Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 2047–2050

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Discovery of a New Class of p38 Kinase Inhibitors

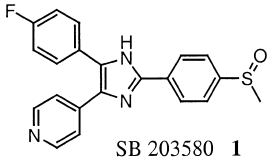
Jacques Dumas,[a,*] Robert Sibley,[a] Bernd Riedl,[a] Mary Katherine Monahan,[a] Wendy Lee,[a] Timothy B. Lowinger,[a] Anikó M. Redman,[a] Jeffrey S. Johnson,[a] Jill Kingery-Wood,[a] William J. Scott,[a] Roger A. Smith,[a] Mark Bobko,[a] Robert Schoenleber,[a] Gerald E. Ranges,[b] Timothy J. Housley,[b] Ajay Bhargava,[b] Scott M. Wilhelm[b] and Alka Shrikhande[b]

[a]*Department of Chemistry Research, Bayer Research Center, 400 Morgan Lane, West Haven, CT 06516, USA*
[b]*Department of Cancer and Osteoporosis Research, Bayer Research Center, 400 Morgan Lane, West Haven, CT 06516, USA*

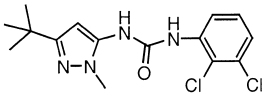
Received 16 March 2000; accepted 2 May 2000

Abstract—The MAP kinase p38 has been implicated in cytokine signaling, and its inhibitors are potentially useful for the treatment of arthritis and osteoporosis. Novel small-molecule inhibitors of p38 kinase were derived from a combinatorial chemistry effort and exhibit activity in the nanomolar range. Very steep structure–activity relationships are observed within this class. © 2000 Elsevier Science Ltd. All rights reserved.

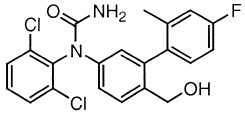
p38 Kinase belongs to the family of MAP kinases, and has been implicated in cytokine signaling.[1] p38 Inhibitors from the pyridyl-imidazole class,[2,3] such as SB203580 (**1**, Fig. 1), can disrupt cytokine signaling in cells, and are effective in in vivo models of endotoxin shock and arthritis.[4] As part of a combinatorial chemistry effort the novel pyrazole **2** was identified as a reversible p38 inhibitor.[5]



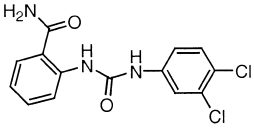
SB 203580 **1**
p38 IC_{50} = 20 nM



2
p38 IC_{50} = 53 nM



3
p38 IC_{50} = 2 nM



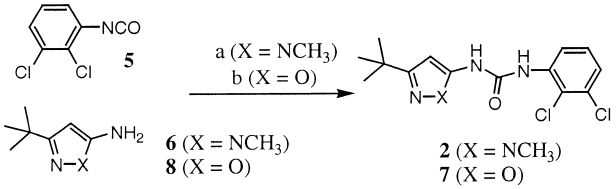
4
p38 IC_{50} = 100 nM

Figure 1. p38 Kinase inhibitors.

At the outset of this program, urea substructures were relatively unknown in the field of kinase inhibition, as most of the patent activity was concentrated on other structural types.[6] Only one urea example could be found in the literature, describing broad tyrosine kinase inhibitory activity with 1-phenyl-3-thienyl ureas.[7] More recently, Vertex has also reported aryl and bis-aryl ureas such as **3** and **4** as inhibitors of p38 kinase.[8]

Chemistry

The goal of this program was to develop SARs around the screening hit **2**. Pyrazolyl ureas are easily accessible in moderate to good yields by the reaction of commercially available 2-methyl-3-amino-5-*tert*-butylpyrazole (**6**) with isocyanate **5**[9] (Fig. 2). The corresponding isoxazolyl ureas, such as **7**, are obtained from 3-*tert*-butyl-5-amino isoxazole (**8**) in the same way.

