Bioorganic & Medicinal Chemistry Letters 20 (2010) 1059–1062

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Piperidine-based heterocyclic oxalyl amides as potent p38α MAP kinase inhibitors

Babu J. Mavunkel, John J. Perumattam, Xuefei Tan *, Gregory R. Luedtke, Qing Lu, Don Lim, Darin Kizer, Sundeep Dugar, Sarvajit Chakravarty, Yong-jin Xu, Joon Jung, Albert Liclican, Daniel E. Levy *, Jocelyn Tabora

Departments of Medicinal Chemistry and Biology, Scios, Inc., 6500 Paseo Padre Parkway, Fremont, CA 94555, USA

ARTICLE INFO

Article history:
Received 22 October 2009
Revised 4 December 2009
Accepted 7 December 2009
Available online 11 December 2009

Keywords:
p38
Piperidine
Heterocyclic
MAP
Kinase
Inhibitor
Oxalyl
Amide
Inflammatory

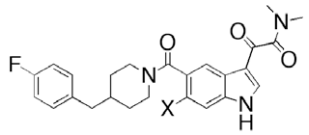
ABSTRACT

The design and synthesis of a new class of p38α MAP kinase inhibitors based on 4-fluorobenzylpiperidine heterocyclic oxalyl amides are described. Many of these compounds showed low-nanomolar activities in p38α enzymatic and cell-based cytokine TNFα production inhibition assays. The optimal linkers between the piperidine and the oxalyl amide were found to be [6,5] fused ring heterocycles. Substituted indoles and azaindoles were favored structural motifs in the cellular assay.

The pro-inflammatory cytokines, tumor necrosis factor alpha (TNFα), and interleukin 1 beta (IL-1β) are known to be involved in the pathogenesis of inflammatory disorders such as rheumatoid arthritis (RA), Crohn's disease, and psoriasis.[1–4] The FDA approval of biologics (Enbrel®, Remicade®, Humira®, and Kineret®) specifically targeting these cytokines by mimicking their receptors[5–8] have demonstrated the effectiveness of this treatment paradigm. However, like many biological agents, these drugs have disadvantages relating to high costs and inconvenient dosing regimens. Thus, there remain unmet needs for developing safe, effective, and orally active small molecule inhibitors that serve the same therapeutic functions.

p38 MAP kinase is a serine–threonine protein kinase and is identified initially as the molecular target of a pyridylimidazole class of compounds. These compounds are known to inhibit the biosynthesis of TNFα and IL-1β in lipopolysaccharide (LPS)-stimulated human monocytes.[9] Among the four known isoforms of p38 (α, β, γ, and δ), the α and β isoforms are the most widely expressed. While the function of the β isoform is not well understood, the α isoform has been shown to be a control point which, when activated, translates multiple stimuli to multiple responses and results in the release of a pro-inflammatory cassette of cytokines including IL-1β, IL-6, and TNFα.[10] As p38α activation is not involved in normal physiology, it is believed that inhibition of this kinase target could result in a reduction of the levels of these cytokines that are thought to play a pathophysiological role in many inflammatory diseases. Therefore, p38α MAP kinase is considered to be a potential therapeutic target for the treatment of inflammatory diseases such as RA[11,12] and has been the focus of many clinical candidates by various pharmaceutical companies in the past decades.[13,14]

We previously reported the discovery of a novel series of 4-fluorobenzylpiperidine indole-based p38α MAP kinase inhibitors represented by Figure 1.[15] The proposed binding mode of **1** in the ATP site of p38α MAP kinase (Fig. 2) shows this class of molecules



1, X = Cl, p38α IC_{50} 0.018 μM
2, X = OMe, p38α IC_{50} 0.01 μM

Figure 1. Structures and potencies of 4-fluorobenzylpiperidine indole analogs, **1** and **2**.

