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Potent and selective pyrazole-based inhibitors of B-Raf kinase

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ABSTRACT

Herein we describe a novel pyrazole-based class of ATP competitive B-Raf inhibitors. These inhibitors exhibit both excellent cellular potency and striking B-Raf selectivity. A subset of these inhibitors has demonstrated the ability to inhibit downstream ERK phosphorylation in LOX tumors from mouse xenograft studies.

The Ras/Raf/MEK/ERK signaling pathway is critical for cell survival, growth, and proliferation. This intensely studied signal transduction pathway has been implicated in a number of human cancers. Examinations of cell lines and primary tumor samples have shown constitutive activation of the ERK pathway in cancers of the lung, colon, pancreas, kidney, and ovary.[1]

The serine/threonine kinase B-Raf, when mutated plays an important role in the development of cancer. B-Raf is mutated in approximately 7% of human cancers, and a majority of malignant melanomas (50–70%) and papillary thyroid cancers (40–70%) harbor B-Raf mutations.[2] The substitution of valine for glutamic acid at position 600 (V600E) accounts for around 90% of identified mutations.[3] The basal rate of MEK phosphorylation from ^{V600E}B-Raf is 500-fold greater than wild type B-Raf, providing a strong signal for growth and proliferation to cancer cells.[4] The high prevalence of B-Raf mutations in human cancer and their prominent role in the Ras/Raf/MEK/ERK signaling pathway in tumorigenesis and progression suggests that disruption of this signaling cascade could offer a beneficial approach for cancer chemotherapy.

Several research programs which are currently targeting inhibition of B-Raf have been cited in recent reviews.[5] Figure 1 shows two distinct structural classes of B-Raf inhibitors, SB-590885[6] and RAF-265, of which the later has advanced into phase I clinical trials.[7] We recently became interested in pursuing a research program on B-Raf inhibition, and discovered a substituted pyrazole as a potentially novel lead structure (Fig. 2).

Our studies began with the synthesis of unsubstituted pyrazole **1** which defined our minimum pharmacophore. Methylation of the pyrazole led to a mixture of N1 and N2 methyl isomers. The N2 methylated pyrazole **2b** showed a threefold loss in cellular potency (pERK), whereas the N1 methyl pyrazole **2a** displayed a modest gain in potency relative to **1** (Table 1).[8] This trend was seen for a number of analogs (data not shown), which focused our efforts toward further exploration of N1-modified pyrazoles. Extension of the alkyl chain by one carbon further increased cellular potency (**3**) and in general, increased bulk at this position, such as with the cyclopentyl analog **4**, was well tolerated. Further substitution on the cycloalkyl ring showed a dependence on stereochemistry. While the *cis* hydroxy analog **5** was less potent, the *trans* isomer **6** was nearly sevenfold more active. A further increase in potency was observed in analog **7**, which was slightly more active than its corresponding *trans* isomer **8**. Although ethers (e.g., **9**) were in general less active, incor-

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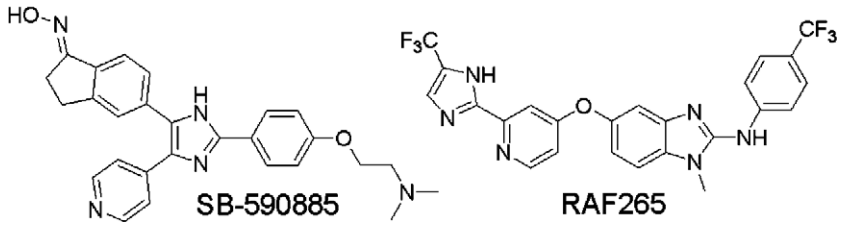


Figure 1. Selected B-Raf inhibitors.

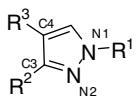
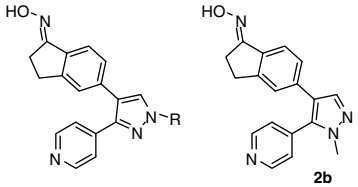
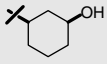
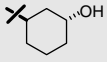


Figure 2. Substituted pyrazoles as B-Raf inhibitors.

