From Imidazoles to Pyrimidines: New Inhibitors of Cytokine Release

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On the basis of model imidazole inhibitors of cytokine release, a series of novel pyridinyl pyrimidine derivatives was prepared and tested on their ability to inhibit the release of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) from peripheral blood mononuclear cells (PBMC) and human whole blood. In the pyrimidine series, structure−activity relationships (SARs) similar to those of the imidazole series were found, although generally pyrimidine compounds were less potent. Modification of the substituent at the 2 position of the pyrimidine led to the most active compound **14** which inhibited release of TNF-α (IC_{50} = 3.2 μM) and IL-1β (IC_{50} = 2.3 μM) from PBMC as effectively as the model imidazole inhibitor ML 3163 (TNF-α, IC_{50} = 3.7 μM; IL-1β, IC_{50} = 0.9 μM). Screening in an isolated enzyme assay revealed both imidazole and pyrimidine compounds as inhibitors of p38 MAP (mitogen-activated protein) kinase.

Introduction

Antagonizing proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) has been recognized as a valid goal in the development of new drug candidates for the treatment of chronic inflammatory conditions such as rheumatoid arthritis[1] and inflammatory bowel disease.[2] The scope of anti-TNF-α strategies has recently been reviewed.[3] A number of small molecules lead to reduced cytokine levels in vitro and in vivo by the inhibition of key enzymes which are involved in the biosynthesis of TNF-α and IL-1β.[4,5] Among these enzymes, a serine-threonine kinase termed p38 MAP (mitogen-activated protein) kinase has become one of the main targets, because it is required for the biosynthesis and release of both TNF-α and IL-1β.[6,7] Exemplified by SB 203580 (Table 1), several inhibitors of p38 MAP kinase contain vicinal pyridin-4-yl-/4-fluoro-phenyl groups attached to a core imidazole ring.[8−10] Because of these structural requirements, SB 203580 binds in the ATP pocket of p38 MAP kinase[11−13] where the pyridin-4-yl moiety forms a crucial hydrogen bond with the amide NH of Met109. Various other five-membered heterocycles (e.g., pyrrole or pyrazolone) have been reported to efficiently replace the imidazole system of SB 203580.[14] However, no unambiguous SAR could be established concerning the interaction of the core heterocycle with p38 MAP kinase. No data have been published so far on six-membered heterocycles as suitable scaffolds for the pyridin-4-yl-/4-fluoro-phenyl pharmacophore. Benzylsulfanyl imidazole ML 3163 combines structural features of cytokine release inhibitors SK&F 86002[15] and SB 203580 (Table 1). To elucidate the contribution of the central ring system to inhibition of cytokine release, we have prepared a series of pyrimidine analogues of ML 3163. Herein, we report the synthesis of these pyrimidines and their ability to inhibit cytokine release from human whole blood and peripheral blood mononuclear cells (PBMC). Additional data for inhibition of p38 MAP kinase by selected compounds are provided.

Chemistry

4,5-Bisubstituted pyrimidine-2-thiones **7**−**9,** which served as key intermediates in the synthesis of 2-benzylsulfanyl pyrimidine derivatives, were obtained following the Bredereck methodology[16] (Scheme 1): Reaction of suitably substituted ketones **1**−**3** with dimethylformamide-dimethylacetale gave enaminones **4**−**6**. Subsequent condensation of pyridin-4-yl- and pyridin-3-yl-substituted enaminones **4**−**6** with thiourea afforded the corresponding 4,5-bisubstituted pyrimidine-2-thiones **7**−**9**. This approach, however, was not suitable for the synthesis of the pyridin-2-yl-substituted pyrimidine-2-thione, where reaction of the corresponding enaminone under basic conditions led to immediate decomposition of the starting material. 2-Benzylsulfanyl pyrimidine derivatives **10**−**12a**−**c**, **13a**, and **14** were obtained from pyrimidine-2-thiones **7**−**9** by nucleophilic substitution of the appropriate benzyl chloride derivatives. The analogue **16**, which bears an additional hydrogen donor at the pyrimidine ring similar to the ring NH of the imidazole compounds, was prepared from thiouracil **15** (Scheme 2). Condensation of ethyl 2-(4-fluorophenyl)-3-oxo-3-pyridin-4-yl-propionate[14] with thiourea gave thiouracil **15** which, upon reaction with 1-chloromethyl-4-methanesulfinyl-benzene, furnished pyrimidin-4-ol derivative **16**. IR and ^{13}C-NMR data show that **16** predominantly exists as the pyrimidin-4-ol and not as the tautomeric pyrimidin-4-one. Three regioisomeric benzyl chloride derivatives were applied in the final step of the general synthesis outlined in Scheme 1. While 1-chloromethyl-2-methanesulfinyl-benzene[17] and 1-chloromethyl-4-methanesulfinyl-benzene[18] were synthesized according to literature procedures, 1-chloromethyl-3-methanesulfinyl-benzene **19** was prepared by the following route (Scheme 3): $LiAlH_4$ reduction of 3-methylsulfanyl-benzoic acid[19] yielded phenyl-methanol deriv-

