Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 4400–4404

p38 MAP kinase inhibitors. Part 3: SAR on 3,4-dihydropyrimido-[4,5-*d*]pyrimidin-2-ones and 3,4-dihydropyrido[4,3-*d*]-pyrimidin-2-ones

Swaminathan R. Natarajan,[a,*] David D. Wisnoski,[a] James E. Thompson,[b] Edward A. O'Neill[b] and Stephen J. O'Keefe[b]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*
[b]*Department of Inflammation Research, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*

Received 10 April 2006; revised 13 May 2006; accepted 15 May 2006
Available online 5 June 2006

Abstract—p38 inhibitors based on 3,4-dihydropyrimido[4,5-*d*]pyrimidin-2-one and 3,4-dihydropyrido[4,3-*d*]pyrimidin-2-one platforms were synthesized and preliminary SAR explored. Among the pyrimido-pyrimidones the emergence of two sub-types of analogs—C7-amino-pyrimidines such as **24** and C7-amino-piperidines such as **42**—characterized with good p38 inhibition and better off-target profiles in terms of ion channel activities was significant. Representative compound **54** in the pyrido-pyrimidone class was found to be equipotent with corresponding analog in the quinazolinone series.
© 2006 Elsevier Ltd. All rights reserved.

VX-745[1] (**1**, Fig. 1), harbinger of a new class of exquisitely selective[2] p38 MAP kinase inhibitors, inspired the development of two novel series[3] of inhibitors typified by dihydro-pyridopyrimidones **3** and **4**, and dihydro-quinazolinones **5** and **6** (Fig. 1).

Dihydro-quinazolinone analogs such as **5** (p38α IC_{50} = 1 nM), **6** (p38α IC_{50} = 6 nM), **7** (p38α IC_{50} = 2 nM), and **8** (p38α IC_{50} = 2 nM) that are devoid of a C7-piperidine substituent were found to lack significant cellular and functional activity. Introduction of a solubilizing amine at the C7-position imparted functional activities to this scaffold. Benchmark compounds[3b] **9** (0.2 nM) and **10** (0.2 nM) were not only very potent in vitro, they also showed good functional activity (human whole blood IC_{50}s were 10 nM and 20 nM, respectively) and oral bioavailability. Compounds in this class bearing the basic amine appendage such as the piperidine moeity in **9** and **10** also displayed potent ion channel activities which correlate with increased cardiovascular risk. Compounds in chronic usage necessitate greater cardiovascular safety profiles. VX-745 (**1**)

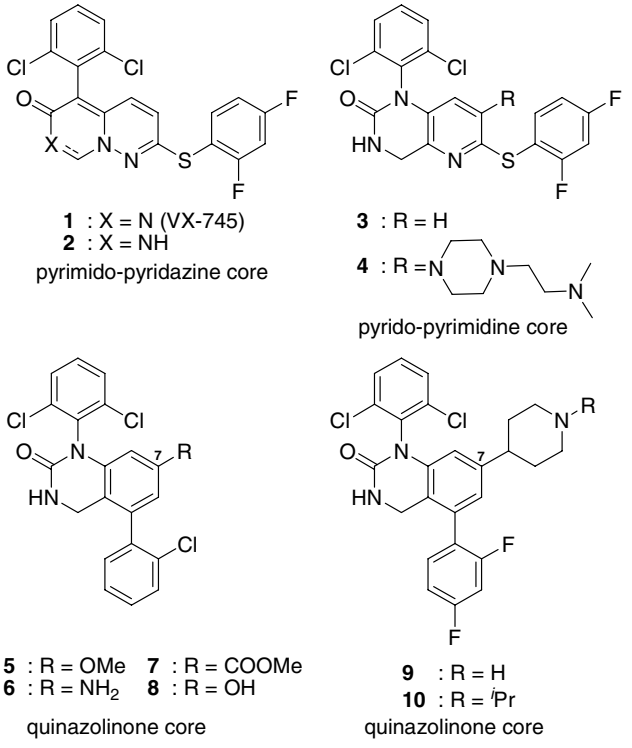


Figure 1. Novel p38 inhibitors, VX-745 and homologs.

