Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 5383–5387
Bioorganic & Medicinal Chemistry Letters

A novel series of p38 MAP kinase inhibitors for the potential treatment of rheumatoid arthritis

Dearg S. Brown,* Andrew J. Belfield, George R. Brown, Douglas Campbell, Alan Foubister, David J. Masters, Kurt G. Pike, Wendy L. Snelson and Stuart L. Wells

AstraZeneca, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK

Received 18 May 2004; revised 30 July 2004; accepted 5 August 2004
Available online 28 August 2004

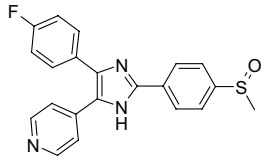
Abstract—A novel p38 MAP kinase inhibitor structural class was discovered through selectivity screening. The rational analogue design, synthesis and structure–activity relationship of this series of bisamide inhibitors is reported. The inhibition in vitro of human p38α enzyme activity and lipopolysaccharide-induced tumour necrosis factor-α release is described for the series. The activity in vivo and pharmacokinetic properties are exemplified for the more potent analogues.

Rheumatoid arthritis (RA) is a chronic disease causing joint pain, swelling and stiffness. Progression of RA leads to joint damage and serious disability, making treatment an important objective. Drug treatment to date has primarily focused on the use of nonsteroidal anti-inflammatory drugs (NSAIDS)[1,2] and disease-modifying anti-rheumatic drugs (DMARDS) such as methotrexate,[2–4] sulfasalazine,[3] leflunomide,[2,4,5] corticosteroids,[2] and various combination protocols,[6] in spite of these tending to be poorly tolerated in long-term clinical use. More recently novel biological products that modify pro-inflammatory cytokines have gained clinical approval as DMARDS. These new biological treatments act through the sequestration of either tumour necrosis factor-α (TNF-α) (e.g. the neutralising antibody infliximab[7–9] and the soluble TNF receptor etanercept[8,9]) or interleukin-1 (IL-1) (e.g. the receptor antagonist anakinra[9]). As these biological drugs have shown that the lowering of pro-inflammatory cytokine levels is a valid treatment for RA patients our aim was to identify small molecule drugs with a similar cytokine-inhibiting profile. Modern small-molecule approaches to the treatment of RA have targeted cell signalling systems, in particular the inhibition of the mitogen-activated protein (MAP) p38 kinase cell-signalling pathway.[10] A potential advantage of this approach over the biological agents is that p38 kinase has been implicated in the induction of cyclooxygenase-2 (COX-2) in lipopolysaccharide (LPS) induced monocytes.[11] The inducible COX-2 enzyme is a key enzyme in the arachidonic acid enzyme cascade that leads to the production of prostaglandins, which are associated with inflammation and pain. Inhibition of p38 kinase is thus an attractive approach to the treatment of both pain and inflammation in RA patients. Pyridylimidazoles such as the prototypic **SB203580** are the most studied class of p38 inhibitors,[12,13] but other structural series have been reported.[13,14] In seeking an alternative series of p38 inhibitors we elected not to pursue the extensively developed pyridylimidazole series but instead sought a more novel starting point.

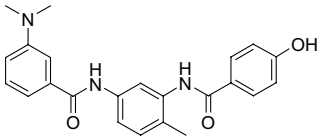
During the screening of the company compound library at AstraZeneca the bisamide **1** was identified as a potent inhibitor of the protein kinase c-raf (IC_{50} 17 nM).[15] Subsequent selectivity testing amongst 19 typical kinases in vitro showed that **1** only inhibited the two isoforms p38α and p38β (p38α IC_{50} 210 nM)[16] and, in addition, it was shown that compound **1** had no effect on the c-raf pathway in cells.[17] Thus compound **1** was a novel p38α kinase inhibitor with considerable selectivity over other protein kinases, with the exception of the closely related protein kinase c-raf, and it had suitable lead-like physical properties (log *D* 2.7 at pH 7.4, solubility 10 μM in pH 7.4 buffer, human protein binding 99.7% bound). Herein we describe novel p38α inhibitor analogues of the bisamide **1** as orally active, small molecule cytokine inhibitors for the potential treatment of RA.

