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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 378–381

The identification of potent and selective imidazole-based inhibitors of B-Raf kinase

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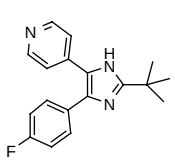
Received 28 July 2005; revised 23 September 2005; accepted 28 September 2005
Available online 2 November 2005

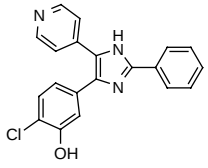
Abstract—A novel triarylimidazole derivative, SB-590885 (**33**), bearing a 2,3-dihydro-1*H*-inden-1-one oxime substituent has been identified as a potent and extremely selective inhibitor of B-Raf kinase.

Mitogen-activated protein (MAP) kinases are a family of serine/threonine protein kinases that participate in signal transduction pathways controlling numerous intra-cellular events.[1] MAP kinases are regulated by phosphorylation cascades whereby activation of an upstream kinase leads to phosphorylation of a downstream substrate which itself has protein kinase activity. Typically in such MAP kinase cascades, three protein kinases are sequentially activated in response to appropriate extracellular stimuli and allow for signal amplification at each step of the cascade.

The RAF-MEK-ERK MAP kinase cascade appears to be intimately involved in the regulation of cell cycle progression and apoptosis. Indeed, activating mutations in B-Raf, one of the Raf family members, are reported to be present in 66% of malignant melanomas.[2] Disruption of this signaling cascade could thus offer a novel approach for cancer chemotherapy.[3] Conversely, increased activation of ERK1/2 has been reported in a number of in vitro models of neuronal cell death and following focal cerebral ischemia in in vivo rodent models of stroke.[4] Furthermore, inhibition of the cascade at the MEK level has proved to be neuroprotective leading to a significant reduction in infarct volume in such animal models.[5]



(**1**) B-Raf IC_{50} 900nM



(**2**) B-Raf IC_{50} 10nM

We sought to identify inhibitors of B-Raf, the likely major Raf isoform in the central nervous system, to assess their potential as neuroprotective agents in the treatment of stroke.

Screening of the SmithKline Beecham compound bank identified the tri-substituted imidazole (**1**) as a submicromolar inhibitor of B-Raf. Concurrently we became aware of a poster publication from Merck disclosing a related tri-aryl imidazole **2** (L-779,450) as a highly potent low nanomolar inhibitor of Raf.[6] Compound (**2**)

Keywords: B-Raf; Imidazole.
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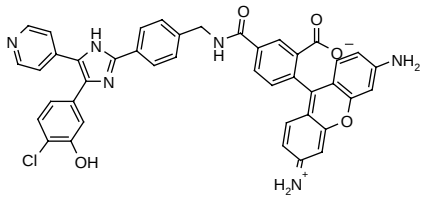
doi:10.1016/j.bmcl.2005.09.072

was reported to demonstrate good selectivity over other kinases tested, with the exception of p38 where the selectivity was approximately 30-fold. Unfortunately **2** is poorly soluble in aqueous systems (~0.005 mg/ml in pH 5 buffer) thus precluding its use as an in vivo tool where administration via iv infusion is desirable. We sought to explore the SAR of the imidazole leads whilst being mindful of the selectivity and developability issues described above.

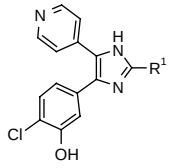
Our studies began with the synthesis of the hybrid molecule **3** which displayed comparable activity to **2**. Further investigation demonstrated that the imidazole C2 position was tolerant of a variety of substituents (Table 1). Heteroaryl derivatives such as **4** and **5** showed comparable potency with **2**, whereas the unsubstituted derivative **6** only showed a modest reduction in potency. Introduction of a basic amine substituent on either the C2 aromatic or *tert*-butyl groups was well tolerated (e.g., compounds **7** and **8**) as was the carboxamide linked derivative **9**. Such derivatives lead to enhanced aqueous solubility but also provide a handle for further substitution (e.g., **10**, **11**) and modulation of the physicochemical properties of the series.

