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Pyridazine Based Inhibitors of p38 MAPK

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Abstract—Trisubstituted pyridazines were synthesized and evaluated as in vitro inhibitors of p38 MAPK. The most active isomers were those possessing an aryl group α and a heteroaryl group β relative to the nitrogen atom in the 2-position of the central pyridazine. Additionally, substitution in the 6-position of the central pyridazine with a variety of dialkylamino substituents afforded a set of inhibitors having good (p38 IC_{50} 1–20 nM) in vitro activity.

The discovery of new agents for the treatment of cytokine mediated inflammatory diseases such as rheumatoid arthritis (RA) or Crohn's disease has been the driving force behind our search for novel, selective, small molecule inhibitors of p38 mitogen-activated protein kinase (p38 MAPK).[1] By inhibiting p38 MAPK, the intracellular signaling cascade leading to excessive production of the pro-inflammatory cytokine, tumor necrosis factor α (TNF-α), is suppressed.[2] Reduction of TNF-α levels by treatment with either anti-TNF-α antibodies (Remicade®) or a soluble TNF-α receptor protein (Enbrel®) has proved to be disease modifying in RA therapy.[3]

Many small molecule inhibitors of p38 MAPK have been reported that contain a central trisubstituted π-electron excessive aromatic heterocycle such as pyrazole, pyrrole, furan, or imidazole.[4] Additionally, potent inhibitors of p38 MAPK were developed that were based on the tetrasubstituted imidazole heterocycle, **1** (Fig. 1).[1]

A common feature of tetrasubstituted imidazole inhibitors (**1**, p38 IC_{50} 0.11 nM; **2**, p38 IC_{50} 12,000 nM) is the presence of both pendant aryl and heteroaryl functionalities which are positioned distal to a 4-substituted piperidine. An important element of imidazole containing inhibitors is that the isomer (**1**) having the greatest intrinsic activity is that which has an aromatic ring in the α-position and the attached nitrogen containing aromatic heterocycle located β to the unsubstituted nitrogen in the imidazole ring.[1,5] Furthermore, X-ray crystallography has demonstrated the necessity of a hydrogen bond acceptor on the central ring in order to achieve high affinity for the ATP-binding pocket of p38 MAPK.[6]

Unlike the five-membered π-electron excessive heterocycles, there are limited reports of p38 MAPK inhibitors that possess a π-electron deficient central ring.[7] In an effort to better delineate the nature of small molecule inhibitors of p38 MAPK, pyridazine based inhibitors analogous to the tetrasubstituted imidazole class were prepared (Schemes 1–3). In each case an amine containing substituent was installed distal to the aryl and heteroaryl functionalities. Like the tetrasubstitued imidazoles, the nitrogen atoms of pyridazine based inhibitors possess the ability to function as a hydrogen bond acceptor in the ATP-binding pocket of p38 MAPK.

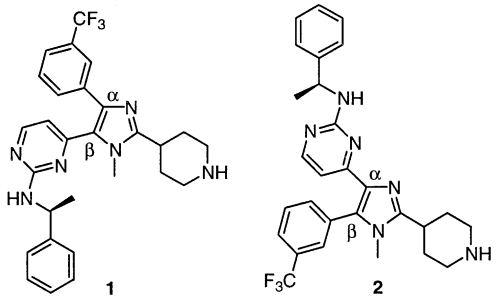

Figure 1.

