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Imidazopyrimidines, Potent Inhibitors of p38 MAP Kinase

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Abstract—The MAP kinase p38 is implicated in the release of the pro-inflammatory cytokines TNF-α and IL-1β. Inhibition of cytokine release may be a useful treatment for inflammatory conditions such as rheumatoid arthritis and Crohn's disease. A novel series of imidazopyrimidines have been discovered that potently inhibit p38 and suppress the production of TNF-α in vivo.

The pro-inflammatory cytokines TNF-α and IL-1β play important roles in chronic inflammatory diseases such as rheumatoid arthritis, Crohn's disease, and psoriasis.[1] Enzymatic activity of the mitogen-activated protein (MAP) kinase p38 is required for the release of TNF-α and IL-1β from monocytes and is also necessary for signal transduction through the cell-surface receptors for TNF-α and IL-1β.[2] Therefore it is not surprising that inhibitors of p38 (such as the prototypical SB 203580 (**1**)) are effective in in vivo models of inflammatory conditions.[3]

We have previously disclosed several different structural classes of p38 inhibitors having improved in vitro and in vivo activity such as the pyrrolopyridine **2**[4] and the pyrrolobenzimidazole **3**[5] (Fig. 1). In our ongoing effort to discover novel, potent inhibitors of p38, we now report the discovery and development of a series of imidazopyrimidines showing excellent in vitro and in vivo potency. Our research approach was to develop novel heterocyclic scaffolds that maintain the key binding elements of known inhibitors such as the 4-pyridyl ring and the 4-fluorophenyl ring in the appropriate spatial relationship. In development of the next generation series of inhibitors related to **2**, we synthesized the imidazopyrimidine **4**.[6] Our screening regime consisted of a p38α enzyme inhibition assay,[7] LPS induced TNF-α inhibition in human peripheral blood mononuclear cells,[4a] and LPS induced TNF-α inhibition in mice.[4a] While compound **4** had somewhat modest activity in the in vitro assays, it showed excellent potency in the in vivo mouse model and compared more favorably in vivo with **2** and **3** (Table 1).

Given this data, a research program was launched to optimize this chemical series for both in vitro potency and in vivo efficacy.

The synthesis of **4** is straight forward (Scheme 1). Deprotonation of 4-picoline (**5**) and addition of the resulting anion to ethyl 4-fluorobenzoate (**6**) produces the ketone **7**. Bromination of **7** occurs readily to afford the α-bromoketone **8**. Treatment of **8** with excess 2,4-diaminopyrimidine (**9**) produces **4** regiospecifically.

It has been shown that incorporating substitution on the 4-pyridyl ring can improve potency.[8] The 4-pyridyl ring of **4** lent itself to substitution (Scheme 2).

Commercially available 2-bromo-4-methylpyridine (**10**) underwent palladium catalyzed coupling with amines to cleanly afford the 2-substituted-4-methyl pyridines **11**. It was necessary to protect the nitrogen of **11** if primary amines were used in the palladium amination. Deprotonation of **11** and reaction of the resulting anion with ethyl benzoates (**12**) gave the corresponding ketones. Deprotection and bromination gave the α bromoketones **13**. Cyclization of **13** with excess 2,4-diaminopyrimidine **9** gave the desired substituted pyridine analogues **14a–d**.

The SAR of the substituted pyridine analogues is outlined in Table 2. Introduction of the benzylamine substituent

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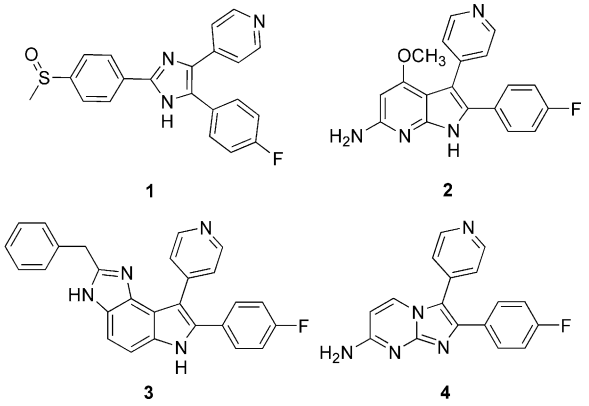


Figure 1. p38 Kinase inhibitors.

