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The identification of potent, selective and CNS penetrant furan-based inhibitors of B-Raf kinase

Andrew K. Takle [a,*], Mark J. Bamford [a], Susannah Davies [a], Robert P. Davis [b], David K. Dean [a], Alessandra Gaiba [a], Elaine A. Irving [b], Frank D. King [a], Antoinette Naylor [a], Christopher A. Parr [a], Alison M. Ray [b], Alastair D. Reith [d], Beverley B. Smith [c], Penelope C. Staton [b], Jon G. A. Steadman [a], Tania O. Stean [b], David M. Wilson [a]

[a] Department of Medicinal Chemistry, Neurology and GI Centre of Excellence for Drug Discovery, GlaxoSmithKline Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK
[b] Department of Biology, Neurology and GI Centre of Excellence for Drug Discovery, GlaxoSmithKline Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK
[c] Department of Drug Metabolism and Pharmacokinetics, Neurology and GI Centre of Excellence for Drug Discovery, GlaxoSmithKline Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK
[d] Discovery Technology Group, GlaxoSmithKline Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK

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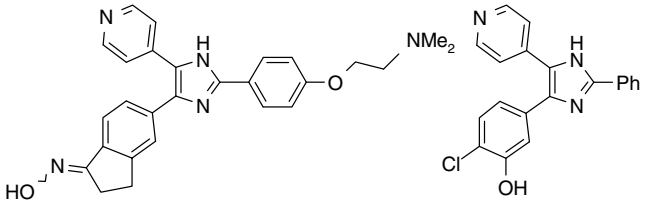
ABSTRACT

Modification of the potent imidazole-based B-Raf inhibitor SB-590885 resulted in the identification of a series of furan-based derivatives with enhanced CNS penetration. One such compound, SB-699393 (**17**), was examined in vivo to challenge the hypothesis that selective B-Raf inhibitors may be of value in the treatment of stroke.

Mitogen-activated protein (MAP) kinase signalling cascades are responsible for controlling numerous intracellular processes.[1] Typically such cascades consist of three tiers of protein kinases that are sequentially activated in response to appropriate extra-cellular stimuli. This leads to signal transduction and allows for amplification to occur at each step of the cascade. The RAF-MEK-ERK MAP kinase cascade is intimately involved in the regulation of cell cycle progression and apoptosis, and has been implicated in both proliferative and degenerative disease states. Activating mutations in B-Raf, one of the Raf family members, are reported to be present in 66% of malignant melanomas,[2] whereas increased activation of ERK1/2 has also been reported in a number of in vitro and in vivo models of neuronal cell death.[3,4] Furthermore, inhibition of the cascade at the MEK level has led to a reduction in infarct volume in rodent models of stroke.[4]

We have previously reported our initial efforts to identify potent inhibitors of B-Raf in order to assess their potential in the treatment of stroke.[5] These studies resulted in the identification of the potent and extremely selective triaryl imidazole SB-590885 (**1**). Here, we wish to report the result of further studies to identify inhibitors with enhanced CNS penetration.



SB-590885 (**1**)
B-Raf Kd 0.3nM

L-779,450 (**2**)
B-Raf Kd 2.4nM

SB-590885 was initially assessed for its ability to inhibit B-Raf-mediated ERK phosphorylation in rat pheochromocytoma (PC12) cells following nerve growth-factor (NGF) stimulation, as a marker of cellular pathway inhibition (Table 1). In this assay, SB-590885 showed a robust, dose-dependant inhibition of ERK phosphoryla-

* Corresponding author. Tel.: +44 (0)1279 627686; fax: +44 (0)1279 622260.
E-mail address: Andy.K.Takle@gsk.com (A.K. Takle).

