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p38 MAP kinase inhibitors: Metabolically stabilized piperidine-substituted quinolinones and naphthyridinones

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Abstract—Quinolinones and naphthyridinones with C7 *N*-*t*-butyl piperidine substituents were found to be potent p38 MAP kinase inhibitors. These compounds significantly suppress TNF-α release in both cellular and LPS-stimulated whole blood assays. They also displayed excellent PK profiles across three animal species. Quinolinone **4f** at 10 mpk showed comparable oral efficacy to that of dexamethasone at 1 mpk in a murine collagen-induced arthritis model.

The p38 mitogen-activated protein (MAP) kinase plays a key role in the release of proinflammatory cytokines TNF-α and IL-1β from monocytes.[1] An excess level of these cytokines is associated with a number of inflammatory diseases, including rheumatoid arthritis (RA). In the last decade, considerable efforts have been devoted to discovering potent and selective p38 inhibitors as potential therapy for treatment of RA.[2–5]

We have recently reported the discovery of a series of quinazolinones (e.g., **1a**–**c**), quinolinones (e.g., **2a**), and naphthyridinones (e.g., **2b** and **3**) as potent p38 MAP kinase inhibitors (Fig. 1).[6–8] These compounds displayed excellent selectivity for inhibition of p38 over other kinases. Quinazolinone **1a** was rapidly metabolized, but increasing the steric bulkiness of the R group, as in **1b** and **1c**, markedly improved oral bioavailability in the

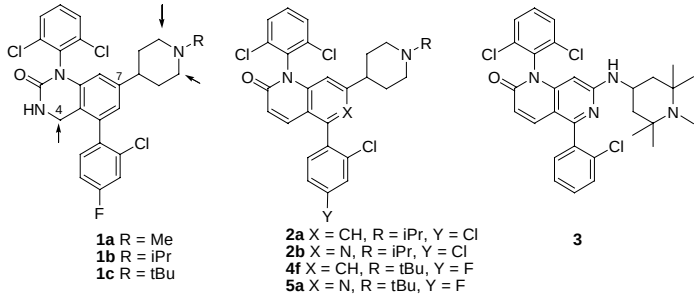

Figure 1. Inhibitors of p38 activity.

Keywords: P38; Kinase; Inflammatory disease; Rheumatoid arthritis; Quinolinone; Naphthyridinone.
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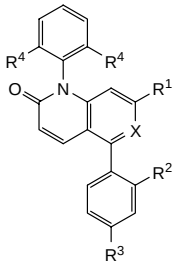
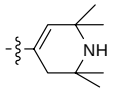
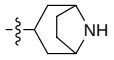
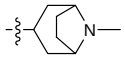
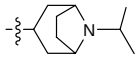
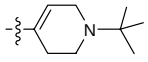
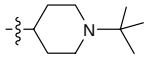
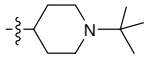
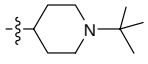
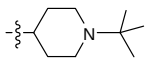
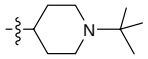
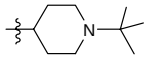
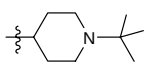
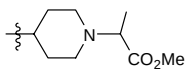
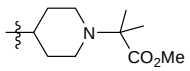
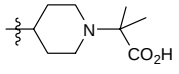
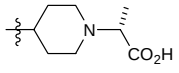
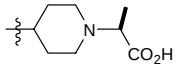
Table 1. Activities and PK profiles of p38 inhibitors in rats

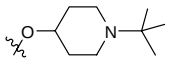
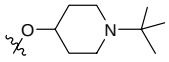
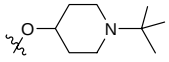
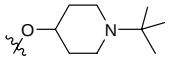