Acylation of the C2 benzylamine **7** was also of value in the generation of a fluorescent derivative **12** (SB-477790) which proved to be suitable for the development of a B-Raf fluorescent ligand binding assay (FP). The two B-Raf assay formats showed a good correlation in rank order potency but, due to increased throughput, the FP format was used to generate all subsequent SAR.



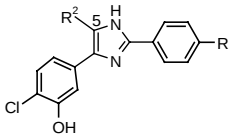
SB-477790 (**12**)

Table 1. B-Raf activity of C2 modified imidazoles[7]



Compound	R^1	B-Raf IC_{50} nM	B-Raf (FP) K_d nM
2	Ph	10	2.4
3	*t*Bu	12	2
4	3-Pyridyl	6	—
5	2-Furyl	13	4.2
6	H	33	39
7	4-(H_2NCH_2)Ph	8	5.3
8	$Me_2CCH_2NH_2$	6	4.8
9	$CONH(CH_2)_3NMe_2$	40	24
10	$Me_2CCH_2NHSO_2Me$	14	10
11	$Me_2CCH_2NHCONHPh(4\text{-}Cl)$	13	21

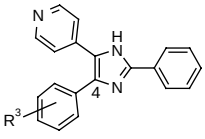
Table 2. B-Raf activity of C5 modified imidazoles



Compound	R	R^2	B-Raf (FP) K_d nM
2	H	4-Pyridyl	2.4
13	H	Phenyl	>200
14	H	3-Pyridyl	>200
15	H	4-Pyrimidinyl	14
16	H	4-Pyridazinyl	87
17	CN	2-Amino, 4-pyrimidinyl	5.7

Our investigation of the imidazole C5 substituent showed trends similar to those reported in the p38 kinase area (Table 2).[8] Replacement of the 4-pyridyl C5 substituent of **2** by phenyl- or 3-pyridyl groups (**13** and **14**, respectively) produced compounds that were essentially devoid of activity in the FP assay, thus implying that an appropriately positioned H-bond acceptor at C5 is essential for activity. This is consistent with the well-established binding mode of such imidazole-based kinase inhibitors where the C5 substituent forms an H-bond to the 'hinge' region of the protein.[8] As with p38, B-Raf activity can be retained if the C5 pyridine group is replaced by an alternative H-bond acceptor such as in the pyrimidinyl- and pyridazinyl-derivatives **15** and **16**, albeit with a 5- to 30-fold reduction in potency. The potency drop can be mitigated through the introduction of a 2-amino-4-pyrimidinyl substituent as in **17**, which is postulated to pick up a second H-bonding interaction with the hinge region of the protein.

Table 3. B-Raf activity of C4 modified imidazoles[a,b]



Compound	R^3	B-Raf (FP) K_d nM
2	3-OH, 4-Cl	2.4 (10)
18	3-OH	8
19	4-OH	316[c] (339)
20	3-OMe	>196
21	4-CH_2OH	71
22	3-CH_2OH	>196[c] (1585)
23	3-OH, 4-CH_2OH	22
24	3-Cl, 4-CH_2OH	132
25	4-CH=NOH	5
26	3-OH, 4-CH=NOH	27
27	3-Cl, 4-CH=NOH	6
28	4-C(Me)=NOH	11
29	4-$C(NH_2)$=NOH	53

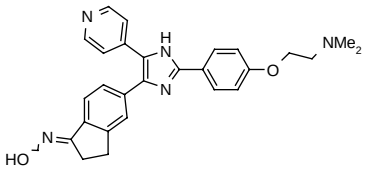
[a] Data from the B-Raf kinase assay is shown in parentheses (see Ref. 7).

[b] Oxime derivatives isolated as mixtures of *E* and *Z* isomers.

[c] Data derived from a variant of the FP binding assay and hence is not directly comparable.

