Bioorganic & Medicinal Chemistry Letters 18 (2008) 5428–5430

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

The identification of pyrazolo[1,5-*a*]pyridines as potent p38 kinase inhibitors

Mui Cheung *, Philip A. Harris, Jennifer G. Badiang, Gregory E. Peckham, Stanley D. Chamberlain †, Michael J. Alberti, David K. Jung, Stephanie S. Harris, Neal H. Bramson, Andrea H. Epperly, Stephen A. Stimpson, Michael R. Peel ‡

GlaxoSmithKline, Five Moore Drive, Research Triangle Park, NC 27709, USA

ARTICLE INFO

Article history:
Received 18 July 2008
Revised 4 September 2008
Accepted 9 September 2008
Available online 12 September 2008

Keywords:
Pyrazolopyridine
p38 Inhibitor

ABSTRACT

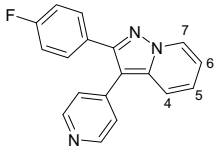
A series of pyrazolo[1,5-*a*]pyridine derivatives was designed and synthesized as novel potent p38 kinase inhibitors. Our approaches towards improving in vitro metabolism and in vivo pharmacokinetic properties of the series are described.

P38, also known as cytokine-suppressive anti-inflammatory drug binding protein (CSBP), is a member of the mitogen activated protein (MAP) kinase family that is involved in stress and inflammatory response signal transduction pathways.[1,2] A large number of small molecular inhibitors of p38 have been reported, and many are undergoing clinical trials for the treatment of inflammatory and autoimmune diseases such as rheumatoid arthritis, Crohn's disease, psoriasis and surgery-induced tissue injury.[3–5] Recently, the potential utility of inhibiting p38 for the treatment of ischemic heart disease was also proposed.[6] The most clinically advanced p38 MAP kinase inhibitors include SCIO-469 (talmapimod), VX-702 and PS-540446 which are currently in phase 2 clinical trials for the treatment of rheumatoid arthritis and additional indications.

As part of a lead optimization program, 2-(4-fluorophenyl)-3-(4-pyridinyl)pyrazolo-[1,5-*a*]pyridine (**1**) was identified as a promising p38 kinase inhibitor with an IC_{50} of 0.12 μM.[7] Compound **1** inhibited TNF release from human peripheral blood mononuclear cells (PBMC) following stimulation with LPS with an IC_{50} of 0.3–1.3 μM. In addition, **1** showed 65% inhibition of murine TNF production in mice following an LPS challenge when dosed at 30 mg/kg orally.[8] As an initial lead this was a very attractive starting point. The main area for improvement was the pharmacokinetic profile of **1** which possessed high in vivo clearances in both rat and dog of 49 and 34 mL/min/kg, respectively, which would not support a target of once daily dosing. Herein we report our approach to improve the pharmacokinetic properties of this series by identification of a key potential site of oxidative metabolism on the pyrazolo-[1,5-*a*]pyridine core and attachment of various substitutions at that site to inhibit metabolism.

Our initial approach was to investigate the structure–activity relationship (SAR) at the pyrazolopyridine heterocycle targeting the open 4-, 5-, 6-, and 7-positions (see Fig. 1). This approach was chemically flexible and would provide useful information about potency and in vitro stability of the template. A parallel strategy was to examine the potential metabolites of **1** by incubation in the S9 liver microsomes and then follow up with metabolite identification using MS/MS and NMR spectroscopy. The aim was to identify the potential sites of metabolism of the molecule and use this information to design appropriate substitutions to inhibit this process.

The syntheses of pyrazolo-[1,5-*a*]pyridines have been described elsewhere.[8–10] As shown in Scheme 1, compounds **1–10** were pre-



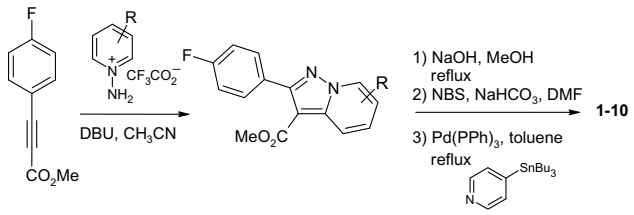
1

Figure 1. Structure of **1**.

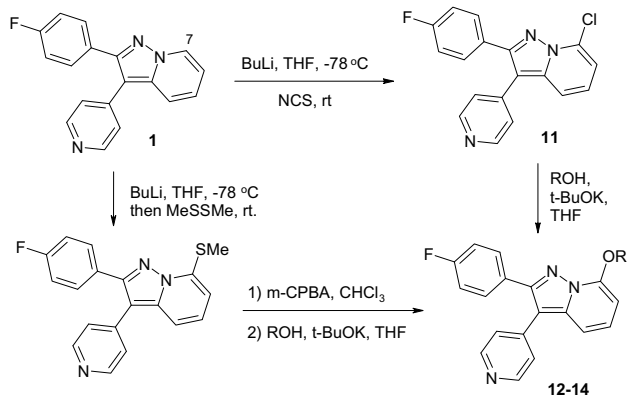
Abbreviations: CSBP, cytokine-suppressive anti-inflammatory drug binding protein; MAP kinase, mitogen-activated protein kinase; PBMNC, human peripheral blood monouclear cells; SAR, structure–activity relationships.

