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SAR of 3,4-Dihydropyrido[3,2-*d*]pyrimidone p38 Inhibitors

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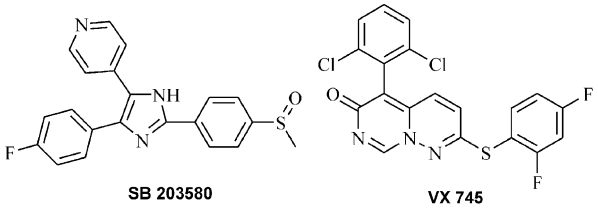
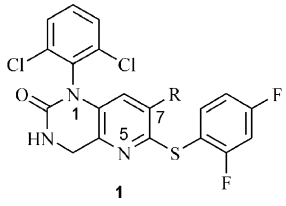
Abstract—Development for a class of potent 3,4-dihydropyrido(3,2-*d*)pyrimidone inhibitors of p38a MAP kinase is described. Modification of N-1 aryl and C-6 arylsulfide in 3,4-dihydropyrido(3,2-*d*)pyrimidone analogues for the interaction with the hydrophobic pockets in p38 active site is also discussed.

Tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) are proinflammatory cytokines produced in response to infection and other cellular stresses. MAP kinase p38 is a key player in the intracellular signal transduction cascade required for the biosynthesis of the inflammatory cytokines IL-1 and TFN-α.[1,2] The identification of SB-203580[1,3] as a p38 MAP kinase inhibitor led to the development of trisubstituted imidazoles as promising class of p38 antagonists by several groups.[4–6] Potent, selective and functionally active pyrido-pyrimidones, such as **1**, derived from VX-745, have been previous described.[7] In this case, substitutents at the 7-position enhanced activity through increased rigidity, pre-orienting the aryl sulfide in a favorable disposition, and possibly formed a salt bridge with ASP-168 in p38 active binding site.[8,9]





X-ray crystallographic and mutagenesis studies of p38a with trisubstituted imidazoles as molecular probes have revealed key binding interaction in the p38 active site.[10,11] The pyridine nitrogen forms a hydrogen bond with the amide N–H of Met 109 and the aryl group penetrating into a hydrophobic pocket not accessed by ATP (Fig. 1). A mnemonic constructed to rationalize the observed SAR in the imidazole series was utilized[8] to predict the relative binding orientations of **1** in the p38 active site. The primary contact is made through the carbonyl oxygen to form a hydrogen bond with amide Met-109. The 2,4-difluoroaryl sulfide at C-6 position penetrates into the hydrophobic pocket-1. The 2,6-dichlorophenyl substituent at N-1 position is assumed to occupy the hydrophobic pocket-2 in the active site.

In this communication, we would like to report SAR efforts that help characterize hydrophobic pockets 1 and 2 in the enzyme active site with insights gleaned through molecular modeling and X-ray crystallographic analysis.

The general preparation procedures were summarized in Scheme 1. We used a modified synthetic method as reported before.[8] Diazotization of amine **3** in HF-pyridine gave 2-fluoropyridine moiety **4**. NBS bromination