Keywords: VX-745; Cellular and functional activity; Pyrimido-pyrimidone.
* Corresponding author. Tel.: +1 732 5940939; e-mail: ravi_natarajan@merck.com

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.05.045

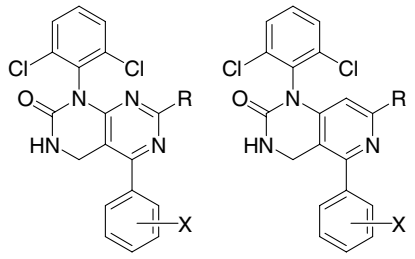


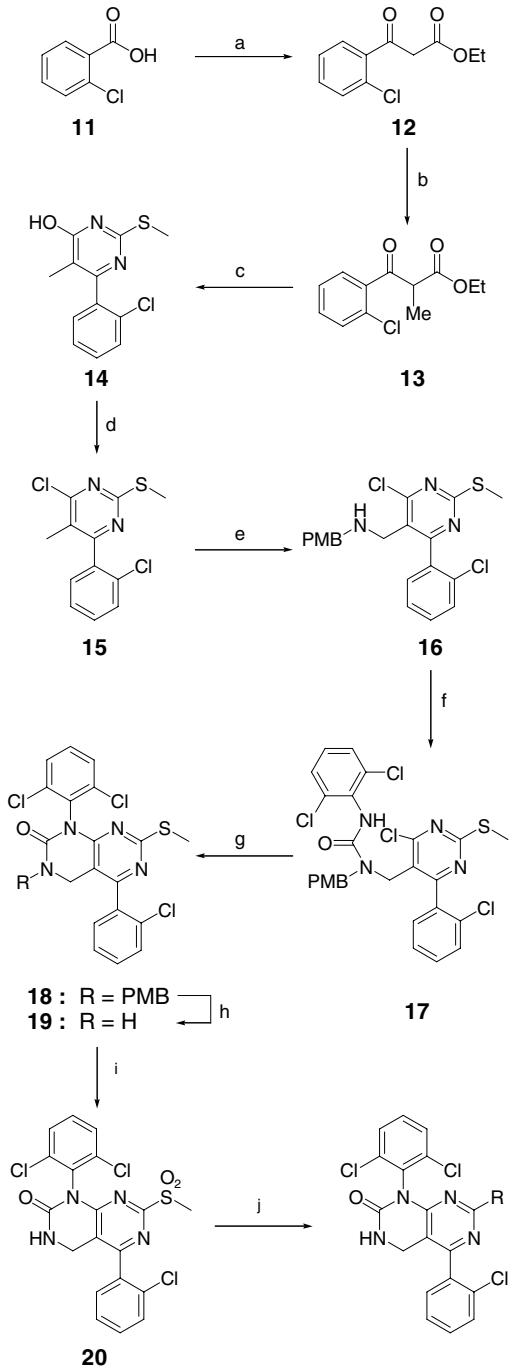
Figure 2. Pyrimido[4,5-*d*]pyrimidone and pyrido[4,3-*d*]pyrimidones as heterocyclic versions of quinazolinone p38 inhibitors.

did not display significant binding affinity to either the potassium or calcium ion channels. The use of heterocyclic templates was explored in the hopes of dialing out ion channel activities while maintaining p38 inhibitory properties. This communication describes synthetic routes to the pyrimido-pyrimidinone- and pyrido-pyrimidinone-based p38 inhibitors along with a preliminary assessment of their drug profiles (Fig. 2).

The synthesis of pyrimido-pyrimidone scaffold is shown in Scheme 1. Masamune homologation of 2-chlorobenzoic acid gave β-keto ester **12**, which was monoalkylated with methyl iodide to give **13** in meager yield. Compound **13** could also be synthesized by carbo-ethoxylation of 2-chlorophenyl ethyl ketone. Pyrimidine **14** was assembled by the condensation of **13** with thiourea followed by selective S-alkylation. The cyclization precursor **17** was obtained in three steps from **14** by chlorination with $POCl_3$, followed by benzylic bromination, displacement of bromide with PMB amine, and urea formation by treatment with 2,6-dichloro phenyl isocyanate. The key cyclization step was efficient, yielding **18** in 85% yield. Removal of PMB group followed by oxidation of the methyl sulfide with MMPP gave desired sulfone **20**.

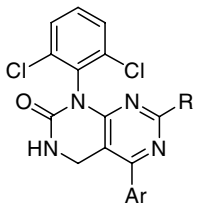
For profiling the pyrimido-pyrimidine-templated p38 inhibitors the Ar-substituent chosen was either a 2-chlorophenyl or a 2,4-dichlorophenyl group, both of which contributed equally to potency. The analogs in Table 1, generated by displacement of sulfone in **20**, can be loosely rank-ordered based on the impact of C7-substituent on either overall polarity (log *D*'s) and/or aqueous solubility of resulting p38 inhibitors. Compound **19** with a C7 methyl sulfide substituent inhibits p38 with good potency. However, in cell-based assays (THP-1 cells) a 70-fold shift was observed and there was no discernable inhibition of p38 in human whole blood. This apparent loss in functional activity arises due to some combination of poor aqueous solubility and the lipophilic nature of this analog. Compound **20** with a methyl sulfone substituent at the C7-position behaved in a similar manner. Since both **19** and **20** were weak blockers of the calcium (DLZ) and potassium (iKr) ion channels. It was gratifying to show that the pyrimido-pyrimidine scaffold in itself did not contribute to increased ion channel binding affinities.

