Hybrid-Designed Inhibitors of p38 MAP Kinase Utilizing *N*-Arylpyridazinones

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Abstract: Imidazo[1,2-*a*]pyridyl *N*-arylpyridazinones were hybridized from the classic pyridinylimidazoles and the more recent dual hydrogen bond acceptors, resulting in a new structural class of selective p38 MAP kinase inhibitors.

Introduction. TNFα is one of several cytokines that plays a significant role in the development of an acute or chronic inflammatory response. The success of the soluble TNFα receptor fusion protein Enbrel (etanercept)[1] and the monoclonal TNFα antibody Remicade (infliximab)[2] in the treatment of rheumatoid arthritis and Crohn's disease has provided a proof of concept for the treatment of these autoimmune diseases. These biologics are generally well tolerated to date but have drawbacks related to patient cost, efficiency of production, and administration by injection. Therefore, an orally active small-molecule drug that blocks or modulates circulating TNFα remains an attractive therapy. Of the different approaches toward this end, inhibition of p38 MAP kinase results in the suppression of not only TNFα but also IL-1β, another significant proinflammatory cytokine.[3]

The biosynthesis of TNFα and IL-1β occurs predominately in activated monocytes and macrophages via an intracellular signaling cascade that involves the dual phosphorylation (via MKK3 and MKK6) of Thr180 and Tyr182 within a TGY motif of p38 kinase. Phosphorylated p38 subsequently phosphorylates a variety of substrates, including kinases and transcription factors.[4] Two of these substrates that are activated by p38 are MAPKAP kinases 2 and 3, which in turn phosphorylate heat shock protein HSP 27, ultimately leading to gene transcription and protein translation. This cascade of events can be initiated by a wide variety of extracellular stimuli or stresses such as endotoxin bacterial lipopolysaccharide and cytokines, or osmotic shock, heat shock, UV light, ionizing radiation, and oxidative stress. Four isozymes of p38 have been cloned and characterized to include the ubiquitously expressed p38α and p38β; p38γ is primarily expressed in skeletal muscle, and p38δ is highly distributed within lung, kidney, endocrine glandular, and small intestinal tissues.[5]

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Historically, SmithKline Beecham demonstrated early on that an orally active small molecule, SB203580 (**1**), could reduce TNFα levels in vivo, validating the pyridinylimidazole structural class.[6] Significant improvements to the kinase selectivity and whole blood potency of **1** were subsequently accomplished at Merck with the discovery of the (*S*)-*sec*-phenethylamine moiety represented in the second-generation pyridinylimidazole **2**.[7] Within a few years, Vertex demonstrated that even greater kinase selectivity could be obtained with VX-745 (**3**).[8] Human clinical trials with **3** have been reported,[9] reviewed,[10] and claimed to have achieved proof of concept, although this press release lacks scientifically reviewed data.[11] In an effort to increase the whole blood potency of **3**, chemists at Merck designed inhibitors such as **4** that retained the structural attributes of **3** in addition to the basic saturated heterocycle present in **2**.[12]

Design. The modeling view in Figure 2 is based on the X-ray crystal structure of p38α in complex with the soaked inhibitor **4**.[12] Hybrid **6** was energy-minimized outside the protein and then docked into the enzyme active site using the binding orientation of **4** as a guide.[13] The carbonyl oxygen of the urea moiety of **4** and that of the pyridazinone ring of **6** are able to accept two hydrogen bonds from the backbone amide NHs of Met109 and Gly110. The 2-chloro-4-fluorophenyl ring of **6** appears to superimpose quite well with the 2,4-difluorophenyl ring of **4** near Thr106.[14] However, the tolyl moiety of **6** is situated in the binding pocket near Val30 differently from the 2,6-dichlorophenyl ring of **4**.

Within the MAP kinase family, the JNK Ser/Thr protein kinases bear high homology to p38.[15] Crystal structure data reveal that inhibitors with a dual hydrogen bond motif at Met109 and Gly110 of p38α induce a peptide flip at Gly110 (Figure 2). Since JNK1−3 kinases contain an aspartic acid at a position equivalent to the position of Gly110 in p38α, the Ramachandran space of this Asp residue is much more restricted. This rotational restriction could result in a hindered peptide flip, an inefficient dual hydrogen bond, and reduced affinity for these inhibitors.[16] VX-745 (**3**) was reported to be more than 1000-fold selective for p38α over JNK kinases.[17] Since one of the closest homologues to p38α kinase is JNK2β2, we counterscreened this JNK isozyme for inhibitor selectivity.