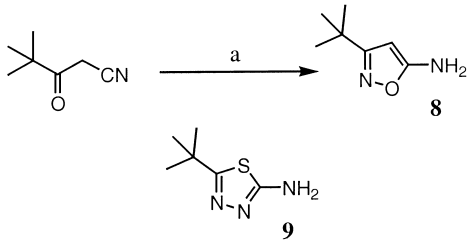


(a) $PhCH_3$, reflux, 18 h, 67%. (b) $PhCH_3$, reflux, 24 h, 34%.

Figure 2. Synthesis of isoxazolyl and pyrazolyl ureas.

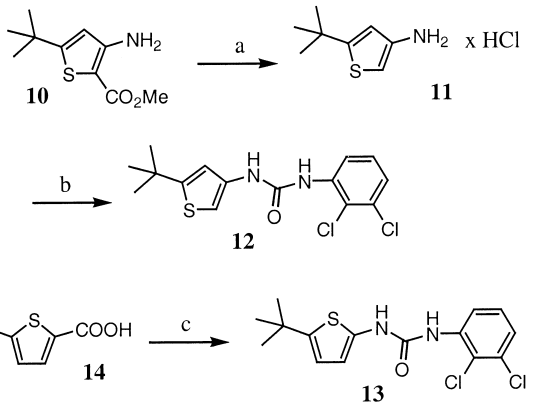
*Corresponding author. Tel.: +1-203-812-5204; fax: +1-203-812-2650; e-mail: jacques.dumas.b@bayer.com

0960-894X/00/$ - see front matter © 2000 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(00)00270-5



(a) Hydroxylamine hydrochloride, NaOH, water, 50 °C, 2.5 h.

Figure 3. 2-Aminoisoxazoles and 2-aminothiadiazoles.



(a) NaOH, EtOH, reflux, 2 h then concd. HCl, rt, 1 h (100% crude). (b) 2,3-dichlorophenyl isocyanate, pyridine, CH_2Cl_2, rt, 18 h, 34%. (c) DPPA, NEt_3, toluene, 80 °C, 2 h, then 2,3-dichloroaniline, 80 °C, 2 h, 24%.

Figure 4. Synthesis of thienyl ureas.

This method can also be used for the preparation of the isomeric isoxazolyl ureas (from commercially available 3-amino-5-*tert*-butylisoxazole), and thiadiazolyl ureas. Isoxazole **8** (Fig. 3) results from hydroxylamine condensation with commercially available 4,4-dimethyl-3-oxo-pentanenitrile. This method is also very flexible, and allows variation of the isoxazole substituent by the use of other cyanoketones. 2-Amino-5-*tert*-butylthiadiazole (**9**) can be obtained according to previously published procedures.[10]

Preparation of analogues in which the pyrazole ring is replaced by a thiophene is depicted in Fig. 4. The known methyl 3-amino-5-*tert*-butyl-2-thiophene-carboxylate (**10**) is saponified with sodium hydroxide in ethanol, then decarboxylated upon HCl treatment. The resulting 3-amino-5-*tert*-butylthiophene HCl salt **11** can be converted to the urea **12** without purification. The isomeric thienyl urea **13** is obtained by Curtius rearrangement of 2-carboxy-5-*tert*-butylthiophene **14**, followed by in situ treatment with 2,3-dichloroaniline.

Rapid generation of urea libraries was achieved in our combinatorial chemistry effort by conducting the amine-isocyanate reaction in anhydrous DMF (80–95 °C, 18 h). A modular parallel-synthesis work station, incorporating Gilson 215 robotic liquid handling and a J-KEM reaction block,[11] allowed the preparation of approximately 1000 analogues, typically as 10×10 reaction matrices.[12]

Results and Discussion

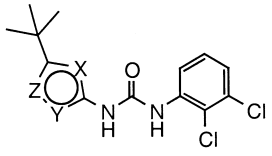
Table 1 summarizes the results of the heterocycle variations developed with the original 2,3-dichlorophenyl urea substituent.

The pyrazole unit of **2** can be successfully replaced by an isoxazole (the two possible isomers **7** and **15** are active in the same range) or by a thiophene (the two prepared isomers **12** and **13** display similar, but slightly lower potency in the p38 α2 assay). The thiadiazole analogue **16** is inactive.