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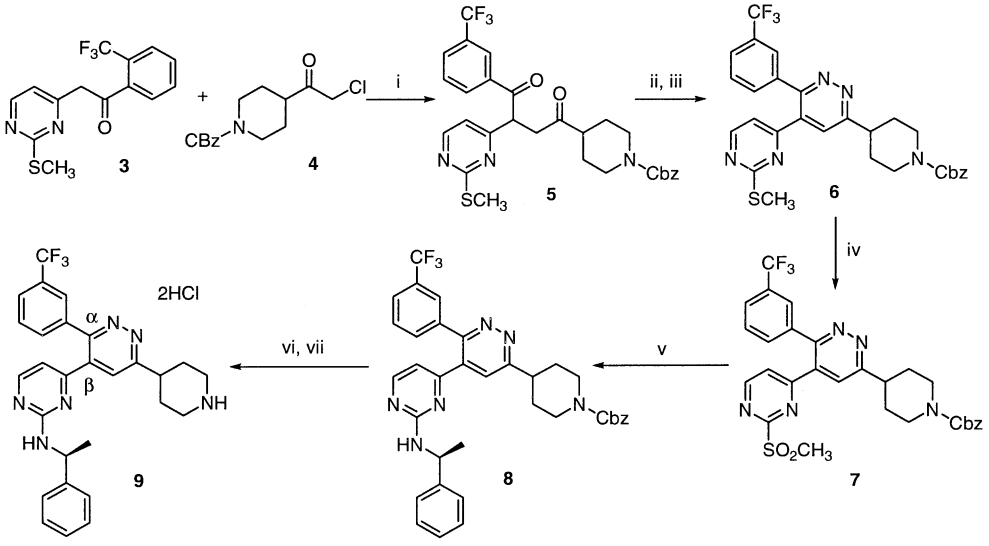
The preparation of the required 3,4,6-trisubstituted pyridazines was accomplished using a classical synthetic approach, which relied on the reaction of hydrazine with either a 1,4-diketone or a 1,4-keto acid.[8] Subsequent aromatization of the heterocycle followed by pendant group modifications gave the compounds desired for testing. Schemes 1–3 summarize three strategies that were used for the preparation of 3,4,6-trisubstituted pyridazine analogues.[9]

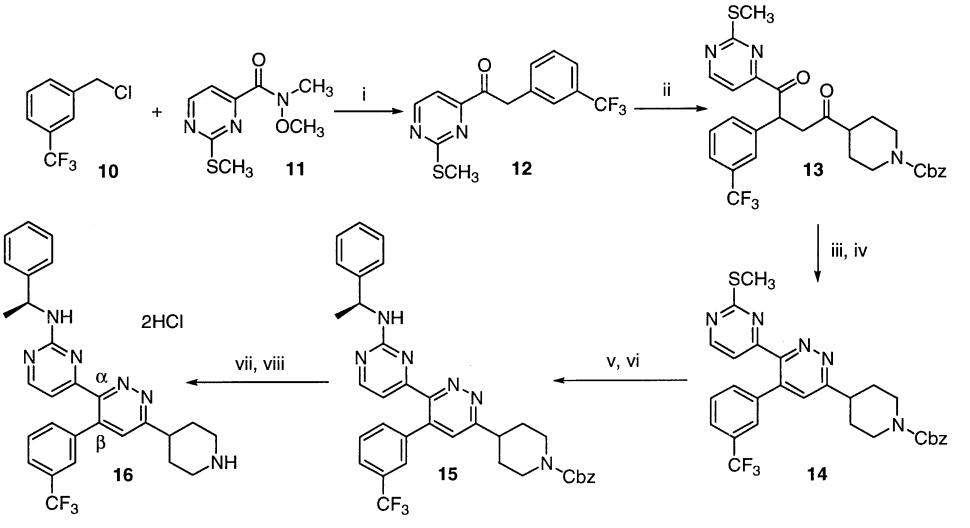
The synthesis of **9**[7c] is illustrated in Scheme 1. Alkylation of the enolate of **3**[1] with the Cbz protected 4-(2-chloroacetyl)piperidine (**4**[7c]) gave the 1,4-diketone, **5**. Cyclization with hydrazine in ethanol gave the 3,4-dihydropyridazine, which was aromatized with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[10] in toluene to give **6**. Selective oxidation of the sulfide (**6**) to the sulfone (**7**) was accomplished by heating at reflux a solution of **6**, 30% hydrogen peroxide, sodium tungstate, methanol and ethyl acetate.[11] Nucleophilic displacement[1] with neat (*S*)-(−)-α-methylbenzylamine gave **8**, which was deprotected using 30% hydrogen bromide in acetic acid to furnish, after conversion to the dihydrochloride salt, the desired pyridazine, **9**.