4a-4i, 6b-6g, 7c -7f, X = CH
5a-5c, X = N

Compound	R^1	R^2	R^3	R^4	P38α IC_{50} (nM)	TNF-α cell[a] IC_{50} (nM)	TNF-α WB IC_{50} (nM)	$t_{1/2}$ (h)(iv)	Vd (L/kg)	nAUC(PO) (μMh/mg/kg)	Clp (mL/min/kg)	*F* (%)
4a		Cl	F	Cl	0.26	3.32	24.2	3.17	7.32	0.37	30.1	36
4b		Cl	F	Cl	0.45	0.89	14.5	2.56	6.20	0.11	37	13
4c		Cl	F	Cl	0.61	0.82	7.71	3.12	6.87	0.34	27	30
4d		Cl	F	Cl	0.51	0.99	8.51	3.36	5.23	0.71	18	44
4e		Cl	F	Cl	1.15	0.74	50.8	—	—	—	—	—
4f		Cl	F	Cl	0.31	0.27	2.6	3.5	3.84	1.02	14	47.7
4g		F	F	Cl	0.14	1.7	2.4	4.13	3.82	2.02	11.9	69.3
4h		Cl	H	Cl	0.1	1.0	6.4	—	—	—	—	—
4i		Cl	F	F	0.3	0.42	1.0	9.96	3.77	1.01	18.5	57
5a		Cl	F	Cl	0.38	1.6	5.3	3.69	11.4	0.51	39.6	67.3
5b		F	F	Cl	0.51	0.69	5.2	9.05	24	0.59	32.3	62
5c		Cl	H	Cl	1.4	—	6.8	5.1	10.1	0.83	25	66
6b		Cl	F	Cl	8.1	1.3	28	—	—	—	—	—
6d		Cl	F	Cl	6.7	2.59	130	—	—	—	—	—
6e		Cl	F	Cl	0.84	3.75	79.1	—	—	—	—	—
6f		Cl	F	Cl	3.2	3.8	25	—	—	—	—	—
6g		Cl	F	Cl	1.3	3.2	28	0.44	0.70	0.001	59	0.11

(*continued on next page*)

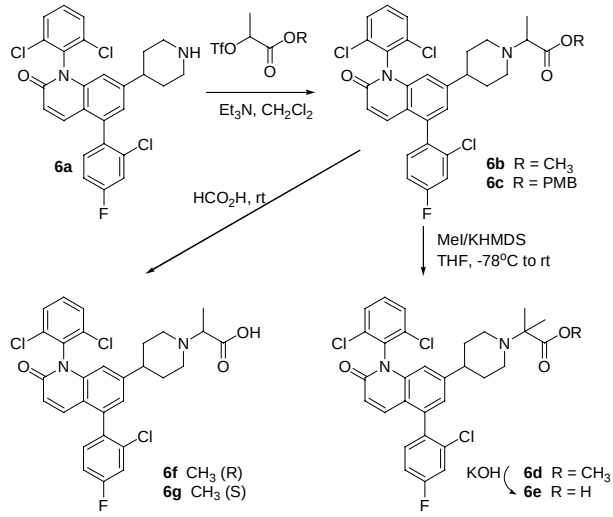
Table 1 (*continued*)

Compound	R^1	R^2	R^3	R^4	P38α IC_{50} (nM)	TNF-α cell[a] IC_{50} (nM)	TNF-α WB IC_{50} (nM)	$t_{1/2}$ (h)(iv)	Vd (L/kg)	nAUC(PO) (μMh/mg/kg)	Clp (mL/min/kg)	*F* (%)
7c		Cl	F	Cl	7.1	2.4	16.2	—	—	—	—	—
7d		F	F	F	10.5	2.2	18.4	—	—	—	—	—
7e		Cl	H	Cl	1.6	1.8	13	2.33	6.17	0.29	34.1	32.3
7f		F	F	Cl	1.87	0.77	—	—	—	—	—	—