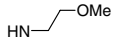
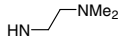
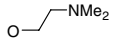
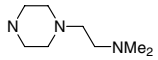
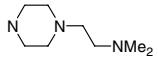
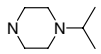
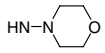
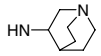
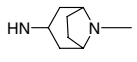
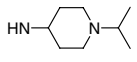
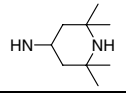
A series of amino-pyrimidines **24**–**29** were subsequently investigated. Although aqueous solubility did not improve, the resulting amino-pyrimidine derivatives were more polar than **19** and **20** as judged by TLC and HPLC retention times as well as calculated log *D* values. Com-



Scheme 1. Synthesis of pyrimido[4,5-*d*]pyrimidone-based p38 inhibitors. Reagents and conditions: (a) 1.1 equiv of imidazole, 2 h then 0.75 equiv of $Mg(COOEtCH_2COO)_2$, THF, rt, 10 h, 75%; (b) 1.2 equiv of NaO-*t*-Bu, 2 equiv of MeI, THF, 45%; (c) i—1.1 equiv of thiourea, 2.2 equiv NaOEt, EtOH, 85 °C, 2 h, 90%; ii—1.1 equiv of KOH, 1.0 equiv of MeI, H_2O, 95%; (d) $POCl_3$, 120 °C, 85%; (e) i—1.1 equiv of NBS, 0.1 equiv of Bz_2O_2, CCl_4,95 °C, 95%; (ii) 2.2 equiv of PMB-NH_2, CH_2Cl_2, rt, 70%; (f) 1.1 equiv of 2,6-dichloro phenyl isocyanate, 95%; (g) 1.5 equiv of K_2CO_3, 1.3 equiv of CuI, py, 150 °C, 0.5 h, 85%; (h) TFA, CH_2Cl_2, 100 °C, 78%; (i) 1.5 equiv of MMPP, THF, 84%; (j) 1.5 equiv of amine, DMF, 150 °C, 0.5 h.

Table 1. p38 inhibitory properties for pyrimido-pyrimidines



Compound	R	p38[b] (nM)	THP-1[b] (nM)	WB[b] (nM)	Ca^{2+} (μM)	iKr (μM)
19	SMe	10.0	700	NA	6.0	6.8
20	SO_2Me	4.2	500	NA	>10.0	9.0
24	NH_2	5.3	81	2000	9.2	7.2
25	NH–Me	2.0	22	850	10.0	6.4
26	NMe_2	3.0	NA	NA	>10.0	6.0
27		1.1	38	NA	10.0	4.5
28		0.8	21	1000	>30	10.0
29		1.9	28	500	>30	10
30	OH	4.0	170.0	NA	7.0	4.6
31[a]		2.0	97.0	270.0	>10.0	3.0
32[a]		5.6	20.0	32.0	1.0	0.5
33		4.2	21.0	250.0	8.8	10.0
34		0.5	2.3	100.0	1.7	3.8
35[a]		1.0	22.0	42.0	0.22	2.3
36[a]		20.0	330.0	1940.0	0.7	0.2
37		4.0	99.0	1000.0	7.0	6.9
38		2.0	500.0	NA	6.0	5.0
39[a]		2.0	500.0	NA	6.0	5.0
40		1.2	34.0	360.0	1.3	5.8
41		0.5	2.6	17.0	2.2	8.9
42		0.6	1.0	15.0	3.3	6.4
43		0.7	2.6	38.0	1.2	4.5

For all analogs Ar is 2-chlorophenyl. Those marked with 'a' Ar is 2,4-di-chlorophenyl.
NA, not active below 15 μM.
[b] Refs. 3a and 5.