This study describes a new class of p38 inhibitors structurally hybridized from the prototypical pyridinylimidazoles and the dual hydrogen bond acceptors represented by **3** and **4**. The pyridazinone moiety[18] was selected as a dual hydrogen bond acceptor because of its diversity based on available arylhydrazines. The imidazo[1,2-*a*]pyridine core[19] was chosen for its basicity to suppress serum albumin binding.[20] On the basis of this design, compounds such as **5** and **6** were anticipated to be highly selective for p38 over JNK kinases and more potent in whole blood relative to VX-745 (**3**).

Chemistry. One highly convergent approach to the synthesis of these molecules is shown in Scheme 1. Intermediate **7** was prepared[21] and then selectively cyclized to the energetically preferred six-membered

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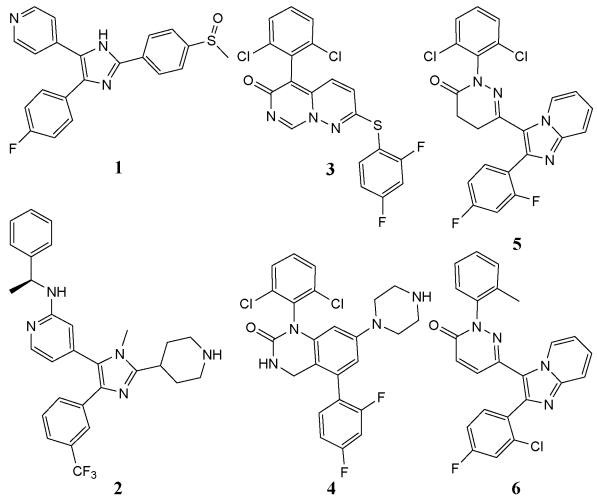


Figure 1. Selected p38 inhibitors.

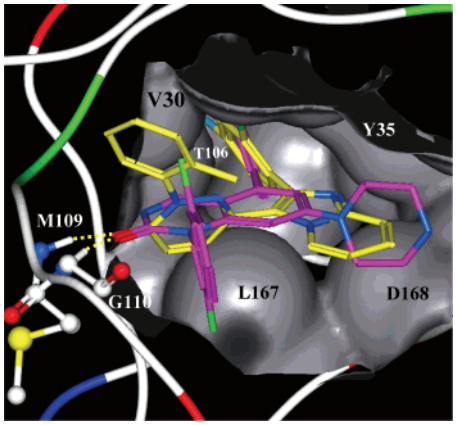
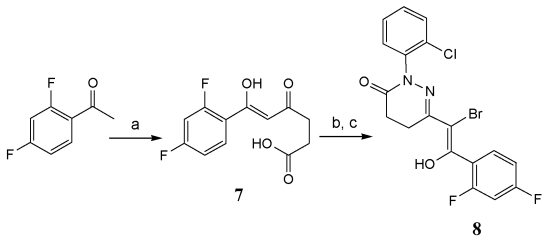


Figure 2. Modeling view of **6** (yellow) overlaid on the crystal structure of p38α MAP kinase in complex with **4** (magenta).

Scheme 1[a]

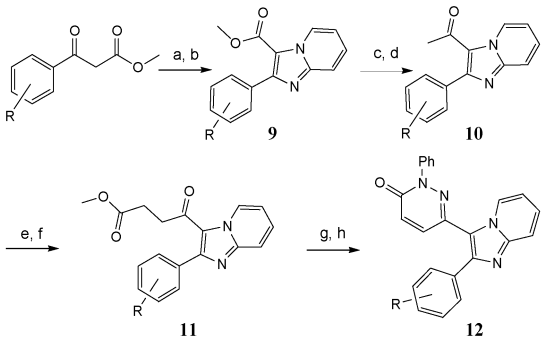


[a] (a) LiHMDS, succinic anhydride, THF, −78 °C → 23 °C, 2 h, 20%; (b) 2-chlorophenylhydrazine hydrochloride, NaOAc, HOAc, 110 °C, 2 h, 50%; (c) *n*-Bu_4NBr_3, 2:1 CH_2Cl_2/CCl_4, 0 °C → 23 °C, 4 h, 99%.

ring dihydropyridazinone. After bromination to form **8**, a Chichibabin based imidazopyridine synthesis was envisioned using 2-aminopyridine. This reaction failed repeatedly apparently because of the absence of any microscopic tautomerization of **8** to the required α-bromoketone.