Keyword: p38 MAP kinase inhibitors.
* Corresponding author. Tel.: +44 1625 516321; fax: +44 1625 510823; e-mail: dearg.brown@astrazeneca.com

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.08.006



SB203580

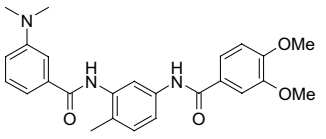


1

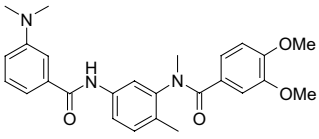
Compounds were tested in vitro for inhibition of human p38α enzyme activity and for the inhibition of TNF-α production in LPS-stimulated human peripheral blood mononuclear cells (PBMCs) and human whole blood using the literature p38 inhibitor, **SB203580**, as a positive control.[18] In this study analogue synthesis was restricted to alkoxy-substitution in place of the hydroxyl in **1** in order to avoid the potential for phenolic β-glucuronidation in vivo, should new analogues later proceed to animal tests.

The bisamides were prepared as exemplified by the synthesis of **18** in Scheme 1. The 3-morpholinobenzoic acid **31** was prepared either via palladium-catalysed amination chemistry from ethyl 3-bromobenzoate[19] or by direct nucleophilic aromatic substitution of 3-fluorobenzonitrile[20] with subsequent hydrolysis of the resulting intermediates. Conversion to the acid chloride, reaction with 4-methyl-3-nitroaniline, and reduction, all under standard conditions, provided the key benzamidoaniline intermediate **32**. Subsequent acylation and deprotection afforded the phenol **33**, which was then alkylated under mild basic conditions. This product **18**[21] and other analogues made in similar manner were characterised by LCMS, ^{1}H NMR and mass spectrometry.

Significant inhibition of p38α was found for a variety of substituted alkoxy-compounds **1**–**17** (Table 1). Simple *O*-alkylation of **1** gave **2** with comparable potency versus the kinase but neither had significant inhibition of TNF-α production in PBMCs. Addition of a 2-methoxy (**3**) had little effect but a 3-methoxy (**4**) resulted in modest cellular potency and the 3,4,5-trimethoxy analogue **5** had a similar profile although these analogues were significantly weaker p38α inhibitors than **SB203580**. Attempts to improve inhibitory potency by varying the methyl group at R1, **6**–**9**, confirmed the requirement for a substituent (e.g. methyl or chloro) at that position. Similarly, transposing the methyl group to the other side of the central ring in **10** and amide *N*-methylation (**11**) both resulted in loss of potency (p38α $IC_{50} > 20\,\mu M$). Changing 3,4-dimethoxy to 3,4-diethoxy (**12**) increased activity slightly but it was found that a single *para*-substituent larger than methoxy (**13**–**17**) was sufficient to provide micromolar activity in the PBMC assay. However, none of the compounds in Table 1 (**1**–**17**) were inhibitors in human whole blood below 50 μM. Although these early simple alkoxy compounds gave little or no exposure in rat pharmacokinetic studies, the inclusion of a 2-pyridylmethoxy **17** resulted in modest blood levels following oral dosing and further work therefore focused on these systems.