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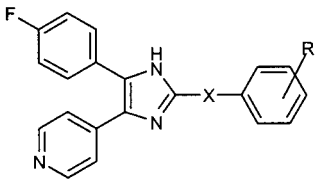
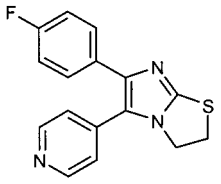
Table 1. Inhibition of Cytokine Release by Imidazole Compounds



SB 203580, ML 3163, 21b, 22

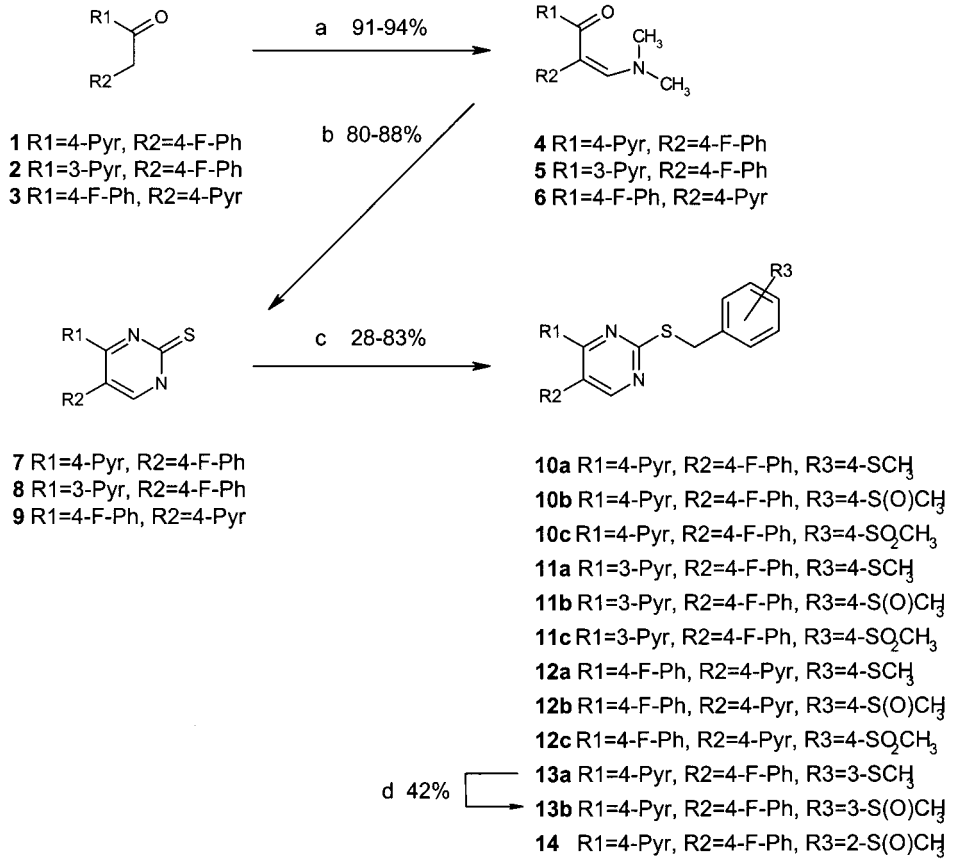


SK&F 86002

			$IC_{50} \pm SEM$ (μM)			
			whole blood		PBMC	
compd	X	R	TNF-α	IL-1β	TNF-α	IL-1β
SB 203580	bond	4-S(O)CH_3	0.94 ± 0.14 (12)[a]	0.35 ± 0.09 (12)	0.59 ± 0.09 (21)	0.037 ± 0.006 (20)
SK&F 86002	–	–	12.0 ± 0.5 (6)	27.0 ± 1.4 (6)	5.5 ± 1.0 (2)	0.52 ± 0.03 (2)
ML 3163	SCH_2	4-S(O)CH_3	20.3 ± 4.8 (2)	2.78 ± 0.13 (2)	3.65 ± 1.73 (6)	0.88 ± 0.34 (6)
21b	SCH_2	3-S(O)CH_3	33.5 ± 6.4 (4)	4.1 ± 3.0 (3)	23.5 ± 6.5 (2)	1.0 ± 0.4 (2)
22	SCH_2	2-S(O)CH_3	47.5 ± 16.5 (2)	4.0 ± 2.8 (2)	15.0 ± 0.5 (2)	0.90 ± 0.10 (2)

[a] Number in brackets denotes number of experiments.