* Corresponding authors. Tel.: +1 650 704 3051 (D.E.L.); +1 011 86 22 66282937 (X.T.).
E-mail addresses: Xuefei827@gmail.com (X. Tan), del345@gmail.com (D.E. Levy).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.12.031

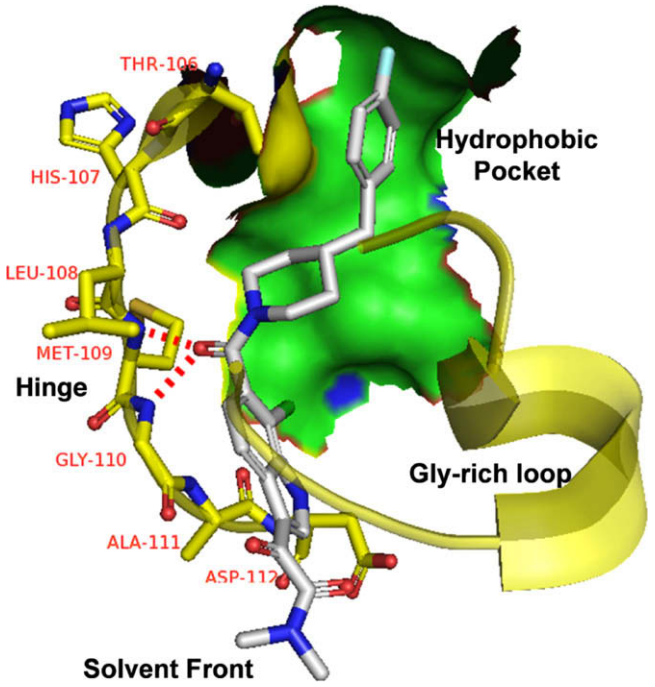


Figure 2. Proposed binding mode of piperidine indole analog **1** in the ATP site of p38α MAP kinase.

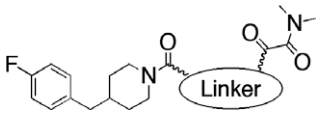
forming a critical hydrogen bond with the kinase backbone at hinge amino acid Met-109.[16] Additionally, the 4-fluorobenzyl group occupies an adjacent hydrophobic pocket that is both critical for activity and, as shown in the literature,[17–19] is a crucial region relating to kinase specificity. Substitutions such as chlorine, methoxy and methyl ortho to the amide moiety at indole position 6 were found to improve p38α enzymatic potency presumably due to the restricted conformation along the carbonyl aryl C–C bond. An oxalyl amide modification at C-3 of the indole was found to further improve enzyme activity. While findings in several of these areas will be the focus of future publications, this Letter describes structure–activity data relating to the heterocyclic core only.

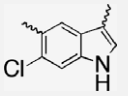
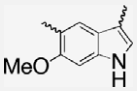
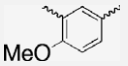
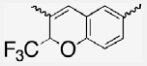
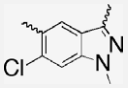
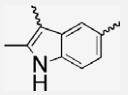
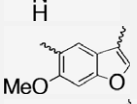
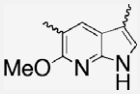
Due to a lack of X-ray crystallographic data at the time of our studies, the exact role of the oxalyl amide was unknown and attention was focused on whether an indole was the optimal linker joining the piperidine amide to the oxalyl amide. Thus, a number of heterocyclic analogs with either different lengths or different electronic properties relative to indole were proposed. Representative analogs, compounds **4–9**, are illustrated in Table 1.

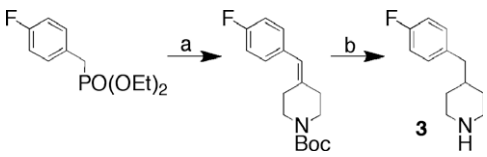
4-Fluorobenzylpiperidine **3** was a common intermediate used in the synthesis of each analog, and was prepared by reacting diethyl 4-fluorobenzylphosphonate with BOC-protected piperidone via a Horner–Emmons reaction, followed by catalytic hydrogenation of the double bond and Boc-deprotection (Scheme 1). Where direct acylation of heteroaromatics with oxalyl chloride could be utilized, the preparation of target compounds was straightforward as illustrated in Scheme 2 for compounds **1**, **2**, and **9**.