Scheme 2 shows the preparation of pyridazine **16**,[7c] which is, due to the transposition of the aryl and heteroaryl substituents, isomeric with pyridazine **9**. Intermediate **12** was prepared by reacting the Grignard reagent[12] derived from 3-trifluoromethylbenzyl chloride (**10**) with Weinreb amide **11**. The remaining steps in the synthesis of **16** were carried out in a manner similar to that described for the preparation of **9** (Scheme 1).

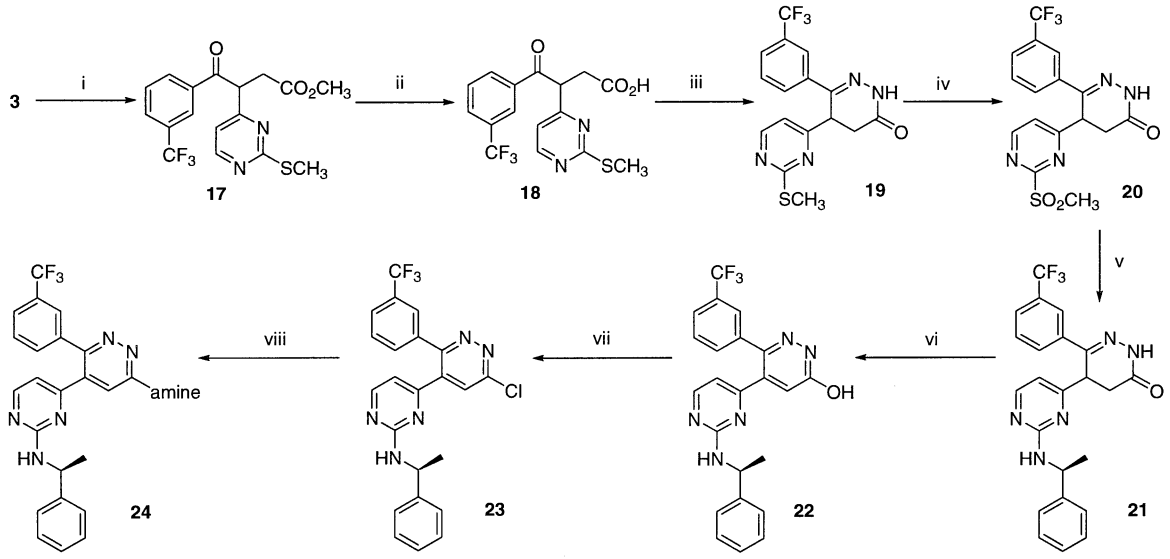
Scheme 3 illustrates a complementary synthetic pathway which provided analogues with an amine directly



Scheme 1. Reagents and conditions: (i) 95% NaH, DMSO, rt, 1 h then **4**, rt, 18 h, 71%; (ii) N_2H_4, EtOH, 24 h, rt; (iii) DDQ, toluene, rt 24 h, 82%; (iv) 30% H_2O_2, $Na_2WO_4{\cdot}2H_2O$, MeOH, EtOAc, reflux, 24 h, 72%; (v) (*S*)-(−)-α-methylbenzylamine, 125 °C, 3 h, 86%; (vi) 30% HBr–HOAc, rt, 1 h, 67%; (vii) HCl–Et_2O (1 N), 0.5 h rt, 41%.