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Table 1
Cell-based pathway inhibition and rat in vivo pharmacokinetic parameters for SB-590885 (**1**) and L-779,450 (**2**)[a7]

Compound	Pathway inhibition (% inhibition)		Blood clearance (ml/min/kg)	Brain:blood ratio (BB)
	1 μM	3 μM		
1	99	101	45	0.14:1
2	–	100	77	<0.1:1

[a] The brain:blood (BB) ratio was calculated as the ratio of brain:blood concentrations following a constant rate (3.5–5 ml/kg/h) *iv* infusion to steady-state (6–16 h) and at a target dose level of between 1 and 3 mg free base/kg/h. Blood clearance was calculated as dose-rate/steady-state blood concentration.

tion similar to that seen with the early lead molecule L-779,450 (**2**).[6] However, due to the inter assay variability, all novel compounds were assessed relative to the activity of a standard 3 μM (micromolar) concentration of L-779,450 as an internal control (i.e., 100% refers to equivalent inhibition to that produced by 3 μM of L-779,450 in that particular assay).[7]

Having demonstrated the ability of our tool compounds to inhibit B-Raf-mediated signalling in a cellular context, we next examined their in vitro neuroprotective activity. Compounds were tested for their ability to protect rat hippocampal slice cultures from death induced by oxygen and glucose deprivation (OGD).[8] We were encouraged to find that both compounds displayed potent neuroprotective activity in this assay; L-779,450 showing approximately 80% protection at 10 μM and SB-590885 showing a comparable degree of protection at 0.1 μM.

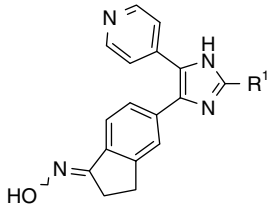
Unfortunately, despite its encouraging in vitro profile, SB-590885 showed moderate blood clearance and poor CNS penetration in the rat (Table 1). Whilst the blood clearance rate was acceptable, since any therapy would likely be administered by continuous intra venous (*iv*) infusion, the poor penetration of the CNS (brain:blood ratio (BB) 0.14:1) was sub-optimal for this indication.

We thus set out to improve the CNS penetration of the series (target BB $\geqslant$ 0.5:1) whilst being mindful of the need to retain solubility enhancing groups to meet the likely requirements of a putative *iv* agent. Our initial approach was to modify the imidazole C2 position since we had previously shown that B-Raf was tolerant of a wide variety of substitutions at this position.[5] Whilst potent B-Raf binding was achieved with a number of aryl-, alkyl-, and carboxamido-C2 substituents bearing solubilising basic amino groups, the cellular activity of these derivatives was variable (Table 2). More disappointing was the finding that the CNS penetration of this series of compounds was routinely poor.

In an effort to overcome the problem of inadequate CNS penetration of this series of molecules, we turned our attention to modifying the core heterocycle and in so doing reducing the number of H-bond donors (HBD) and the polar surface area (PSA) of the molecules (Table 3).[9] We were pleased to find that replacement of the central imidazole core by either isomeric furan (**10** and **12**) or pyrrole (**11** and **13**) groups afforded molecules with potent B-Raf affinity, demonstrable cellular activity and enhanced CNS penetration. This was most notable for the furan derivatives, which benefited from both reduced PSA and HBD count, and especially so for the C2-(4-pyridyl)furan isomer (**10**).

Having identified this series of furan-based inhibitors with intrinsically superior CNS penetration, we sought to tune the overall properties of the molecules through a re-investigation of the SAR of the C5 position (equivalent to the C2 position of the imidazole core) of the C2-(4-pyridyl)furans (Table 4). The C5 position was once again tolerant of a variety of diverse aryl (not shown), alkyl, and carboxamido-substituents that retained potent B-Raf affinity and good cellular activity. It was notable, however, that whilst im-

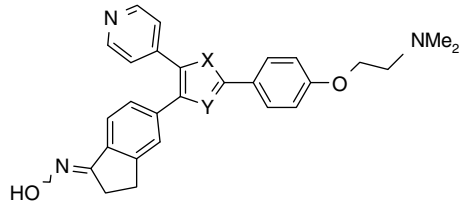
Table 2
In vitro and in vivo activities of C2 modified imidazoles[a]