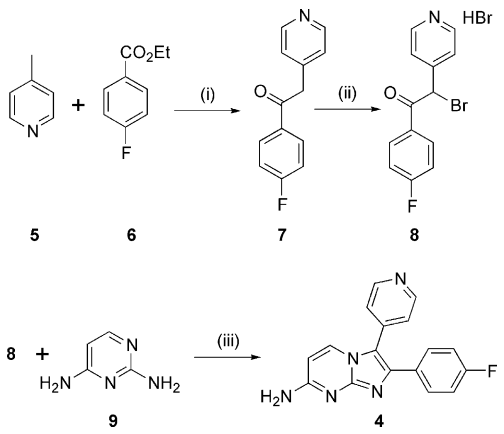
Table 1. Data comparison of **2**–**4**

Compd	p38α Enzyme IC_{50}	TNF-α Inhibition (Cells) IC_{50}	TNF-α Inhibition (Mouse)% Inhibition 10 mg/kg
2	1.5 μM	7 nM	75
3	22 nM	60 nM	70
4	570 nM	40 nM	97

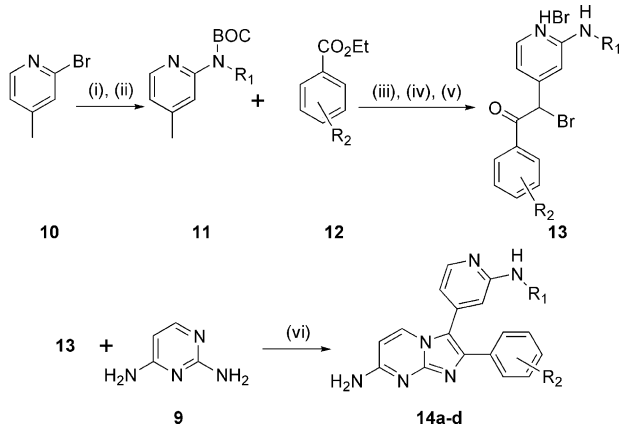
14a provided a profound increase in p38α enzyme activity as well as cellular inhibition of TNF-α compared to **4**. Compound **14a** inhibited p38α with an IC_{50} of 6 nM versus 570 nM for **4**, and had an IC_{50} of 6 nM in the cellular assay versus 40 nM for **4**. We postulate that this increase in potency may result from both a lipophilic interaction of the benzyl group with the enzyme as well as an additional hydrogen bonding interaction from the amino group. Further study would be required to verify this however. Surprisingly **14a** was less potent in vivo than **4**, 42% inhibition versus 97% inhibition at 10 mg/kg. This reduction in activity could be due to unfavorable metabolism at the *N*-benzyl group. Attempts to block metabolism by introduction of a *N*-α-methylbenzyl group possessing *S* stereochemistry gave a further increase in potency over **14a**. Compound **14b** had an IC_{50} of 1 nM in the p38α enzyme assay versus 6 nM for **14a**, as well as increased potency in the cellular assay 2 nM versus 6 nM. A much larger increase in potency was observed in whole animals. Compound **14b** inhibited TNF-α production by 96%; similar to the potency originally observed for **4**.

It was not necessary to incorporate substitution on the aryl ring adjacent to the substituted pyridine. Compound **14c** bearing an unsubstituted phenyl ring and (*S*)-1-methoxy-2-propylamine substitution on the pyridine ring was a potent inhibitor of the enzyme, as well as in the cellular and in vivo assays. Incorporation of the chiral methyl group into a ring as demonstrated in **14d** did not affect the potency in the enzyme and cellular assays, but had a profound effect on in vivo potency, completely failing to inhibit TNF-α production in mice.

Since replacement of the pyridine of **4** with substituted pyridines gave increases in vitro and in vivo potency,



Scheme 1. Synthesis of **4**. (i) NaHMDS, THF, 100%; (ii) Br_2, HBr, AcOH, 97%; (iii) ethanol, reflux, 33%.



Scheme 2. Synthesis of substituted pyridine analogues. (i) $Pd_2(dba)_3$, H_2NR_1, BINAP, 50–75%; (ii) $(BOC)_2O$, *t*BuONa, *t*BuOH, 50–75%; (iii) NaHMDS, THF, 75–100%; (iv) HCl, THF, 90%; (v) Br_2, HBr, AcOH, 70–85%; (vi) ethanol, reflux, 20–30%.

our next goal was to elaborate the series further by replacing the substituted pyridines with a series of substituted pyrimidines. Positive effects with this type of structural modification have been seen with other inhibitors.[8,9] A similar synthesis was developed beginning with 2-mercapto-4-methyl pyrimidine (**15**) (Scheme 3). Alkylation of **15** with iodomethane gave 4-methyl-2-(thiomethyl)pyrimidine (**16**). Deprotonation and reaction of the anion of **16** with ethyl benzoate gave the ketone **17**. Bromination of **17** occurred smoothly to give the α-bromoketone **18**. Cyclization of **18** with 2,4-diaminopyrimidine **9** afforded the thiomethylpyrimidine analogue **19**.