Subsequently a more linear synthesis was developed that provided the targeted imidazopyridyl *N*-arylpyridazinones (Scheme 2). Readily available benzoyl acetates were brominated under ionic conditions, and the α-monobromides were subjected to the Chichibabin based imidazopyridine synthesis using 2-aminopyridine to form haloaryl analogues of **9**.[22] The methyl esters were converted to the Weinreb amides under basic conditions,[23] followed by methyl ketone generation to

Scheme 2[a]



[a] (a) *n*-Bu_4NBr_3, CH_2Cl_2, 0 °C → 23 °C, 3 h; (b) 2-aminopyridine, EtOH, 60 °C, 14 h, ∼70% (two steps); (c) Me(MeO)NH/HCl, *i*-PrMgCl, THF, −10 °C, 1 h, ∼74%; (d) MeMgBr, THF, 0 °C, 30 min, ∼97%; (e) LiHMDS, $BrCH_2CO_2t$-Bu, THF, −78 °C → 23 °C, 2 h; (f) TFA, CH_2Cl_2, 0 °C → 23 °C, 4 h, then HCl_g, MeOH, 0 °C, 1 h, ∼40% (two steps); (g) arylhydrazine hydrochloride, NaOAc, HOAc, H_2O, 130 °C, 20 h, ∼30%; (h) $CuCl_2$, CH_3CN, 85 °C, 72 h, ∼50%.

form **10** in good overall yield from the starting benzoyl acetates. The methyl ketones (**10**) were then homologated to the γ-ketoesters (**11**) in modest yield, followed by cyclization with several different phenylhydrazines to form the *N*-aryldihydropyridazinones. Oxidation proved to be extremely slow and often incomplete, yet absent of any byproducts to provide the desired pyridazinones (**12**).

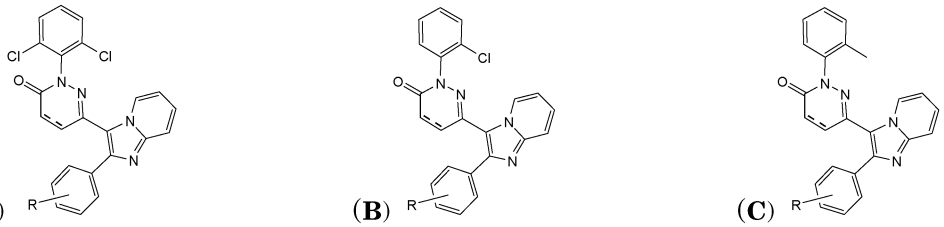
In Vitro SAR. The biological data for the *p*-fluoro derivative **13** represent a baseline for SAR in the *N*-(2,6-dichlorophenyl)pyridazinone series (**A**, Table 1). Unlike pyridinyl imidazole **2**, the *m*-trifluoromethyl group was not tolerated in **14** at the level of the enzyme, indicating a different binding orientation from **2**. This result is consistent with the activity of the dual hydrogen bond acceptor inhibitors relative to the pyridinylimidazoles. Likewise the *o,m*-dichloro analogues **15** and **16** displayed poor enzyme inhibition. As with **4**,[12] combined substitution at the ortho and para positions was ideal for enzyme affinity and is exemplified by the 2-chloro-4-fluoroaryl analogues **17** and **18**.

To reduce the lipophilicity of **17** and **18**, the 2,4-difluoroaryl analogues **5** and **19** were prepared.[24] As anticipated, the IC_{50} shift from enzyme inhibition to THP-1 cellular activity was reduced with **5** relative to **17**. However, the oxidation of **5** to **19** resulted in an overall decrease in activity observed not only in whole blood but also in the p38 inhibition assay.