Table 1
B-Raf activity of N1-modified pyrazoles: alkyl and hydroxylalkyl substituents



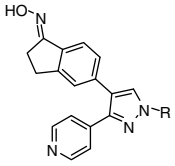
Compound	R	B-Raf IC_{50}[a] (nM)	pERK IC_{50} (nM)
1	H	0.15	53
2a	CH_3	0.02	33
2b	—	0.48	165
3	CH_2CH_3	0.57	18
4	Cyclopentyl	3.46	57
5		3.91	245
6		0.33	37
7		0.04	7
8		0.09	17
9	$CH_2CH_2OCH_3$	1.16	569
10	CH_2CH_2OH	0.13	63
11	$CH_2CH_2CH_2OH$	0.05	26
12	$CH_2CH(OH)CH_2OH$	0.87	22
13	$CH(CH_2OH)_2$	0.56	171

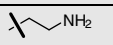
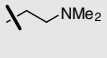
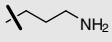
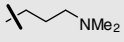
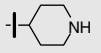
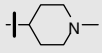
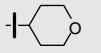
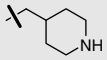
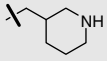
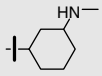
[a] IC_{50} values reflect the average from at least three separate experiments.

poration of alcohols was well tolerated (e.g., **10–13**). Such derivatives led to increased aqueous solubility and provided a convenient handle for further substitution and modulation of physiochemical properties.

In addition to solubilizing tails such as hydroxyl alkyls, a number of tethered amino substituents were examined (Table 2). The ethyl-linked amines were tolerated, but less active in the cellular assay than their propyl-linked counterparts (**14, 15** vs **16**, **17**). In both chain lengths, methylation of the terminal nitrogen caused a decrease in potency (**14** vs **15** and **16** vs **17**). Cyclic amines, such as piperidine **18**, led to increased potency, and further substitution (**19**) maintained comparable cellular activity. Extending the cyclic amines with a one-carbon spacer caused a reduction in potency (**21** and **22**) while extension of amines directly from the carbocycle displayed good activity in both enzymatic and cellular assays (e.g., **23**).

Table 2
B-Raf activity of N1-modified pyrazoles: amino substituents

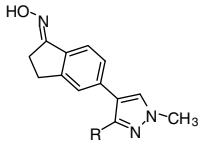


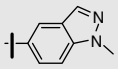
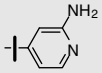
Compound	R	B-Raf IC_{50}[a] (nM)	pERK IC_{50} (nM)
14		2.4	119
15		4.02	607
16		0.39	20
17		0.47	122
18		0.03	9
19		0.34	10
20		0.22	48
21		1.2	332
22		0.85	154
23		0.23	18

[a] IC_{50} values reflect the average from at least three separate experiments.

Although modifications at N1 simultaneously increased solubility and potency, some compounds in this series displayed an undesirable CYP inhibition profile. To explore this property, several 4-pyridyl replacements were made (Table 3). Under the assay conditions, both indazole **24** and the 3-pyridyl **25** analogs showed no detectable cellular activity, while the oxazole **26** showed a 70-fold loss in activity relative to **2**. This seemed consistent with a theory that improved binding occurs when the

Table 3
B-Raf activity of C3-modified pyrazoles



Compound	R	B-Raf IC_{50}[a] (nM)	pERK IC_{50}[a] (nM)
24		969.6	–
25		433.8	–
26		4.1	2400
27		2.1	1000
28		2.9	800
29		3.1	1500

[a] IC_{50} values reflect the average from at least three separate experiments.