Thiomethylpyrimidine **19** proved to be a useful intermediate. The thiomethyl substituent of **19** could be removed reductively with Raney nickel to give the unsubstituted pyrimidine **20** or oxidized with oxone to the methylsulfone pyrimidine **21**. The sulfone of **21** could then be displaced by both oxygen and nitrogen nucleophiles to give compounds **22**–**4** (Scheme 4).

Table 2. SAR of substituted pyridine analogues

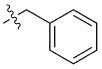
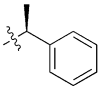
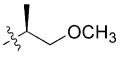
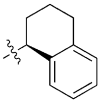
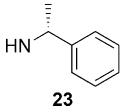
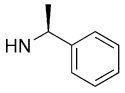
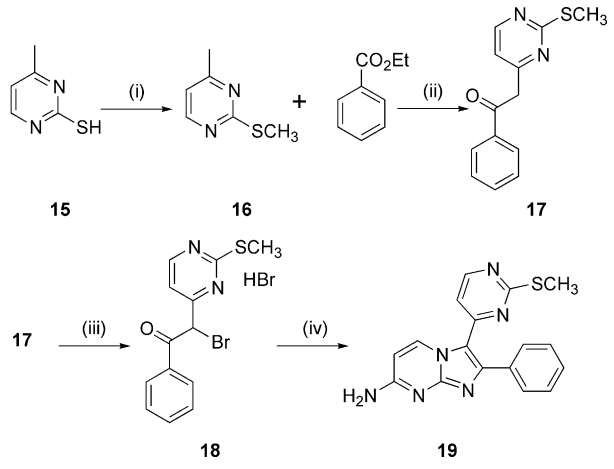
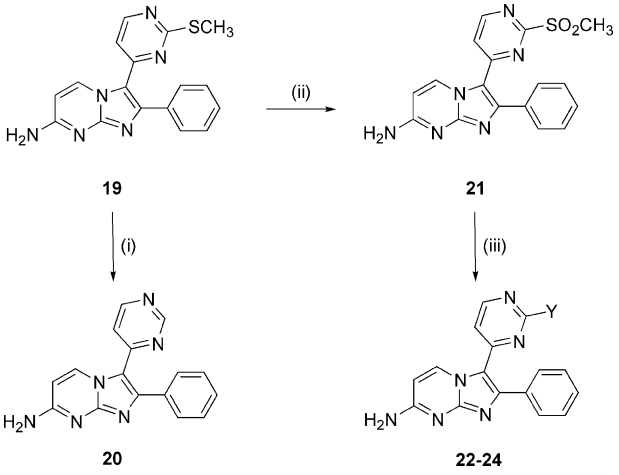
R_1	R_2	p38α Enzyme IC_{50} (nM)	TNF-α (cells) IC_{50} (nM)	TNF-α (Mice) % Inhib. 10 mg/kg
 **14a**	4-F	6	6	42
 **14b**	4-F	1	2	96
 **14c**	H	2	0.5	93
 **14d**	H	8	3	Inactive

Table 3. SAR of substituted pyrimidine analogues

Y	p38α Enzyme IC_{50} (nM)	TNF-α (cells) IC_{50} (nM)	TNF-α (Mice)% inhib. 10 mg/kg
H **20**	990	304	73
SCH_3 **19**	457	28	23
OCH_3 **22**	210	90	87
 **23**	37	2.3	66
 **24**	8	0.6	100



Scheme 3. Synthesis of substituted pyrimidine analogues. (i) NaOH, CH_3I, H_2O, 80%; (ii) NaHMDS, THF, 90%; (iii) Br_2, HBr, AcOH, 80%; (iv) ethanol, reflux, 30%.



Scheme 4. Synthesis of substituted pyrimidine analogues. (i) Raney Ni, EtOH, H_2O, 44%; (ii) Oxone, MeOH, H_2O, 70%; (iii) Y = N,O nucleophiles, heat, 40–60%.

