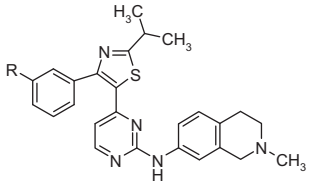
Table 1a
Headgroup linker SAR: Imidazopyridine core

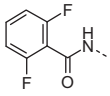
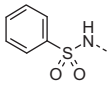


Entry	R	B-Raf^{V600E} IC_{50}[a] (nM)	pERK EC_{50}[a] (nM)	SKMEL28 EC_{50}[b] (nM)
1[c]		9	>10,000	5316
9		5	>20,000	6336
10[c]		12	>2200	NT[d]
11		132	99	1109

[a] B-Raf and pERK IC_{50} values are means of 2–4 experiments; individual values are within ±30% of the reported mean value.
[b] SKMEL28 IC_{50} values are means of two experiments; individual values vary approximately ±2-fold.
[c] Compounds tested as HCl salts.
[d] NT = not tested.

Table 1b
Alkylthiazole core



Entry	R	B-Raf^{V600E} IC_{50}[a] (nM)	pERK EC_{50}[a] (nM)	SKMEL28 EC_{50}[b] (nM)
12		4	580	2753
13		12	52	287

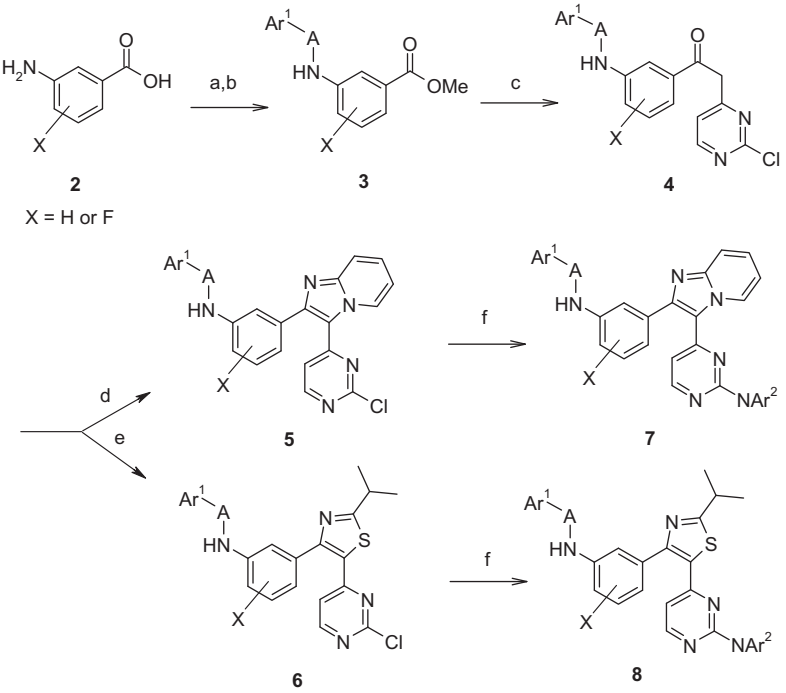
[a] B-Raf and pERK (B-Raf^{V600E} SKMEL28 cells) IC_{50} values are means of 2–5 experiments; individual values are within ±50% of the reported mean value.
[b] SKMEL28 IC_{50} values are means of two experiments; individual values are within ±5% of the reported mean value.

ester was condensed with the lithium anion of 2-chloro-4-methylpyrimidine to generate ketone **4**. Bromination of the ketone with NBS followed by cyclization with either 2-aminopyridine (**5**) or 2-methylpropylthioamide (**6**) afforded the desired heterocyclic core. The aniline 'tail' was then added to the chloropyrimidine using acidic conditions in a microwave to generate the desired analogs (**7** and **8**).

Evaluation of several different headgroup linkers (**1** and **9**–**11**) revealed that the sulfonamide-containing analog **11** showed a substantial improvement in cellular potency, particularly in the pERK mechanistic assay run in B-Raf^{V600E} mutant SKMEL28 cells. Interestingly, sulfonamide **11** had similar potency in the cellular pERK assay and B-Raf biochemical assay, although activity in the SKMEL28 anti-proliferative assay was still inadequate.

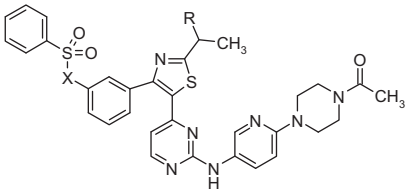
Replacing the imidazopyridine with a thiazole core, while keeping the benzamide headgroup, also increased potency in cellular assays (compare **12** with **1**). Combining these two observations generated the potent B-Raf inhibitor **13**, showing dramatic improvements in both pERK potency (52 nM) and anti-proliferative potency (287 nM) (Table 1b).