Scheme 2. Reagents and conditions: (i) Mg, Et_2O, I_2 (cat), reflux, 0.5 h, then **11**, reflux, 4 h, 15%; (ii) 95% NaH, DMSO, rt, 0.25 h then **4**, rt, 18 h, 21%; (iii) N_2H_4, EtOH, 5 h, rt; (iv) DDQ, toluene, rt 0.5 h, 81%; (v) 30% H_2O_2, $Na_2WO_4{\cdot}2H_2O$, MeOH, EtOAc, reflux, 18 h, 49%; (vi) (*S*)-(−)-α-methylbenzylamine, 125 °C, 3 h, 94%; (vii) 30% HBr–HOAc, rt, 1 h, 30%; (viii) HCl–Et_2O (1 N), 0.5 h rt, 22%.



Scheme 3. Reagents and conditions: (i) 60% NaH, DMSO, then methyl bromoacetate, rt, 2 h, 86%; (ii) dioxane, 6 N hydrochloric acid, rt, 24 h, 78%; (iii) N_2H_4, EtOH, reflux, 4 h, 41%; (iv) 30% H_2O_2, $Na_2WO_4{\cdot}2H_2O$, MeOH, EtOAc, reflux, 24 h, 92%; (v) (*S*)-(−)-α-methylbenzylamine, reflux, 24 h, 91%; (vi) DDQ, MeCN, rt, 24 h, 92%; (vii) $POCl_3$, 125°C, 24 h, 83%; (viii) amine, 90 °C, 24 h.

attached to the pyridazine heterocycle in the 6-position. Addition of methyl bromoacetate to the previously employed enolate of ketone **3** gave the alkylated product, **17**. Hydrolysis of the ester and cyclization with hydrazine in ethanol provided pyridazinone **19**. Intermediate **22** was subsequently synthesized from **19** using an approach similar to those described in Schemes 1 and 2. Further elaboration to the key chloropyridazine, **23**, was effected using phosphorus oxychloride.[13] Treatment of **23** with primary or secondary amines[14] gave a series of pyridazines possessing a nitrogen-linked substituent in the 6-position.

The trend observed for the tetrasubstituted imidazole series[1] of p38 MAPK inhibitors, regarding the arrangement of substituents around the central heterocycle, was found to be true for the analogous 3,4,6-trisubstituted pyridazines **9** and **16**. In each case a piperidine substituent was positioned distal to the pendant aryl and heteroaryl groups. Similarly, the pyridazine with the aryl ring α and the heteroaryl ring β to the pyridazine nitrogen in the 2-position showed greater in vitro inhibitory activity for p38 MAPK (**9**, p38 IC_{50} 0.15 nM) than the isomeric compound where the aryl and heteroaryl rings were interchanged (**16**, p38 IC_{50} 4.81%@1000 nM).[15] Table 1 summarizes p38 MAPK inhibition data for the two isomeric tetrasubstituted imidazoles and their trisubstituted pyridazine counterparts.

The 3-trifluoromethylphenyl and the 2-substituted pyrimidine substituents were previously optimized in the tetrasubsituted imidazole series of inhibitors. These substituents were found to impart both activity and selectivity for p38 MAPK over other protein kinases such as c-Raf.[1] While retaining these two key elements of the tetrasubstituted imidazoles and using the pyridazine scaffold, we sought a rapid and flexible approach that would allow for the preparation of final products having a variety of nitrogen-linked substituents in the 6-position (see Scheme 3). Table 2 shows a set of secondary amines that were thus installed on the pyridazine framework. This strategy allowed for the quick assessment of the requisite structural and electronic attributes of the pendant group in the 6-position of the central pyridazine.

