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p38 MAP kinase inhibitors. Part 5: Discovery of an orally bio-available and highly efficacious compound based on the 7-amino-naphthyridone scaffold

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Abstract—A new sub-class of p38 inhibitors represented by 7-amino-naphthyridone have been discovered. Benchmark compound **16** potently inhibited p38 in vitro, was functionally active, and displayed excellent pharmacokinetic profiles in two animal species. Compound **16** reduced inflammation in animal disease models at EC_{50} doses as low as 0.2 mpk.

In preceding communications[1] the development of a novel series of p38 MAP kinase inhibitors originating from quinazolinones were described. This class of p38 MAP kinase inhibitors was originally inspired by seminal work done at Vertex Pharmaceuticals which yielded them a clinical candidate VX-745 (**1**).[2] The subject of this communication is an SAR analysis on the naphthyridone platform and the subsequent discovery of a new sub-class, the 7-amino-naphthyridones, as potent, orally bio-available, and efficacious p38 inhibitors (see Fig. 1).

The pyrido[3,2-*d*]pyrimidone core (**3** and **4**) is an isomeric scaffold derived from VX-745 (**1**). Significant potency could only be attained via the installation of a C7-substituent which predisposes the pendant sulfide moiety in order to enable a facile entry into the hydrophobic pocket past Thr-106 in the p38 ATP binding site. Based on predicted binding mode of these compounds in the

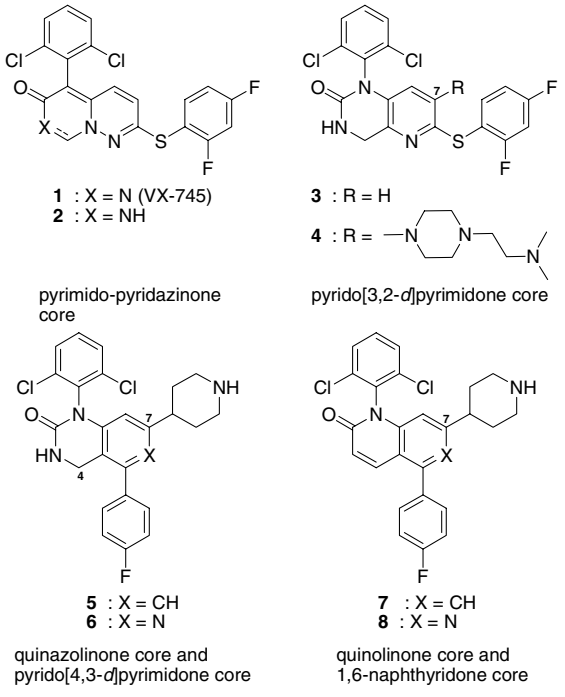

Figure 1. Novel p38 inhibitors: VX-745 and homologs.

Keywords: 7-Amino-naphthyridones; p38 MAP kinase inhibitors; Peptide flip; Rat LPS challenge.
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active site this design was further refined by direct attachment of the aryl substituent onto the scaffold. The resulting quinazolinones (**5**) and pyrido[4,3-*d*]pyrimidones (**6**) represented a promising new series of p38 inhibitors. The viability of this class of inhibitors critically depended on a C7-substituted piperidine appendage for functional activity. On account of metabolic lability represented by the pendant piperidine appendage and the C4-benzyllic moiety, these compounds generally suffered from high clearances and poor overall pharmacokinetic profiles in all animal species. The latter liability was addressed by a scaffold re-design resulting in the quinolinones (**7**) and 1,6-naphthyridones (**8**).[3] The liability arising from the pendant piperidine appendage, which is critical for functional activity, still remained. Key compounds in this class are typified by **9** and **10**, where the introduction of a bulky *iso*-propyl group on the piperidine slowed down metabolism and enabled efficacy. Although **9** and **10** embody many desirable features of a clinical compound, there still remained much room for improvement over the above compounds and a solution to restore functional activity to these p38 inhibitors based on the naphthyridone platform without making use of a piperidine appendage was highly sought after (see Fig. 2).