Compound	R^1	B-Raf (FP) K_d (nM)	Pathway inhibition (% inhibition)		BB ratio
			1 μM	3 μM	
3	4-[2-(2-Dimethylamino propyloxy)]pyrimidine	0.5	88	100	0.1:1
4	$C(Me_2)CH_2NH_2$	4.9	61	96	
5	4-Piperidine	1.7	36	59	
6	4-Piperidine-1-$(CH_2)_2OMe$	6.3	41	33	0.09:1
7	4-Morpholino-methyl	15.8	−10	−8	
8	$CONH(CH_2)_2$-morpholine	1.4	61	59	
9	$CONHPh(3\text{-}CH_2NMe_2)$	1.3	77	98	0.09:1

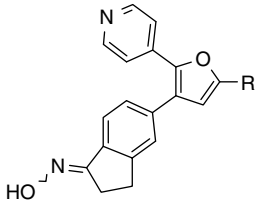
[a] Compound potency is expressed as a B-Raf binding affinity (K_d) derived from a fluorescent ligand binding assay (FP). See Ref. 8 for details.

Table 3
In vitro and in vivo activities of core modified B-Raf inhibitors



Compound	X	Y	B-Raf (FP) K_d (nM)	Pathway inhibition (% inhibition)		PSA (HBD)	BB ratio
				1 μM	3 μM		
1	NH	N	0.3	99	101	87 (2)	0.14:1
10	O	CH	0.5	75	87	71 (1)	1.33:1
11	NH	CH	0.7	66	74	74 (2)	0.27:1
12	CH	O	0.9	60	85	71 (1)	0.56:1
13	CH	NH	2.4	17	66	74 (2)	0.33:1

Table 4
In vitro and in vivo activities of C5 substituted furans



Compound	R	B-Raf (FP) K_d (nM)	Pathway inhibition (% inhibition)		Blood clearance (ml/min/kg)	BB ratio
			1 μM	**3** μM		
14	4-Piperidine	2.4	93	100	78	0.24:1
15	4-Piperidine-1-$(CH_2)_2OMe$	2.7	83	96	100	1.39:1
16	$CONH(CH_2)_2\text{-}NMe_2$	0.5	93	NT	91	0.1:1
17	4-Morpholino-methyl	7.2	91	95	54	0.8:1

NT, not tested.

Table 5
Selectivity profiling of SB-590885 (**1**) and SB-699393 (**17**)[a]

Compound	AMPK	Chk1	CK2	GSK3 β	JNK1	Lck	MAPK2	MAPKAP-K2	MEK1	MSK1	p70 S6K	Phos.K	PKA	PKBα	PKCα	PRAK	ROCK-II	p38α	p38β	p38γ	p38δ	SGK
1	14	9	8	25	19	39	11	9	14	38	13	3	−2	8	10	18	26	46	9	10	10	17
17	6	7	9	−9	−2	3	7	4	12	7	−3	−8	−4	5	3	11	11	12	12	8	0	0

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

proved CNS penetration was a general feature of the furan derivatives, the incorporation substituents bearing additional HBDs, such as the unsubtituted piperidine (**14**) or carboxamide derivative (**16**), once again resulted in a reduction in the brain:blood ratio. It was also notable that blood clearance rates could be modulated by modification of the furan C5 position, although in this case SAR was difficult to interpret. As a result of our efforts, the C5 morpholino-methyl derivative **17** (SB-699393) was identified as a molecule possessing the optimal balance of potency, cellular activity and pharmacokinetic properties. When assessed against a panel of 21 protein kinases, SB-699393 (**17**) also showed an excellent selectivity profile comparable to that of the imidazole lead SB-590885 (**1**) (Table 5).

With a potent, selective and CNS penetrant molecule in hand (SB-699393), we next set out to assess its activity in vivo. As a measure of pharmacodynamic activity in the brain, we chose to assess the compounds ability to inhibit hippocampal ERK phosphorylation in rats subjected to cold water stress.[11] We were gratified to find that pretreatment with a 3 or 10 mg/kg *iv* bolus of SB-699393, 5 min prior to cold water stress, was successful in reducing the induced hippocampal ERK phosphorylation (Fig. 1). In order to assess its neuroprotective activity, SB-699393 was administered as a 5 h continuous *iv* infusion at doses ranging from 0.3 to 10 mg/kg/h, starting 1 h post-insult, in a permanent middle cerebral artery occlusion model of stroke in normotensive rats.[12] In this model, efficacy would be determined by an improvement in neurological deficit and/or a reduction in infarct lesion volume relative to vehicle. Unfortunately, SB-699393 failed to demonstrate a significant improvement in either parameter when assessed 24 h post-injury. These results, whilst apparently at odds to earlier reports that link prolonged ERK activation to neuronal cell death,[4] suggest that selective inhibition of B-Raf alone is not sufficient to elicit significant neuroprotection in rodent models of stroke.