Another approach to reduce the lipophilicity of **17** and **18** was to remove one chlorine atom and prepare the *N*-(2-chlorophenyl)pyridazinone series (**B**, Table 1). Although the IC_{50} shift between enzyme affinity and THP-1 activity was attenuated for compounds **20** and **21** relative to **17** and **18**, a corresponding reduction in p38 affinity was also observed, resulting in THP-1 activity similar to that of **17** and **18**. The *m*-trifluoromethylaryl analogues **22** and **23** displayed weak activity similar to that of **14**.

On the basis of the patent literature[25] and the SAR of **4**, the *N*-(2-chlorophenyl)pyridazinone (**B**) binding subunit was replaced with an *N*-(2-tolyl)pyridazinone moiety (**C**, Table 1). Although a reduction in lipophilicity was anticipated, a potential reduction in p38 affinity relative to the *N*-(2,6-dichlorophenyl)pyridazinone series

Table 1. In Vitro Activity of *N*-Arylpyridazinone Analogues[a]



compd	R	bond order	IC$_{50}$ (nM) p38α[b]	p38β[b]	JNK2β2[c]	THP-1[d]	hWB[e]
(**A**) *N*-(2,6-Dichlorophenyl)pyridazinone Analogues							
13	4-F	2	49	153	40% @ 10000	70	60% @ 4000
14	3-CF_3	2	1000	50% @ 5000	0% @ 10000	41% @ 4000	
15	2,3-diCl	1	2000	57% @ 5000	26% @ 5000	59% @ 4000	
16	2,3-diCl	2	270	890	14% @ 5000	65% @ 4000	
17	2-Cl−4-F	1	42	670	0% @ 10000	180	
18	2-Cl−4-F	2	14	64	23% @ 10000	161	
5	2,4-diF	1	8	63	22% @ 10000	10	205
19	2,4-diF	2	22	112	11% @ 5000	89% @ 4000	1000
(**B**) *N*-(2-Chlorophenyl)pyridazinone Analogues							
20	2-Cl−4-F	1	208	2600	0% @ 10000	400	
21	2-Cl−4-F	2	76	171	0% @ 10000	161	
22	3-CF_3	1	171	355	0% @ 10000	72% @ 4000	
23	3-CF_3	2	344			48% @ 4000	
(**C**) *N*-(2-Tolyl)pyridazinone Analogues							
24	2,3-diCl	1	36% @ 100	33% @ 100	0% @ 1000	170	
25	2,3-diCl	2	22	44	15% @ 10000	6	500
26	2-Cl−4-F	1	14	94	4% @ 5000	22	220
6	2-Cl−4-F	2	9	28	16% @ 5000	20	100
27	2,4-diF	1	8	78	4% @ 10000	14	440
28	2,4-diF	2	26	140	15% @ 5000	68	870

[a] Values are an average of two or greater individual assays. On average, repeat determinations differed by ±20%. [b] Filter plate binding of compound versus [γ-^{33}P]ATP in active recombinant murine FLAG-p38 fusion protein (GST-ATF2).[7] [c] HTRF detection of GST-ATF2 phosphorylated by recombinant human flag-tagged JNK2β2 fusion protein. [d] LPS-induced human TNFα inhibition detected by ELISA in THP-1 cell supernatants. [e] LPS-induced human TNFα inhibition in human whole blood quantified by immunoassay.[7]

(**A**) was a concern. In practice, the 2-tolyl analogues **24**−**26** and **6** were more active in functional assays and enzyme inhibition relative to the corresponding 2,6-dichlorophenyl analogues **15**−**18**. Analogues **26** and **6** were also superior to the related 2-chlorophenyl derivatives **20** and **21**.

For in the 2-tolyl series, attention was turned to the 5- to 10-fold IC$_{50}$ shift observed between the THP-1 and whole blood assays of **6** and **26**. The 2,4-difluoroaryl analogues were targeted again, this time toward the preparation of **27** and **28**. Once again, a reduction in affinity and functional activity was observed between the 2,4-difluoroaryl analogues **27** and **28**, similar to that between the 2,4-difluoroaryl analogues **5** and **19**.[26] Last, the p38 inhibitors described in Table 1 show consistent p38α selectivity relative to JNK2β2. The most active hybrids, **5** and **6**, demonstrated >1000-fold and >500-fold kinase selectivity, respectively.