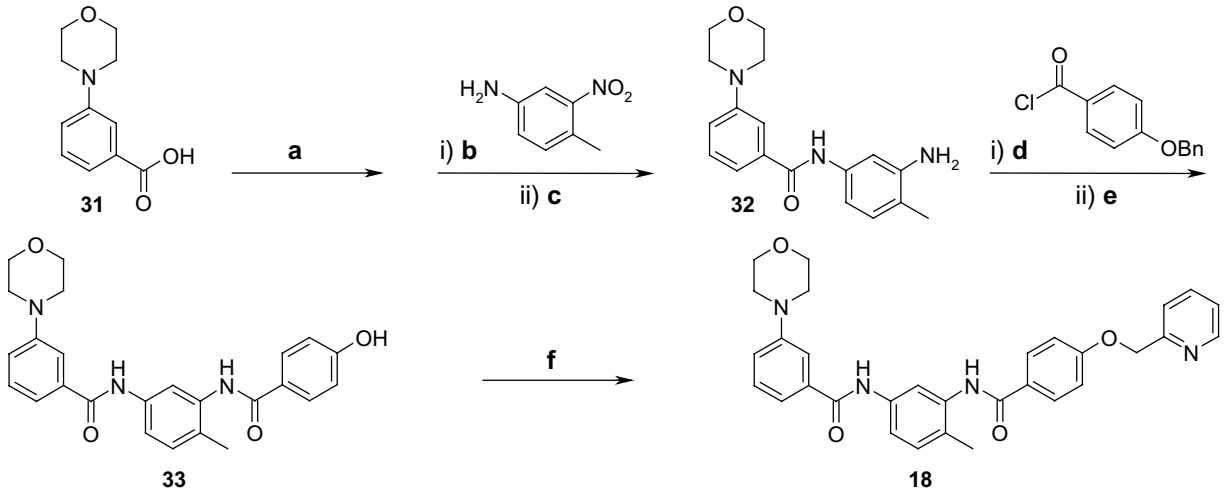


10



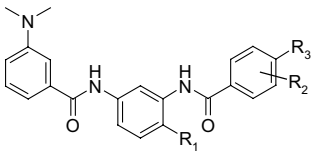
11

Replacement of the dimethylamino with a morpholino substituent (**18**) resulted in a modest increase in kinase inhibition with similar cellular potency (PBMC IC_{50} 1.2 μM), but encouraging activity in human whole blood. Whole blood activity was observed for a number



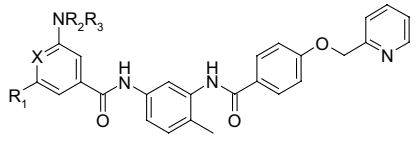
Scheme 1. Preparation of **18**. Reagents and conditions: (a) $(COCl)_2$, $DMF_{(cat)}$, CH_2Cl_2, rt (quantitative); (b) Et_3N, CH_2Cl_2, rt (80%); (c) 10% Pd on carbon, H_2, MeOH, rt (95%); (d) Et_3N, CH_2Cl_2, rt (61%); (e) 10% Pd on carbon, H_2, MeOH, rt (84%); (f) 2-(chloromethyl)pyridine, K_2CO_3, DMA, 60 °C (55%).

Table 1. Inhibition of human p38α enzyme activity and LPS-induced TNF-α release in human PBMCs by dimethylamino-substituted bisamides in vitro



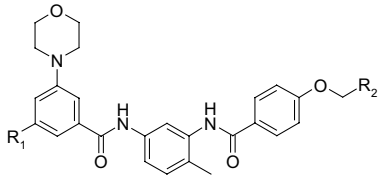
No.	R^1	R^2	R^3	p38α IC_{50} (μM)	PBMC IC_{50} (μM)
1	Me	H	HO	0.18	>50
2	Me	H	MeO	0.18	>50
3	Me	2-MeO	MeO	0.10	>50
4	Me	3-MeO	MeO	0.18	6.4
5	Me	3,5-$(MeO)_2$	MeO	0.23	3.9
6	H	3-MeO	MeO	>20	—
7	F	3-MeO	MeO	1.90	22.0
8	Cl	3-MeO	MeO	0.22	6.4
9	Br	3-MeO	MeO	0.34	9.5
12	Me	3-EtO	EtO	0.05	4.5
13	Me	H	EtO	0.06	7.0
14	Me	H	*i*-PrO	0.17	4.6
15	Me	H	$MeO(CH_2)_2O$	0.27	3.4
16	Me	H	$EtO(CH_2)_2O$	0.31	9.5
17	Me	H	2-Pyridyl-CH_2O	0.051	1.4
SB203580				0.026	0.19

Table 2. Inhibition of human p38α and LPS induced TNF-α release in human whole blood by *para*-(pyrid-2-ylmethoxy)-substituted bisamides in vitro



No.	X	R^1	NR^2R^3	p38α IC_{50} (μM)	HWB IC_{50} (μM)
17	HC	H	NMe_2	0.051	>40
18	HC	H	Morpholinyl	0.030	3.7
19	N	H	Morpholinyl	0.010	2.8
20	HC	F	Morpholinyl	0.064	11.2
21	HC	CF_3	Morpholinyl	0.020	10.2
22	HC	F	Pyrrolidinyl	0.025	14.1
SB203580				0.026	4.2

Table 3. In vitro activity and rat PK AUCs for a normalised 1 mg/kg dose for a series of morpholino-substituted bisamides



No.	R^1	R^2	p38α IC_{50} (μM)	HWB IC_{50} (μM)	AUC (μMh)
23	H	Phenyl	0.037	40.0	—
18	H	2-Pyridyl	0.030	3.7	1.38
24	H	3-Pyridyl	0.095	14.7	—
25	H	4-Pyridyl	0.022	3.3	0.13
26	F	4-Pyridyl	0.017	3.4	<0.02
27	H	4-Thiazolyl	0.018	1.5	<0.12
28	F	4-Thiazolyl	0.016	2.5	0.71
29	H	2-Methyl-4-thiazolyl	0.028	14.6	2.03
30	F	2-Methyl-4-thiazolyl	0.097	2.8	0.73

