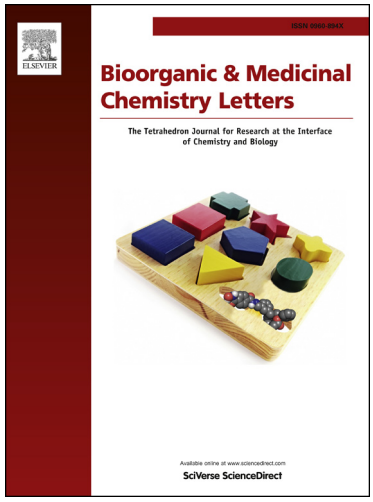

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Imidazo[4,5-b]pyridine inhibitors of B-Raf kinase

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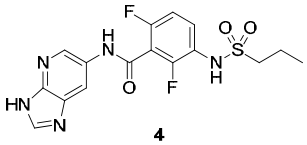

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ABSTRACT

This report details the synthesis and evaluation of imidazo[4,5-b]pyridines as inhibitors of B-Raf kinase. These compounds bind in a DFG-in, αC-helix out conformation of B-Raf, which is a binding mode associated with significant kinase selectivity. Structure activity relationship studies involved optimization of the ATP-cleft binding region of these molecules, and led to compound **23**, an inhibitor with excellent enzyme/cell potency, and kinase selectivity.

The mitogen activated protein kinase (MAPK) signaling pathway has garnered much interest in the past several years as a target for therapeutic intervention, specifically in oncology.[1] This pathway consists of the Ras-Raf-MEK-ERK cascade, which is known to regulate cell growth, proliferation, differentiation, and survival.[2] Of particular interest is the serine/threonine protein kinase Raf, which exists in three isoforms, A-Raf, B-Raf, and C-Raf. Mutations in the B-Raf gene lead to constitutive activation of B-Raf and amplification of the cell signaling pathway,[3] and are observed in many cancer types. The glutamic acid for valine substitution (V600E), accounts for over 90% of B-Raf mutations, and correlates with poor survival,[4] thereby making B-Raf an attractive anti-cancer target.

The approval of PLX-4032 (vemurafenib, Zelboraf®),[5] a potent and selective B-Raf inhibitor for the treatment of melanoma, provides clinical validation of B-Raf as an anticancer target (Figure 1). Other small molecule B-Raf inhibitors (Figure 1) in various stages of clinical development include XL-281 (BMS-908662), RAF265, and GSK2118436.[6] Recent reports from our group describe the synthesis and evaluation of potent and selective pyrrolopyridine,[7] pyrazolopyridine,[8] and pyrazolopyrimidine[9] B-Raf inhibitors (Figure 2, compounds **1a**-**3a**). As an extension of this "scaffold-hopping"[10] strategy, we prepared a series of imidazo[4,5-b]pyridine analogs (Figure 2, compound **4**) in order to exploit the C-2 position, which was inaccessible with the pyrazolopyridine series.

The X-ray crystal structure of compound **4** (Figure 3)[11] in complex with B-Raf revealed that the kinase adopts a DFG-in configuration[12] in which **4** occupies the ATP cleft with the

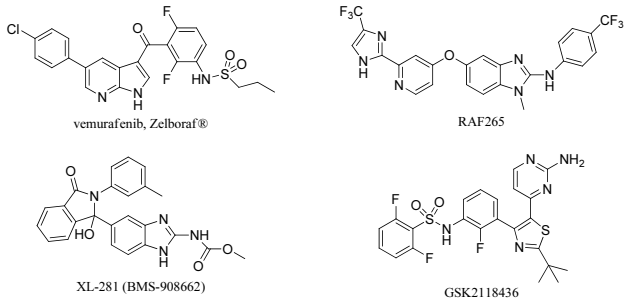
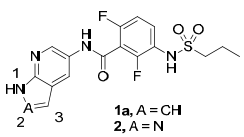
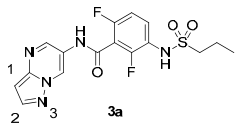
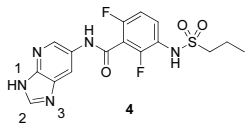


Figure 1. B-Raf inhibitors in clinical development.