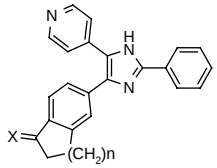
Early investigation of the imidazole C4 position SAR demonstrated that the 3-hydroxy phenyl substituent was key for potent B-Raf activity (Table 3). The mono-substituted 3-hydroxy derivative **18** showed comparable affinity to **2**, whereas the 4-hydroxy isomer **19** and 3-methoxy derivative **20** showed significantly reduced B-Raf affinity. These data suggested that the 3-hydroxy group was acting as an H-bond donor and so we embarked upon a search to identify alternative groups to perform this role. We were encouraged to find that the 4-hydroxymethyl derivative **21** showed modest affinity in the FP assay (K_d 71 nM), which was in contrast to the regioisomeric 3-hydroxymethyl derivative **22**. Further substitution of the C4 phenyl ring was tolerated as exemplified by the 3-hydroxy- and 3-chloro-4-hydroxymethyl derivatives **23** and **24** but this had only a modest effect on potency. Transformation of **21** into the oxime derivative **25** however produced a 15-fold increase in potency (K_d 5 nM). Once again, substitution with hydroxyl- or chloro-substituents in the 3-position was tolerated (**26** and **27**) although the hydroxyl derivative **26** showed a 5-fold drop in potency compared to **25**. Further exploration of the oxime unit demonstrated that substitution on the oxime carbon atom was also tolerated in the form of the ketoxime **28** (K_d 11 nM), although the amidoxime derivative **29** showed a 10-fold drop in potency relative to **25**.

Cyclization between the 3 and 4 positions of the C4 substituent to form the 2,3-dihydroindene and 3,4-dihydronaphthalenone derivatives (**30**–**32**) also provided some interesting SAR findings (Table 4). Whilst the racemic 1-hydroxy 2,3-dihydroindene **30** showed weak affinity, the oxime **31** (K_d 1.3 nM) was extremely potent with activity at least equal to that of the early lead **2**. The ring expanded 3,4-dihydro-1(2*H*)-naphthalenone oxime **32** (K_d 17 nM) also showed significant activity, although this was 10-fold lower than that of **31**. SB-590885 (**33**, K_d 0.3 nM), the C2 (4-dimethylaminoethoxy) phenyl analogue of **31**, was prepared to improve the aqueous solubility (>1 mg/ml in pH 5 buffer) but also showed enhanced potency, being some 8-fold more potent than the early lead **2**.



SB-590885 (33)
B-Raf Kd 0.3nM

Table 4. B-Raf activity of cyclic oximes



Compound	X	*n*	B-Raf (FP) K_d nM
30	H, OH (*R*/*S*)	1	>200
31	HON	1	1.3
32	HON	2	17

The selectivity profiles of SB-590885 (**33**) and **2** were assessed against a panel of 21 protein kinases (Table 5). The data confirmed the generally good selectivity profile

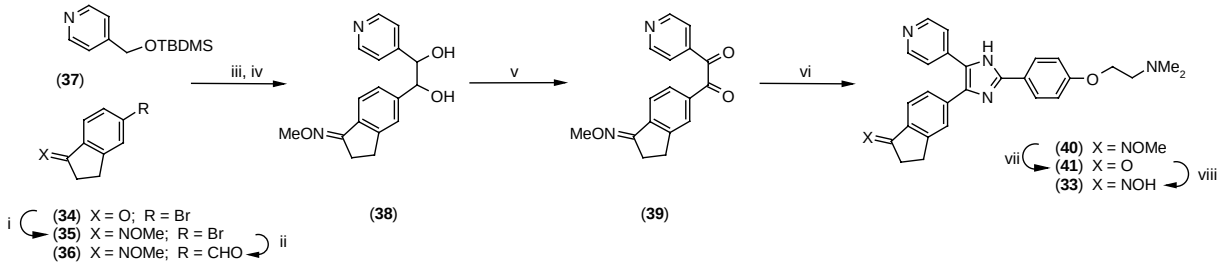
Table 6. Selectivity of SB-590885 (**33**) and (**2**) versus p38α, GSK3β and lck

Compound	Fold selectivity versus B-Raf		
	p38α	GSK3β	lck
2	7	30	70
33	>1000	>1000	>1000