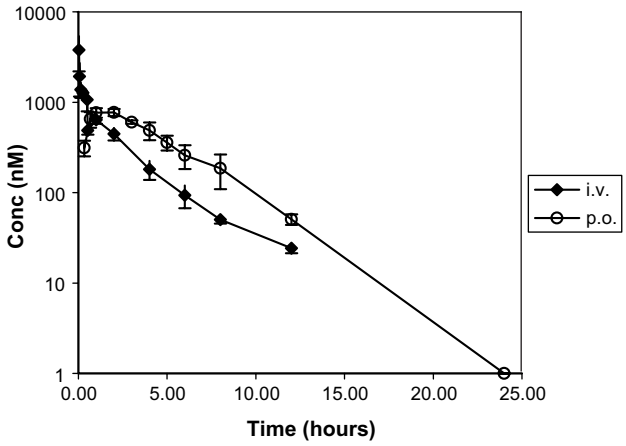
Table 4. Inhibition of TNF-α production in SEB-stimulated Balb/C mice in vivo[24]

No.	HWB	AUC	% Inhibition		
	IC_{50} (μM)	(μM h)	30 mg/kg	10 mg/kg	3 mg/kg
18	3.7	1.38	59	—	—
20	11.2	0.13	43	—	—
21	10.2	0.04	55	4	—
22	14.1	0.07	7	3	
25	3.3	0.13	75	43	14
26	3.4	<0.02	40	24	—
27	1.5	<0.12	56	44	28
28	2.5	0.71	81	37	—
29	14.6	2.03	71	44	33
30	2.8	0.73	35	27	0
SB203580			50	47	0

of other morpholino systems including the pyridyl analogue **19**, the fluoro and trifluoromethyl substituted benzamides **20** and **21**, and a pyrrolidinyl analogue **22** (Table 2). There seemed to be no clear explanation for this significant improvement in whole blood activity in moving from the dimethylamino to the cyclic amines; kinase activity and lipophilicity (e.g. log *D*'s 2.8 for **17**, 3.2 for **18**) were similar. In this instance the human protein binding (0.3% and 3.8% free for **17** and **18**, respectively) could be a factor but the fraction free was less for other compounds that exhibited good blood potency (e.g. **19** has only 0.11% free). Furthermore the morpholino oxygen atom did not appear to be acting as a hydrogen bond acceptor in view of the similar inhibitory potencies of TNF-α in blood of **20** and **22**.

A range of further heterocyclic compounds was investigated all of which were clearly more potent inhibitors in blood than the corresponding phenyl compound **23**, presumably due to the reduced lipophilicity and protein binding in whole blood (Table 3). The best substituents included the pyridyl isomers, 4-thiazolyl, and 2-methyl-4-thiazolyl **24**–**30**. In order to confirm that the series still retained the original attribute of excellent selectivity for p38α the methylthiazolyl **29** was evaluated against a range of kinases and exhibited no significant activity at 10 μM.[22] As the compounds in Table 3 generally exhibited good metabolic stability during incubation of the compounds with rat hepatocytes in vitro the more potent derivatives were dosed orally in rats ($n = 2$ animals) to assess systemic exposure. Pharmacokinetic (PK) evaluation was carried out using a cassette dosing protocol and area under the curve (AUC) normalised for a dose of 1 mg/kg (compounds were dosed at ~2 mg/kg in a propyleneglycol formulation).[23]

Bisamides analogues with IC_{50} values <15 μM in human whole blood were examined for inhibition of TNF-α release in Balb/C mice challenged with staphylococcal enterotoxin B (SEB). Compounds **18**, **21**, **25** and **27**–**29** inhibited circulating TNF-α levels by greater than 50% at 30mg/kg, thus establishing in vivo efficacy for this series. At this stage a PK–PD relationship was not established as this would require mouse PK and whole blood data (Table 4).



Graph 1. PK profile for compound **19** in rat; dosing at 3 mg/kg po ($n = 2$) and at 1 mg/kg iv ($n = 3$).