Cmpd	B-Raf IC_{50} nM	pERK IC_{50} nM
1a	42	94
2	18	62
3a	247	488
4	61	2386

Figure 2. Pyrrolo, pyrazolo pyridines **1a** and **2,** pyrazolpyrimidines **3a,** and imidazo[4,5-b]pyridine **4.**

imidazopyridine core forming hydrogen bonds with the NH and carbonyl of Cys532, as previously observed with the pyrazolo- and pyrrolopyridine templates. The sulfonamide moiety forms hydrogen bonds with the DFG backbone, while the propyl side chain inserts into a small lipophilic pocket created by outward movement of α-helix C. Since the NH of the imidazo core was desirable for hydrogen bonding to the hinge, we sought to explore substitution of the 2-position into the exterior of the ATP cleft.

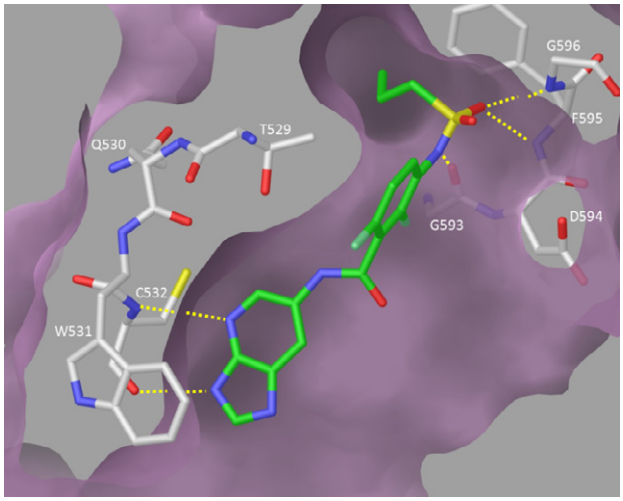


Figure 3. X-ray crystal structure of **4** (3.6Å resolution) in complex with B-Raf. The enzyme surface is illustrated in violet; the hinge residues and the Gly-DFG sequence at the N-terminus of the activation loop are explicitly displayed. Hydrogen bonds are depicted as dashed yellow lines.

Compound **4** (Table 1) showed promising enzyme activity (61 nM), however its cellular potency[13] was less than ideal (2.4 μM). Substituting the 2-position of the imidazo core with various alkyl groups (linear and branched, compounds **5-8**) failed to improve the cellular potency. Piperidine incorporation (compounds **10** and **11**) essentially rendered these analogs inactive in the cellular assay, most likely due to its basic nature.

To further probe the ATP cleft, the 2-phenyl imidazopyridine analog **12** (Table 2) was prepared. We were delighted to observe significant enhancement in both enzyme and cell activity versus the hydrogen, alkyl and piperidine substituted compounds **4-11** (Table 2). Cellular potency, however, was still 4- to 11-fold lower than **3b** and **1b**. A trend emerged in 4-halo substituted compounds **13, 14,** and **15**. Enzyme and cellular activities improved as the halogen size increased (the opposite was observed with 2-halo substituted compounds **19-21,** confirming that co-planarity between the phenyl ring and imidazo core is advantageous), but **13** and **14** were still less active in the cell than **3c/1c** and **3d/1d** respectively.

Of the 3-halo substituted compounds **16-18**, 3-chlorophenyl compound **17** proved to be the most tolerated. Preparation of the 3,4-dihalo analogs **22** and **23** essentially remained equipotent to its mono halo counterparts **16** and **17**, respectively.

The effects of electron donating and withdrawing groups on the phenyl ring are outlined in Table 3. Compounds **24-31** all showed excellent enzyme potency, with the 4-Me compound **25** proving to be the most potent in the cell. Interestingly, compound **27** with its $-OCF_3$ electron withdrawing group had slightly weaker cell potency but the most favorable cell-to-enzyme shift. Good enzyme potency was observed with pyridyl analogs **32** and **33**, however these heterocycles were not tolerated in the cell assay.

The gains in enzyme potency of aromatic groups over alkyl groups could be rationalized by the proposed binding mode in Figure 4. The expected orientation for compound **23** in B-Raf shows that multiple lipophilic contacts are available (such as Ile463, Trp531, Glu533, and the main chain of Gly534, and Ser535). A beneficial aromatic stacking contact is made between the Trp531 residue and the imidazole portion of the core and 2-aryl ring.

The diminished cell potencies and larger enzyme to cell shifts[14] observed could arise from physiochemical properties of the molecule. A strong correlation between Polar Surface Area (PSA) and and cellular activity has been noted in the literature.[15] PSA calculations on the imidazopyridine series (data not shown)[16] show it to be 10-15 points higher than its pyrrolopyridine and pyrazolopyrimidine counterparts. In addition, relative to the pyrazolopyrimidine series, the imidazopyridines have an additional hydrogen bond donor.