Scheme 1[a]



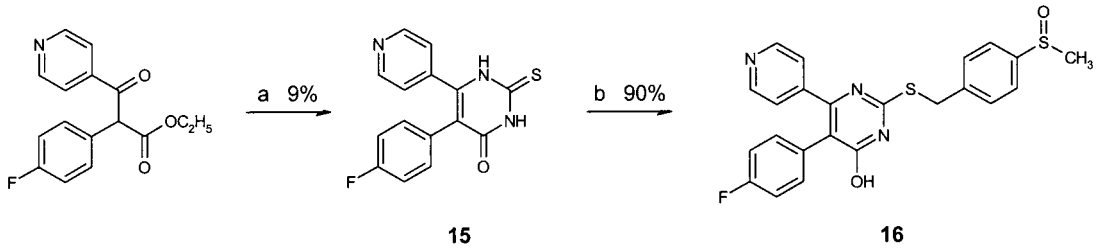
[a] Reagents: (a) DMF-DMA, toluene, reflux; (b) thiourea, $NaOC_2H_5$/EtOH, reflux; (c) R_3BzCl, Na_2CO_3, THF/EtOH, reflux; (d) 35% H_2O_2, acetic acid, 10 °C then rt.

ative **17**. Subsequent chlorination of **17** with $SOCl_2$ gave 1-chloromethyl-3-methylsulfanyl-benzene **18** which was readily oxidized with H_2O_2 to yield sulfoxide **19**. Nucleophilic replacement of **19** with pyrimidine-2-thione **7** did not, however, provide 5-(4-fluorophenyl)-2-(3-methanesulfinyl-benzylsulfanyl)-4-pyridin-4-yl-pyrimidine **13b** in an acceptable state of purity. The synthesis of **13b** went smoothly when **18** was reacted with pyrimidine-2-thione **7**, and the resulting 3-methylsulfanyl-benzylsulfanyl pyrimidine **13a** was oxidized to the corresponding sulfoxide **13b** by means of 1 equiv of H_2O_2. Benzylsulfanyl imidazoles ML 3163, **21b**, and **22** were prepared from 4-(4-fluorophenyl)-5-pyridin-4-yl-1,3-dihydro-imidazole-2-thione[20] (**20**) by nucleophilic substitution of appropriate benzyl chloride derivatives. In the preparation of **21b**, the same synthetic strategy had to be applied as for the synthesis of **13b** (Scheme 3).

Biological Results and Discussion

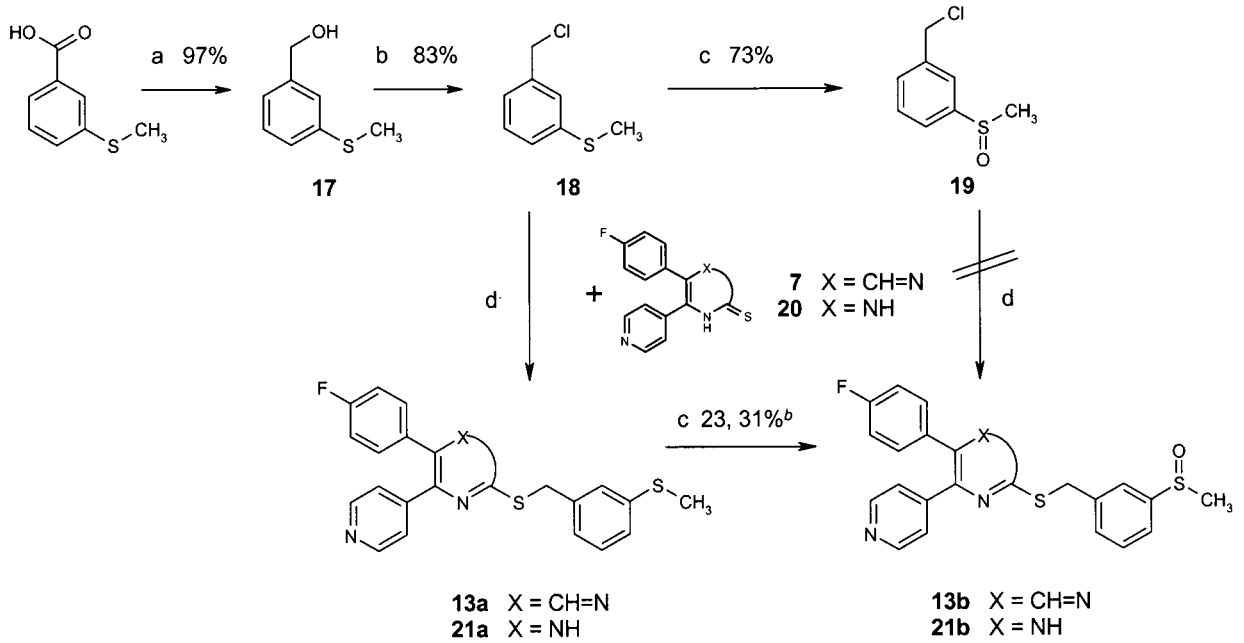
Imidazole lead compounds SB 203580, SK&F 86002, and ML 3163 inhibited cytokine release from human whole blood as well as from PBMC in the sub-micro-

Scheme 2[a]



[a] Reagents: (a) thiourea, neat, 180 °C; (b) 4-methylsulfinyl-benzyl chloride, Na_2CO_3, THF/EtOH, reflux.