* Corresponding author. Tel.: +1 610 270 7782; fax: +1 610 270 4490.
E-mail address: mui.h.cheung@gsk.com (M. Cheung).
† Present address: Inhibitex Inc., Alpharetta, GA, USA.
‡ Present address: Scynexis Inc., Research Triangle Park, NC, USA.

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.09.040



Scheme 1. Synthesis of **1**–**10**.



Scheme 2. Synthesis of **11**–**14**.

pared by cyclization of methyl 3-(4-fluorophenyl)-2-propynoate with the appropriately substituted *N*-aminopyridines to form the pyrazolopyridine methyl ester. Hydrolysis of methyl ester followed by in situ decarboxylation and bromination afforded the bromide intermediate which underwent Susuki coupling with 4-(tributylstannyl)pyridine to provide pyrazolopyridines **1–10**. Compounds **11–14** were synthesized by functionalization of the 7-position of **1** as described in Scheme 2. Deprotonation of **1** with *n*-butyl lithium and subsequent trapping of the resulting anion with electrophiles such as *N*-chlorosuccinimide or *p*-toluenesulfonyl chloride provided compound **11**. Compounds **12–14** were prepared by trapping the anion of **1** with dimethyl disulfide to form the 7-S-Me derivative, which was then oxidized with meta-chloroperoxybenzoic acid to yield the 7-methyl sulfoxide. Subsequent displacement of the 7-methyl sulfoxide with alkoxide nucleophiles afforded the 7-alkoxy substituted compounds (**12–14**). Alternatively, compounds **12–14** could be prepared by direct displacement of 7-chloride compound (**11**) with alkoxides.

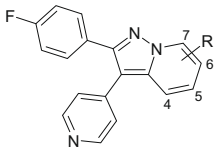
As shown in Table 1, substitutions at the pyrazolopyridine ring with electron-donating groups (compounds **4–7**, **12–13**) and electron-withdrawing groups (compounds **2–3**, **8–11**) are well-tolerated.

When evaluating the metabolic stability of pyrazolopyridines in the S9 microsomal fraction, pyrazolopyridines with electron rich groups attached (**5–7**) tended to have a higher metabolic turnover than those with electron-withdrawing groups (**2** and **3**). As shown in Table 2, after 30 min of incubation in the liver S9 fractions, the 6-methylated compound (**6**) was highly metabolized in rat and dog when compared to the 6-fluoro compound (**3**).

The pyrazolo[1,5-*a*]pyridine core is known as a site of metabolism.[11,12] To identify the potential metabolites, **1** was incubated in liver S9 microsomes and then metabolite identification was achieved using MS/MS and NMR spectroscopy. Following analysis of samples of compound **1** in mouse, rat, dog and human liver microsomes, dihyroxylated compounds with molecular weights of 324, which is +34 of the parent compound **1**, were identified as the major metabolites. NMR spectroscopy identified the metabolite structures as compounds **15** and **16** where dihydroxylation occurred at the 6- and 7-positions, and the 4- and 7-positions, respectively, in a 60:40 ratio. The likely pathway is initial epoxidation at the 6-/7-position, followed by epoxide-opening nucleophilic addition of water at either the 4- or 6-positions (Scheme 3). This in vitro metabolism pathway would support the earlier observation that electron deficient substituents on the pyrazolopyridine ring reduced S9 metabolism, as reduction of electron density at the pyridine ring will lower the propensity for the initial epoxidation.

Based on the earlier SAR and the metabolite information, we expected that metabolism of the pyrazolopyridine core could be reduced by either increasing steric hindrance or incorporation electron-withdrawing groups at the 6- or 7-positions. As shown in Table 3, incorporation of the electron-withdrawing group at the 6-position such as 6-CF_3 (**8**) and bulky groups at the 7-position such as 7-OCH_2CF_3 (**14**) and 7-*O*-cyclopentane (**12**) significantly reduced metabolite liability when compared to the parent compound (**1**) with no observed turnover in both rat and dog S9 microsomes.