For heterocycles inert to treatment with oxalyl chloride, an aldehyde was chosen as a precursor for the oxalyl moiety and one of two coupling protocols were utilized. In the first set of conditions, reaction of an aldehyde with TMSCN followed by treatment with acid and subsequent base hydrolysis provided an α-hydroxy aryl acetic acid. After first forming a methyl ester, PCC oxidation of the hydroxyl group provided the α-keto ester. Hydrolysis with NaOH and coupling with dimethyl amine then gave the desired α-keto amide. Both compounds **4** and **5** were prepared using this route (Scheme 3).

Table 1
p38α enzyme and cellular activity for compounds **1**, **2**, and **4-9**



Compd	Linker	p38α IC_{50}[25] (μM, $n = 3$)	dWBA IC_{50}[26] (μM, $n = 3$)
1		0.018	0.10
2		0.010	0.03
4		0.945	17%@1 μM
5		>10	ND
6		0.10	26%@0.3 μM
7		0.090	ND
8		0.60	10%@1 μM
9		0.06	0.048

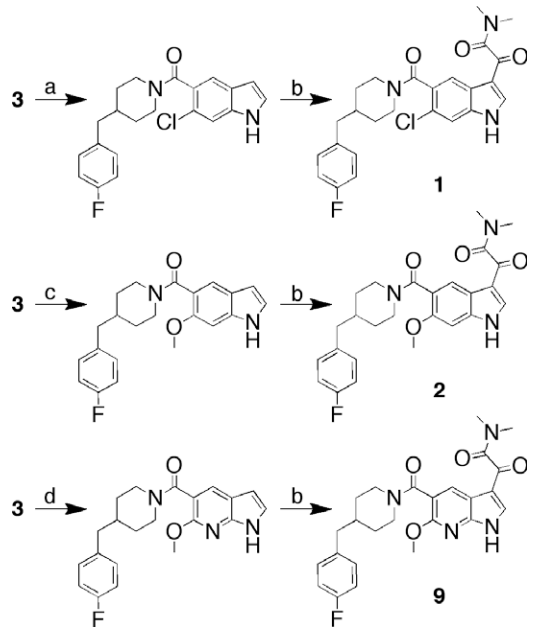


Scheme 1. Preparation of 4-fluorobenzylpiperidine. Reagents and conditions: (a) NaH, DMF then 4-oxopiperidine-1-*tert*-butyl carbamate, 48%; (b) H_2, Pd/C, then HCl/ether, 98%.

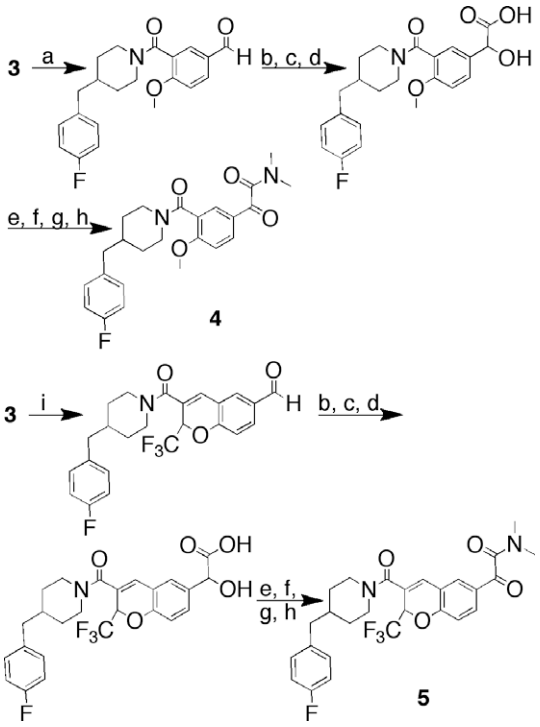
For the second set of conditions, a mild basic transformation was developed for the preparation of compound **6** (Scheme 4). As shown, the indazole 3-aldehyde intermediate was prepared in a one step process from its corresponding indole derivative via double nitrosation followed by ring rearrangement.[24] The aldehyde group was then protected as a dithiane prior to introduction of the methyl group at N-1 of the indazole. Treatment with butyl lithium followed by quenching with dimethylcarbamyl chloride gave the protected oxalyl amide. Upon deprotection, compound **6** was obtained.

