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p38 MAP Kinase Inhibitors. Part 1: Design and Development of a New Class of Potent and Highly Selective Inhibitors Based on 3,4-Dihydropyrido[3,2-*d*]pyrimidone Scaffold

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Abstract—A new class of p38 antagonists based on 3,4-dihydropyrido[3,2,-*d*]pyrimidine scaffold has been developed. These inhibitors exhibit unprecedented selectivity towards p38 over other very closely related kinases. Compounds **25**, **33**, and **34** were identified as benchmark analogues for follow-up studies. They show good potency for enzyme inhibition and excellent functional activity.

p38 is a newly identified member of the mitogen activated protein (MAP) kinase superfamily.[1,2] MAP kinases are important signaling molecules which are activated by a number of extracellular stress stimuli. The actual activation of p38 occurs by dual phosphorylation of conserved threonine and tyrosine residues by upstream activators such as MAP kinase kinases (MKKs) upon receiving extracellular signals.[3] The downstream events that are subsequently regulated by p38 lead to the production of cytokines such as TNF-α and IL-1β and these are the causative agents for rheumatoid arthritis and other inflammatory diseases. A small molecule inhibitor that can selectively block p38 will prevent this signal transduction cascade from producing harmful cytokines and eventually alleviate the onset of disease.[4]

The validation of p38α as a target for modulating the production of both TNF-α and IL-1β came as a result of incidental discovery that SB-203580 prevented LPS stimulated cytokine production.[1] This led to the development of trisubstituted imidazoles as a promising class of p38 antagonists by several groups.[5]

More recently, researchers at Vertex[6] and Bayer[7] have disclosed the discovery and development of new p38 inhibitors. The Vertex effort has resulted in a clinical candidate, VX-745 (**1**) (Fig. 1). VX-745 and its congeners are particularly interesting in light of the unprecedented level of selectivity they exhibit for p38α over a variety of other closely related kinases tested. This exquisite selectivity shown by the Vertex class of inhibitors was the inspiration behind our design. VX-745 is derived from a pyrimido-pyridazinone core and is characterized by the presence of an extended vinylogous amide system that is critical for functional potency. An isomeric core based on a pyrido-pyrimidone (**2**, Fig. 1) was envisioned to be a suitable alternative for the development of potential new inhibitors. Scaffolds such as **2** were reasoned to be stable in biological systems and hence desirable as substrates for drug design.

Scheme 1 outlines a synthetic strategy[8] for assembly of the pyrido-pyrimidone derived scaffold **2**. Treatment of amino pyridine **5** with sodium nitrite in 6 N HCl solution resulted in chloro **6**. Benzylic bromination followed

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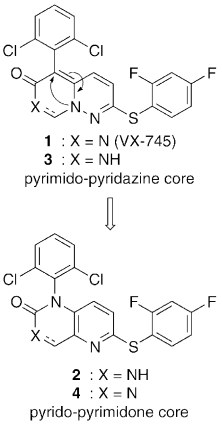
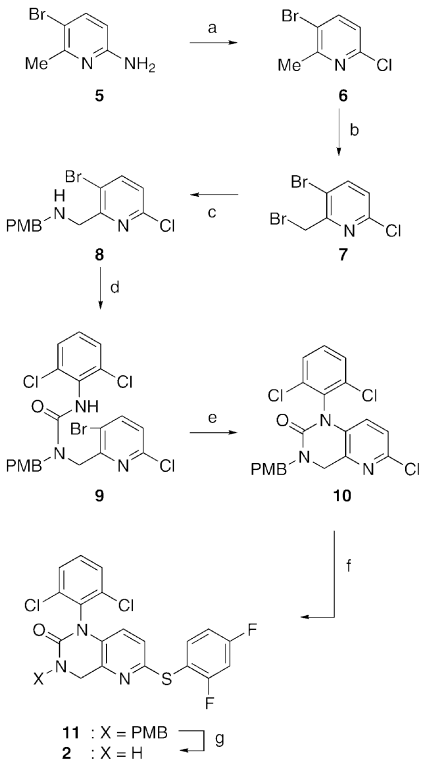


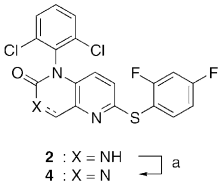
Figure 1. Derivation of pyrido-pyrimidone core (**2**) from VX-745.