The introduction of nitrogen-linked amine substituents in the 6-position of the central pyridazine afforded a set of compounds which, like the carbon-linked piperidine, **9**, were potent in vitro inhibitors of p38 MAPK. The maintenance of inhibitory activity in going from a carbon-linked to a nitrogen-linked functionality suggests a

Table 1. Summary of p38 MAPK inhibition data

Compd	p38 IC_{50} (nM)
1	0.11
2	12,000
9	0.15
16	4.81%@1000

Table 2. Amine derivatives of **24**

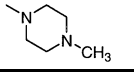
Compd	Amine	p38 IC_{50} (nM)
24a		2.5
24b		1.4
24c		20.1
24d		7.4
24e		2.6
24f		0.6
24g		1.9

Table 3. Kinase selectivity for compound **24e**

Kinase	p38	c-Raf	JNK2α1	JNK2α2	LCK
IC_{50}	2.6 nM	29%@5000 nM	820 nM	1400 nM	22%@15,000 nM

remarkable tolerance in the 6-position for a range of substituents, each possessing distinctive structural and electronic features. One of the first compounds prepared in this series, the morpholine derivative **24e**, was tested to evaluate its selectivity for p38 MAPK over several other protein kinases. These results are illustrated in Table 3.[16]

In addition to the kinase selectivity experiments, **24e** was assessed in two cell-based assays of TNF-α release. A substantial decrease in activity (human monocyte[17] IC_{50} 292 nM, human whole blood[1] IC_{50} 1200 nM) versus the value for p38 MAPK inhibition (IC_{50} 2.6 nM) was observed which suggests this pyridazine derivative was not effectively penetrating cells.

In summary, although **24e** showed favorable in vitro potency and selectivity versus p38 MAPK, additional SAR studies directed towards increasing activity in the cell-based assays would be required before a trisubstituted pyridazine could advance as a small molecule drug candidate for intervention in TNF-α mediated disorders.