SAR investigations began with C7-unsubstituted naphthyridones (Table 1). Synthesis of these derivatives was achieved readily by Pd-mediated reduction of corresponding 7-bromo compound.[4] Table 1 depicts activities of three C7-unsubstituted naphthyridinone analogs. Although compound **11** displayed potent in vitro activity, functional activity was modest in human whole blood. It was encouraging to note that compounds **12** and **13** showed similar potent functional activity. It can be reasoned that substitution with fluorines contributed to lower calculated log *D*s and enabled greater aqueous solvation or increased fraction of protein unbound drug which translated into observed functional potencies. Compound **11** inhibited TNF-α release in LPS-stimulated human whole blood with an IC_{50} of 100 nM. In rats **11** was 57% orally bioavailable with an AUC (PO/1 mpk) of 1.5 μM, C_{max} of 1 μM, and $t_{1/2}$ of 2 h. With the above rat pharmacokinetic profile it was anticipated that **11** could effectively inhibit TNF-α production in a rat LPS challenge model at 3 mpk oral QD dose. Thus, rats were treated with a 3 mpk oral dose of **11** and were injected with LPS 1.5 h prior to euthanasia. At the end of the experiment plasma samples were pooled and the levels of circulating TNF-α were determined at three different time points. A 61% inhibition of TNF-α observed at the 6 h time point gave rise to optimism. However, inhibition at the 16 and 24 h time points was only 37% and 27%, respectively. The lack of sustained inhibition was disappointing.

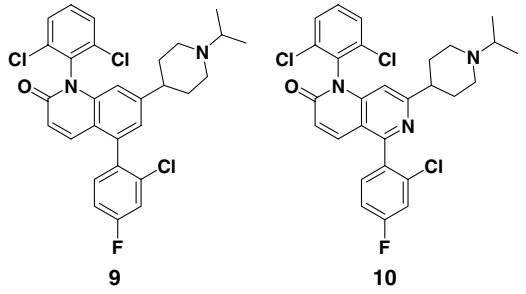
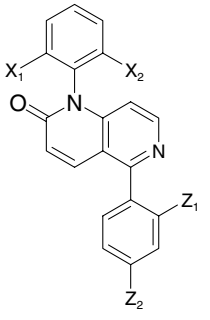


Figure 2. Key quinolinone and 1,6-naphthyridone p38 inhibitors.

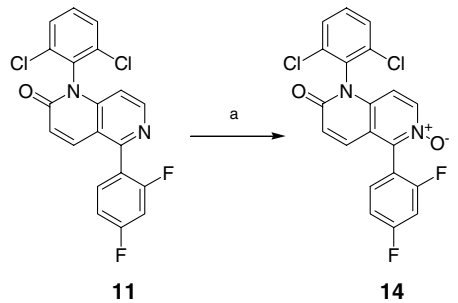
Table 1. p38 inhibitory properties for 1,6-naphthyridones



Compound	X_1	X_2	Z_1	Z_2	p38 (nM)	THP-1 (nM)	WB (nM)
11	Cl	Cl	F	F	1.5	6.6	101
12	Cl	H	F	F	9.3	40.5	86.1
13	F	F	F	F	9.7	7.0	145

Treatment in a liver microsomal assay revealed that the unsubstituted naphthyridine analogs above (Table 1) were turned over rapidly. In the case of compound **12** it was hypothesized that formation of corresponding N-oxide derivative **14** could be a facile first step in the metabolic process. Accordingly an authentic sample of proposed N-oxide **14** was synthesized by oxidation of **11** with MCPBA as shown in Scheme 1.

Compound **14** displayed remarkable rat pharmacokinetic profile. Thus, a 1 mpk po dose of **14** in SD rats showed 98% bioavailability with an AUC of 49 μM, C_{max} of 3.5 μM, and half-life of 9.9 h. Since N-oxides can be reduced in vivo, the above oral dose experiment was also monitored for the formation of **11** and no evidence for the reductive process could be observed. N-oxide **14** inhibited p38 with an IC_{50} of 12 nM and TNF-α release from LPS stimulated monocytes with an IC_{50} of 200 nM. Given the good oral bioavailability and potency, it was not surprising that a single 3 mpk



Scheme 1. Synthesis of pyrimido[4,5-*d*]pyrimidone-based p38 inhibitors. Reagents and conditions: (a) 1.5 equiv of MCPBA, MeCN, rt, 5 h, 85%.

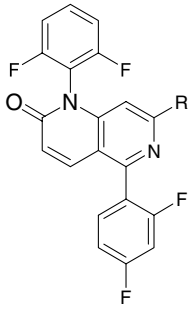
oral dose of **14** could effectively lower TNF-α production by 80% for 24 h in a rat LPS challenge PD assay. From the observed profile of **11** and **14** above it became obvious that metabolism at the naphthyridinone N-atom was a key factor contributing to poor therapeutic efficacy of the C7-unsubstituted naphthyridinone compounds. Alternative means to modulate the character of naphthyridinone scaffold in order to improve metabolic profiles was therefore sought.