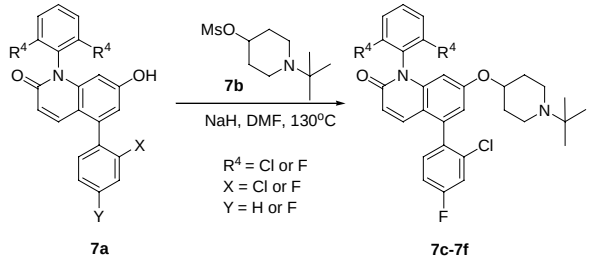
[a] THP-1 cells.

rat.[7] Unfortunately, clearance of these quinazolinones remained high, and PK profiles were poor in other animal species. Metabolic studies revealed the benzylic C4 and the two positions α to the piperidine nitrogen as metabolic hot spots. Replacement of the quinazolinone core with quinolinone and naphthyridinone structures eliminated the lability at the C4 position.[8] However, limited success was reported in addressing the metabolic stability at the positions α to the piperidine nitrogen. Compound **3**, with an α,α′-stabilized piperidine, had a satisfactory PK profile in rats, but this profile did not translate to other animal species. The key pathways for metabolic degradation of **3** were shown to be related to N-demethylation.[8] Consequently, we have prepared a series of quinolinones and naphthyridinones with stabilized C7 piperidine substituents. The *t*-Bu piperidines (e.g., **4f** and **5a**) are particularly potent inhibitors of p38 activity and TNF-α release with excellent oral bioavailability in rat, dog, and rhesus monkey. Remarkably, in a accelerated murine collagen-induced arthritis (CIA) model, compound **4f** proved comparable to dexamethasone, the 'gold standard' for arthritis studies.

Compounds **4a**–**4i** and **5a**–**5c** (Table 1) were prepared according to methods reported in previous papers.[6–8] Compounds **6b**–**6g** were prepared according to Scheme 1. Alkylation of the C7 piperidine **6a** with the properly protected triflate afforded methyl ester **6b** or PMB ester **6c**. Compound **6b** was alkylated with iodomethane to give **6d**, which upon hydrolysis furnished zwitterion **6e**. The PMB-protecting group of **6c** was removed with formic acid to afford optically active compounds **6f** and **6g** (derived from the appropriate optically active lactate esters).



Scheme 1. Preparation of esters and zwitterions **6b**–**6g**.



Scheme 2. Preparation of aryl ethers **7c**–**7f**.

Aryl ethers **7c**–**7f** (Table 1) were prepared from phenol **7a** and mesylate **7b** (Scheme 2). The yields were generally low (below 20%).

Metabolic studies indicated that one of the major pathways for clearance of quinolinones and naphthyridinones with C7 piperidine substituents proceeds through oxidation of the α carbon that attached to the piperidine N. Subsequent decomposition of the resulting carbinolamine generates dealkylated metabolite. We hoped that the tetramethyl piperidine derived from reduction of **4a** would completely eliminate this pathway; however, attempts to reduce the double bond in the dihydropiperidine ring were not successful, presumably due to the steric bulkiness of the tetramethyl substituents. Compared to compound **3** (Fig. 1), **4a** shows high clearance and moderate oral bioavailability in rats.

Tropanes were introduced to replace the piperidine at C7, with the expectation that both the bulkiness and the rigidity of the tropane system would impede rapid

Table 2. Dog and Rhesus PK profiles of selected p38 inhibitors

Compound	Dog					Monkey				
	$t_{1/2}$ (h)(iv)	Vd (L/kg)	nAUC(PO) (μM h/mg/kg)	Clp (mL/min/kg)	*F* (%)	$t_{1/2}$ (h)(iv)	Vd (L/kg)	nAUC(PO) (μM h/mg/kg)	Clp (mL/min/kg)	*F* (%)
4f	5.59	23.80	0.99	26.75	83	13.60	52.80	0.28	52.85	49.0
4g	19.77	8.99	2.29	5.50	36.2	9.7	26.25	0.60	29.25	56.5
4i	—	—	—	—	—	9.55	28.65	0.29	35.15	31.50
5a	—	—	—	—	—	5.32	22.10	0.21	47.50	33.0
5b	15.55	23.75	1.35	20.50	99.3	6.5	21.65	0.16	41.45	23.00
5c	5.42	15.10	0.078	37.60	9.35	—	—	—	—	—