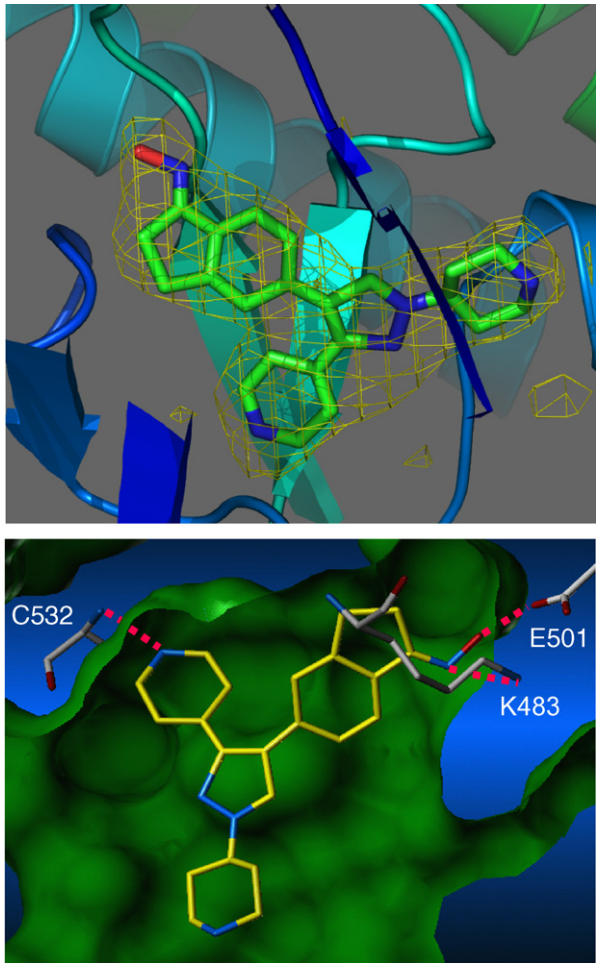


Figure 3. (a) X-ray crystal structure of **18** bound to B-Raf, at 2.8 Å resolution. The electron density shows an $F_o - F_c$ omit map, contoured at 2.5sigma. The structure is viewed from the N-terminal domain of B-Raf, looking into the ATP-binding site. The "hinge" region of B-Raf is on the left of the figure. (b) Cut away view of **18** on surface of B-Raf. Hydrogen bonds are depicted as dashed red lines.

C3 substituent is aligned to accept an H-bond from the hinge region of the protein (Fig. 3). Attempts to retain potency by keeping an H-bond acceptor in this region (e.g., **27–29**) were ineffective.

H-bond interactions made by the oxime moiety were revealed in the X-ray structure of the B-Raf complex co-crystallized with inhibitor **18** (Fig. 3a).[9] Our SAR evaluation at the C4 position of the pyrazole ring was aimed toward retaining these contacts and simultaneously improving the poor aqueous stability at low pH that characterized the oxime series.[10] While the hydroxyamidine analogs **30–32** had the potential H-bonding network intact, only analog **31** displayed good potency. This suggests that, in certain derivatives, potency increases with both a proper display and appropriate filling of the hydrophobic pocket with which the indane moiety makes contact (Fig. 1b). A similar SAR trend was also observed for pyrazole analogs **33–35** in which conformational restraint and additional hydrophobic contact serve to increase potency. Both the lactam **36** and phenol isostere[11] **37** lost potency relative to **2** (Table 4).

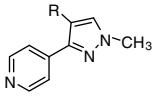
The synthesis used to access analogs such as **10** is outlined in Scheme 1. Pyrazole **39**[12] was prepared from 4-acetylpyridine via cyclization of the intermediate enaminoketone with hydrazine and subsequent bromination. Alkylation of the pyrazole at N1 with halides or tosylates was substrate-dependant, but generally afforded selectivity better than 6:1. Other electrophiles such as cyclic epoxides and enones, (e.g., **5–8**) reacted with exclusive N1 selectivity. Palladium-mediated coupling of **40** and commercially available **41** followed by treatment with hydroxylamine provided the *E*-oxime analogs.

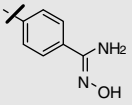
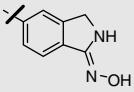
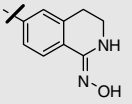
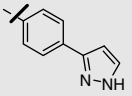
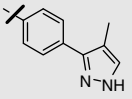
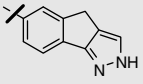
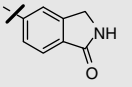
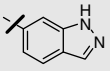
Four compounds were selected for further evaluation based upon physiochemical (Table 5)[13] and in vivo properties (data not shown). Female nude mice bearing established subcutaneous LOX tumor xenografts were given a single 25 mg/kg po dose of compound in 50% PEG400/0.05 N HCl vehicle. At 1 and 4 h post dose, blood was collected for analysis of the plasma level of the compound. Tumors were harvested, then processed for Western blot quantitation of phosphorylated ERK (p44/42, thr202/tyr204) and total ERK (Fig. 4). Compound **18** had no detectable plasma levels and correspondingly, no effect on pERK levels. Compounds **2**, **7**, and **10** all showed plasma levels at the 1 hour time-point, which correlated well with pERK inhibition.