Scheme 1. Synthesis of **2**. (a) 1.2 equiv $NaNO_2$, 6 N HCl, 70%; (b) 1.2 equiv NBS, 0.1 equiv of Bz_2O_2, 80 °C, CCl_4, sun lamp, 1 h, 95%; (c) 2 equiv of PMB–NH_2, CH_2Cl_2, 65%; (d) Ar–NCO, CH_2Cl_2, 98%; (e) 1.2 CuI, 1.5 equiv K_2CO_3, py, 160 °C, 1 h, 35%; (f) 1.5 equiv Ar–SH, 2 equiv TEA, DMF, 160 °C 10 h, 30%; (g) TFA, 110 °C, 78%.

by displacement with 4-methoxybenzyl amine gave **8**. Treatment of PMB amine **8** with 2,6-dichlorophenyl isocyanate gave urea **9** and set the stage for an intramolecular Ullmann–Goldberg reaction.[9] Thus, treatment of **9** with 1.6 equivalents of copper(I) iodide in pyridine gave the desired cyclized product **10** in 35% yield. Displacement of chloride was carried out by simply heating **10** with 2,4-difluorothiophenol in DMF at 160 °C. The desired product **11** was obtained in 30% yield. Removal of the PMB group by treatment with TFA gave final compound **2**.

The conversion of **2** to its corresponding dehydro derivative was achieved by treatment with NBS and a radical



Scheme 2. Oxidation of **2**: (a) 1.2 equiv NBS, 0.1 equiv of Bz_2O, 80 °C, sun lamp, 1 h, CCL_4, 65%.

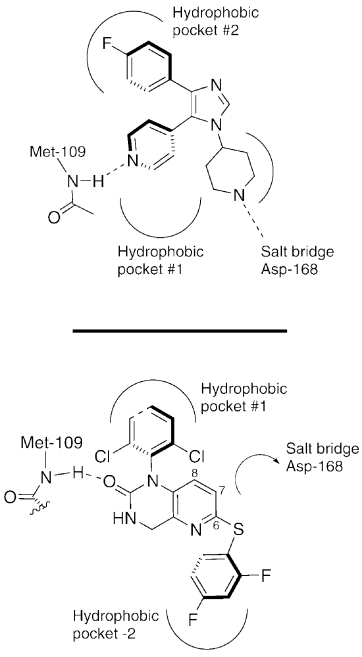


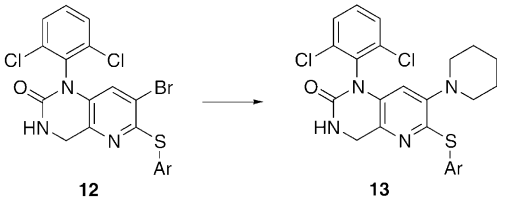
Figure 2. Schematic representation of binding of VK-19911 to p38 and predicted binding of **2** to p38.

initiator such as benzoyl peroxide. The in vitro potencies[10] of **2** and **4** were determined to be 25 and 600 nM respectively. Compared to VX-745 (**1**, 10 nM) and **3** (0.8 nM) the inhibitors based on the pyrido-pyrimidone core were only modestly potent. It was reasoned that the increased potency of the Vertex class of inhibitors was directly related to the ability of the electron rich carbonyl oxygen in **1** and **3** to form a stronger hydrogen bond with Met-109 amide along the peptide backbone of the enzyme. The urea substructure in **2** and **4** on the other hand formed a weaker hydrogen bond. In THP-1 cell assay[11] designed to measure functional activity, **2** was found to have an IC_{50} of 700 nM, while **4** was active only in the high micro molar range. In addition, **2** and **4** were not active in whole blood assay.[5b] Clearly the pyrido-pyrimidone based inhibitors were not viable at this stage (Scheme 2).

In order to augment potency and functional activity in the urea based scaffolds a serious analysis of the mode of binding of these compounds in the enzyme active site was undertaken. The enzyme active site has been previously characterized by Goldsmith[12] and Su[13] utilizing the trisubstituted imidazoles as molecular probes. A pneumonic constructed to rationalize the observed SAR in the imidazole series (Fig. 2) was utilized to predict the mode of binding of designed pyrido-pyrimidine derived

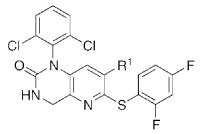
inhibitors like **2** in the hopes that any insight gleaned would enable infusion of greater potency and functional activity. By homology the binding of **2** can be postulated as follows: (1) the primary contact is made through the carbonyl oxygen which accepts a hydrogen bond from amide Met-109; (2) the 2,6 dichloro phenyl substituent is assumed to occupy the closest hydrophobic pocket to Met-109 in the active site; (3) the aryl sulfide is envisioned to occupy the second hydrophobic pocket. The entrance to this hydrophobic pocket is usually lined by amino acids with larger side chains such as methionine in closely related kinases such as ERKs and JNKs and so on. In p38, however, this pocket is lined by a smaller Threonine-106 which allows the aryl sulfide to slide into the hydrophobic pocket. This is one of the important factors for observed selectivity. Apart from these primary contacts the tri-substituted imidazoles were observed to augment potency by way of an additional substituent such as a piperidine in VK-19911 (Fig. 2). It was thought that such a hydrophobic amine substituent would be best introduced at the 7-position in the pyrido-pyrimidone scaffolds (Fig. 2) for two reasons. An appropriate piperidine-type substituent at this position would have the potential to derive additional potency by favorable hydrophobic interactions with the additional possibility of forming a salt bridge with proximal Asp-168. More importantly a suitable 7-substituent would restrict the degrees of free rotation about the diaryl sulfide moiety thereby pre-orienting the aryl sulfide in a favored binding mode leading to enhanced potencies.