PD Profile and in Vitro Metabolism of Compound 6. After the in vitro data shown in Table 1 were obtained, analogue **6** was further evaluated against VX-745 (**3**) in a murine MAPKAP pharmacodynamic (PD) assay (Table 2). In this study, both **3** and **6** were each dosed ip at 3 mg/kg in five mice, resulting in mMAPKAP inhibition of 29% and 42%, respectively. The mMAPKAP inhibition with **6** was statistically significant ($p < 0.05$) relative to vehicle. Importantly, the compound exposure in mice was different for **3** relative to **6**. Plasma concentrations of 1743 and 810 nM for **3** and **6**, respectively, were measured from the same experiment by an ex vivo p38 kinase inhibition assay.[27]

Table 2. Biological Profiles for **3** and **6**

compd	in vitro IC$_{50}$ (nM) p38α	THP-1	hWB	ex vivo mMAPKAP[a] % inhibition	plasma concn (nM)
3	22	150	700	29	1734
6	9	20	100	42	810

[a] BALB/c mice **3** ($n = 5$) and **6** ($n = 5$) were dosed ip at 3 mg/kg in a vehicle of DMSO/EtOH/PEG400/saline (10:10:60:20). Blood was collected for MAPKAP inhibition and plasma concentration at 1 h after dosing.

An additional comparison of **3** with **6** regarding in vitro metabolism served to correlate these plasma concentrations with microsomal stability. Both **3** and **6** were incubated with mouse liver microsomes, resulting in a respective 46% and 9% compound presence after 10 min (Supporting Information). From both this microsomal data and the in vivo plasma concentrations, it is apparent that **6** is more readily metabolized in the mouse relative to **3**, yet **6** appears to be equally, if not slightly more, efficacious relative to **3** in the mMAPKAP PD assay. This result is consistent with the greater in vitro activity of **6** relative to **3** (Table 2).

Conclusion. In summary, a new structural class of p38 kinase inhibitors was designed based ultimately on SB203580 and VX-745. Representative hybrids include imidazopyridyl *N*-arylpyridazinones **5** and **6**, both of which demonstrated low nanomolar enzyme inhibition, ≥1000-fold p38α/JNK2β2 kinase selectivity, and efficacy

in human whole blood. Analogue **6** was evaluated ex vivo in mice, providing 42% inhibition of mMAPKAP activity and further validating this class of p38 inhibitors. Relative to the clinically used VX-745 (**3**), analogue **6** displayed similar efficacy in this pharmacodynamic mouse model.

Supporting Information Available: Methods for molecular modeling, experimental procedures for compound preparation and characterization data, biological assay protocols, and liver microsome stability plots. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Pugsley, M. K. Etanercept: Immunex. *Curr. Opin. Invest. Drugs* **2001**, *2*, 1725.

(2) Bondeson, J.; Maini, R. N. Tumour necrosis factor as a therapeutic target in rheumatoid arthritis and other chronic inflammatory diseases: The clinical experience with infliximab. Remicade. *Int. J. Clin. Pract.* **2001**, *55*, 211.

(3) Chen, Z.; Gibson, T. V.; Robinson, F.; Silvestro, L.; Pearson, G.; Xu, B.; Wright, A.; Vanderbilt, C.; Cobb, M. H. MAP kinases. *Chem. Rev.* **2001**, *101*, 2449.

(4) Ono, K.; Han, J. H. The p38 signal transduction pathway: Activation and function. *Cell. Signalling* **2000**, *12*, 1.

(5) Jiang, Y.; Gram, H.; Zhao, M.; New, L.; Gu, J.; Feng, L.; Di Padova, F.; Ulevitch, R. J.; Han, J. Characterization of the structure and function of the fourth member of p38 group mitogen-activated protein kinases: p38δ. *J. Biol. Chem.* **1997**, *272*, 30122 and references therein.