Scheme 3[a,b]



[a] Reagents: (a) $LiAlH_4$, THF, 0 °C then rt; (b) $SOCl_2$, DCM, reflux; (c) 35% H_2O_2, acetic acid, 10 °C then rt; (d) Na_2CO_3, THF/EtOH, reflux. [b] Yields over 2 steps.

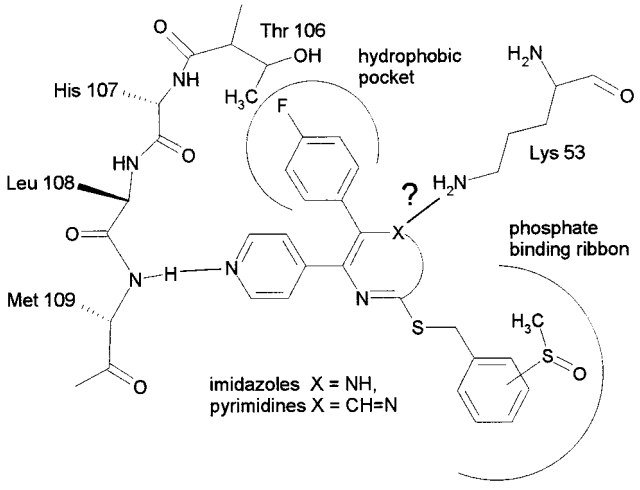
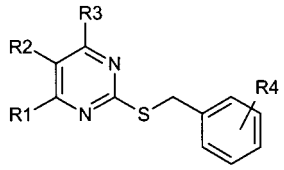


Figure 1. Sites of interaction between pyridin-4-yl heterocycles and p38 MAP kinase.[8]

molar to low-micromolar range (Table 1). It has been demonstrated for several inhibitors which bind in the ATP site of p38 MAP kinase that a hydrophobic pocket and the amide NH of Met109 are the main sites of interaction[11−13] (Figure 1). Therefore, a 4-fluoro-phenyl and a pyridin-4-yl group are regarded as essential for biological activity in this class of compounds. Both the formation of a hydrogen bond with the side chain of Lys53 and the absence of any negative interactions with this residue have been suggested to explain the efficacy of different heterocyclic scaffolds for the pyridin-4-yl-/4-fluoro-phenyl pharmacophore. As a result of the appropriate orientation of the core heterocycle, a substituent at the 2 position may interact with the phosphate-binding ribbon of the ATP binding site of p38 MAP kinase. Enlargement of the core imidazole heterocycle to a six-membered ring led to a decrease in biological activity for ML 3163 pyrimidine analogues **10a**−**c**, **11a**−**c**, and **12a**−**c** in an initial whole blood screen (columns 6 and 7 of Table 2). The compounds **10a**, **10c**, **11a**−**c**, and **12a**−**c** exhibited less than 50% inhibition of cytokine release at a concentration of 100 μM. Only sulfoxide **10b** exhibited moderate anti-IL-1β and weak anti-TNF-α activity. These results prompted us to examine SAR in the PBMC assay (columns 8 and 9 of Table 2). In the pyrimidine series, the oxidation state of the terminal sulfur atom significantly affected inhibition of TNF-α release but not that of IL-1β. Sulfone **10c** and sulfoxide **10b** were about 4 times more active than sulfide **10a**, demonstrating that only polar substituents at the 4 position of the benzylsulfanyl moiety increase inhibitory potency. This correlation has also been described for other inhibitors of cytokine release which exert their biological activity by inhibition

Table 2. Inhibition of Cytokine Release by Pyrimidine Compounds



					$IC_{50} \pm SEM$ (μM)			
					whole blood		PBMC	
compd	R1	R2	R3	R4	TNF-α	IL-1β	TNF-α	IL-1β
10a	4-Pyr	4-F−Ph	H	4-SCH_3	>100 (2)[a]	>100 (2)	60.0 ± 10.0 (2)	7.4 ± 0.2 (2)
10b	4-Pyr	4-F−Ph	H	4-S(O)CH_3	95.5 ± 64.5 (2)	45.5 ± 11.5 (2)	14.1 ± 4.4 (4)	5.6 ± 0.4 (2)
10c	4-Pyr	4-F−Ph	H	4-SO_2CH_3	>100 (2)	>100 (2)	17.8 ± 8.2 (2)	7.7 ± 4.2 (2)
11b	3-Pyr	4-F−Ph	H	4-S(O)CH_3	>100 (2)	>100 (2)	54.0 ± 20.0 (2)	75.5 ± 12.5 (2)
12b	4-F−Ph	4-Pyr	H	4-S(O)CH_3	>100 (2)	>100 (2)	29.9 ± 4.4 (4)	17.8 ± 4.1 (4)
13b	4-Pyr	4-F−Ph	H	3-S(O)CH_3	nd[b]	nd	6.5 ± 0.0 (2)	5.4 ± 0.0 (2)
14	4-Pyr	4-F−Ph	H	2-S(O)CH_3	nd	nd	3.2 ± 0.5 (2)	2.3 ± 0.6 (2)
16	4-Pyr	4-F−Ph	HO	4-S(O)CH_3	>100 (2)	>100 (2)	27.0 ± 2.0 (2)	25.5 ± 4.0 (2)

[a] Number in parentheses denotes number of experiments. [b] Not determined.