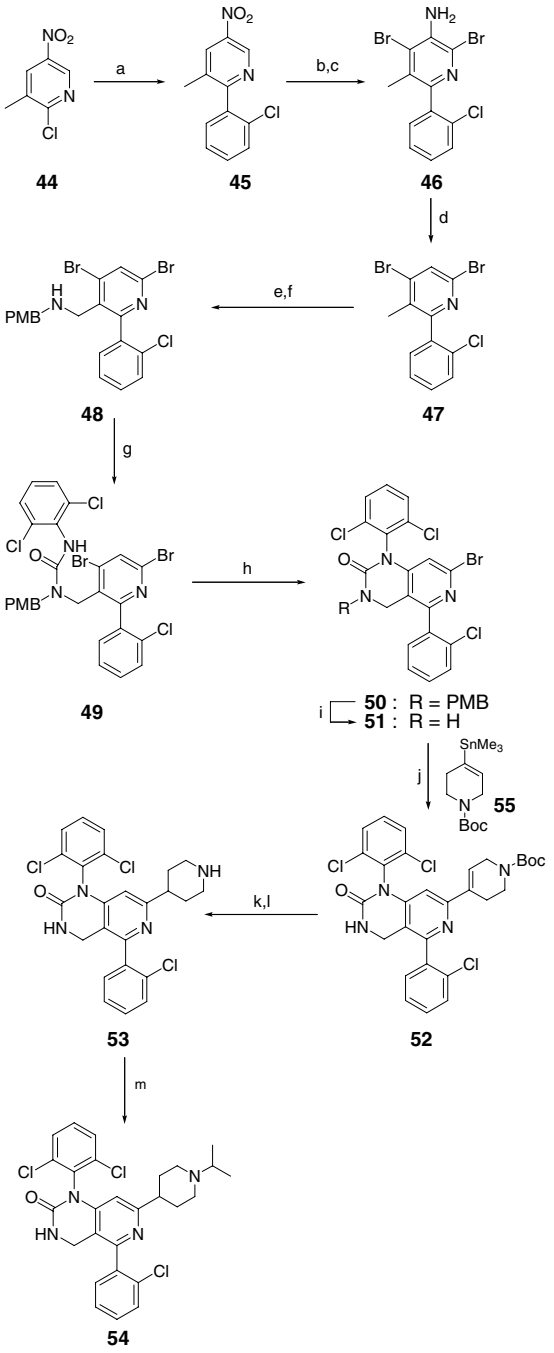
pound **24** displayed excellent in vitro potency, was active in cell-based assays losing potency only 20-fold, and inhibited TNF-α release in LPS-stimulated human whole blood with an IC_{50} of 2000 nM. It was encouraging to note that the increased functional activities among the amino-pyrimidines were not accompanied

with a concomitant increase in their ion-channel affinities. In rats **24** was 100% orally bioavailable with a half-life of 1.1 h, low clearance, and an AUC (po/1 mpk) of 3.5 μM. Amino-pyrimidines **25**–**28** were similarly active in vitro with excellent cellular transport properties and appreciable functional activity in whole blood. Compound **29** was particularly interesting on account of its good activity in human whole blood and lack of activity against ion channels. It was disappointing to find pyridone **30** to be functionally inactive unlike the amino-pyrimidines.

Compounds **31**–**39** represented various 1,2-diamine or 1,2-oxo-amine derivatives. Although most of these compounds were potent in vitro and functionally active, compounds **32** and **35** inhibited p38 in human whole blood with an IC_{50} of 32 and 42 nM, respectively, they were potent blockers of ion channels. The pharmacokinetic profile of **35** in rats was unremarkable, high clearance coupled with poor absorption resulted in very low oral bioavailability of 3%. The piperazine and morpholine series of analogs did not evoke enough curiosity to warrant further work.

A series of 4-amino piperidines were also profiled. Compound **42**, a 4-amino piperidine capped with an iso-propyl group, showed excellent potency in whole blood at the same time offering greater than 100-fold safety window vis-à-vis affinity toward ion channels. Compound **42** exhibited modest pharmacokinetic properties when dosed in rats with a $t_{1/2}$ of 2 h, moderate clearance, and 24% oral bioavailability. The synthesis of **40**, **41**, and **43** was motivated by the need to attenuate piperidine metabolism and further improve PK profiles. However, **40** did not display adequate functional potency, while **41** and **43** though equally active did not withstand metabolism anymore than **42**.