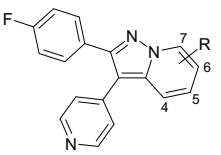
Table 1
P38 kinase activity of pyrazolopyridines



Compound	R	p38 enzyme IC_{50}[a] (μM)
1	H	0.12
2	4-F	0.24
3	6-F	0.16
4	4-Me	0.28
5	5-Me	0.16
6	6-Me	0.15
7	7-Me	0.15
8	6-CF_3	0.20
9	6-CN	0.14
10	6-Cl	0.15
11	7-Cl	0.15
12	7-*O*-Cyclopentane	0.17
13	7-*O*-Et	0.05
14	7-OCH_2CF_3	0.07

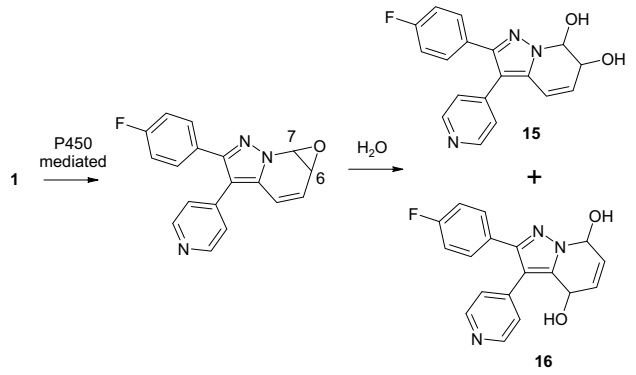
[a] Values are means of greater than two experiments.

Table 2
In vitro metabolic stability (% metabolized) of p38 inhibitors in liver S9 fractions in rat and dog



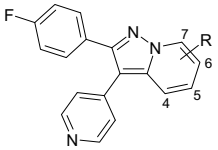
Compound	R	Rat S9[a]	Dog S9[a]
1	H	27	81
2	4-F	21	39
3	6-F	1	23
5	5-Me	60	ND
6	6-Me	99	81
7	7-Me	43	93

[a] Values are means of ⩾2 incubations. Substrate concentration is 15 μM with 5 mg protein/mL. Readout is % turnover of parent compound after 30 min incubation in liver S9 fractions. ND, no data.



Scheme 3. Potential metabolites for compound **1**.

Table 3
In vitro metabolic stability (% metabolized) of p38 inhibitors in liver S9 fractions in rat and dog



Compound	R	Rat S9[a]	Dog S9[a]
1	H	27	81
7	7-Me	43	93
8	6-CF_3	0	0
11	7-Cl	37	84
12	7-*O*-Cyclopentane	8	18
14	7-OCH_2CF_3	0	0

[a] Values are means of ⩾2 incubations. Substrate concentration is 15 μM with 5 mg protein/mL. Readout is % turnover of parent compound after 30 min incubation in liver S9 fractions. ND, no data.

Table 4
In vivo pharmacokinetic properties of p38 inhibitors in male Lewis rats

Compound	Dose (mg/kg)	CL (mL/min/kg)	V_{dss} (L/kg)	$t_{1/2}$ (h)	AUC (μg h/mL)	%*F*
1	5 (iv)[a]	49	3.2	1.2	1.7	50
5	1 (iv)[a]	83	2.0	1.2	0.2	ND
8	1 (iv)[b]	34	5.4	2.5	0.5	84
14	1 (iv)[b]	40	5.4	2.1	0.5	72

[a] $n = 3$; iv formulation: solution in 10% HP-β-cyclodextrin in 0.05 M acetic acid; oral formulation: suspension in HPMC with Tween 80.
[b] $n = 2$. ND, no data.

Unexpectedly, compound with the electron-withdrawing group at the 7-position (**11**) did not improve metabolite stability when compared to parent compound (**1**) and compound with the electron-donating group at the 7-position (**7**).

The improvement in the in vitro metabolic stability also translated into increased in vivo metabolic stability. As shown in Table 4, compounds **8** and **14** with no observed turnover in rat S9 microsomes have significantly lower clearance in rats than compound **5** where 60% turnover was observed. In addition, both compounds **8** and **14** have improved half-lives and excellent bioavailability in rat.

Table 5
In vivo pharmacokinetic properties of p38 inhibitors in male Beagle dogs

Compound	Dose (mg/kg)	CL (mL/min/kg)	V_{dss} (L/kg)	$t_{1/2}$ (h)	AUC (μg h/mL)	%*F*
1	1 (iv)[a]	34	3.8	1.9	0.5	18
14	1 (iv)[b]	17	8.2	6.5	1.5	32

[a] $n = 3$; iv formulation: solution in 10% HP-β-cyclodextrin in 0.05 M acetic acid; oral formulation: suspension in HPMC with Tween 80.
[b] $n = 2$.

The pharmacokinetic properties of compound **14** were evaluated in the dog. As shown in Table 5, compound **14** showed a reduced clearance, longer half-life, and improved AUC and oral bioavailability in the dog compared to the patent compound (**1**).