Flipped indole analog **7** was prepared according to Scheme 5 by first reacting 4-nitrophenylglyoxylic acid with dimethyl amine followed by $SnCl_2$ reduction to give corresponding aniline. Reductive amination with 1,1-dimethoxyacetone followed by treatment with $AlCl_3$ resulted in cyclization forming the desired indole core. Subsequent reaction with phosgene followed by quenching with 4-fluorobenzylpiperidine gave the desired oxalyl amide.

Finally, regarding heterocyclic systems such as benzofuran that could not be incorporated utilizing an aldehyde route, synthesis was achieved through oxidation of a methylene group to an oxalyl moiety (Scheme 6). Beginning with methyl 2,4-dihydroxybenzoate,

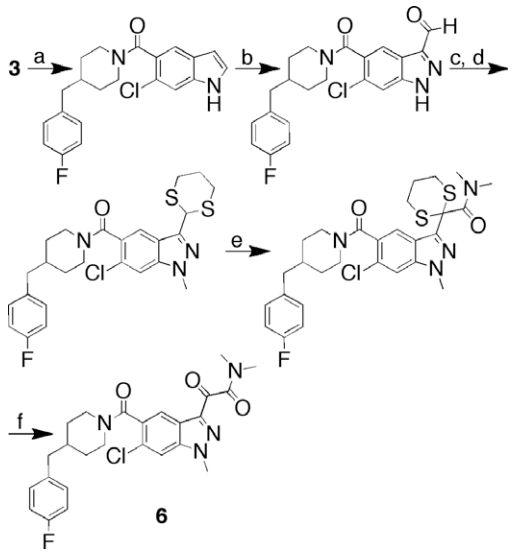


Scheme 2. Reagents and conditions: (a) 6-chloro-1*H*-indole-5-carboxylic acid,[20] EDCI, DMAP, DIPEA, DCM, 81%; (b) oxalyl chloride then dimethyl amine, 72–87%; (c) 6-methoxy-1*H*-indole-5-carboxylic acid,[20] EDCI, DMAP, DIPEA, DCM, 90%; (d) TBTU, 6-methoxy-1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxylic acid,[21] Et_3N, DMF, 83%.

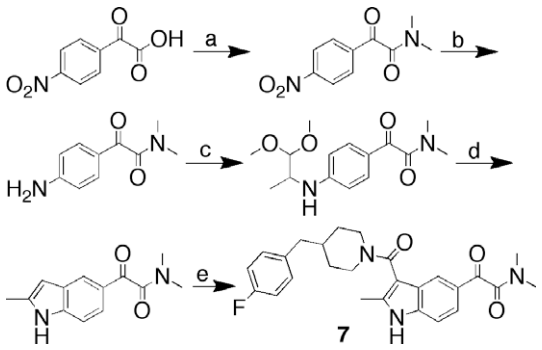


Scheme 3. Reagents and conditions: (a) EDCI, DMAP, 5-formyl-2-methoxy-benzoic acid,[22] Et_3N, DMF, 86%; (b) TMSCN, *n*-BuLi; (c) concd HCl, heat; (d) KOH, MeOH, 75–84% (three steps); (e) HCl, MeOH, reflux; (f) PCC, DCM; (g) NaOH, MeOH–H_2O; (h) EDCI, dimethyl amine, 30–50% (four steps); (i) EDCI, DMAP, 5-formyl-2-trifluoromethyl-2*H*-chromene-3-carboxylic acid,[23] Et_3N, DMF, 81%.