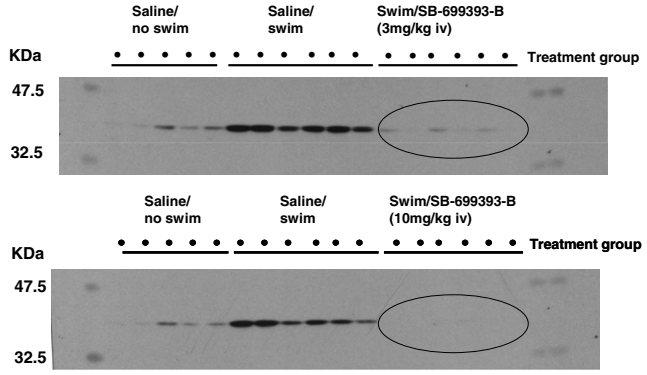
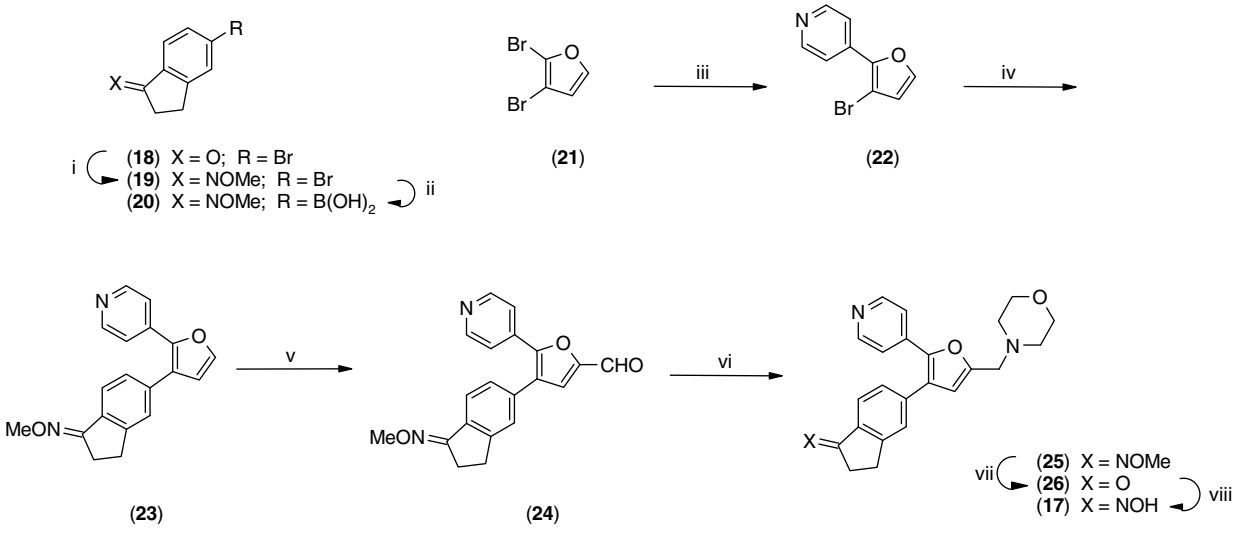


Figure 1. Inhibition of cold water stress-induced hippocampal ERK phosphorylation by SB-699393 (**17**). An equivalent loading of tissue samples is used in each lane.