A full PK profile was carried out in rat for the morpholinopyridine **19**, which had shown good potency in human whole blood, excellent metabolic stability (human microsomes, rat and human hepatocytes) in vitro and high oral exposure in rat PK (Graph 1). From the iv profile (dosing 1 mg/kg) the compound exhibited low clearance (10 mL/min/kg) and moderate volume of distribution (1.6 L/kg) resulting in a half-life of 2.9 h. Based on the oral arm (dosing at 3 mg/kg) bioavailability was determined to be 46%. This compound had rather high human protein binding (0.1% free) and very low solubility (<1 μM) and was not progressed further but subsequent studies have shown that these issues can be addressed within the series.

In conclusion, the novel bisamide series of p38α kinase inhibitors has afforded novel compounds with micromolar activity in whole blood cytokine production assays in vitro and significant inhibition of TNF-α production in SEB-stimulated Balb/C mice in vivo. Several analogues have good exposure from oral dosing and a leading analogue has excellent PK parameters in rat. Further structural modification in the bisamide series may afford improved physical properties leading to compounds suitable for the treatment of RA.

Acknowledgements

The authors thank our colleagues Lucy C. Ashman, Carol L. Biddulph, Karen M. Burns, and Brian Ellershaw for developing and carrying out the biological assays, Dermot McGinnity for the PK study on compound **19**, and Graham Crawley for assistance in producing the manuscript.

References and notes

1. Sundy, J. S. *Mod. Ther. Rheum. Dis.* **2002**, 23.
2. (a) Pisetsky, D.; St. Clair, E. W. *JAMA—J. Am. Med. Assoc.* **2001**, *286*(22), 2787; (b) Hughes, L. B.; Moreland, L. W. *BioDrugs* **2001**, *15*(6), 379.
3. Capell, H. *J. Rheumatol. Suppl.* **2002**, *66*, 38.