the desired methyl 4-hydroxy-2-methoxybenzoate was prepared by sequential protection of two hydroxyl groups through benzylation and methylation. Following deprotection of the benzyl group and ring iodination, the ester was hydrolyzed and the resulting acid was coupled with 4-fluorobenzyl piperidine. Subsequent



Scheme 4. Reagents and conditions: (a) EDCI, DMAP, 6-chloro-1*H*-indole-5-carboxylic acid,[20] Et_3N, DMF, 81%; (b) 6 N HCl, $NaNO_2$, 56%; (c) $HS(CH_2)_3SH$, BF_3, 90%; (d) NaH, DMF then MeI, 94%; (e) *n*-BuLi, THF then $ClCONMe_2$, 73%; (f) $(CF_3CO)_2IPh$, CH_3CN–H_2O, 90%.

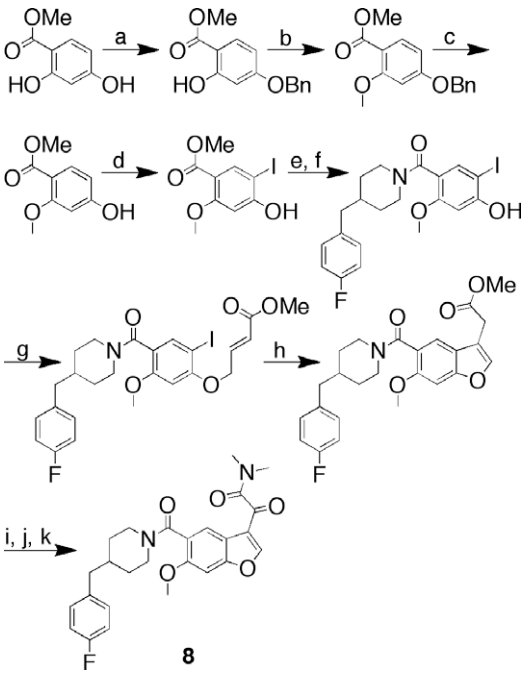


Scheme 5. Reagents and conditions: (a) dimethyl amine, EDCI, THF, 79%; (b) $SnCl_2{\cdot}H_2O$, concd HCl, 60 °C, 85%; (c) 1,1-dimethoxyacetone, anhydrous Na_2SO_4, AcOH, $NaBH(OAc)_3$, 87%; (d) $AlCl_3$, DCM, 60 °C, 24%; (e) phosgene, pyridine, 0 °C then 4-fluorobenzypiperidine, 58%.

reaction with 4-bromo-but-2-enoic acid methyl ester followed by palladium-mediated cyclization yielded the desired benzofuran core. SeO_2 oxidation of the β-carbon then gave the methyl oxalate. Finally, hydrolysis with LiOH and coupling with dimethyl amine gave compound **8**.

The p38α activities for analogs **1, 2**, and **4**–**9** are shown in Table 1. Compared to the initial 6-substituted indole leads, analogs with different length of linkers such as phenyl (**4**) and 2*H*-chromene (**5**) showed a significant decrease in p38α enzymatic activity, indicating the [6,5] fused ring systems maintained the preferred spacing between the amide and oxalyl amide. Furthermore, the flipped 2-methyl-1*H*-indole analog (**7**) still had reasonably good p38α enzyme activity, when compared to the initial indole analogs (**1** and **2**).