The data for the pyrimidine analogues is shown in Table 3. The unsubstituted pyrimidine analogue **20** was substantially weaker than **4** in the enzyme and cellular assays, having an IC_{50} of 990 nM versus 570 nM in the enzyme assay and 304 nM versus 40 nM in the cellular assay. Pyrimidine **20** was also slightly weaker in vivo, inhibiting TNF-α production in mice by 73% as compared to 97% for **4**. The intermediate **19** also displayed activity, but only weakly inhibited TNF-α production in vivo by 23%. The oxygen analogue **22** was slightly more potent than **19** in the enzyme assay but significantly more potent in vivo inhibiting TNF-α production by 87%. Compound **23** containing the (*R*)-α-methylbenzylamino group showed a further increase in enzyme and cellular activities 37 nM and 2.3 nM respectively, but a slight decrease in whole animal activity to 66% inhibition. The enantiomer of **23**, compound **24** resulting from the reaction of **21** with (*S*)-α-methylbenzylamine proved to be the most potent of the substituted pyrimidine analogues both in vitro and in vivo.

Pyrimidine **24** inhibited p38α with and IC_{50} of 8 nM, and inhibited TNF-α production in cells with an IC_{50} of 0.6 nM. Compound **24** was also extremely potent in vivo, inhibiting TNF-α production in mice completely when administered orally at 10 mg/kg. Since the (*S*)-α-methylbenzylamine substituted pyridine **14b**, and pyrimidine **24**, were both extremely potent in vivo, the administered doses were lowered to achieve differentiation between the two compounds. Both compounds were dosed orally to mice at 2 mg/kg. Pyridine **14b** inhibited TNF-α production by 34% while pyrimidine **24** was still extremely effective, inhibiting by 83%. This data suggests that the substituted pyrimidine series of imidazopyrimidines has greater in vivo efficacy than that of the substituted pyridine analogues (Table 4).

In summary, a new series of potent p38 kinase inhibitors having excellent enzymatic, cellular, and in vivo activities has been developed. The synthesis is brief and scalable, allowing for a variety of analogues to be prepared.

Table 4. Comparison of **14b** and **24**

Analogue	p38α Enzyme IC_{50} (nM)	TNF-α (cells) IC_{50} (nM)	TNF-α (Mice)% Inhib. 10 mg/kg	TNF-α (Mice)% Inhib. 2 mg/kg
14b	1	2	96	34
24	8	0.6	100	83

Replacement of an unsubstituted pyridine ring with a substituted pyridine or pyrimidine ring provided inhibitors having increased in vitro potency while maintaining excellent potency in whole animals. The most potent substituted pyridine analogue, **14b**, inhibited p38α with an IC_{50} of 1 nM and nearly completely inhibited TNF-α production in mice at 10 mg/kg,while pyrimidine **24** inhibited p38α with an IC_{50} of 8 nM and completely inhibited TNF-α production in mice at 10 mg/kg. This compound remained effective at even lower doses, inhibiting TNF-α production in vivo by 83% at 2 mg/kg. The excellent in vitro and in vivo potency of this series warrants further investigation in more advanced models of inflammatory disease.

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7. Recombinant, activated, 6×His-tagged mouse p38α enzyme was purified from osmotically shocked Drosophila S2 cells in our laboratory, using a p38α clone generously provided by Dr. Richard Ulevitch, Scripps Research Institute, La Jolla, CA. p38 was incubated in kinase reaction buffer (25 mM HEPES pH 7.5, 10 mM $MgCl_2$, 10 mM $MnCl_2$) containing 25 μM ATP, with 60 μg myelin basic protein (MBP) as substrate (Life Technologies, Gaithersburg, MD) and 1μCi γ-^{33}P-ATP (3000 Ci/mmol, Amersham Life Science, Arlington Heights, IL), with or without test compounds or vehicle (DMSO, 2% final concentration), in a total volume of 60 μL, in a round-bottom polypropylene 96-well plate. After 30 min at 30 °C, reactions were stopped and proteins precipitated by the addition of 60 μL/well of 50% trichloroacetic acid (TCA), and the precipitates transferred to a 96-well Durapore membrane filterplate (Millipore, Bedford, MA). Wells were filtered using a Millipore vacuum manifold, washed 5× with 200 μL/well of 10% TCA/10 mM sodium phosphate, and briefly air-dried. Thirty μL/well of Microscint-20 scintillant (Packard, Meriden, CT) was added, the plate sealed with plastic film (Packard), and counted in a Packard TopCount microplate scintillation counter.
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