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LETTER

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The Discovery of VX-745: A Novel and Selective p38α Kinase Inhibitor

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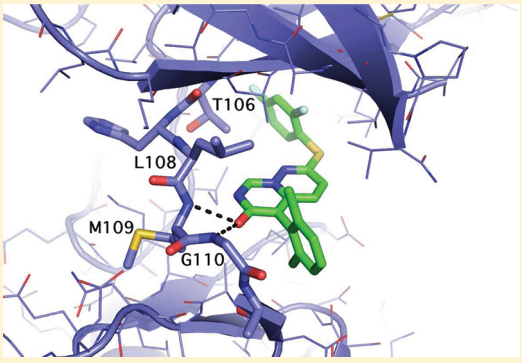
Supporting Information

ABSTRACT: The synthesis of novel, selective, orally active 2,5-disubstituted 6H-pyrimido[1,6-*b*]pyridazin-6-one p38α inhibitors is described. Application of structural information from enzyme−ligand complexes guided the selection of screening compounds, leading to the identification of a novel class of p38α inhibitors containing a previously unreported bicyclic heterocycle core. Advancing the SAR of this series led to the eventual discovery of 5-(2,6-dichlorophenyl)-2-(2,4-difluorophenylthio)-6H-pyrimido[1,6-*b*]pyridazin-6-one (VX-745). VX-745 displays excellent enzyme activity and selectivity, has a favorable pharmacokinetic profile, and demonstrates good in vivo activity in models of inflammation.



KEYWORDS: p38 inhibitor, VX-745, inflammatory diseases

Mammalian mitogen-activated protein (MAP) kinases are serine/threonine kinases that mediate pro-inflammatory cytokine biosynthesis at the transcriptional and translational level.[1−3] Tumor necrosis factor α (TNFα) and interleukin-1β (IL-1β) are cytokines involved in various cellular functions and, when present in elevated levels, are associated with the pathogenesis of a variety of inflammatory diseases, including rheumatoid arthritis (RA).[4] It is known that small molecule inhibitors of p38α MAP kinase (i.e., inhibitors of the p38α and p38β isoforms) suppress the production of TNFα and IL-1β in vitro and in animal models,[5−8] suggesting that the stress-activated signal transduction pathway leading to these cytokines is at least partially regulated by p38. The clinical success in the treatment of RA patients by targeting TNFα mediated inflammatory pathways using, for example, the soluble TNFα receptor fusion protein etanercept (Enbrel) and the monoclonal anti-TNFα products infliximab (Remicade) and adalimumab (Humira) provides the rationale of targeting these cytokines for therapeutic effect.[9−14] Thus, novel orally administered small molecules that selectively inhibit p38 would be desirable to provide therapeutic intervention for inflammatory disorders and to more clearly define the physiological role of this protein kinase-mediated pathway.

First disclosed in 1994, pyridinylimidazole compounds, exemplified by SB 203580 (**1**) and VRT-19911 (**2**) (see Figure 1), block the production of TNFα and IL-1β from monocytes stimulated by bacterial lipopolysaccharides (LPS) through the inhibition of p38 MAP kinase.[2] Compound **1** has been shown to be effective in animal models of arthritis, bone resorption, and endotoxin shock.[15] Compound **1** is a selective inhibitor of p38α and p38β (IC_{50} = 0.1 and 0.43 μM respectively, but does not inhibit the closely related p38γ or p38δ kinases or other MAP kinase family members such as the extracellular-signal regulated kinases (ERK) and most isoforms of the c-Jun N-terminal kinases (JNKs).[16−18]

Compound **2** has been shown to bind in the ATP binding site of p38α and is a selective and potent inhibitor with a K_i of 60 nM.[19] However, it has been previously reported that the presence of the pyridyl moiety in **1** and **2** leads to significant inhibition of hepatic cytochrome p450 isozymes in vitro,[20] rendering these compounds unsuitable for development in the treatment of chronic disease.

Analysis of our previously reported crystal structure of unphosphorylated p38α MAP kinase bound to **2**[21] facilitated the early design and eventual discovery of novel p38 inhibitors. We utilized virtual screening and a diverse selection of shape similarity methods to search commercially available compound databases for molecules with similar configuration, yet different chemical connectivity when compared to **2**. The selected compounds were screened for p38 inhibition, and based on this approach, we identified several inhibitors of p38α in the range of IC_{50} = 1−30 μM. We report here the optimization of one pyridazine-containing class of inhibitors, leading to the identification of VX-745 (**3**), a first-generation p38 inhibitor clinical candidate.