In our subsequent [6,5] fused ring systems, it is clearly shown that both polarity and electronics play important roles in modulating enzymatic and, in particular, cellular activity. Indoles and azaindoles are clearly preferred systems than indazole and benzofuran in both enzyme and cellular assays. Additionally, 6-methoxy substitutions result in better cellular activities than 6-chloro substitutions. In the case of 7-azaindole analog **9**, the cellular activity is almost the same as its enzymatic activity. An anomaly in the SAR is the



Scheme 6. Reagents and conditions: (a) K_2CO_3, Bu_4NCl, KI, benzyl bromide, acetone, 81%; (b) NaH, MeI, 95%; (c) Pd/C, H_2 (1 atm.), MeOH, 84%; (d) $BTMAICl_2$, Na_2CO_3, 65%; (e) KOH, MeOH–H_2O, 89%; (f) **3**, TBTU, Et_3N, DMF, 93%; (g) 4-bromo-but-2-enoic acid methyl ester, K_2CO_3, acetone, 69%; (h) $Pd(OAc)_2$, Na_2CO_3, HCOONa, $BnEt_3NCl$, DMF, 81%; (i) SeO_2, dioxane, 77%; (j) LiOH, THF–H_2O, 100%; (k) EDCI, Et_3N, dimethyl amine, 87%.

6-methoxy-benzofuran analog **8**, which exhibits a 60-fold reduction in potency in the p38α enzyme assay compared to indole **2**.

In summary, we designed and synthesized a number of 4-fluorobenzylpiperidine based heterocyclic oxalyl amides as potent p38α MAP kinase inhibitors. The optimal linker length between the piperidine amide and oxalyl amide as well as the significance of electronic properties of different heterocycles were studied in both p38α enzymatic and cellular based assays and confirm the importance of the indole core.