Table 5. Selectivity profiling of SB-590885 (**33**) and (**2**)[a]

Compound	AMPK	Chk1	CK2	GSK 3β	JNK1	lck	MAPK2	MAPK AP-K2	MEK1	MSK1	p70 S6K	Phos. K	PKA	PKB a	PKC a	PRAK	ROCK-II	p38 α	p38 β	p38 γ	p38δ	SGK
2	12	1	−3	**83**	0	**88**	−3	−1	5	7	26	17	12	44	2	19	3	**95**	**81**	5	−15	2
33	14	9	8	25	19	39	11	9	14	38	13	3	−2	8	10	18	26	46	9	10	10	17

[a] Values are %inhibition at 10 μM drug concentration in kinase activity assays in the presence of 100 μM ATP (see Ref. 9 for details).



Scheme 1. Reagents and conditions: (i) $MeONH_2$ HCl, pyridine, ethanol (97%); (ii) *n*BuLi, DMF, THF −60 °C (65%); (iii) LDA, THF −60 °C, add **36**; (iv) TBAF, THF (64% over two steps); (v) DMSO, oxalyl chloride, triethylamine dichloromethane −60 °C to room temperature (94%); (vi) 4-(2-dimethylaminoethoxy)benzaldehyde, ammonium acetate, acetic acid 100 °C (30%); (vii) 5 M HCl, acetone, dioxane 100 °C (56%); (viii) 50% aqs NH_2OH, ethanol, reflux (100%).

of **2** but also identified some activity against GSK3β and lck. SB-590885, on the other hand, appeared to be devoid of significant activity against this panel of enzymes. More in-depth profiling of both compounds against p38α, GSK3β, and lck in the fluorescent binding assay format confirmed the enhanced selectivity profile for SB-590885 (Table 6).

Triaryl imidazoles, such as SB-590885 (**33**), were prepared as outlined in Scheme 1.[10,11] Thus, commercially available 5-bromo-2,3-dihydro-1*H*-inden-1-one **34** was converted to the *O*-methyl oxime **35** and then formylated by treatment with *n*-butyl lithium followed by the addition of DMF, to afford **36**. Reaction of **36** with the anion derived from the 4-hydroxymethylpyridine derivative **37**,[12] followed by desilylation, afforded the racemic 1,2-diol **38**. This was then oxidized to the dione **39** under Swern conditions and converted to the imidazole **40** in modest yield, by treatment with 4-(2-dimethylaminoethoxy) benzaldehyde and ammonium acetate in acetic acid at 100 °C. Hydrolysis of the *O*-methyl oxime, by treatment with 5 M hydrochloric acid and acetone in dioxane, formed the ketone **41** which was converted to **33** by treatment with aqueous hydroxylamine.

In summary, SB-590885 (**33**), a potent and extremely selective inhibitor of B-Raf kinase, was identified following an evaluation of the SAR of a series of imidazole based leads. SB-590885 represents an excellent molecule with which to investigate the role of B-Raf in neurodegenerative and other disease states.

Acknowledgments

The authors thank colleagues from the departments of Gene Expression and Protein Biochemistry for the provision of human recombinant reagents and Screening and Compound Profiling for kinase activity data. We also acknowledge the Division of Signal Transduction Therapy, University of Dundee, for the generation of kinase selectivity data.

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7. Compound activity is determined as either the ability of test compounds to inhibit B-Raf mediated phosphorylation of kinase dead MEK (data expressed as IC_{50}) or as a B-Raf-binding affinity derived from the fluorescent ligand displacement assay (FP, data expressed as K_d). For assay details, see Dean, D. K.; Takle, A. K.; Wilson, D. M. PCT Int. Appl. WO 02/24680.
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10. All novel compounds gave satisfactory ^1H NMR and LC/MS data in full agreement with their proposed structures.
11. SB-590885 was isolated as a 4 to 11:1 mixture of *E* and *Z* isomers. The configuration of the major (*E*) isomer was confirmed by NMR.
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