For the variation of the *tert*-butyl group, the isoxazole urea **7** was used as a reference compound (Table 2). Very steep structure–activity relationships are observed, with significant potency losses when one carbon atom is either added, as in isoxazoles **19** and **24**, or removed (entry **21**). The same trend is observed when the geometry of the *tert*-butyl group is modified, keeping the number of atoms constant as in entries **17** and **20**. The 4-position of the isoxazole (R_2 in Table 2) does not tolerate substitution very well, as even the addition of a methyl group results in weaker compounds (entries **22**, **23**, and **25**). The phenyl group substitution of ureas **15** (Type I) and **7** (Type II) is explored in Table 3. The observed trends are quite consistent across the two structural types.

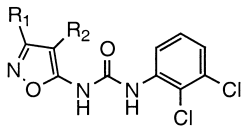
Deletion of the two chlorine atoms, exemplified in **27**, results in inactive compounds. Replacement of the chlorine atoms by methyl groups (similar sizes and lipophilicities, but different electronic effects) results in weaker compounds (**35** and **42**). Introduction of polar, hydrogen bonding groups in the *meta* and *para* positions does not seem to be tolerated (compounds **30**, **31** and **40**, **41**). Clearly, halogens and trifluoromethyl substituents properly attached to the phenyl ring, which

Table 1. Variation of the heterocyle



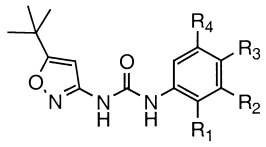
Compound	X	Y	Z	% Inhibition (500 nM)	p38 α2 IC_{50} (nM)
15	CH	N	O	88	58
7	CH	O	N	93	36
2	CH	NCH_3	N		53
16	S	N	N	1	
13	S	CH	CH	82	120
12	CH	CH	S	76	160

Table 2. Isoxazole alkyl substituent

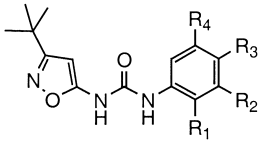


Compound	R_1	R_2	% Inhibition (500 nM)	p38 α2 IC_{50} (nM)
7	*tert*-Bu	H	93	36
17	Cyclobutyl	H	57	370
18	Adamantyl	H	9	
19	2,2-Dimethylpropyl	H	81	120
20	*n*-Bu	H	33	1100
21	*i*-Pr	H	62	290
22	*tert*-Bu	CH_3	18	
23	*i*-Pr	CH_3	34	920
24	1,1-Dimethylpropyl	H	83	120
25	1,1-Dimethylpropyl	CH_3	18	
26	CH_3	H	4	

Table 3. Substitution of the phenyl moiety



Type I



Type II

Compound	Type	R_1	R_2	R_3	R_4	% Inhibition (500 nM)	p38 α2 IC_{50} (nM)
27	I	H	H	H	H	33[a]	
28	I	CF_3	H	Cl	H	9	
29	I	H	Br	H	Br		710
30	I	H	NHAc	H	H	18	
31	I	H	H	NHAc	H	14	
32	I	H	H	F	H	74	190
33	I	CF_3	H	H	H	36	
34	I	H	H	CF_3	H	39	
15	I	Cl	Cl	H	H	88	58
35	I	CH_3	CH_3	H	H	54	
36	II	H	H	F	H	41	
37	II	H	Cl	Cl	H	62	220
38	II	H	H	Cl	H	68	260
39	II	H	H	CF_3	H	77	200
7	II	Cl	Cl	H	H	93	36
40	II	H	NHAc	H	H	11	
41	II	H	H	NHAc	H	20	
42	II	CH_3	CH_3	H	H	49	

combine lipophilicity and electron withdrawing effects, yield potent p38 inhibition within this series. The 2,3-dichlorophenyl ureas **2** and **15** were selected for further in vitro characterization. Cytokine induction of IL-6 in chondrocytes is believed to proceed through a signal transduction pathway, which includes p38 kinase.[3] Ureas **2** and **15**, as well as SB203580 (**1**) inhibit IL-6 production in SW1353 cells treated with cytokines IL-1 and TNF.[13] A dose-responsive inhibition was observed with all three agents ($IC_{50} = 0.05$ μM, 0.82 μM and 1.2 μM, respectively, for **1**, **2**, and **15**), although **2** and **15** were much less potent than **1**.