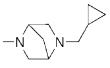
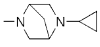
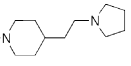
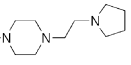
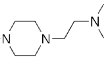
Bromide **12** served as a convenient starting point for exploration of substituent effects at the 7-position. The synthesis of **12** was achieved in a similar fashion as described in Scheme 1 starting from 2-amino-3,5-dibromo-6-methyl pyridine. **12** was then subjected to Buchwald–Hartwig couplings[14] with a variety of amines (Scheme 3). Out of the various substituents appended at the 7-position, it became quickly obvious (Table 1) that cyclic derivatives brought about an instant increase in potency. Analogue **13** bearing a piperidine appendage gave an IC_{50} value of 4 nM against the enzyme. Although analogues such as **13** had low nanomolar potency against the enzyme there was a considerable loss in potency in functional assays. To address this issue the piperidine moiety was replaced with more polar cyclic derivatives such as morpholines and piperazines. The morpholine derivative **15** still suffered a considerable shift in potency in the whole blood assay. Piperazine analogues **18** and **20**, on the other hand, were very potent against the enzyme and maintain much of



Scheme 3. Buchwald–Hartwig coupling conditions. (Ar = 2,4-difluoro phenyl). 1.5 equiv of piperidine, 0.1 equiv of $Pd_2(dba)_3$, 0.2 equiv of BINAP, 1.1 equiv of Na–O*t*Bu, dioxane, 90 °C, 10 h, 65%.

Table 1. p38 inhibitory activity for designed pyrido-pyrimidones: 7-substituted analogues[a]



Compd	R^1	p38[a]	THP-1[b]	WB[c]
12	Br	16 nM	NA	NA
13		4.2 nM	500 nM	NA
14		5.3 nM	500 nM	NA
15		2.0 nM	22 nM	400 nM
16		3.0 nM	25 nM	72 nM
17		25 nM	NA	NA
18		2.0 nM	5 nM	20 nM
19		44 nM	NA	NA
20		600 pM	1.5 nM	11.2 nM
21		7.0 nM	7.0 nM	170 nM
22		11 nM	11.0 nM	63 nM
23		4.0 nM	9.0 nM	17 nM
24		9.0 nM	1.3 nM	42 nM
25		3.0 nM	1.2 nM	51 nM
26		3.0 nM	8.2 nM	220 nM
27		2.0 nM	1.6 nM	65 nM
28		6.0 nM	7.3 nM	940 nM
29		1.1 nM	2.0 nM	9.0 nM
30		600 pM	700 pM	14 nM

[a]Enzyme activity.[5b,10]
[b]Cell assay.[11]
[c]Ref 5b. NA, not active below 10 μM.

their potency in whole blood. Based on the enzyme activity of the above-mentioned analogues, it was clear that the 7-substituent brought about increased potency primarily by steric rigidity and hydrophobic interactions. In order to explore a potential salt forming interaction with the proximal Asp-168, a polar amine was extended from the piperazine. The marginal increase in potency observed for **29** and **30** was not conclusive with formation of a salt bridge. In order for a transparent carryover of in vitro enzyme activity to functional efficacy, it was observed that a basic amine was required in all cases.

This new class of compounds when tested against a variety of other closely related kinases including JNK2, ERK1 and ZAP70 did not show any appreciable activity. Insights gained in the development of this class of p38 inhibitors will aid in the design of second and third generation of analogues with further improved potency and functional activity. A comprehensive study encompassing the development of good pharmacokinetic profiles for this series is underway and will be the subject of a future publication.

Acknowledgements

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10. Enzyme activity was measured by two methods: The filter-binding assay is described in ref 5b. A SPA-bead based assay was carried out using mouse p38. Compounds were serially diluted into a 96 well plate containing a MOPS based p38 assay buffer. The assay was initiated by addition of cold ATP, ^{33}P ATP (gamma) and biotin labeled GST-ATF2 substrate (4 μM). After incubation at 30 °C for 3 h, the reaction was stopped by addition of a PBS based quench buffer with 2× moles of SPA beads over the amount of substrate used. The extent of phosphorylation of GST-ATF2 was measured using a topcount reader and subtracted from background. IC_{50}s are means of two experiments.
11. Anti human TNF-α was coated on immulon 4 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.
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