Three targetted analogs, pyridone **15**, amino-pyridine **16**, and aminomethyl-pyridine **17**, were synthesized (Table 2) in order to ameliorate purported in vivo metabolism at the naphthyridone N-atom. Treatment of bromide **18** with potassium hydroxide at 110 °C yielded a meager 10% of desired pyridone **15**. Although **15** potently inhibited TNF-α release in human whole blood (IC_{50} = 121 nM), only a 70% reduction in circulating TNF-α levels was observed at the 3 h time point with a 10 mpk oral dose in a rat LPS challenge assay. The hydroxy pyridine moiety could have contributed to poor absorption or enabled conjugation and rapid clearance.

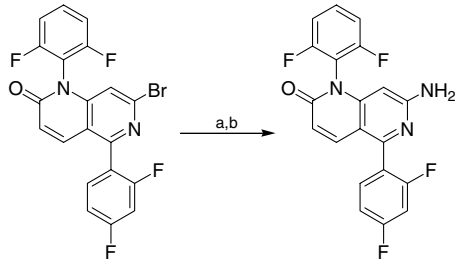
Amino-pyridine derivative **16** and aminomethyl-pyridine **17** potently inhibited TNF-α release in human monocytes. Speculations over metabolic demethylation of **17** to **16** re-directed our attention to the simple amino-pyridine derivative for further study. The synthesis of compound **16** is shown in Scheme 2. Bromo derivative **18** was synthesized in an analogous manner as described previously. Direct displacement of bromide in **18** with ammonia did not yield **16** in appreciable yield. Access to **16** was achieved indirectly by first displacement of the bromide with PMB amine by simply heating at 150 °C. The PMB group was removed by treatment with TFA to give desired analog **16**. It was gratifying to note that amino-pyridine derivative **16** was stable in microsomal assays and displayed excellent pharmacokinetic profiles in rats and monkeys.

In male SD rat **16** showed a 68% oral bioavailability when dosed as a 0.5% methylcellulose suspension. AUC at 1 mpk was 12 μM with a half life of 6.5 h,

Table 2. p38 inhibitory properties for 1,6-naphthyridones



Compound	–R	p38 (nM)	THP-1 (nM)	WB (nM)
15	OH	1.6	4.5	121
16	NH_2	0.69	1.0	34
17	NH	0.51	7.3	73



18 **16**

Scheme 2. Synthesis of amino-pyridine **16**. Reagents and conditions: (a) 3.0 equiv of PMB-NH_2, MeCN, 150 °C, 5 h, 85%; (b) TFA, reflux, 10 h, 79%.

low clearance of 1.6 mL/min/kg, C_{max} of 3.5 μM and at 0.9 L/kg, a small volume of distribution. Conjecture has it that a small volume of distribution could be thought of as a safety feature. The compound would be expected to localize in the vasculature and it is speculated that p38-mediated processes in the rest of the body not pertaining to the arrest of inflammation could be spared. In male rhesus macaques the oral bioavailability was 22% with a half-life of 11.6 h, a low clearance of 1.3 mL/min/kg, and similar low volume of distribution of 0.9 L/kg.

For in vivo evaluation **16** was initially screened in a mouse LPS challenge assay.[4] 12- to 16-week-old female Balb/c mice were used in these studies. The compound was formulated in 0.5% methylcellulose and administered orally 22.5 h prior to LPS challenge. The animals were sacrificed 1.5 h after challenge and the resulting plasma samples collected were analyzed by ELISA to measure amount of circulating TNF-α. It was found that **16** was very effective at suppressing the production of TNF-α (>90% inhibition) for up to 24 h at doses as low as 0.3 mpk. A similar LPS challenge experiment was carried out in rats using dexamethasone as a positive control. One panel of rats was given a 3 mpk oral dose of **16**, while a second panel of rats was given a 1 mpk dose of dexamethasone. Rats in both panels were injected with LPS at three time points. At the 6 h, 16 h, and 24 h time points a 3 mpk dose of **16** inhibited TNF-α production by 97%, 95%, and 92%, respectively, while a 1 mpk dose of dexamethasone inhibited TNF-α production, 92%, 30%, and 28%, respectively. In another rat LPS challenge study doses of 0.1, 0.3, 1, and 3 mpk of **16** inhibited TNF-α production, 53%, 75%, 79%, and 92%, respectively, at the 24 h time point (Fig. 3). It was thus inferred that **16** was equally efficacious, if not better than dexamethasone at arresting LPS induced inflammatory processes in rats.