Structure **4** (see Figure 2), a commercially procured sample, emerged as a p38α enzyme inhibitor screening hit[22] selected for further evaluation. In an attempt to resynthesize **4**, we performed

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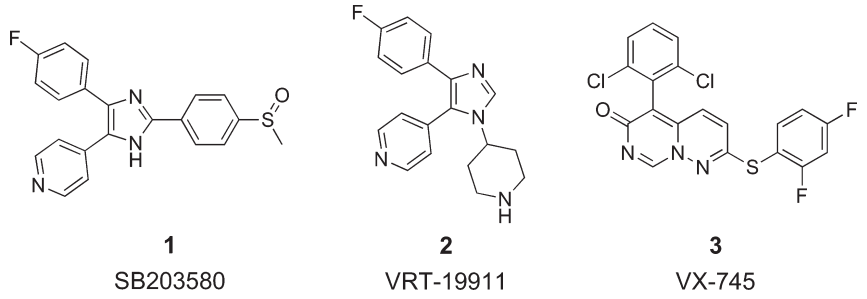

Figure 1. Inhibitors of p38 MAP kinase.

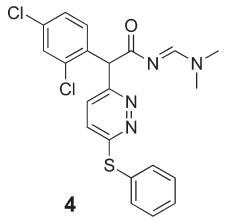

Figure 2. Structure of screening hit, as reported by vendor.

the synthesis outlined in Scheme 1.[23] 2,4-Dichlorophenylacetonitrile (**5**) was treated with sodium *tert*-butoxide followed by 3,6-dichloropyridizine to give the corresponding pyridazinylphenylacetonitrile (**6**). Displacement of the second chloro group with phenylthiosodium gave the 3,6-disubstituted pyridazine (**7**). Compound **7** was subsequently hydrated to amide **8** using concentrated sulfuric acid. The attempted synthesis of **4** from **8** using DMF−DMA in toluene at 100 °C did not result in the desired product but afforded a product in which the *N*,*N*-dimethyl group was absent from the ^{1}H NMR spectrum. This was confirmed by mass spectrometry, which showed m/z = 400 (M + H), not 445 as might be expected, indicating the loss of dimethylamine. We hypothesized the *in situ* formation of compound **4**, and tautomerization of the α-pyridazine ring system to compound **9** allowed for nucleophilic collapse of the pyridazine ring nitrogen on the ethanamide carbon to afford compound **10**.

Small molecule X-ray crystallography[24] of a closely related analogue (**23**), also prepared via Scheme 1, confirmed the structure we pursued was not **4**, as assigned by the vendor, but 5-(2,4-dichlorophenyl)-2-(phenylthio)-6H-pyrimido[1,6-*b*]pyridazin-6-one (**10**). The core bicyclic system, 6H-pyrimido[1,6-*b*]pyridazin-6-one, was, at this time, unreported in the literature.

Having developed a practical synthetic route, we initiated exploration of the SAR of the two pendant aryl rings. Initial studies focused on the substitutions of the directly attached phenyl ring (5-position substituent of pyrimido[1,6-*b*]pyridazin-6-one: Table 1), prepared from commercially available phenylacetonitriles, by the same methods shown in Scheme 1. Evaluation of the products **10**−**21** (Table 1) indicated that the unsubstituted phenyl (**11**) and the 4-substituted systems **16**, **17**, and **18** showed poor enzyme inhibitory activity. Introduction of a 2-substituent (**12**−**15**) showed modest potency, similar to the screening hit **10**, suggesting that, of the two substituents, the 2-chloro is the key to enzyme activity. The 2,6-dichloro substitution emerged as optimal, as indicated by compound **21**, which also demonstrated corresponding inhibition of IL-1β and TNFα release from lipopolysaccharide (LPS) treated human peripheral blood mononuclear cells (PBMCs). For other 2,6-disubstituted systems examined (**19**, **20**), potency was approximately 10-fold weaker than that of **21**.