References and notes

1. Smolen, J. S.; Steiner, G. *Nat. Rev. Drug Disc.* **2003**, *2*, 473.
2. Smith, R. J. *Drug Discovery Today* **2005**, *10*, 1598.
3. Cominelli, F. *N. Eng. J. Med.* **2004**, *351*, 2045.
4. Sticherling, M. *Drug Discovery Today* **2005**, *2*, 275.
5. Jarvis, B.; Faulds, D. *Drugs* **1999**, *57*, 945.
6. Choy, E. H.; Panayi, G. S. *N. Eng. J. Med.* **2001**, *344*, 907.
7. Lichtenstein, G. R.; Panaccione, R.; Mallarkey, G. *Ther. Adv. Gastroenterol.* **2008**, *1*, 43.
8. Furst, D. E. *Clin. Ther.* **2004**, *26*, 1960.
9. Lee, J. C.; Laydon, J. T.; McDonnell, P. C.; Gallagher, T. F.; Kumar, S.; Green, D.; McNutly, D.; Blumenthal, M. J.; Heyes, J. R.; Landvatter, S. W.; Strickler, J. E.; McLaughlin, M. M.; Siemens, I. R.; Fisher, S. M.; Livi, G. P.; White, J. R.; Adams, J. L.; Young, P. R. *Nature* **1994**, *372*, 739.
10. Foster, M. L.; Halley, F.; Souness, J. E. *Drug News Perspect.* **2000**, *13*, 488.
11. Kumar, S.; Boehm, J.; Lee, J. C. *Nat. Rev. Drug Disc.* **2003**, *2*, 717.
12. Saklatvala, J. *Curr. Opin. Pharmacol.* **2004**, *4*, 372.
13. Dominguez, C.; Powers, D. A.; Tamayo, N. *Curr. Opin. Drug Discov. Devel.* **2005**, *8*, 421.
14. Lee, M.; Dominguez, C. *Curr. Med. Chem.* **2005**, *12*, 2979.
15. Mavunkel, B. J.; Chakravarty, S.; Perumattam, J. J.; Luedtke, G. R.; Lu, Q.; Dugar, S.; Laney, M.; Henson, M.; Protter, A.; Xu, Y.-J.; Schreiner, G. F.; Almirez, R.; Scott, B.; Lewicki, J.; Moore, A.; Lee, S.; Brahn, E.; Liu, D. Y. International Symposium on Advances in Synthetic, Combinatorial and Medicinal Chemistry, Moscow, Russia, 2004.
16. Docking studies were conducted using a high-resolution p38α X-ray structure (PDB ID: 1ove). The extra-precision mode of Glide, a ligand–receptor docking tool (Schrodinger, LLC), was used to generate a poses for piperidine indole analog **1**. Poses were further filtered by visual inspection.
17. Davies, S. P.; Reddy, H.; Caivano, M.; Cohen, P. *Biochem. J.* **2000**, *351*, 95.
18. Wang, Z.; Canagarajah, B. J.; Boehm, J. C.; Kassisa, S.; Cobb, M. H.; Young, P. R.; Abdel-Meguid, S.; Adams, J. L.; Goldsmith, E. J. *Structure* **1998**, *6*, 1117.
19. Lisnock, J.; Tebben, A.; Frantz, B.; O'Neill, E. A.; Croft, G.; O'Keefe, S. J.; Li, B.; Hacker, C.; De Laszlo, S.; Smith, A.; Libby, B.; Liverton, N.; Hermes, J.; LoGrasso, P. *Biochemistry* **1998**, *37*, 16573.
20. Mavunkel, B. J.; Chakravarty, S.; Perumattam, J. J.; Dugar, S.; Lu, Q.; Liang, X. WO 2000/071535, 2000.
21. Mavunkel, B. J.; Perumattam, J. J.; Lu, Q.; Dugar, S.; Goyal, B.; Wang, D. X.; Chakravarty, S.; Luedtke, G. R.; Nashashibi, I.; Tester, R.; Tan, X. US Patent Application 2005/288299, 2005.
22. Nomura, M.; Takahashi, Y.; Tanase, T.; Miyachi, H.; Tsunoda, M.; Ide, T.; Murakami, K. WO 2000/ 075103, 2000.
23. Carter, J. S.; Obukowicz, M. G.; Devadas, B.; Talley, J. J.; Brown, D. L.; Graneto, M. J.; Bertenshaw, S. R.; Rogier, D. J., Jr.; Nagarajan, S. R.; Hanau, C. E.; Hartmann, S. J.; Ludwig, C. L.; Metz, S. WO 98/47890, 1998.
24. Büchi, G.; Lee, G. C. M.; Yang, D.; Tannenbaum, S. R. *J. Am. Chem. Soc.* **1986**, *108*, 4115.
25. Procedure for human p38α kinase assay: Compounds were assayed using an in vitro method measuring the incorporation of radiolabeled ATP into a peptide substrate. Compounds were dissolved in DMSO and diluted into water to the desired concentrations. Compounds were mixed with the enzyme reagent, and the reactions were initiated by the addition of a 4× substrate cocktail containing 200 μM biotin–peptide substrate and 0.6 mM ATP (+100 μCi/ml γ-^{32}P-ATP). Final assay conditions were 25 mM MOPS, pH 7.0, 26.25 mM β-glycerol phosphate, 80 mM KCl, 22 mM $MgCl_2$, 3 mM $MgSO_4$, 1 mg/ml gelatin, 0.625 mM EGTA, 1 mM DTT, 50 μM peptide substrate, 150 μM ATP, and 5 nM p38α. After 60 min incubation at 30 °C, the reactions were stopped by the addition of 10 μl per reaction of 1.5% phosphoric acid. A portion of each of the reactions was transferred into the well of a streptavidin-coated Flash Plate (Perkin Elmer). The plates were washed 3× in PBS containing 0.01% Tween, and sealed. Counts incorporated were determined on a scintillation counter. Relative enzyme activity at each compound concentration was calculated by subtracting background counts (counts measured in the absence of enzyme) from each result, and comparing the resulting counts to those obtained in the absence of inhibitor. The IC_{50} was determined to be the concentration of compound which reduced the incorporation of ATP into the substrate by 50%, when compared with control reactions containing no inhibitor.
26. The LPS/TNFα Human Whole Blood Assay was run as described in: Mavunkel, B.; Dugar, S.; Luedtke, G.; Tan, X.; McEnroe, G. US Patent 6,696,443, 2004.