metabolism. Tropanes **4b**–**4d** indeed proved to be potent inhibitors of p38 activity and TNF-α release, and the data in Table 1 show a clear trend in improvement of both clearance and oral bioavailability with increasing steric bulkiness at the tropane nitrogen. Unfortunately, the PK profiles of these compounds clearly show that the rigid tropane bridge does not completely prevent metabolism at the carbons α to nitrogen.

Methyl esters and zwitterions **6b**–**6g** (Table 1) displayed reduced inhibitory activity in both the p38 binding assay and TNF-α release assay in whole blood (compared to the analogous basic compound **4f**). In addition, an unsatisfactory PK profile for compound **6g** prevented further consideration of these zwitterion compounds. Aryl ethers **7c**–**7f** were generally less potent inhibitors of p38 activity and TNF-α release (particularly in whole blood assays) than the corresponding carbon-linked analogues **4f**–**4i**. Compound **7e** showed a moderate PK profile in rats.

Compounds **4f**–**4i** and **5a**–**5c**, with *t*-Bu piperidine substituents at C7 of the quinolinone and naphthyridinone cores, respectively, proved to be our most potent p38 inhibitors. Their potencies for inhibition of TNF-α release in human whole blood are especially outstanding. The quinolinones proved slightly more potent than the corresponding naphthyridinones in the p38α binding assay and in the whole blood assay. Among the quinolinones, compound **4i**, which has a 2,6-difluorophenyl moiety at N1, is the most potent inhibitor in our human whole blood assay.

As expected from our previous results with α,α′-stabilized piperidines,[8] quinolinones **4f**–**4i** and naphthyridinones **5a**–**5c** generally have excellent PK properties in the rat. Differences in the rates of clearance among these *t*-Bu piperidine analogues are consistent with our metabolism studies, indicating secondary metabolism at the aryl rings. Unlike our previous results, these *t*-Bu-stabilized piperidines also have excellent PK profiles in the dog and the rhesus monkey (Table 2).

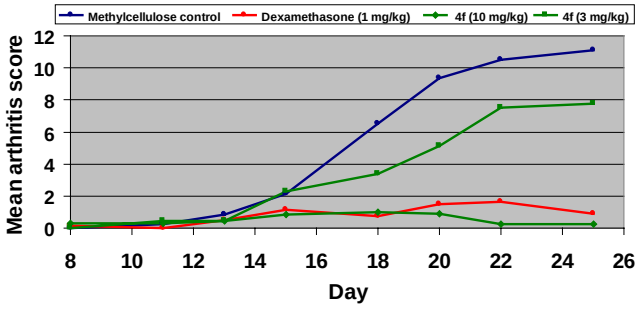


Figure 2. Clinical arthritis scores of orally administered **4f** and dexamethasone in a Murine CIA Model.

The murine collagen-induced arthritis (CIA) model is widely used as a model of human rheumatoid arthritis. To evaluate the in vivo efficacy of our quinolinone and naphthyridinone p38 inhibitors against RA, quinolinone **4f** was tested in an accelerated murine CIA model (Fig. 2).[9] When administered orally at 10 mpk, compound **4f** is at least equal to, if not superior to, dexamethasone at 1 mpk in this arthritis model. These data clearly demonstrate that our p38 inhibitors may offer a promising way to stop the progression of rheumatoid arthritis.