The importance of the sulfonamide N–H for B-Raf potency in the thiazole core series was further investigated and is shown in Table 2. For this set of analogs, the *N*-methyl-tetrahydroisoquinoline tail of **13** was replaced with an *N*-acetylpiperazinylpyridine tail to form **14** (Table 2). This tail had been shown to marginally improve potency in the B-Raf cellular assays.[7] The sulfonamide N–H was then either methylated (**15**), or replaced with a methylene group (**16**). The modification or loss of the sulfonamide N–H in these two analogs caused a dramatic reduction in their in vitro



Scheme 1. General synthetic route for imidazopyridine and thiazole inhibitors. Reagents and conditions: (a) H_2SO_4, MeOH; (b) for amide linker (A = CO): ArCOCl, TEA, DCM; for urea linker (A = NHCO): ArNCO, THF; for sulfonamide linker (A = SO_2): $ArSO_2Cl$, pyridine, DCM; (c) 2-chloro-4-methylpyrimidine, LiHMDS; (d) NBS, DCM then 2-aminopyridine, dioxane; (e) NBS, DMF then 2-methyl-propanethioamide; (f) aniline or aminopyridine tail, HCl, 2,2,2-trifluoroethanol, microwave, 180 °C.

Table 2
Sulfonamide N–H substitution



Entry	X	R	B-Raf^{V600E} IC_{50}[a] (nM)	pERK EC_{50}[a] (nM)	SKMEL28 EC_{50}[b] (nM)
14	NH	CH_3	3.6	7	24
15	NMe	H	1970	4731	8619
16	CH_2	H	1358	>20,000	11,091

[a] B-Raf and pERK (B-Raf^{V600E} SK-MEK-28 cells) IC_{50} values are means of two experiments; individual values are within ±50% of the reported mean value.
[b] SKMEL28 IC_{50} values are means of two experiments; individual values are within ±10% of the reported mean value.

activity. Thus, the sulfonamide N–H appeared to be a key pharmacophore for potent in vitro activity in this series.

The interactions between the sulfonamide group and the kinase were evaluated in a model of a compound from the sulfonamide thiazole series docked into a B-Raf^{V600E} crystal structure (Fig. 2).[8] We speculate that the arylsulfonamide nitrogen exists as a deprotonated species, making hydrogen bonding interactions with the backbone NH of Asp-594. This is similar to the binding modes observed for the sulfonamide groups in the B-Raf inhibitors PLX4720[8] and PLX4032.[4b] This model is also consistent with calculated pK_a data[9] showing the sulfonamide NH having a pK_a ~7, while the pK_a for the amide and urea analogs ranged from 10 to 12. This difference in pK_a, and thus the ability of the NH to ionize at the pH that the in vitro assays are conducted at (7.2–7.4), may explain the large potency differences observed between these linkers. Finally, the ammonium ion of Lys-483 is in close proximity to the sulfonamide anion, and can form a hydrogen bond with one of the sulfonamide oxygens whereas the other sulfonamide oxygen forms a hydrogen bond with the backbone NH of Phe-595.

The B-Raf potency of this series was further optimized through an SAR study of the aryl headgroup in sulfonamide **14**, summarized in Table 3. Addition of a fluorine in either the 2- or 3-position of the aryl ring (compounds **17** and **18**) was found to improve both the B-Raf enzyme activity and the potency in the SKMEL28 proliferation assay. Fluorine substitution in the 4-position of the ring (**19**), or a larger substituent, such as a chlorine (**20**), led to a moderate reduction in potency in both the enzyme and cellular assays. The difluorinated analogs **21** and **22** mirrored the high potencies observed for the mono-fluorinated analogs **17** and **18**.

The in vitro intrinsic clearances of compounds in the headgroup SAR series in rat liver microsomes are also listed in Table 3. Although the clearances are high for almost all compounds in the table, fluorination of the headgroup did marginally improve the metabolic stability of this series. The only analog found to show a moderate intrinsic clearance in rat microsomes was the 2,6-difluorinated sulfonamide **22**. This headgroup provided the best combination of potency and metabolic stability, and was thus used for further SAR exploration in other regions of the molecule.

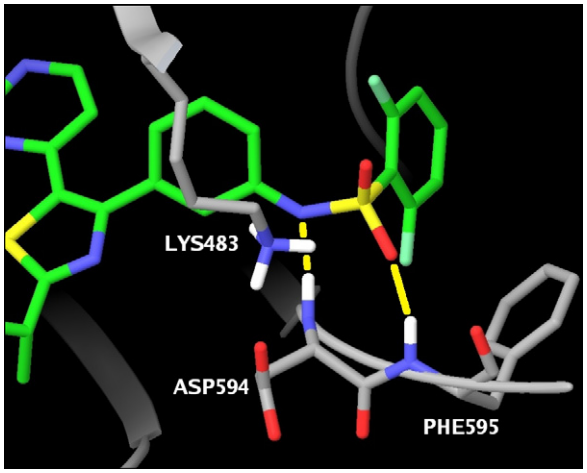
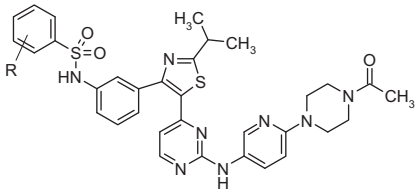


Figure 2. Model of sulfonamide inhibitor in the active site of B-Raf^{V600E}.