Table 3. p38 MAP Kinase Inhibition Data

compd	$IC_{50} \pm SEM$ (μM)	compd	IC_{50} (μM)
SB 203580	0.36 ± 0.07 (7)[a]	**10b**	36 (1)
ML 3163	4.0 ± 1.0 (2)	**13b**	19 ± 1.4 (2)
		14	5.1 ± 2.0 (2)

[a] Number in parentheses denotes number of experiments.

of p38 MAP kinase.[5,21] As can also be observed with various inhibitors of p38 MAP kinase,[22] replacement of the pyridin-4-yl moiety in **10b** with a pyridin-3-yl ring in **11b** resulted in a reduced inhibition of cytokine release. This loss of potency can be explained by a disturbance of the pivotal hydrogen bond between the pyridinyl moiety and Met109. The weaker inhibitory potency of **12b** compared to the regioisomeric pyrimidine **10b** underlines the importance of the core heterocycle in providing an optimum scaffold for both the pyridin-4-yl- and 4-fluoro-phenyl moiety. This purpose is better served by the pyrimidine nucleus in **10b** than in **12b**, albeit to a lesser extent than by the imidazole in ML 3163. Apart from their different geometries, ML 3163 and **10b** also differ in their hydrogen-bonding properties. Among potent inhibitors of p38 MAP kinase hydrogen-donating as well as hydrogen-accepting functionalities are found at various positions of the central heterocycle. In the pyrimidine series, biological activity decreased when an additional hydrogen-donating substructure was introduced at position 4 of the core pyrimidine (compound **16**) to mimic the ring NH of the imidazole compounds. This result can be attributed to an unfavorable interaction with Lys53. Upon examination of SAR at the methanesulfinyl-benzylsulfanyl moiety in the pyrimidine series, the 3-methanesulfinyl isomer **13b** and the 2-methanesulfinyl isomer **14** showed a 2- and 4.5-fold better inhibition, respectively, of TNF-α release than **10b**. The same order of efficacy was observed when regioisomers **10b**, **13b**, and **14** were tested for inhibition of isolated p38 MAP kinase (Table 3). This contrasts remarkably with the corresponding imidazole derivatives where a reversed correlation was observed. Placement of the methanesulfinyl substituent at the 3 or 2 position of the benzylsulfanyl moiety resulted in reduced bioactivity of compounds **21b** and **22** compared to ML 3163 (Table 1). Similar results have been obtained for the corresponding SB 203580 regioisomers.[5]

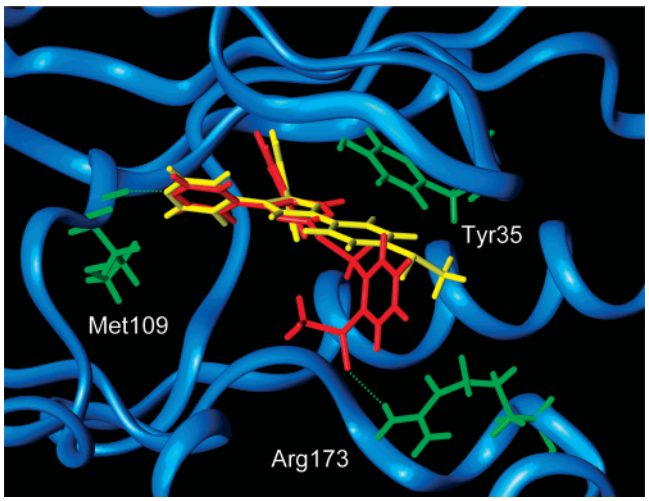


Figure 2. Superposition of SB 203580 (yellow) in complex with p38 MAP kinase[13] and compound **14** (red);[23] Thr35, Met109, and Arg173 are depicted in green.