In order to test the efficacy in an animal disease model, **16** was dosed in rats in a therapeutic manner after disease progression was induced by means of suitable adjuvant. Seven-week-old female lewis rats were used. On day 0 rats were weighed, paw volumes measured, and radiographed. Adjuvant was injected into the left hind paw. Onset of disease was allowed to occur for 12 days when the animals were re-weighed, the paw volumes re-measured, and radiographed.

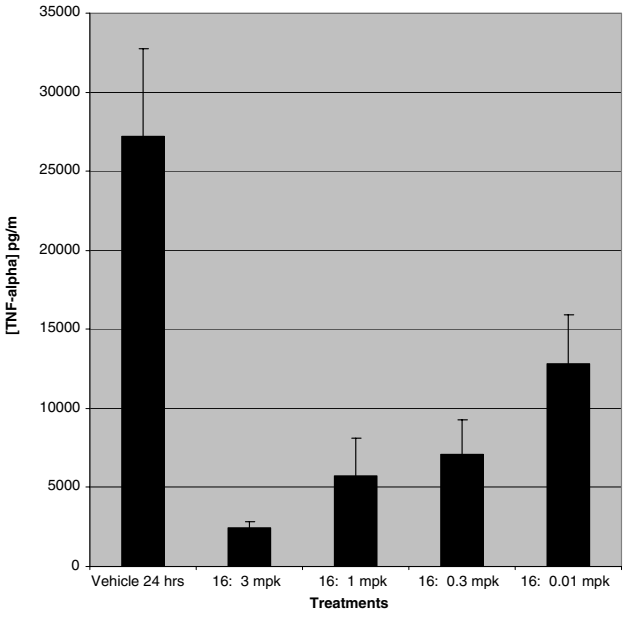


Figure 3. Dose titration of **16** in a rat LPS challenge assay.

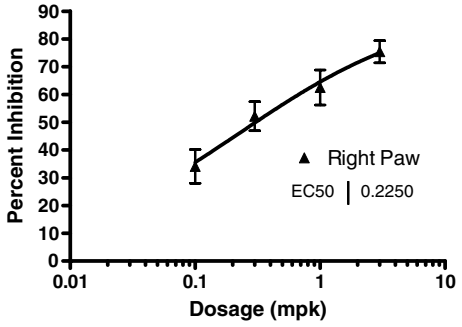


Figure 4. Dose titration of **16** for inhibition of paw swelling in AIA animal disease model.

The animals were orally dosed at 0.1, 0.3, 1, and 3 mpk once daily for the next ten days. On day 21 animals were re-weighed, paw volumes measured, and radiographed. A dose dependent inhibition of swelling bserved in the secondary paw at the four doses was 38%, 54%, 65%, and 80% respectively. The ED_{50} for the observed therapeutic efficacy in the AIA disease model was determined to be 0.22 mpk (Fig. 4). It is also worthy to note that **16** did not display affinities for ion channels and this bodes well from a cardiovascular safety point of view for this class of inhibitors.

In summary, the goal of achieving functional activities on the naphthyridinone platform without resorting to use of a piperidine appendage has been demonstrated. Compound **16** is a highly effective compound for blocking p38-mediated inflammatory processes. Follow-up studies and identification of appropriate drug candidates in this area are underway.

References and notes

1. (a) 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. M.; Schmatz, D. M.; Doherty, J. B. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 273 (and references within); (b) Stelmach, J. E.; Liu, L.; Patel, S. B.; Pivnichny, J. V.; Scapin, G.; Singh, S. B.; 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; (c) Natarajan, S. R.; Wisnoski, D. D.; Thompson, J. E.; O'Neill, E. A.; O'Keefe, S. J.; Zaller, D. M.; Doherty, J. B. *Bioorg. Med. Chem. Lett.* 2, in press.
2. Bemis, G. W.; Salituro, F. G.; Duffy, J. P.; Harrington, E. M. U.S. Patent 2000, 6,147,080.
3. Hunt, 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.
4. (a) Ali, A.; Thompson, C. F.; Balkovec, J. M.; Graham, D. W.; Hammond, M. L.; Quraishi, N.; Tata, J. R.; Einstein, M.; Ge, L.; Harris, G.; Kelly, T. M.; Mazur, P.; Pandit, S.; Santoro, J.; Sitlani, A.; Wang, C.; Williamson, J.; Miller, D. K.; Thompson, C. M.; Zaller, D. M.; Forrest, M. J.; Carballo-Jane, E.; Luell, S. *J. Med. Chem.* **2004**, *47*, 2441; (b) Gonzales, J. C.; Johnson, D. C.; Morrison, D. C.; Freudenberg, M. A.; Galanos, C.; Silverstein, R. *Infect. Immun.* **1993**, *61*, 970.