(6) Gallagher, T. F.; Seibel, G. L.; Kassis, S.; Laydon, J. T.; Blumenthal, M. J.; Lee, J. C.; Lee, D.; Boehm, J. C.; Fier-Thompson, S. M.; Abt, J. W.; Soreson, M. E.; Smietana, J. M.; Hall, R. F.; Garigipati, R. S.; Bender, P. E.; Erhard, K. F.; Krog, A. J.; Hofmann, G. A.; Sheldrake, P. L.; McDonnell, P. C.; Kumar, S.; Young, P. R.; Adams, J. L. Regulation of stress-induced cytokine production by pyridinylimidazoles. Inhibition of CSBP kinase. *Bioorg. Med. Chem.* **1997**, *5*, 49.

(7) Liverton, N. J.; Butcher, J. W.; Claiborne, C. F.; Claremon, D. A.; Libby, B. E.; Nguyen, K. T.; Pitzenberger, S. M.; Selnick, H. G.; Smith, G. R.; Tebben, A.; Vacca, J. P.; Varga, S. L.; Agarwal, L.; Dancheck, K.; Forsyth, A. J.; Fletcher, D. S.; Frantz, B.; Hanlon, W. A.; Harper, C. F.; Hofsess, S. J.; Kostura, M.; Lin, J.; Luell, S.; O'Neill, E. A.; Orevillo, C. J.; Pang, M.; Parsons, J.; Rolando, A.; Sahly, Y.; Visco, D. M.; O'Keefe, S. J. Design and synthesis of potent, selective and orally bioavailable tetrasubstituted imidazole inhibitors of p38 mitogen-activated protein kinase. *J. Med. Chem.* **1999**, *42*, 2180.

(8) Bemis, G. W.; Salituro, F. G.; Duffy, J. P.; Cochran, J. E.; Harrington, E. M.; Murcko, M. A.; Wilson, K. P.; Su, M.; Galullo, V. P. (Vertex Pharmaceuticals, Inc.). Substituted nitrogen containing heterocycles as inhibitors of p38 protein kinase. Patent WO 98/27098, 1998.

(9) Salituro, F. G. VX-745. Presented at the 11th RSC-SCI Medicinal Chemistry Symposium, Churchill College, Cambridge, U.K., September 9−12, 2001.

(10) Boehm, J. C.; Adams, J. L. New inhibitors of p38 kinase. *Expert Opin. Ther. Pat.* **2000**, *10*, 25.

(11) Vertex press release (News Wire, September 24, 2001): 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.

(12) 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. Design and synthesis of potent, orally bioavailable dihydroquinazolinone inhibitors of p38 MAP kinase. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 277.

(13) See Supporting Information for modeling details. Since the glycine-rich loop of p38 (containing Val30 and Tyr35) is known to change conformation significantly, depending on the inhibitor in the active site, the structure of p38 used here is only an approximation of the active site for **6**. Furthermore, it appears that the protein would need to adjust at Val30 to accommodate the tolyl moiety of **6**.

(14) Wang, Z.; Canagarajah, B. J.; Boehm, J. C.; Kassisa, S.; Cobb, M. H.; Young, P. R.; Abdel-Meguid, S.; Adams, J. L.; Goldsmith, E. J. Structural basis of inhibitor selectivity in MAP kinases. *Structure* **1998**, *6*, 1117 and references therein.

(15) Lisnock, J. M.; Tebben, A.; Frantz, B.; O'Neill, E. A.; Croft, G.; O'Keefe, S. J.; Li, B.; Hacker, C.; De Laszlo, S.; Smith, A.; Libby, B.; Liverton, N.; Hermes, J.; LoGrasso, P. Molecular basis for p38 protein kinase inhibitor specificity. *Biochemistry* **1998**, *37*, 16573 and references therein.

(16) p38 G110A and G110D mutants support this observation. Fitzgerald, C. E.; O'Keefe, S. J.; Scapin, G.; et al. Manuscript in preparation. Scapin, G. Selectivity in homologous protein families: protein kinase inhibition. Protein Kinases as Therapeutic Targets in Drug Discovery and Development. Presented in Cambridge, U.K., October 28−29, 2002. Kinase selectivity of p38 over JNK can also be achieved through exploiting the larger hydrophobic pocket of p38α at T106 versus the smaller hydrophobic pocket of JNK, which has a methionine residue in this position.[14,15]

(17) Salituro, F. G. Presented at the 27th National Medicinal Chemistry Symposium; Kansas City, MO, June 13−17, 2000.