Conclusion

We have shown that 2-benzylsulfanyl imidazole compounds are effective inhibitors of cytokine release from PBMC and human whole blood. Replacement of the core imidazole ring with pyrimidine leads to a decrease in biological activity (ML 3163 vs compound **10b**). This is due to the different geometry which is provided by imidazole and pyrimidine scaffolds for the pyridin-4-yl-/4-fluoro-phenyl pharmacophore and the substituent in position 2. A hydrogen-donating substructure at the core heterocycle itself is not essential for inhibition of cytokine release (compound **16**). In both the imidazole and pyrimidine series, elongation of the linker between the core heterocycle and the methylsulfinylphenyl ring diminished inhibitory potency compared to SB 203580. As illustrated by the superposition of pyrimidine **14** and SB 203580 in complex with p38 MAP kinase,[13] this can be attributed to the benzyl moiety being forced out of the favorable $\pi-\pi$ stacking with Tyr35 (Figure 2). The

disadvantageous geometry of the pyrimidine derivatives can be overcome to some extent if the contribution of the methanesulfinyl substituent to bioactivity is enhanced by its placement at the 3 or 2 position of the benzylsulfanyl moiety (compounds **13b** and **14**). We suggest that the benefits from this regioisomeric modification in the pyrimidine series may arise from an additional interaction between Arg173 and the sulfoxide functionality in **14** which may not be formed by the corresponding imidazole regioisomer **22** (Figure 2). Several other SAR in the pyrimidine series parallel those of known imidazole inhibitors of p38 MAP kinase. These observations, together with the enzyme data for selected compounds (Table 3), suggest a mode of action for pyrimidine inhibitors of cytokine release similar to those for model imidazole compounds.

Experimental Section

General. All reagents and solvents were of commercial quality and used without further purification. All reactions were carried out under an inert atmosphere of argon. Melting points were determined on a Buechi Melting Point B-545 apparatus and are thermodynamically corrected. ^{1}H- and ^{13}C-NMR spectra were obtained on a Bruker Spectrospin AC 200 instrument at 200 MHz. Chemical shifts are reported in parts per million relative to TMS as the internal standard. Infrared spectra were recorded using KBr pellets on a Nicolet Impact 410 or by ATR technique on a Perkin-Elmer Spectrum One spectrometer. TLC analyses were performed on fluorescent silica gel 60 plates (Macherey-Nagel Art.-Nr. 805021). Spots were visualized under 254 nm UV illumination. HPLC analyses were carried out on Merck Hitachi (Darmstadt) equipment, using a LiChrospher 100 RP-18 column (5 μm) and eluting with MeCN/NaH_2PO_4 buffer at pH 3.5 (70:30) and a flow rate of 1.00 mL/min at 25 °C (UV detection at 254 nm). HPLC results are presented as retention times (min) and relative purity (%). Microanalyses were carried out on Perkin-Elmer EA 240 and 2400 instruments. The following compounds were prepared according to literature procedures: imidazole-2-thione **20**,[20] SB 203580,[21] SK&F 86002,[15] 1-chloromethyl-4-methylsulfanyl-benzene,[18] 1-chloromethyl-2-/4-methanesulfinyl-benzene,[17,18] 1-chloromethyl-4-methanesulfonyl-benzene,[18] and ketones **1**−**3**.[5,20]

3-Dimethylamino-2-(4-fluoro-phenyl)-1-pyridin-4-yl-propenone (4). To a suspension of 2-(4-fluoro-phenyl)-1-pyridin-4-yl-ethanone **1**[20] (5.0 g, 23.3 mmol) in toluene (30 mL) was added dimethylformamide-dimethylacetale (5.5 mL, 41 mmol). The mixture was heated to reflux for 2.5 h. Removal of the solvent and excess dimethylformamide-dimethylacetale gave a dark-brown solid which, upon trituration with *tert*-butylmethyl ether, afforded 5.7 g (91%) of **1**: ^{1}H-NMR ($CDCl_3$, δ) 2.78 (bs, 6H, 2 × CH_3), 6.98−7.02 (m, 2H, 4-F−Ph), 7.08−7.11 (m, 2H, 4-F−Ph), 7.27−7.29 (m, 2H, 4-Pyr), 7.37 (s, 1H, CH), 8.55−8.57 (m, 2H, 4-Pyr); ^{13}C-NMR (DMSO-d_6, δ) 43.2, 108.9, 113.9, 114.4, 122.1, 132.8, 133.6, 133.8, 149.1, 149.3, 154.5, 158.3, 163.2, 190.7; IR (KBr) 1627 (C═O), 1216 cm^{-1} (C−F).

3-Dimethylamino-2-(4-fluoro-phenyl)-1-pyridin-3-yl-propenone (5). This compound was prepared from ketone **2**[20] as described in the synthesis of **4** with a yield of 1.2 g (94%): ^{1}H-NMR ($CDCl_3$, δ) 2.78 (bs, 6H, 2 × CH_3), 6.93−7.02 (m, 2H, 4-F−Ph), 7.09−7.16 (m, 2H, 4-F−Ph), 7.23−7.28 (m, 1H, 3-Pyr C^5-H), 7.39 (s, 1H, CH), 7.71−7.74 (m, 1H, 3-Pyr C^6-H), 8.55 (dd, 1H, 1.2/4.8 Hz, 3-Pyr C^4-H), 8.62 (d, 1H, 1.2 Hz, 3-Pyr C^2-H); IR (ATR) 1683 (C═O), 1223 cm^{-1} (C−F).