With **21** representing optimal substitution of the 5-aryl ring, we focused on the S-linked phenyl ring at the pyrimido-[1,6-*b*]pyridazin-6-one 2-position (Table 2) using commercially available thiophenols. Addition of a 4-fluoro group, exemplified by **23**, resulted in a slight improvement in enzyme and cellular activity versus the corresponding unsubstituted thiophenyl analogue **21** (Table 1). Small alkyl groups or halogens at the *meta* position resulted in a reduction of affinity, as in **24**, **25**, **26**, **30**, **31**, and **32** without regard to the nature of the substituent at the *para* position. Small polar functionalities at the *ortho* position were tolerated, as in **29** and **33**. However, the most significant improvement was observed with a 2,4-dihalo substitution of the thiophenol. The 2-chloro-4-fluoro compound **34** showed excellent potency, while the 2,4-difluoro exhibited the best potency, resulting in **3**. As before, cellular data tracked enzyme inhibition. Compound **3** exhibits PBMC IL-1β and TNFα IC_{50} values of 45 and 51 nM, respectively. Compound **3** is also effective in whole blood, blocking IL-1β and TNFα release with IC_{50} values of 150 and 180 nM, respectively. Conversely, compound **3** does not affect proliferation of phytohemaglutinin-stimulated PBMCs up to 20 μM, indicating cellular selectivity for p38 versus other kinases.

The X-ray cocomplex of **3** and p38α at 2.4 Å resolution (Figure 3) shows favorable van der Waals interactions that stabilize the interaction of the 2,6-dichloro phenyl ring with p38.[25] Each chlorine atom occupies a hydrophobic pocket with contact to residues V30, L108, A157, and L167. The phenyl ring itself makes favorable van der Waals interactions with backbone residue atoms G110, A111, and D112. The *para* position of the ring faces bulk solvent. The 2,4-difluorophenyl ring of **3** occupies a hydrophobic pocket at the gatekeeper (T106) residue and makes extensive van der Waals contacts. In addition, the enzyme facilitates the binding of **3** by flipping the G110 backbone.[26] This flip is possible because glycine lacks an α-carbon substitution. This unique enzyme conformation allows the carbonyl oxygen of **3** to accept an additional H-bond from the p38 backbone. This combination of interactions is a key determinant of enzyme specificity.[19,21]

The lead compound, **3**, was tested against the different p38 isozymes and closely related MAP kinases (ERK, JNK) as well as a panel of 50 other kinases to examine its selectivity profile. Compound **3** showed a promising selectivity profile, with 20-fold selectivity for p38α over p38β (K_i = 220 nM),[27] and no significant inhibition of other MAP kinases (with the exception of MKK6, itself an activator of p38)[28] or any of the other

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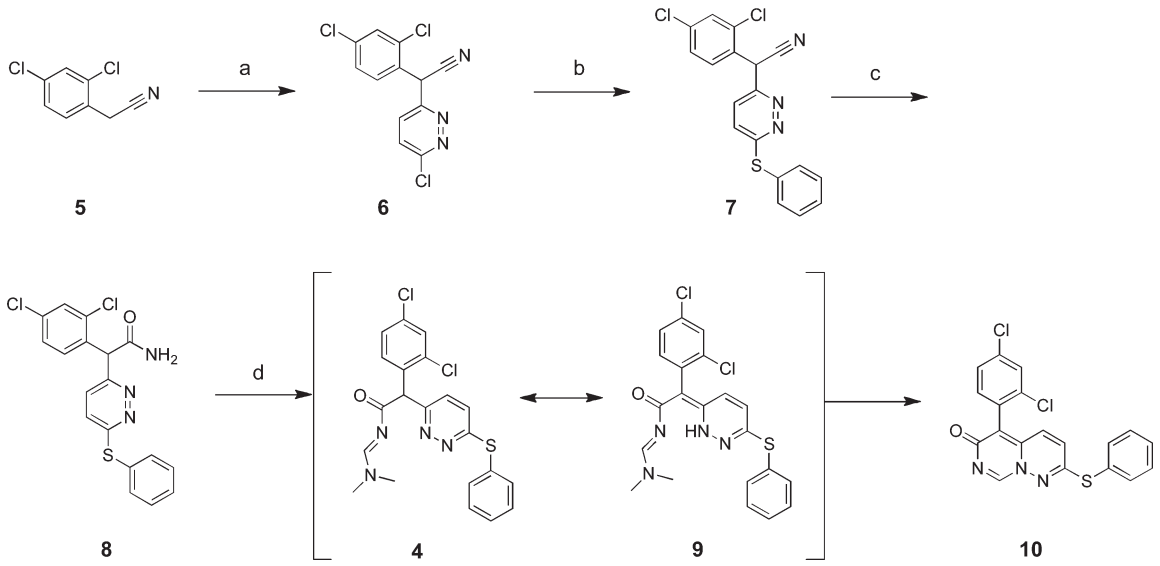
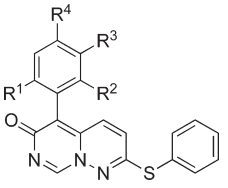
Scheme 1[a]



[a] Reagents and conditions: (a) 3,6-dichloropyridazine, NaO^{t}Bu, THF, 15−54%; (b) thiophenol, K_2CO_3, THF, reflux, 40−92%; (c) cH_2SO_4, 100 °C, 45−100%; (d) DMF−DMA, 100 °C, 41%.