(18) Lubsen, J.; Just, H.; Hjalmarsson, A. C.; La Framboise, D.; Remme, W. J.; Heinrich-Nols, J.; Dumont, J. M.; Seed, P. Effect of pimobendan on exercise capacity in patients with heart failure: main results from the pimobendan in congestive heart failure (PICO) trial. *Heart* **1996**, *76*, 223.

(19) Claiborne, C. F.; Claremon, D. A.; Liverton, N. J.; Nguyen, K. T. (Merck & Co., Inc.). Substituted imidazoles having cytokine inhibitory activity. Patent WO 01/22965, 2001. Revesz, L. (Novartis AG). Thiazole and imidazo[4,5-*B*]pyridine compounds and their pharmaceutical use. Patent WO 01/30778, 2001. Dodd, J. H.; Henry, J. R.; Rupert, K. C. (Ortho-McNeil Pharmaceutical, Inc.). Substituted 2-aryl-3-(heteroaryl)-imidazo[1,2-*a*]pyrimidines and related pharmaceutical compositions and methods. Patent WO 01/34605, 2001.

(20) Comparable to imidazole ($pK_a = 7.0$), the pK_a for imidazo[1,2-*a*]pyridine is 6.8. Gilchrist, T. L. *Heterocyclic Chemistry*, 3rd ed.; Addison-Wesley Longman Ltd.: Harlow, U.K., 1997; p 298. Joule, J. A.; Mills, K.; Smith, G. F. *Heterocyclic Chemistry*, 3rd ed.; Stanley Thornes Ltd.: England, 1998; p 437. Albumin, although not exclusive, is a major constituent in whole blood and is a highly lipophilic and lysine-rich basic protein. van de Waterbeemd, H.; Smith, D. A.; Beaumont, K.; Walker, D. K. Property-based design: optimization of drug absorption and pharmacokinetics. *J. Med. Chem.* **2001**, *44*, 1313.

(21) Murray, W. V.; Wachter, M. P. Synthesis and properties of aryl-1,3-dioxo carboxylic acids. *J. Org. Chem.* **1990**, *55*, 3424.

(22) Trapani, G.; Franco, M.; Ricciardi, L.; Latrofa, A.; Genchi, G.; Sanna, E.; Tuveri, F.; Cagetti, E.; Biggio, G.; Liso, G. Synthesis and binding affinity of 2-phenylimidazo[1,2-*a*]pyridine derivatives for both central and peripheral benzodiazepine receptors. A new series of high-affinity and selective ligands for the peripheral type. *J. Med. Chem.* **1997**, *40*, 3109.

(23) Williams, J. M.; Jobson, R. B.; Yasuda, N.; Marchesini, G.; Dolling, U.-H.; Grabowski, E. J. J. A new general method for preparation of *N*-methoxy-*N*-methylamides. Application in direct conversion of an ester to a ketone. *Tetrahedron Lett.* **1995**, *36*, 5461.

(24) The observed IC_{50} shifts from enzyme inhibition to the THP-1 cellular assay to the suppression of TNFα in whole blood were partly attributed to protein binding in these functional assays. Serum protein binding measurements with related structural classes supported this hypothesis.

(25) Salituro, F. G.; Bemis, G. W.; Cochran, J. E. (Vertex Pharmaceuticals, Inc.). Inhibitors of p38. Patent WO 99/64400, 1999.

(26) It is speculated that the smaller *o*-fluorine atom of **19** and **28** allows the rotation of the aryl ring into the plane of the imidazopyridine, both moieties of which may delocalize π-electron density more easily into the oxidized pyridazinone ring and *N*-aryl moiety. This increased planarity, based on a lowered ground-state energy of the molecule, could result in a higher energy barrier toward enzyme binding conformation that requires orthogonal aryl rings (Figure 2).

(27) Blood plasma concentrations of **3** and **6** were calculated by bioassay. The free compound p38 inhibition IC_{50} is multiplied by 1 over the dilution factor of the plasma required to reach 50% inhibition in the p38 assay (EC_{50}). This method does not address active metabolites.

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