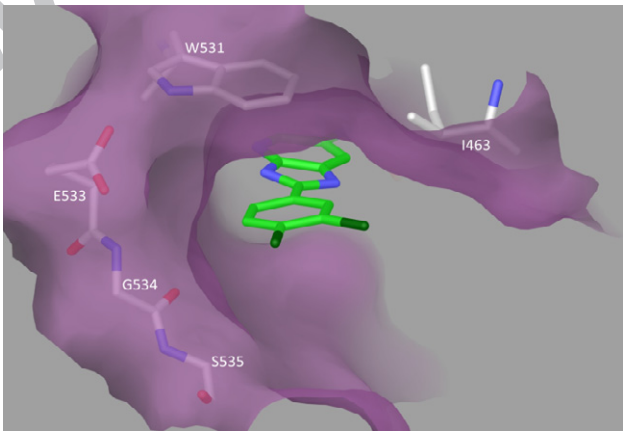
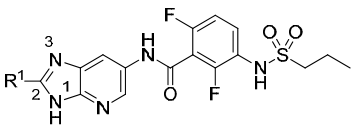


Figure 4. Compound **23** docked into B-Raf. Residues that make lipophilic contacts to the dichlorophenyl ring are explicitly shown.

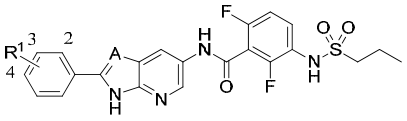
Table 1.
Hydrogen, alkyl, and piperidine substituted analogs **4-11**.



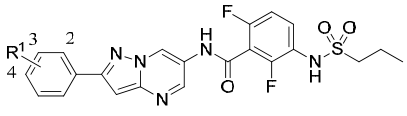
Compound	R^1	B-Raf IC_{50} (nM)	pERK IC_{50} (nM)	Cell/Enzyme
4	H	61	2386	39
5	Me	40	1710	43
6	Et	59	1768	30
7	i-Pr	60	1728	29

8	t-Bu	69	1560	23
9	cyclobutyl	31	3332	107
10	4-piperidine	107	>10,000	>93
11	3-piperidine	167	>10,000	>60

Table 2.
Phenyl and halogen substituted aryl analogs **12-23 and 3 b-d**. Compounds **1b-d** and **3b-d** were included for comparison.



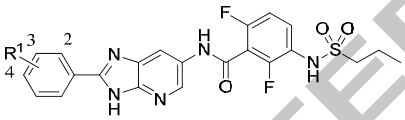
12-23



3 b-d

Compound	R^1	A	B-Raf IC_{50} (nM)	pERK IC_{50} (nM)	Cell/Enzyme
12	H	N	3.6	132	37
1b	H	CH	1.0	32	32
3b	H	---	4.6	12	2.6
13	4-F	N	4.4	1052	240
1c	4-F	CH	1.1	20	18
3c	4-F	---	11	54	4.9
14	4-Cl	N	2.2	157	71
1d	4-Cl	CH	2.4	20	8.4
3d	4-Cl	---	8.2	90	11
15	4-Br	N	2.2	61	28
16	3-F	N	3.1	298	97
17	3-Cl	N	1.1	68	62
18	3-Br	N	0.76	172	226
19	2-F	N	8.0	167	21
20	2-Cl	N	27	557	21
21	2-Br	N	27	948	35
22	3,4-di-F	N	3.4	194	57
23	3,4-di-Cl	N	1.5	81	54

Table 3. Electron donating and withdrawing group substitution and pyridyl analogs.



Compound	R^1	B-Raf IC_{50} (nM)	pERK IC_{50} (nM)	Cell/Enzyme
12	H	1.6	165	103
24	4-MeO	1.1	137	125
25	4-Me	1.3	49	38
26	4-CF_3	1.4	89	62
27	4-CF_3O	2.5	86	34
28	4-CN	2.7	239	88
29	4-$MeSO_2$	1.4	728	527
30	3-MeO	1.2	271	219
31	3-CF_3	1.0	113	112
32	4-pyridyl	3.2	1909	596
33	3-pyridyl	3.0	1125	375

Compound **23** was selected for further characterization; a panel screen of 26 kinases showed no significant activity at 1μM, with the expected exception of C-Raf. No cytochrome P450 inhibition was observed (data not shown).