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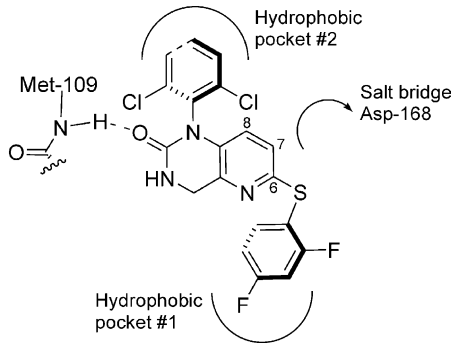
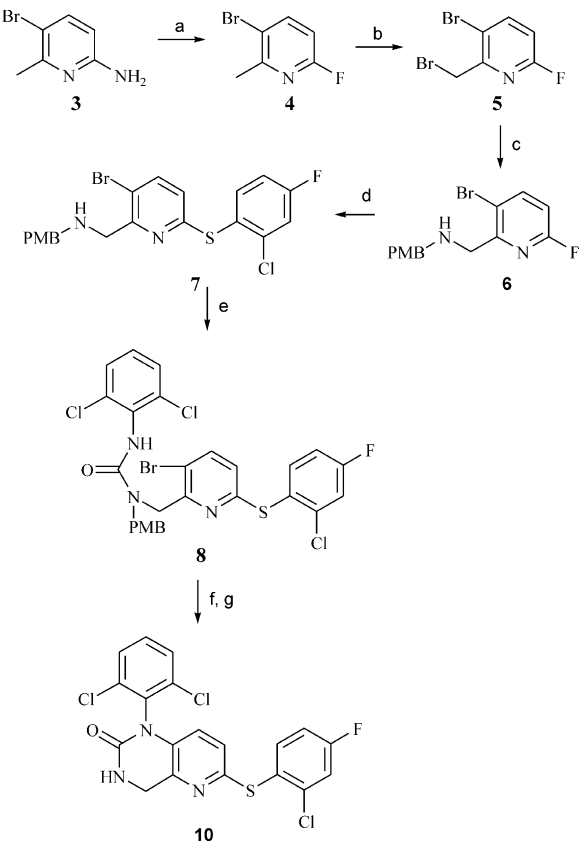


Figure 1. Relative binding orientations of **11** to p38.



Scheme 1. Synthesis of **10**: (a) 1.2 equiv $NaNO_2$, HF-pyridine, 70%; (b) 1.2 equiv NBS, 0.1 equiv Bz_2O_2, 80 °C, CCl_4, sun lamp, 1 h, 95%; (c) 2 equiv PMB–NH_2, CH_2Cl_2, 91%; (d) 1.5 equiv Ar–SH, 2 equiv DIPEA, 1,4-dioxane, reflux, 4 h, 71%; (e) Ar–NCO, DIPEA, CH_2Cl_2, 82%; (f) 1.2 equiv CuI, 2 equiv DIPEA, DMF, 150 °C, 2 h, 33%; (g) TFA, 110 °C, 80%.

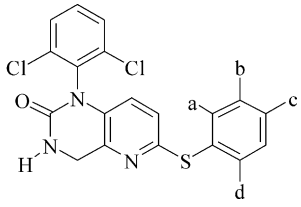
produced **5**. Displacement of bromine with 4-methoxybenzyl amine gave **6**. Replacement of fluorine with thiophenol gave **7**. Treatment of **7** with 2,6-dichlorophenyl isocyanate gave urea **8**. Cyclization of **8** under copper iodide catalysis yield **9**. Deprotection by refluxing in trifluoroacetic acid (containing 10-25% anisole) gave the corresponding pyridine-urea analogue **10** (Scheme 1).

Our binding model of **11** proposes a tight fit of the thiophenol substituent in a hydrophobic pocket 1. The model suggests that no large substituents will be tolerated in the *para* and *meta* position of the thiophenol. However, larger substituents should be tolerated in the *ortho* position. This model is consistent with the observed SAR in Table 1.

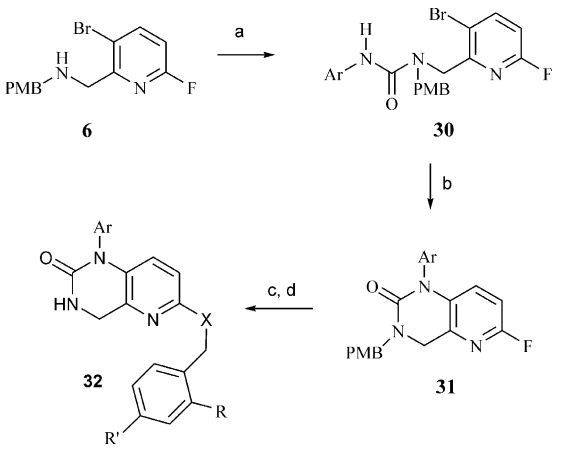
The data from p38α/β kinase assay[8] (Table 1) shows that *meta*- and *para*-substituted thiophenols gave low to modest activities. The *ortho*-substituented compounds **23**, **24** and **25** are less active compared to compound **18**, but compounds **14**, **15** and **16** are more potent implying methyl or chloro is preferred and there might be size or electronic restrictions at this site. All *meta*-substituted thiophenols, such as **20**, **26**, and **28** gave low to modest activities. Substituents larger than a chlorine group in the *para* position, such as **27** and **29**, give reduced activity. The experimental data from the p38α/β kinase assay is thus consistent with the prediction from our computer modeling studies. The 2,4-disubstituted thiophenols, such as **10**, **11** and **12**, showed significantly improved activity.