3-Dimethylamino-1-(4-fluoro-phenyl)-2-pyridin-4-yl-propenone (6). This compound was prepared from ketone **3**[5] as described in the synthesis of **4** with a yield of 1.17 g (92%): ^{1}H-NMR ($CDCl_3$, δ) 2.80 (s, 6H, 2 × CH_3), 6.94−7.07 (m, 4H, 4-Pyr and 4-F−Ph), 7.28−7.43 (m, 3H, CH and 4-F−Ph), 8.48−8.50 (m, 2H, 4-Pyr); IR (ATR) 1679 (C═O), 1227 cm^{-1} (C−F).

5-(4-Fluoro-phenyl)-4-pyridin-4-yl-1*H*-pyrimidine-2-thione (7). To a freshly prepared solution of sodium (0.29 g, 12.6 mmol) in ethanol (30 mL) were consecutively added compound **4** (1.0 g, 3.7 mmol) and thiourea (0.31 g, 4.1 mmol). The mixture was heated to reflux for 4 h. The solvent was removed, and the residue was dissolved in H_2O (15 mL). The dark-red aqueous solution was neutralized with 8% aqueous H_3PO_4. A precipitate formed which was collected by filtration, washed with H_2O, and dried in vacuo to give 0.92 g (88%) of **7**: ^{1}H-NMR (DMSO-d_6, δ) 7.16−7.27 (m, 6H, 4-Pyr and 4-F−Ph), 8.18 (s, 1H, Pyr and CH), 8.55−8.58 (m, 2H, 4-Pyr), NH not detected; ^{13}C-NMR (DMSO-d_6, δ) 115.3, 115.8, 121.1, 123.4, 130.3, 131.3, 131.5, 144.9, 148.1, 149.6, 159.4, 164.3, 164.6, 179.6; IR (KBr) 1509 (HN−C═S), 1218 cm^{-1} (C−F).

5-(4-Fluoro-phenyl)-4-pyridin-3-yl-1*H*-pyrimidine-2-thione (8). This compound was prepared from enaminone **5** as described in the synthesis of **7** with a yield of 0.90 g (86%): ^{1}H-NMR (DMSO-d_6, δ) 7.13−7.42 (m, 5H, 3-Pyr C^5-H and 4-F−Ph), 7.72 (d, 1H, 3-Pyr C^6-H), 8.17 (s, 1H, Pyr CH), 8.45 (m, 1H, 3-Pyr C^4-H), 8.57 (d, 1H, 3-Pyr C^2-H), NH not detected; IR (KBr) 1509 (HN−C═S), 1230 cm^{-1} (C−F).

4-(4-Fluoro-phenyl)-5-pyridin-4-yl-1*H*-pyrimidine-2-thione (9). This compound was prepared from enaminone **6** as described in the synthesis of **7** with a yield of 0.84 g (80%): ^{1}H-NMR (DMSO-d_6, δ) 7.20−7.24 (m, 4H, 4-Pyr and 4-F−Ph), 7.36−7.43 (m, 2H, 4-F−Ph), 8.22 (s, 1H, Pyr CH), 8.48−8.51 (d, 2H, 4-Pyr), NH not detected; ^{13}C-NMR (DMSO-d_6, δ) 115.0, 115.4, 119.1, 123.6, 131.9, 132.0, 132.3, 132.4, 142.4, 148.0, 149.6, 149.8, 164.9, 165.6, 179.8; IR (KBr) 1509 (HN−C═S), 1231 cm^{-1} (C−F).

General Procedure for the Preparation of 2-Benzylsulfanyl Pyrimidines 10a−c, 11a−c, 12a−c, 13a, and **14.** A suspension of the appropriate pyrimidine-2-thione (0.30 g, 1.06 mmol), sodium acetate (0.17 g, 2.1 mmol), and the appropriate benzyl chloride (1.0 mmol) in 9:1 ethanol/THF (15 mL) was heated to reflux for 2 h. After the mixture was cooled to room temperature, it was filtered, and the bright-red filtrate was concentrated in vacuo. The oily residue was purified by column chromatography or crystallization to give 2-benzylsulfanyl pyrimidines **10a**−**c**, **11a**−**c**, **12a**−**c**, **13a**, and **14**.

5-(4-Fluoro-phenyl)-2-(4-methylsulfanyl-benzylsulfanyl)-4-pyridin-4-yl-pyrimidine (10a). Compound **10a** was prepared from 1-chloromethyl-4-methylsulfanyl-benzene and **7** according to the general procedure described above. Purification by column chromatography (basic alumina, DCM) yielded 0.23 g (61%) of **10a**: mp 117 °C; ^{1}H-NMR ($CDCl_3$, δ) 2.47 (s, 3H, CH_3), 4.43 (s, 2H, CH_2), 7.05−7.39 (m, 10H, 4-Pyr, 4-F−Ph, and 4-MeS−Ph), 8.56−8.59 (m, 3H, 4-Pyr and Pyr CH); ^{13}C-NMR ($CDCl_3$, δ) 15.8, 35.1, 116.0, 116.4, 123.8, 126.6, 128.0, 129.5, 131.0, 131.1, 134.2, 137.4, 144.7, 149.8, 158.9, 160.3, 160.9, 165.5, 171.0; IR (KBr) 1397 (N═C−S), 1224 cm^{-1} (C−F); HPLC 6.14 min, 98.8%. Anal. ($C_{23}H_{18}FN_3S_2$) C, H, N.