SB-699393 (**17**) was prepared as shown in Scheme 1.[13] Thus, commercially available 5-bromo-2,3-dihydro-1*H*-inden-1-one **18** was converted to the *O*-methyl oxime **19** then to the boronic acid **20** by treatment with *n*-butyl lithium followed by the addition of trimethyl borate. Meanwhile, 2,3-dibromo furan was reacted with 4-pyridyl boronic acid under Suzuki cross-coupling conditions to regioselectively generate the 2-(4-pyridyl)furan **22** in 54% yield.[14] Suzuki coupling between the furan **22** and the boronic acid **20** furnished the disubstituted furan **23** (74%) which was then formylated, by treatment with LDA followed by the addition of DMF, to afford **24** in 56% yield. Reductive amination with morpholine and polymer-bound trimethylammonium cyanoborohydride afforded the trisubstituted precursor **25**. Hydrolysis of the *O*-methyl oxime with 5 M hydrochloric acid and acetone in dioxane formed the ke-



Scheme 1. Preparation of SB-699393 (**17**).[13] Reagents and conditions: (i) $MeONH_2$ HCl, pyridine, ethanol (96%); (ii) *n*BuLi, trimethyl borate, THF −78 °C to room temperature (58%); (iii) 4-pyridyl boronic acid, $Pd(OAc)_2$, PPh_3, K_2CO_3, 2:1 DME–water, reflux (54%); (iv) (**20**), $Pd(OAc)_2$, PPh_3, K_2CO_3, 2:1 DME–water, reflux (74%); (v) LDA, DMF, THF −78 °C (56%); (vi) morpholine, polymer-bound trimethylammonium cyanoborohydride, methanol, acetic acid (61%); (vii) 5 M HCl, acetone, dioxane 100 °C; (viii) 50% aqueous NH_2OH, ethanol, reflux (64% for two steps).

tone **26**, which was converted to **17** by treatment with aqueous hydroxylamine in 64% yield.

In conclusion, we have extended our understanding of the SAR associated with this series of extremely potent and selective 2,3-dihydro-1*H*-inden-1-one oxime substituted heterocyclic B-Raf inhibitors. In so doing, we have identified the furan derivative SB-699393 (**17**) which possesses enhanced CNS penetration; however, this molecule failed to show significant neuroprotective effects when evaluated in a rodent model of stroke.

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7. PC12 cells were pretreated with compounds for 60 min prior to stimulation with NGF. After 10 min cells were lysed and the lysates separated by SDS–PAGE. Following transfer to nitrocellulose membranes, levels of phosphorylated-ERK1/2 were determined following antibody probing. Pretreatment with 3 μM L-779,540 (**2**) routinely caused approx. 90% inhibition of NGF-stimulated ERK1/2 phosphorylation.
8. For details of the B-Raf fluorescent ligand binding assay (FP) and the OGD in vitro neuroprotection assay, see Dean, D. K.; Takle, A. K.; Wilson, D. M. PCT Int. Appl. WO 02/24680.
9. Molecular polar surface area (PSA) has long been recognised as a contributing factor to the brain penetration of drug-like molecules. See Kelder, J.; Grootenhuis, P. D. J.; Bayada, D. M.; Delbressine, L. P. C.; Plemen, J.-P. *Pharm. Res.* **1999**, *16*, 1514; PSA values quoted in this paper are calculated using '2D' methods. See Ertl, P.; Rohde, B.; Selzer, P. *J. Med. Chem.* **2000**, *43*, 3714.
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11. Rats were subjected to a cold water swim (18 °C) for 10 min. The test animals were then sacrificed, the brains removed, dissected and hippocampal phospho-ERK levels assessed by Western blotting. Test compounds were administered as an *iv* bolus 5 min prior to the cold water swim.
12. See Ref. 3, and references therein, for details of the rat, permanent middle cerebral artery occlusion model of stroke.
13. All novel compounds gave satisfactory ^{1}H NMR and LC/MS data in full agreement with their proposed structures. Oximes were isolated as mixtures of *E* and *Z* isomers in which the *E* isomer predominated. For experimental details of the synthesis of SB-699393 (**17**) see Bamford, M. J.; Dean, D. K.; Naylor, A.; Takle, A. K.; Wilson, D. M. PCT Int. Appl. WO 03/022840.
14. Precedence exists for the regioselective coupling of 2,3-dihalo-substituted furans, see, for example, Bach, T.; Kruger, L. *Eur. J. Org. Chem.* **1999**, 2045. Nonetheless, the structure of **22** was also confirmed by HMBC NMR spectroscopy.