In order to further probe the size of hydrophobic pocket 1, we adjusted the linker one carbon longer between the aromatic group and the core ring. The synthetic procedure for the linker modification is summarized in Scheme 2. Treatment of **6** with 2,6-dichlorobenzylisocyanates gave urea **30**. Cyclization of **30** under copper iodide catalysis yields **31**. Replacement of fluorine with a range of nucleophiles followed by deprotection by refluxing in trifluoroacetic acid (containing 10–25% anisole) gave the corresponding pyridine-urea analogues **32**. (Scheme 2). The corresponding p38α/β data for the products are summarized in Table 2.

Table 1. p38 Inhibitory activities for designed pyrido-pyrimidones: C-6 arylsulfides

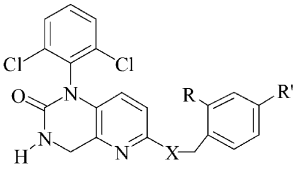


Compd	a	b	c	d	p38a	p38b
					IC_{50} (nM)	IC_{50} (nM)
10	Cl	H	F	H	11	57
11	F	H	F	H	25	250
12	Cl	H	Cl	H	32	190
13	Cl	H	H	Cl	49	50%@340
14	Cl	H	H	H	94	393
15	Me	H	H	H	110	800
16	Br	H	H	H	120	1480
17	NH_2	H	F	H	200	1340
18	H	H	H	H	270	2370
19	Me	H	H	Me	260	980
20	H	Cl	H	Cl	350	47%@1250
21	H	H	H	F	450	74%@1000
22	H	H	H	Cl	515	1490
23	NH_2	H	H	H	1420	2750
24	CF_3	H	H	H	1850	66%@1000
25	OMe	H	H	H	1990	3430
26	H	Cl	H	H	2190	67@1000
27	H	H	CF_3	H	51%@10,000	31%@10,000
28	H	CF_3	H	H	1815	66%@80,000
29	H	H	-S-4-F-Ph	H	−6%@10,000	6%@10,000



Scheme 2. Synthesis of **32**: (a) Ar-NCO, DIPEA, CH_2Cl_2, 82%; (b) 1.2 equiv CuI, 2 equiv DIPEA, DMF, 150 °C, 2 h, 35%; (c) $BnNH_2$ (or HSBn), DIPEA, 1,4-dioxane, 160 °C, 14 h, 48%; (d) TFA, 110 °C, 80%.

Table 2. p38 Inhibitory activities for **32–39**



Compd	X	R	R′	p38a	p38b
				IC_{50} (nM)	IC_{50}(nM)
32	NH	H	H	113	980
33	NH	F	F	340	42%@1000
34	NH	H	Cl	14%@1000	12%@1000
35	NH	Cl	Cl	9%@1000	15%@1000
36	S	H	H	42%@1000	13%@1000
37	S	F	F	21%@1000	6%@1000
38	S	H	OMe	−8%@1000	3%@1000

As we predicted, because of limited space in hydrophobic pocket 1, especially the tight fit in the para-position of the aromatic group, the p38α/β data for the compounds **36**, **37** and **38** in Table 2 have much lower activities comparing with the corresponding compounds in Table 1. Moreover, bigger *para*-substituent R′ of the aromatic group in both substituted benzyl amines, such as **32**, **33**, **34**, **35**, and benzyl thioethers, such as **36**, **37**, **38**, gave lower activities. Those observations further identified the tight fit in hydrophobic pocket 1 for the phenyl group.