The comparator to benchmark quinazolinone compound **10** in the pyrido-pyrimidone series was accessed via a synthetic route outlined in Scheme 2. Suzuki coupling on **44**[4] with 2-chlorophenyl boronic acid gave biaryl **45**. Reduction of the nitro group in **45** followed by ortho dibromination and deamination gave **47**. The scaffold core **51** was then accomplished in a parallel fashion to the one described for the pyrimido-pyrimidone core in Scheme 1. That the bromide **51** was not functionally active came as no surprise. Compound **51** was coupled to stannane derivative **55** in a Stille reaction, followed by reduction of the double bond, deprotection of the Boc group, and reductive amination on the liberated piperidine with acetone gave comparator compound **54**. While **10** inhibited p38 more potently than **54** in vitro, they were equipotent in inhibiting TNF-α in human whole blood. **54** displayed a remarkably similar pharmacokinetic profile as **10** in rats. Thus, **54** was 18% orally bioavailable with a $t_{1/2}$ of 2.1 h, AUC (po/1 mpk) of 365 nM, and relatively high clearances. Compound **54** displayed significantly better ion channel activity profile and it is thought that this advantage accrued over quinazolinone **10** is attributed to the heterocyclic template and hence generally useful.



Scheme 2. Synthesis of pyrido[4,3-*d*]pyrimidone-based p38 inhibitors. Reagents and conditions: (a) 1.1 equiv of 2-chlorophenyl boronic acid, 2.5 equiv of Cs_2CO_3, toluene/MeOH/H_2O (8:1:1), 0.02 equiv of $Pd(PPh_3)_4$, reflux, 12 h, 88%; (b) Raney Ni, MeOH, rt, 7 h, 85%; (c) 2.1 equiv of Br_2, THF/1 M HCl (1:2), 2 h, 85%; (d) 1.65 equiv of ^{t}amylONO, THF, reflux, 1 h, 80%; (e) 1.1 equiv of NBS, 0.1 equiv of Bz_2O_2, CCl_4, 95 °C, 85%; (f) 2.2 equiv of PMB-NH_2, CH_2Cl_2, rt, 75%; (g) 1.1 equiv of 2,6-dichloro phenyl isocyanate, 95%; (h) 1.5 equiv of K_2CO_3, 1.3 equiv of CuI, py, 150 °C, 0.5 h, 89%; (i) TFA, CH_2Cl_2, 100 °C, 75%; (j) 1.1 equiv of **56**, 0.04 equiv of $Pd(PPh_3)_4$, DMF, 110 °C, 85%; (k) H_2, Pt_2O (10%), 0.15 h, 20 psi, 65%; (l) TFA, rt, 0.5 h, 95%; (m) 2.5 equiv of Me_2CO, 1.5 equiv of $NaCNBH_3$, MeOH, rt, 5 h, 76%.

In conclusion, the pyrimido-pyrimidones and pyrido-pyrimidones were found to be viable platforms for accessing potent p38 inhibitors. Among the

pyrimido-pyrimidone analogs profiled in Table 1 two sub-types evoke particular interest for follow-up discovery. Amino-pyrimidines such as **24** displayed a useful compromise between their p38 inhibitory properties yet poor ion channel affinities. There clearly exists an opportunity to optimize the potency without concomitantly increasing ion channel affinities and thereby identify a safe clinical compound. The amino-piperidine derivative such as **42**, representing the second sub-type, was very potent in human whole blood, offered a slightly diminished safety margin (100-fold) vis-à-vis ion channels. It is reasoned that modulating the basicity of the piperidine would be one way to decrease affinities of these compounds for ion channels, while maintaining good p38-mediated therapeutic activity. Compound **54**, comparator to benchmark quinazolinone compound **9**, was found to be equipotent, orally bioavailable, and displayed much weaker ion channel affinities. In general, the heterocyclic templated p38 inhibitors match quinazolinones in terms of potency and functional activities while offering favorable ion channel profiles. The impetus provided by these findings has directly impacted the goal of finding suitable pre-clinical candidates and will be reported.

References and notes

1. Bemis, G. W.; Salituro, F. G.; Duffy, J. P.; Harrington, E. M. US Patent 6,147,080, 2000.
2. Fitzgerald, C. E.; Patel, S. B.; Becker, J. W.; Cameron, P. M.; Zaller, D.; Pikounis, V. B.; O'Keefe, S. J.; Scapin, G. *Nat. Struct. Biol.* **2003**, *10*, 764.
3. (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.
4. Hawkins, G. F.; Roe, A. *J. Org. Chem.* **1949**, *14*, 328.
5. Anti human TNF-α was coated on immulon four plates. THP-1 cells (density = 2.5×10^6/mL) were suspended into 96-well plates containing a PBS-based medium. Compound was added as solution in DMSO followed by addition of LPS. The reaction was incubated for 4 h at 37 °C under CO_2. TNF-α release was measured in the supernatants by ELISA. Reported IC_{50}s are means from three measurements.