Table 1. Inhibition of p38α and PBMC Cytokine Release by 5-Aryl-2-(phenylthio)-6H-pyrimido[1,6-*b*]pyridazin-6-ones



compd	R^1	R^2	R^3	R^4	p38α IC_{50} (μM)[a]	PBMC IL-1β IC_{50}[b] (μM)	PBMC TNFα IC_{50}[b] (μM)
10	Cl	H	H	Cl	5	20	20
11	H	H	H	H	>20	ND[c]	ND
12	Me	H	H	H	4.4	3.5	9.7
13	OMe	H	H	H	12	2.8	>20
14	CF_3	H	H	H	11	2.1	>20
15	Cl	H	H	H	4.1	5.0	19.9
16	H	H	H	F	>20	15	>20
17	H	H	H	OMe	>20	20	>20
18	H	H	Cl	Cl	>20	>20	>20
19	CF_3	F	H	H	1.7	1.0	3.1
20	F	F	H	H	3.5	3.7	15.3
21	Cl	Cl	H	H	0.25	0.40	0.46

[a] Assayed according to ref 22. [b] See Supporting Information for assay details. [c] ND = not determined.

50 kinases tested at 2 μM concentration (see Supporting Information). Similar selectivity results have been reported elsewhere.[29]

The pharmacokinetic parameters obtained for **3** in three species are summarized in Table 3. Systemic clearance was slightly higher than hepatic blood flow for rat and dog, and **3** appears to have significant extravascular distribution in all three species studied, as indicated by the volume of distribution at steady state. The oral pharmacokinetic profile of **3** in TPGS/PEG-400/water (2:7:1) was characterized in male BALB/c mice, Sprague−Dawley rats, and beagle dogs. The results of these single dose studies showed that **3** has excellent bioavailability in all three species (87%, 56%, and 69%, respectively). Compound **3** demonstrated a longer half-life following oral administration as compared to IV administration, suggesting an absorption-rate limited elimination process in the three species. Compound **3** is neither a significant inhibitor nor an inducer of human hepatic cytochrome p450 isozymes. The IC_{50}

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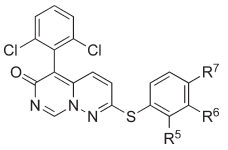
Table 2. Inhibition of p38α and PBMC Cytokine Release by 5-(2,6-Dichlorophenyl)-2-(arylthio)-6H-pyrimido[1,6-*b*]pyridazin-6-ones



compd	R^5	R^6	R^7	p38α IC_{50} (μM)	PBMC IL-1β IC_{50} (μM)	PBMC TNFα IC_{50} (μM)
21	H	H	H	0.25	0.40	0.45
22	H	H	Me	1.4	0.37	0.87
23	H	H	F	0.123	0.099	0.293
24	H	F	H	0.45	0.44	0.72
25	H	Cl	H	0.28	0.35	0.90
26	H	Me	H	1.4	0.21	0.28
27	Et	H	H	0.75	0.98	2.8
28	Me	H	H	0.20	0.33	3.7
29	OH	H	H	0.36	0.14	0.49
30	H	Cl	Cl	0.8	0.97	1.2
31	H	Me	Me	1.9	2.4	3.5
32	H	Cl	F	0.5	0.18	0.48
33	NH_2	H	F	0.22	0.28	1.2
34	Cl	H	F	0.036	0.053	0.275
3	F	H	F	0.009	0.045	0.051
35	Me	H	Cl	0.18	0.18	0.16
36	Me	H	Me	0.26	0.69	0.32

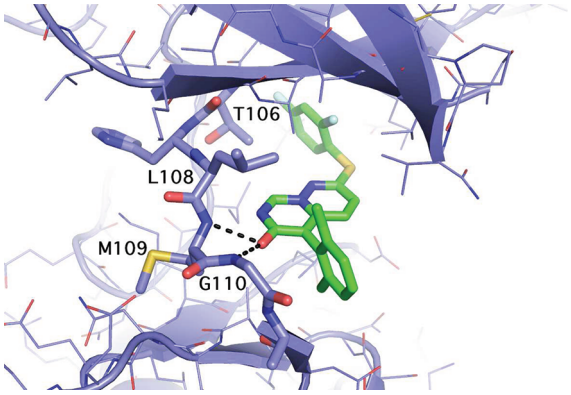


Figure 3. Structure of **3** bound to p38.

values for the inhibition of CYPs 3A4, 2D6, 2C19, 2C9, and 1A2 were determined to be >40 μM. The fraction of **3** bound to plasma protein was 98% and 92% in the rat and the dog, respectively.