A series of pyrazolo[1,5-*a*]pyridine derivatives were identified as potent p38 kinase inhibitors. Initial SAR indicated that the pyrazolopyridine heterocycle could be the site of metabolism which was confirmed by metabolite identification of compound **1**. The improvement in the in vitro and in vivo pharmacokinetic properties of the series was achieved by reducing electron density at the 6-position and increasing steric hindrance at the 7-position of the pyrazolopyridine ring.

Acknowledgments

We thank Kirk Stevens for reviewing the manuscript. We thank Peiyuan Lin for providing the cellular data of **1** in PBMC. We also acknowledge Jamie Conway and Heather Pink for providing the in vivo data of **1** in TNF release model in mice.

References and notes

1. Lee, J. C.; Laydon, J. T.; McDonnell, P. C.; Gallagher, T. F.; Kumar, S.; Green, D.; McNulty, D.; Blumenthal, M. J.; Heys, J. R.; Landvatter, S. W.; Strickler, J. E.; McLaughlin, M. M.; Siemens, I. R.; Fisher, S. M.; Livi, G. P. I.; White, J. R.; Adams, J. L.; Young, P. R. *Nature* **1994**, *372*, 739.
2. Lee, J. C.; Young, P. R. *J. Leukocyte Biol.* **1996**, *59*, 152.
3. Peifer, C.; Wagner, G.; Laufer, S. *Curr. Top. Med. Chem.* **2006**, *6*, 113.
4. Goldstein, D. M.; Gabriel, T. *Curr. Top. Med. Chem.* **2005**, *5*, 1017.
5. Wagner, G.; Laufer, S. *Med. Res. Rev.* **2006**, *26*, 1.
6. Clark, J. E.; Sarafraz, N.; Marber, M. S. *Pharmacol. Ther.* **2007**, *116*, 192.
7. (a) The p38α IC_{50}s reported in this article were determined as follows: the peptide substrate used in the p38 assay was biotin-IPTSPITTTYFFFRRR-amide. The p38 and MEK6 proteins were purified to homogeneity from *E. coli* expression systems. The fusion proteins were tagged at the N-terminus with glutathione-*S*-transferase (GST). The maximum activation was achieved by incubating 20 μL of a reaction mixture of 30 nM MEK6 protein and 120 nM p38 protein in the presence of 1.5 μM peptide and 10 mM $Mg(CH_3CO_2)_2$ in 100 mM Hepes, pH 7.5, added to 15 μL of a mixture of 1.5 μM ATP with 0.08 μCi [g-^{33}P]ATP, with or without 15 μL of inhibitor in 6% DMSO. The controls were reactions in the presence (negative controls) or absence (positive controls) of 50 mM EDTA. Reactions were allowed to proceed for 60 min at rt and quenched with addition of 50 μL of 250 mM EDTA and mixed with 150 μL of Streptavidin SPA beads (Amersham) to 0.5 mg/reaction. The Dynatech Microfluor white U-bottom plates were sealed and the beads were allowed to settle overnight. The plates were counted in a Packard TopCount for 60 s. IC_{50} values were obtained by fitting raw data to $\%I = 100 * (1 - (I - C2)/(C1 - C2))$, where I was CPM of background, $C1$ was positive control, and $C2$ was negative control. (b) Kinase selectivity profile of **1** (all data reported as IC_{50} with $n \geqslant 2$): p38 = 0.12 μM, GSK3b = 2.2 μM, C-Raf/Mek/Erk = 5.8 μM, EGFR = 6.3 μM; AKT3, CDK2/CyclinA, C-FMS, ERBB2, ERBB4, PDHK4, TIE2, and VEGFR2 >10 μM.
8. Alberti, M. J.; Baldwin, I. R.; Cheung, M.; Cockerill, S.; Flack, S.; Harris, P. A.; Jung, D. K.; Peckham, G.; Peel, M. R.; Stanford, J. B.; Stevens, K.; Veal, J. M. WO 0216359, 2002.
9. Alberti, M. J.; Chamberlain, S. D.; Cheung, M.; Gudmundsson, K.; Harris, P. A.; Johns, B. A.; Jung, D. K.; Peel, M. R.; Stanford, J. B. WO 0278700, 2002.
10. Stevens, K. L.; Jung, D. K.; Alberti, M. J.; Badiang, J. G.; Peckham, G. E.; Veal, J. M.; Cheung, M.; Harris, P. A.; Chamberlain, S. D.; Peel, M. R. *Org. Lett.* **2005**, *7*, 4753.
11. Nagatsu, Y.; Higuchi, T.; Hirobe, M. *Chem. Pharm. Bull.* **1989**, *37*, 1410.
12. Awano, K.; Iwase, K.; Nagatsu, Y.; Suzue, S. *Chem. Pharm. Bull.* **1992**, *40*, 639.