In conclusion, a novel series of highly potent p38 kinase inhibitors has been identified by a combinatorial chemistry effort.[14] Replacement of the pyrazole ring of the lead compound **2** broadens the scope of this discovery to isoxazolyl and thienyl ureas. Very steep structure–activity relationships are observed on the bulky alkyl group of the five-membered ring heterocycle, as well as on the phenyl moiety. The original pyrazole hit (**2**) and its isoxazole analogue (**15**) are active in a functional assay of cellular cytokine signaling (TNF and IL-1 induced IL-6 production in SW1353 cells) in the submicromolar range, making this series a very promising starting point for future programs.

Acknowledgements

We would like to thank Christopher Browe, Michael Bombara, Robert Dreyer, David Osterman, Judith Cosgrove, David Brittelli, Charles Maniglia, Robert Carlson, and Thuy Chau for support and helpful discussions.

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.; Stricker, J. E.; McLaughlin, M. M.; Siemens, I. R.; Fisher, S. M.; Livi, G. P.; White, J. R.; Adams, J. L.; Yound, P. R. *Nature* **1994**, *372*, 739. Han, J.; Lee, J.-D.; Bibbs, L.; Ulevitch, R. J. *Science* **1994**, *265*, 808.
2. 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.; Chabot-Fletcher, M. C.; Breton, J. J.; Adams, J. L. *Bioorg. Med. Chem. Lett*. **1995**, *5*, 1171. Boehm, J. C.; Smietana, J. M.; Sorenson, M. E.; Gallagher, T. F.; Sheldrake, P. L.; Bradbeer, J.; Badger, A. M.; Laydon, J. T.; Lee, J. C.; Hillegrass, L. M.; Griswold, D. E.; Breton, J. J.; Chabot-Fletcher, M. C.; Adams, J. L. *J. Med. Chem*. **1996**, *39*, 3929.
3. Wilson, K.; McCaffrey, P.; Hsiao, K.; Pazhanisamy, S.; Galullo, V.; Bemis, G.; Fitzgibbon, J.; Caron, P.; Murcko, M.; Su, M. *Chem. Biol*. **1997**, *4*, 423.
4. Badger, A. M.; Bradbeer, J. N.; Votta, B.; Lee, J. C.; Adams, J. L.; Griswold, D. E. *J. Pharm. Exper. Ther*. **1996**, *279*, 1453.
5. Purified and His-tagged p38 α2 (expressed in *E. coli*) was activated in vitro by MMK-6 to a high specific activity. Using a microtiter format, all reactions were conducted in 100 μL volumes with reagents diluted to yield 0.05 μg/well of activated p38 α2 and 10 μg/well of myelin basic protein in assay buffer (25 mM HEPES 7.4, 20 mM $MgCl_2$, 150 mM NaCl). Test compounds (5 μL of a 10% DMSO solution in water) were prepared and diluted into the assay to cover a final concentration range from 5 nM to 2.5 μM. The kinase assay was initiated by addition of 25 μL of an ATP cocktail to give a final concentration of 10 μM cold ATP and 0.2 μCi [γ-^{33}P] ATP per well (200–400 dpm/pmol of ATP). The plate was incubated at 32 °C for 35 min and the reaction quenched with 7 μL of a 1 N aq HCl solution. The samples were harvested onto a P30 Filtermat (Wallac, Inc.) using a TomTec 1295 Harvester (Wallac, Inc.), and counted in a LKB 1205 Betaplate Liquid Scintillation Counter (Wallac, Inc.). Negative controls included substrate plus ATP alone. SmithKline Beecham's SB203580 (**1**) was used as a standard ($IC_{50} = 20$ nM). At least two independent IC_{50} determinations were performed on each compound, and the mean value is reported. In all cases, standard deviations were less than 50% of the mean IC_{50} value.
6. For recent reviews, see: Boehm, J. C.; Adams, J. L. *Exp. Opin. Ther. Pat*. **2000**, *10*, 25. Henry, J. R.; Cavender, D. E.; Wadworth, S. A. *Drugs Future* **1999**, *24*, 1345.