To evaluate the in vivo therapeutic potential of compound **3**, we employed the murine collagen-induced arthritis model of human rheumatoid arthritis, in which DBA/1J mice were immunized twice with chick type II collagen. Treatment was initiated once an inflammation score of 2 in each paw had been reached, corresponding to focal swelling of the wrist joint. Results showed that mice treated with 2.5, 5, and 10 mg/kg of compound **3** twice daily for 20 days had 27%, 31%, and 44% improvement in the inflammatory scores, respectively, when compared to vehicle-treated mice at the end of treatment

Table 3. Pharmacokinetic Properties of 3

parameter	rat	mouse	dog
IV dose (mg/kg)	4.8	2	13.8
n	3	3	4
$T_{1/2}$ (h)	1.9	2.6	1.5
V_{ss} (L/kg)	3.9	2.2	2.3
AUC (μg·h/mL)	1.26	1.54	6.12
Cl (mL/min/kg)	63	21	39
PO dose (mg/kg)	43	40	33
n	3	3	4
$T_{1/2}$ (h)	4.5	4.5	4.5
C_{max} (μg/mL)	0.89	7.85	1.37
AUC (μg·h/mL)	6.3	26.96	4.34
F (%)	56	87	69

(Table 4; see Supporting Information for details). In addition, histological scores showed a 32−39% protection of bone and cartilage erosion.

Compound **3** (VX-745) and other analogues described in this study represent the first known examples of 5-phenyl-2-(phenylthio)-6H-pyrimido[1,6-*b*]pyridazin-6-ones as p38 inhibitors. These compounds show potent inhibition of the key inflammatory mediators, IL-1β and TNFα, production in vitro in isolated PBMCs and whole blood. Compound **3** has also been shown to demonstrate anti-inflammatory efficacy in an animal model of rheumatoid arthritis and was designated for further development.[30]

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Table 4. Therapeutic Effect of 3 in Type II Murine Collagen-Induced Arthritis (CIA) Model

	clinical score on paw inflammation[a]			histology score[b]		
dose	mean[a]	SEM	% inh	mean[b]	SEM	% inh
vehicle (100% polyethylene glycol)	3.81	0.13	NA	2.04	0.09	NA
compound **3**, 2.5 mg/kg	2.77	0.17	27	1.33	0.09	35
compound **3**, 5 mg/kg	2.65	0.28	31	1.38	0.11	32
compound **3**, 10 mg/kg	2.13	0.13	44	1.25	0.09	39

[a] Clinical scores measured on final day 20. Clinical scores are defined in the Supporting Information. [b] Histology scores are defined in the Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information. Full experimental details for representative compounds synthesized, HRMS results, description of assays, animal efficacy studies, and X-ray crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(22) Compounds were assayed by the coupled assay method described in:Fox, T.; Coll, J. T.; Xie, X.; Ford, P. J.; Germann, U. A.; Porter, M. D.; Pazhanisamy, S.; Fleming, M. A.; Galullo, V.; Su, M. S.; Wilson, K. P. *Protein Sci.* **1998**, *7*, 2249Reactions were carried out in 100 mM HEPES pH 7.6, 10 mM $MgCl_2$, 25 mM NaCl, 1 mM DTT, 20 μg/mL BSA, and 1.5% DMSO. Final substrate concentrations in the assay were 100 μM ATP and 200 μM peptide (KRELVEPLTPSGEAPNQALLR). Assays were carried out at 30 °C using 15 nM p38. The final concentrations of the components of the coupled enzyme system were 2.5 mM phosphoenolpyruvate, 200 μM NADH, 140 μg/mL pyruvate kinase, and 10 μg/mL lactate dehydrogenase.

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(24) See Supporting Information for X-ray crystallographic data.

(25) PDB accession code 3fc1.

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(30) Compound **3** (VX-745) attained clinical proof-of-principle correlating inhibition of p38 with anti-inflammatory effect, but clinical development was suspended following adverse effects findings in nonclinical studies. http://www.prnewswire.com/news-releases/vertex-moves-to-re-allocate-resources-from-vx-745-in-p38-map-kinase-program-to-accelerate-development-of-second-generation-drug-candidates-vx-702-and-vx-850-72104487.html.