In summary, quinolinones and naphthyridinones with C7 *t*-Bu piperidine substituents proved to be potent p38 MAP kinase inhibitors. They have outstanding activity against the release of TNF-α in both cellular and human whole blood assays. These compounds also have excellent PK profiles in the rat, dog, and rhesus monkey. Quinolinone analogue **4f** was orally tested in the murine CIA model and demonstrated excellent efficacy, comparable to that of dexamethasone, the 'gold standard' for arthritis studies.

References and notes

1. Chen, Z.; Gibson, T. B.; Robinson, F.; Silvestro, L.; Pearson, G.; Xu, B.; Wright, A.; Vanderbilt, C.; Cobb, M. H. *Chem. Rev.* **2001**, *101*, 2449.
2. Adams, J. L.; Boehm, J. C.; Gallagher, T. F.; Kassis, S.; Webb, E. F.; Hall, R.; Sorenson, M.; Garigipati, R.; Griswold, D. E.; Lee, J. C. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2867.
3. Collis, A. J.; Foster, M. L.; Halley, F.; Maslen, C.; McLay, I. M.; Page, K. M.; Redford, E. J.; Souness, J. E.; Wilsher, N. E. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 693.
4. Lee, L. C.; Laydon, J. T.; Mcdonnell, P. C.; Gallagher, T. F.; Kumar, S.; Green, D.; Mcnulty, D.; Blumenthal, M. J.; Heys, J. R.; Landervatter, 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.
5. 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.
6. Natarajan, S. R.; Wisnoski, D. D.; Singh, S. B.; Stelmach, J. E.; O'Neill, E. A.; Schwartz, C. D.; Thompson, C. M.; Fitzgerald, C. E.; O'Keefe, S. J.; Kumar, S.; Hop, C. E. C. A.; Zaller, D. S.; Schmatz, D. M.; Doherty, J. B. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 273.
7. Stelmach, J. E.; Liu, L.; Patel, S. B.; Pivnichny, J. V.; Scapin, G.; Singh, S. V.; Hop, C. E. C. A.; Wang, Z.; Strauss, J. R.; Cameron, P. M.; Nichols, E. A.; O'Keefe, S. J.; O'Neill, E. A.; Schmatz, D. M.; Schwartz, C. D.; Thompson, C. M.; Zaller, D. M.; Doherty, J. B. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 277.
8. Hunt, J. J. A.; Kallashi, F.; Ruzek, R. D.; Sinclair, P. J.; Ita, I.; McCormick, S. X.; Pivnichny, J. V.; Hop, C. E. C. A.; Kumar, S.; Wang, Z.; O'Keefe, S. J.; O'Neill, E. A.; Porter, G.; Thompson, J. E.; Woods, A.; Zaller, D. M.; Doherty, J. B. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 467.
9. Experimental details: Male B10RIII mice approximately 8 weeks of age were injected subcutaneously at the base of the tail with a cold 100 μL emulsion of 100 μg bovine Type II collagen (Sigma) and Freund's Complete Adjuvant (BD Biosciences) on day 0. No booster injection was given. Following administration of the emulsion, groups of mice were formed at random and then those groups were assigned at random to the various treatments which started on day 7. Mice were housed individually and given food and water ad lib. Evaluation of inflammation was conducted three times a week for the duration of the trial. At each evaluation, all four paws of each mouse were graded based on the following clinical scale: 0 = no inflammation, 1 = inflammation in at least one digit progressing to the metacarpus or metatarsus, 2 = inflammation progressing to the carpus or tarsus, 3 = inflammation progressing above the carpus or tarsus, 4 = mild to moderate functional ankylosis of the carpus or tarsus, and 5 = severe functional ankylosis (i.e., fusion) of the carpus or tarus. Inflammation in each paw of each mouse was recorded and a summary for each mouse was made by adding the scores of the four paws for each observation period (maximum score for an individual mouse at a given observation was 20). The data are presented as the mean score for each mouse in a treatment group for each observation period.