When subjected to human and mouse microsome and hepatocyte stability assays, compound **23** showed low predicted clearances (Table 5). Interestingly, rat microsome and hepatocyte predicted clearances binned moderate to high, suggesting higher metabolism than other species. Little exposure was observed when **23** was dosed orally in a mouse pharmacokinetic study (30 mg/kg), which was consistent with its low permeability in a Caco-2 assay (Pe AB = 2.0 x 10^{-6} cm/s). As shown in Table 4, the imidazopyridine series in general suffered from low permeability relative to the pyrrolopyridine and pyrazolopyrimidine series.

Table 4. Caco-2 permeability of select compounds.

Compound	Caco-2 Pe AB*	Compound	Caco-2 Pe AB*
1a	7.3	1b	5.6
2	1.3	*15*	*1.3*
3a	11.4	*23*	*2.0*
3b	15.6	*25*	*2.6*
4	*0.6*	*26*	*1.0*

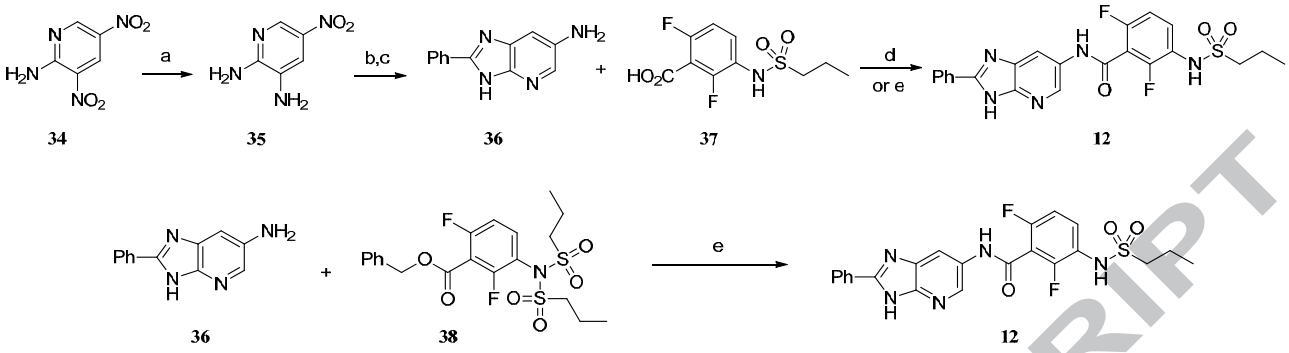
* x 10^{-6} cm/s
Imidazopyridine series compounds are italicized.

Table 5. Predicted microsome and hepatocyte clearances (ml/min/kg) for compound **23.**

	Human	Rat	Mouse
Microsomes	2.5	24.2	10.6
Hepatocytes	3.9	49.7	2.6

The imidazopyridines were generally prepared according to Scheme 1. Selective reduction of 3,5-dinitropyridin-2-amine (**34**) with a 20% aqueous solution of ammonium sulfide in refluxing methanol afforded a quantitative yield of 5-nitropyridine-2,3-diamine (**35**)[17]. Imidazopyridine formation was carried out by condensing **35** with benzoic acid (or equivalent) in refluxing $POCl_3$. Nitro reduction under zinc/ammonium chloride conditions gave the amino imidazopyridine **36**, which was then

coupled with **37**[18] to give the final product **12**. Alternatively, amide coupling can be performed by treatment of **36** with trimethyl aluminum, followed by the addition of ester **38**[15] at 90°C. The resulting bis-sulfonamide is then de-protected in aqueous potassium carbonate-methanol at 60°C to give **12**.



Scheme 1. Preparation of imidazopyridines: (a) 20% aq. ammonium sulfide, MeOH, reflux, 100%; (b) $PhCO_2H$, $POCl_3$, reflux, 43%; (c) zinc/NH_4Cl, EtOH/water, 60%; (d) EDCI, HOBT, DMF, 30-60%; (e) Me_3Al, toluene, 90°C then 2M aqueous K_2CO_3, methanol, 60°C, 25%.

We have prepared a series of imidazo[4,5-b]pyridine B-Raf inhibitors with excellent potency and selectivity. X-Ray analysis of compound **4** shows the imidazo core forming contacts with the hinge binding region of B-Raf, consistent with what has been observed with related series of inhibitors. Probing into the exterior of the ATP-cleft with this series led to **23**, a potent and selective inhibitor. Further progress of these compounds will be reported in due course.

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