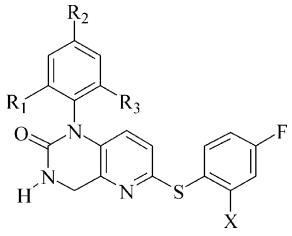
Investigation of hydrophobic pocket 2 has been performed by testing a series of analogues with different substituents in the N-1 aromatic ring. As demonstrated in Table 3, compounds possessing ortho substituted phenyl were dramatically more potent than the corresponding *ortho* unsubstituted **64**. It appears that the primary role of the *ortho* substituent is to maintain an appropriate orientation of the phenyl substituent.

Substituents with active proton at the *ortho* position, such as **50**, **51**, **52**, **53**, **54**, were not tolerated. Bulky group, such as **55**, will also dramatically reduce the activity.

Molecular modeling studies suggested an additional hydrogen bonding interaction with residue Y35 of p38 may be possible if a suitable hydrogen bond acceptor was present in the *para* position of the phenyl. A methyl ester substituent **56** in the *para* position did produce a slight increase activity over the parent compound **60**. A *para* acetyl moiety **57** also produced a slight increase in activity over the parent **60**. Amide analogues **59** and **61** produced same or attenuated activity especially for amides derived from larger amines.

In summary, we have described the results of optimization from our investigation for both hydrophobic

Table 3. p38 Inhibitory activities for designed pyrido-pyrimidones: N-1 arylsulfides



Compd	R1	R2	R3	p38a	p38b	X
				IC_{50} (nM)	IC_{50} (nM)	
39	Cl	COOMe	Me	36	250	
40	Cl	H	Et	38	200	
41	Cl	COMe	Me	47	80%@300	
42	Cl	H	F	48	86%@2000	
43	Cl	H	Me	54	270	
44	Cl	NO_2	Me	66	66%@1000	
45	Cl	H	COOMe	80	67%@1000	
46	Me	H	OMe	100	66%@1000	
47	Cl	H	CF_3	110	950	
48	Me	H	H	180	52%@1000	Cl
49	Cl	CF_3	Cl	230	56%@1000	
50	Cl	H	CH_2OH	46%@1000	23%@1000	
51	Cl	H	$COONH_2$	46%@1000	10%@1000	
52	Cl	H	NH_2	29%@1000	11%@1000	
53	Me	H	OH	20%@1000	16%@1000	
54	Cl	H	COOH	14%@1000	0%@1000	
55	Ph	H	H	28%@1000	16%@1000	
56	Cl	COOMe	Cl	9	73	
57	Cl	COMe	Cl	16	210	
58	Cl	H	Me	19	220	
59	Cl	$COONH_2$	Cl	23	220	
60	Cl	H	Cl	25	250	F
61	Cl	CONH–$(CH_2)_2$–NME_2	Cl	80	1230	
62	Cl	COOH	Cl	96	530	
63	Cl	H	H	69	530	
64	H	H	H	600	26%@10,000	

pocket 1 and hydrophobic pocket 2 in p38 binding site for 3,4-dihydropyrido[3,2-*d*]pyrimidones. In order to fit hydrophobic pocket 2, the best aromatic groups in the N-1 position should have 2,6-disubstituents with size similar to chlorine, such as methyl. The *para* position in those aromatic rings have bigger space and possibly produce an additional hydrogen binding interaction with Y35 of p38 to increase activity, such as **56** and **57**. As for the hydrophobic pocket 1, the optimized substituents in aromatic ring are also *ortho* chlorine or methyl group. The space in *para* and *meta* positions are very small, only fluorine and proton can fit in. Compounds **9**, **10**, **56**, **57**, **58**, **59** and **60** are the best compounds in this analogues with p38α IC_{50} <30 nM. All of those best compounds have optimized substituents in the aromatic rings.

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