The selectivity of a subset of these inhibitors was examined. Compounds **2, 7**, and **10** were screened against a panel of over 70 protein kinases at 1 μM. None of the compounds tested showed greater than 50% inhibition against any of the enzymes with the exception of C-Raf. Furthermore, when compound **10** was screened in an expanded panel of 212 protein kinases, it was again selective, only showing expected activity against C-Raf.

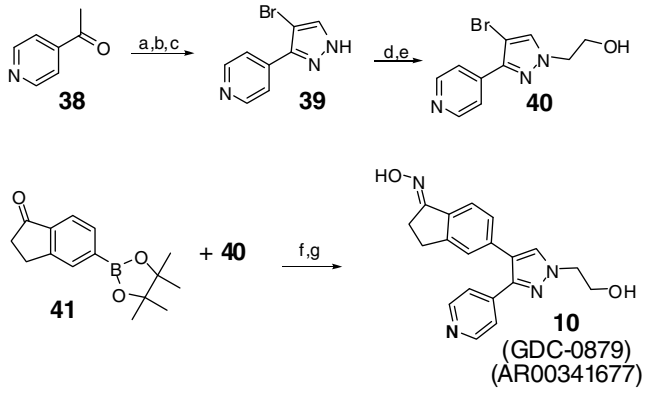
In summary, we have utilized a pyrazole core to produce B-Raf inhibitors with excellent potency, physiochemical properties, and selectivity profiles. A subset of these compounds showed significant in vivo inhibition of pERK in xenograft studies. Further progress on these inhibitors will be reported in due course.

Table 4
B-Raf activity of C4-modified pyrazoles



Compound	R	B-Raf IC_{50}[a] (nM)	pERK IC_{50} (nM)
30		18.4	20,000
31		<2.0	56
32		16.0	10,000
33		68.4	–
34		4.4	18,000
35		1.5	1900
36		68.4	–
37		70.0	–

[a] IC_{50} values reflect the average from at least three separate experiments.



Scheme 1. Reagents and conditions: (a) DMF–DMA, tol, Δ, (78%); (b) hydrazine, EtOH, Δ, (96%); (c) bromine, NaOAc, AcOH, (70%); (d) 2-bromoethyl acetate, potassium carbonate, DMF; (e) water, MeOH, potassium carbonate, Δ, (76%); (f) tetrakis(triphenylphosphine)palladium, potassium carbonate, ACN–water, Δ, (74%); (g) hydroxylamine hydrochloride, EtOH, Δ, (78%).

Acknowledgement

The authors thank Andrew Allen for NMR analysis and Susan Rhodes, Jennifer Otten, and Michelle Livingston for Caco, P450, and solubility determinations.

Table 5
In vitro stability, permeability, and solubility

	2	10	7	18	31
Hepat. Cl[a]	7	6	8.5	7.8	8
Caco-2	High	High	High	Med	High
Sol. @pH6.5[b]	4[c]	76[c]	3[c]	52	590

[a] Human hepatocyte clearance (mL/min/kg).
[b] Aqueous thermodynamic solubility (μg/mL).
[c] Crystalline solid.

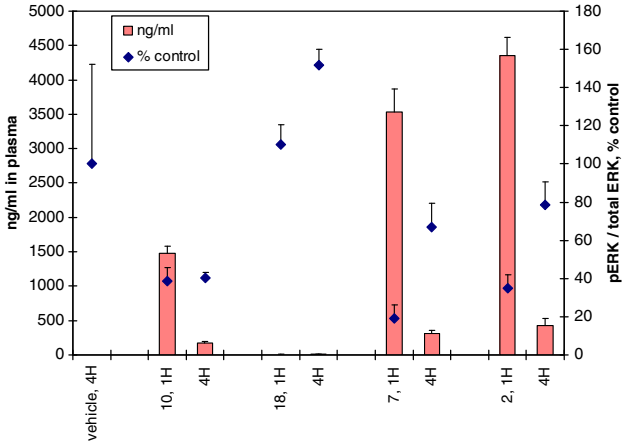


Figure 4. PK/PD evaluation of substituted pyrazoles after 25 mg/kg po dose in LOX tumor-bearing mice. Four per group, error bars are SEM.

References and notes

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