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References and Notes

1. Liverton, N. J.; Butcher, J. W.; Claiborne, C. F.; Claremon, D. A.; Libby, B. E.; Nguyen, K. T.; Pitzenberger, S. M.; Selnick, H. G.; Smith, G. R.; Tebben, A.; Vacca, J. P.; Varga, S. L.; Agarwal, L.; Dancheck, K.; Forsyth, A. J.; Fletcher, D. S.; Frantz, B.; Hanlon, W. A.; Harper, C. F.; Hofsess, S. J.; Kostura, M.; Lin, J.; Luell, S.; O'Neill, E. A.; Orevillo, C. J.; Pang, M.; Parsons, J.; Rolando, A.; Sahly, Y.; Visco, D. M.; O'Keefe, S. J. *J. Med. Chem.* **1999**, *42*, 2180.
2. (a) Lee, J. C.; Laydon, J. T.; Mcdonnell, P. C.; Gallagher, T. F.; Kimar, 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.; White, J. R.; Adams, J. L.; Young, P. R. *Nature* **1994**, *372*, 739. (b) Lee, J. C.; Young, P. R. *J. Leuk. Bio.* **1996**, *59*, 152. (c) Wang, H.; Tracey, K. J. In *Inflammation: Basic Principles and Clinical Correlates*, 3rd ed.; Gallin, J. I., Snyderman, R., Eds.; Lippincott, Williams & Wilkins: Philadelphia, 1999; p 471.
3. (a) *Physicians Desk Reference*, 54th ed.; Medical Economics: Montvale, 2000; pp 927 and 1413. (b) Seymour, H. E.; Worsley, A.; Smith, J. M.; Thomas, S. H. L. *Br. J. Clin. Pharmacol.* **2001**, *51*, 201. (c) Keystone, E. C. *Rheu. Dis. Clin. of North Am.* **2001**, *27*, 427.
4. For review see: Boehm, J. C.; Adams, J. L. *Exp. Opin. Ther. Pat.* **2000**, *10*, 25.
5. Owens, R. J.; Lumb, S. In *Novel Cytokine Inhibitors*; Higgs, G. A., Henderson, B., Eds.; Birkhäuser: Basel, 2000; p 201.
6. (a) Lisnock, J. M.; 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. (b) Lee, J. C.; Kassis, S.; Kumar, S.; Badger, A.; Adams, J. L. *Pharmacol. Ther.* **1999**, *82*, 389.
7. (a) Mantlo, N. B.; Hwang, C. K.; Spohr, U. D. WO9932448; *Chem. Abstr.* **1999**, *131*, 58754. (b) Adams, J. L.; Boehm, J. C.; Hall, R. WO0025791; *Chem. Abstr.* **2000**, *132*, 334471. (c) Claremon, D. A.; Ponticello, G. S. WO0031065; *Chem. Abstr.* **2000**, *133*, 4673. (d) Adams, J. L.; Boehm, J. C. WO0040243; *Chem. Abstr.* **2000**, *133*, 89539.
8. Tišler, M.; Stanovnik, B. In *Comprehensive Heterocyclic Chemistry*; Katritzky, A. R., Rees, C. W., Eds.; Pergamon: Oxford, 1984; Vol. 3, p 45.
9. All final products gave satisfactory ^{1}H NMR, MS, and CHN analysis. Representative data follows for **9** and **24e**. For **9**·2HCl·0.95H_2O: ^{1}H NMR (300 MHz, CD_3OD) δ 7.86 (d, br, 1H), 7.86–7.70 (m, 3H), 7.64–7.52 (m, 3H), 7.36–7.22 (m, 5H), 4.80 (s, br, 1H), 3.66–3.56 (m, 2H), 3.48–3.38 (m, 1H), 3.32–3.18 (m, 2H), 2.38–2.25 (m, 4H), 1.46 (d, br, 3H); MS *m*/*z* 505.25 (MH^+). Anal. calcd for $C_{28}H_{27}F_3N_6$·2HCl·0.95H_2O: C, 56.56; H, 5.24; N, 14.13. Found: C, 56.61; H, 5.27; N, 14.07. For **24e**·HCl·1.55H_2O: ^{1}H NMR (500 MHz, CD_3OD) δ 8.40 (s, br, 1H), 8.06 (s, br, 1H), 7.80 (d, J = 7 Hz, 1H), 7.76 (s, 1H), 7.64–7.57 (m, 2H), 7.30–7.24 (m, 2H), 7.23–7.16 (m, 3H), 6.95 (s, br, 1H), 4.50 (s, br, 1H), 3.90 (m, 4H), 3.83 (m, 4H), 1.35 (s, br, 3H); MS *m*/*z* 507.27 (MH^+). Anal. calcd for $C_{27}H_{25}F_3N_6O$·HCl·1.55H_2O: C, 56.80; H, 5.14; N, 14.72. Found: C, 56.82; H, 4.76; N, 14.54.
10. Buckle, D. R. In *Encyclopedia of Reagents for Organic Synthesis*; Paquette, L. A., Ed.; John Wiley and Sons: Chichester, 1995; Vol. 3, p 1699.
11. Blacklock, T. J.; Sohar, P.; Butcher, J. W.; Lamanec, T.; Grabowski, E. J. J. *J. Org. Chem.* **1993**, *58*, 1672.
12. Schön, U.; Kehrbach, W.; Hachmeister, B.; Buschmann, G.; Kühl, U. G. *U.S.* 4,755,520; *Chem. Abstr.* **1987**, *106*, 156761.
13. Aldous, D. L.; Castle, R. N. In *Pyridazines*; Castle, R. N., Ed.; John Wiley and Sons: New York, 1973; p 221.
14. Nagagome, T. In *Pyridazines*; Castle, R. N., Ed.; John Wiley and Sons: New York, 1973; p 468.
15. The inhibition of p38 kinase was determined according to a published method[18] using an enzyme concentration of 0.5 nM.
16. All enzyme inhibition assays were carried out using methods described in ref 1 and the references cited therein.
17. Chin, J.; Kostura, M. J. *J. Immunol.* **1993**, *151*, 5574.
18. LoGrasso, P. V.; Frantz, B.; Rolando, A. M.; O'Keefe, S. J.; Hermes, J. D.; O'Neill, E. A. *Biochemistry* **1997**, *36*, 10422.