7. Tang, P. C.; McMahon, G. *PCT Int. Appl.* WO 96 40673; *Chem. Abstr.* **1997**, *126*, 139849.
8. Salituro, F. G.; Bemis, G. W.; Green, J.; Kofron, J. L. *PCT Int. Appl.* WO 99 00357; *Chem. Abstr.* **1999**, *130*, 66491. Salituro, F. G.; Galullo, V.; Bellon, S.; Bemis, G.; Cochan, J. *PCT Int. Appl.* WO 99 58502; *Chem. Abstr.* **1999**, *131*, 336949.
9. Preparation of pyrazolyl urea **2**: A mixture of 2,3-dichlorophenylisocyanate (1.9 g) and 2-methyl-3-amino-5-*tert*-butylpyrazole (1.43 g, 9.33 mmol) in dry toluene was refluxed for 18 h, then cooled to room temp., and concentrated. The residue was purified by chromatography on silica gel, eluting with 20–60% EtOAc in hexanes to afford 2.14 g (67%) of urea **2** (white solid, recrystallized from Et_2O/hexanes). ^1H NMR (300 MHz, DMSO-d_6) δ 1.2 (s, 9H), 3.62 (s, 3H), 6.09 (s, 1H), 7.3 (m, 2H), 8.12 (dd, 1H, $J=3$ and 7 Hz), 8.68 (s, 1H), 9.28 (s, 1H); MS (FAB) m/z 341 ($M+H^+$, 100%). Anal. calcd C, 52.80; H, 5.32; N, 16.42. Found: C, 52.88; H, 5.30; N, 16.43.
10. Matti, J.; Ledoux, C.; Kesler, E. *Bull. Soc. Chim. Fr.* **1959**, 477. Chubb, F. L.; Nissenbaum, J. *Can. J. Chem.* **1959**, *37*, 1121.
11. (a) Gilson, Inc., Middleton, Wisconsin, USA. (b) A dry-block heater that holds 100 sample vials and provides orbital shaking was obtained from J-KEM Scientific, Inc., St. Louis, Missouri, USA.
12. General procedure for the parallel synthesis of ureas: Reagent solutions were typically dispensed into reaction vials by using a robotic liquid handler (Gilson 215, custom program). Into each reaction vial was added a stock solution of the amine in anhydrous DMF (0.1 M, 1.0 mL, 0.1 mmol) and a solution of the isocyanate in anhydrous DMF (0.2 M, 0.5 mL, 0.1 mmol). The reaction vial was capped, and the reaction mixture was heated at 80–95 °C with orbital shaking for 18 h. The reaction mixture was cooled to room temperature, and any potential unreacted isocyanate was quenched by the addition of methanol (1.0 mL). After 30 min, solvents were evaporated by using a multiple sample evaporator (Savant SpeedVac). Products were characterized for purity and identity by HPLC/UV/MS. p38 α2 assay and SW 1353 cell assay results reported herein reflect purified (HPLC or LC, >95% purity) and characterized (^1H NMR, MS) samples.
13. SW1353 cells (human chondrosarcoma, ATCC, Bethesda, MD) are seeded (1000 cells/100 μL DMEM 10% FCS/well) into 96-well plates and incubated overnight. After medium replacement, cells are exposed to test compounds for 1 h at 37°C, at which time human IL-1 (1 ng/mL, Endogen, Woburn, WA) and recombinant human TNFα (10 ng/mL) are added. Cultures are incubated for 48 h at 37 °C, then supernatant IL-6 values are determined by ELISA (Endogen, Woburn, WA).
14. Dumas, J.; Khire, U.; Lowinger, T. B.; Paulsen, H.; Riedl, B.; Scott, W. J.; Smith, R. A.; Wood, J. E.; Hatoum-Mokdad, H.; Johnson, J.; Lee, W.; Redman, A. *PCT Int. Appl.* WO 99 32111; *Chem. Abstr.* **1999**, *131*, 87909.