4. Laan, R. F. J. M.; Van Riel, P. L. C. M.; Van De Putte, L. B. A. *Curr. Opin. Rheumatol.* **2001**, *13*(3), 159.
5. (a) Emery, P.; Scott, D. L.; Strand, V. *Mod. Ther. Rheum. Dis.* **2002**, 121; (b) Alldred, A.; Emery, P. *Exp. Opin. Pharmacother.* **2001**, *2*(1), 125.
6. O'Dell, J. R. *Mod. Ther. Rheum. Dis.*, **2002**, 147.
7. (a) Keating, G. M.; Perry, C. M. *BioDrugs* **2002**, *16*(2), 111; (b) Markham, A.; Lamb, H. M. *Drugs* **2000**, *59*(6), 1341.
8. (a) Criscione, L. G.; St. Clair, E. W. *Curr. Opin. Rheumatol.* **2002**, *14*(3), 204; (b) Mikuls, E. R.; Moreland, L. W. *Exp. Opin. Pharmacother.* **2001**, *2*(1), 75.
9. (a) Taylor, P. C. *Curr. Pharm. Des.* **2003**, *9*(14), 1095; (b) Paget, S. A. *Adv. Ther.* **2002**, *19*(1), 27.
10. Kumar, S.; Boehm, J.; Lee, J. C. *Nat. Rev.* (*Drug Discov.*) **2003**, *2*, 717.
11. Dean, J. L. E.; Brook, M.; Clark, A. R.; Saklatvala, J. *J. Biol. Chem.* **1999**, *274*(1), 264.
12. (a) Jackson, P. F.; Bullington, J. L. *Curr. Top. Med. Chem.* **2002**, *2*, 1011; (b) Badger, A. M.; Bradbeer, J. N.; Votta, B.; Lee, J. C.; Adams, J. L.; Griswold, D. E. *J. Pharmacol. Exp. Ther.* **1996**, *279*(3), 1453; (c) Boehm, J. C.; Smietana, J. M.; Sorenson, M. E.; Garigipati, R. S.; Gallagher, T. F.; Sheldrake, P. L.; Bradbeer, J.; Badger, A. M.; Laydon, J. T.; Lee, J. C.; Hillegass, L. M.; Griswold, D. E.; Breton, J. J.; Chabot-Fletcher, M. C.; Adams, J. L. *J. Med. Chem.* **1996**, *39*(20), 3929; (d) Cuenda, A.; Rouse, J.; Doza, Y. N.; Meier, R.; Cohen, P.; Gallagher, T. F.; Young, P. R.; Lee, J. C. *FEBS Lett.* **1995**, *364*(2), 229; (e) Gallagher, T. F.; Fier-Thompson, S. M.; Garigipati, R. S.; Sorenson, M. E.; Smietana, J. M.; Lee, D.; Bender, P. E.; Lee, J. C.; Laydon, J. T.; Griswold, D. E.; Chabot-Fletcher, M. C.; Breton, J. J.; Adams, J. L. *Bioorg. Med. Chem. Lett.* **1995**, *5*(11), 1171.
13. Chakravarty, S.; Dugar, S. *Ann. Rep. Med. Chem.* **2002**, *37*, 177.
14. Cirillo, P. F.; Pargellis, C.; Regan, J. *Curr. Top. Med. Chem.* **2002**, *2*, 1021.
15. Hedge, P. J.; Boyle, F. T. WO 9822103 A1 19980528; *Chem. Abstr. 129*, 45323.
16. Work carried out on behalf of AstraZeneca by Sir Philip Cohen, Division of Signal Transduction Therapy, University of Dundee, Dundee, UK.
17. Hall-Jackson, C. A.; Eyers, P. A.; Cohen, P.; Goedert, M.; Boyle, F. T.; Hewitt, N.; Plant, H.; Hedge, P. *Chem. Biol.* **1999**, *6*(8), 559.
18. For in vitro assay experimental procedures see: Brown, D. S.; Brown, G. R. WO 9959959 A1 19991125; *Chem. Abstr. 131*: 351089.
19. Brown, D. S.; Brown, G. R.; Cohen, P. WO 9915164 A1 19990401; *Chem. Abstr. 130*: 267218.
20. (a) Brown, G. R.; Foubister, A. J.; Roberts, C. A.; Wells, S. L.; Wood, R. *Tetrahedron Lett.* **2001**, *42*(23), 3917; (b) Belfield, A. J.; Brown, G. R.; Foubister, A. J.; Ratcliffe, P. D. *Tetrahedron* **1999**, *55*(46), 13285, Corrigendum *Tetrahedron* **2000**, 56(47), 4107; (c) Brown, G. R.; Foubister, A. J.; Ratcliffe, P. D. *Tetrahedron Lett.* **1999**, *40*(6), 1219.
21. The product gave the following data: NMR (DMSO-d_6) 2.19 (s, 3H), 3.18 (t, 4H), 3.73 (t, 4H), 5.23 (s, 2H), 7.12 (d, 3H), 7.2 (d, 1H), 7.34 (m, 3H), 7.42 (s, 1H), 7.54 (m, 2H), 7.8 (m, 2H), 7.97 (d, 2H), 8.58 (d, 1H), 9.72 (s, 1H), 10.1 (s, 1H); Mass MH^+ 523.
22. For further information on kinases and assay protocols see: Davies, S. P.; Reddy, H.; Caivano, M.; Cohen, P. *Biochem. J.* **2000**, *351*(1), 95. The kinase panel used was the Core panel shown in Table 1.
23. For an example of this type of method see: Korfmacher, W. A.; Cox, K. A.; Ng, K. J.; Veals, J.; Hsieh, Y.; Wainhaus, S.; Broske, L.; Prelusky, D.; Nomeir, A.; White, R. E. *Rapid Commun. Mass Spectrom.* **2001**, *15*(5), 335.
24. Procedure for mouse SEB in vivo model: Female Balb/C mice (18–22 g) were dosed orally with test compounds (30, 10 and 3 mg/kg in 20% DMSO/60% PEG 400/20% water; 0.1 mL volume; 4 animals per group), or vehicle alone, 60 min prior to intraperitoneal (ip) challenge with 50 g staphylococcal enterotoxin B (SEB from Toxin Technology BT202) in 0.2 mL pyrogen-free physiological saline (Phoenix PL 1502/0006R). Control animals were challenged (ip) with 0.2 mL pyrogen-free physiological saline. Animals were sacrificed 2 h post SEB-challenge and bled via the vena cava. Serum was obtained following incubation in serum gel tubes (Sarstedt 41-1500.005) for 30 min at room temperature and centrifugation. Samples were stored at −80 °C prior to determination of mouse TNF-α content by ELISA (Genzyme 80-2802-05). Cyclosporin A (Neoral, Novartis)(20 mg/kg dosed orally in 0.1 mL physiological saline) and SB220025 (100, 25, 6.25 and 1.56 mg/kg in DMSO/PEG400/water formulation; 0.1 mL dosed orally) (synthesised in house) were used as positive controls.