Having achieved excellent potency in the enzyme and cellular assays, our focus turned to further improving the metabolic stability and oral exposure of the series (Table 4). For this effort, the acetylpiperazine moiety in the tail group of **22** was replaced with a morpholine ring to generate **23**, which was found to marginally enhance the in vitro potency of the series. While **23** maintained excellent potency in all in vitro assays, it showed high intrinsic clearance in rat liver microsomes, and poor oral exposure in the rat. In an attempt to improve the metabolic stability of the series, potential metabolic sites were targeted. As such, a fluorine atom was systematically installed around the phenyl ring connecting the thiazole core to the sulfonamide. Analogs **24** and **25**, with a fluorine atom either *ortho* to both the thiazole and the sulfonamide nitrogen or *meta* to both groups, showed little improvement in oral exposure in rat despite showing a modest improvement in intrinsic clearance. However, a fluorine atom *para* to the thiazole (**26**) was found to reduce both the intrinsic clearance and the intravenous clearance, providing an almost 70-fold increase in the rat oral dose-normalized AUC compared to **23**. In comparing the four-fold improvement in iv clearance with the 70-fold improvement in oral

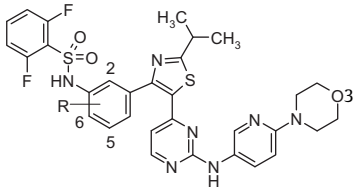
Table 3
Headgroup SAR



Entry	R	B-Raf^{V600E} IC_{50}[a] (nM)	pERK EC_{50}[a] (nM)	SKMEL28 EC_{50}[b] (nM)	Rat Cl_i[c]
14	H	3.6	7	24	20
17	2-F	0.6	4	4	18
18	3-F	0.8	10	8	NT[d]
19	4-F	9.5	9	69	NT[d]
20	3-Cl	34	21	85	12
21	2,5-F	0.6	8	8	15
22	2,6-F	1.3	10	12	7

[a] B-Raf and pERK (B-Raf^{V600E} SK-MEK-28 cells) IC_{50} values are means of 2–5 experiments; individual values are within two-fold of the reported mean value.
[b] SKMEL28 IC_{50} values are means of two experiments; individual values are within ±35% of the reported mean value.
[c] Intrinsic clearance data from liver microsomes, reported in units of mL/min/g liver. Values of <5 are considered low, between 5 and 8 are medium, and above 8 are high.
[d] NT = not tested.

Table 4
Fluorination of the inner-phenyl ring



2

Entry	R	B-Raf^{V600E} IC_{50}[a] (nM)	pERK EC_{50}[a] (nM)	SKMEL28 EC_{50}[b] (nM)	Rat Cl_i[c]	Rat iv Cl[d,e]	Rat po DNAUC[d,e]
23	H	1.2	7	6	19	NT[f]	42
24	2-F	0.5	11	8	10	69	87
25	5-F	0.9	24	14	11	48	58
26	6-F	3.8	23	47	8	12	2933

[a] B-Raf and pERK (B-Raf^{V600E} SK-MEK-28 cells) IC_{50} values are means of 2–5 experiments; individual values are within three-fold of the reported mean value.
[b] SKMEL28 IC_{50} values are means of two experiments; individual values are within two-fold of the reported mean value.
[c] Intrinsic clearance data from liver microsomes, reported in units of mL/min/g liver.
[d] Intravenous clearance reported in units of mL/min/Kg. Oral dose-normalized AUC reported as ng*h/mL/mg/kg.
[e] Data from cassette dosing.
[f] NT = not tested.

exposure, we believe that the 6-F may be improving both oral absorption as well as metabolic stability, perhaps by affording an intramolecular hydrogen bonding interaction with the sulfonamide NH. Although the fluorine in this position also caused a small decrease in the potency of **26** in cellular assays, the enhanced oral exposure represented a key breakthrough for the series. In cellular anti-proliferation assays, the corresponding HCl salt of compound **26** was found to be highly selective for the B-Raf^{V600E} cell line SK-MEL-28 versus a representative non-B-Raf^{V600E} cell line HFF (52 nM versus 15.6 μM). In order to assess its overall kinase selectivity profile, compound **26** was tested in a panel of 52 kinase assays and found to have excellent overall selectivity, showing IC_{50}'s between 10 nM and 100 nM for only 7 out of 52 kinases and greater than 100-fold selectivity (typically greater than 1000-fold) against the remainder. Other than B-Raf^{V600E} itself, the most potent additional kinase activities were ActR2B, Alk5, Btk, ErbB4, Lck, Lyn, and Src1. The combination of excellent enzyme and cell potencies of **26**, along with its promising in vivo oral exposure in rat, represented an excellent platform for further optimization into a drug candidate. Notably, higher species PK remained a major challenge for this series, and efforts within this arylsulfonamide headgroup series to improve higher species PK will be reported in due course.

In conclusion, a very potent series of inhibitors of the B-Raf^{V600E} mutant kinase has been developed, culminating with the identification of **26**. This compound combines excellent enzyme, cellular potency, and good rat oral exposure and served as a key lead for additional optimization of this series.

Acknowledgments

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