5-(4-Fluoro-phenyl)-2-(4-methanesulfinyl-benzylsulfanyl)-4-pyridin-4-yl-pyrimidine (10b). Compound **10b** was prepared from 1-chloromethyl-4-methanesulfinyl-benzene and **7** according to the general procedure described above. Purification by column chromatography (basic alumina, 9:1 DCM/MeCN) yielded 0.21 g (46%) of **10b**: mp 134 °C; ^{1}H-NMR ($CDCl_3$, δ) 2.71 (s, 3H, CH_3), 4.52 (s, 2H, CH_2), 7.01−7.17 (m, 4H, 4-F−Ph), 7.26−7.30 (m, 2H, 4-Pyr), 7.56−7.66 (m, 4H, 4-MeS(O)−Ph), 8.57−8.58 (m, 3H, 4-Pyr and Pyr CH); ^{13}C-NMR ($CDCl_3$, δ) 34.9, 43.9, 116.0, 116.5, 123.7, 128.3, 130.0, 130.8, 130.9, 131.1, 141.2, 144.6, 144.7, 149.8, 159.0, 160.3, 161.1, 165.3, 170.5; IR (KBr) 1398 (N═C−S), 1223 (C−F), 1048 cm^{-1} (S═O); HPLC 1.96 min, 99.6%. Anal. ($C_{23}H_{18}FN_3OS_2$) C, H, N.

5-(4-Fluoro-phenyl)-2-(4-methanesulfonyl-benzylsulfanyl)-4-pyridin-4-yl-pyrimidine (10c). Compound **10c** was prepared from 1-chloromethyl-4-methanesulfonyl-benzene and **7** according to the general procedure described above. Purification by column chromatography (basic alumina, 9:1 DCM/MeCN) yielded 0.14 g (29%) of **10c**: mp 142 °C; ^{1}H-NMR ($CDCl_3$, δ) 3.04 (s, 3H, CH_3), 4.53 (s, 2H, CH_2), 7.01−7.17 (m, 4H, 4-F−Ph), 7.24−7.28 (m, 2H, 4-Pyr), 7.67 (d, 2H, 8.32 Hz,

4-MeS(O)$_2$−Ph), 7.88 (d, 2H, 8.33 Hz, 4-MeS(O)$_2$−Ph), 8.58−8.62 (m, 3H, 4-Pyr and Pyr CH); ^{13}C-NMR ($CDCl_3$, δ) 34.8, 44.5, 116.1, 116.5, 123.7, 127.6, 127.7, 128.4, 130.0, 130.7, 130.8, 131.1, 131.0, 139.4, 144.5, 144.7, 149.8, 159.1, 160.4, 161.2, 165.3, 170.1; IR (KBr) 1399 (N=C−S), 1304 (SO_2), 1220 (C−F), 1149 cm^{-1} (SO_2); HPLC 2.15 min, 95.0%. Anal. ($C_{23}H_{18}FN_3O_2S_2$) C, H, N.

5-(4-Fluoro-phenyl)-2-(4-methylsulfanyl-benzylsulfanyl)-4-pyridin-3-yl-pyrimidine (11a). Compound **11a** was prepared from 1-chloromethyl-4-methylsulfanyl-benzene and **8** according to the general procedure described above. The crude oil was extracted with diethyl ether. The organic extract was washed with H_2O and dried over Na_2SO_4, and the solvent was removed. Trituration of the oily residue with *tert*-butylmethyl ether gave 0.19 g (45%) of **11a**: mp 124 °C; ^{1}H-NMR ($CDCl_3$, δ) 2.47 (s, 3H, CH_3), 4.44 (s, 2H, CH_2), 7.06−7.41 (m, 9H, 3-Pyr C^5-H, 4-F−Ph, and 4-MeS−Ph), 7.72−7.80 (m, 1H, 3-Pyr C^6-H), 8.55 (s, 1H, Pyr CH), 8.61 (m, 1H, 3-Pyr C^4-H), 8.69 (m, 1H, 3-Pyr C^2-H); ^{13}C-NMR ($CDCl_3$, δ) 15.9, 35.1, 116.2, 116.7, 123.4, 126.7, 128.0, 129.5, 131.0, 131.1, 131.2, 133.6, 134.2, 137.5, 138.3, 149.0, 149.3, 158.9, 160.1, 160.3, 165.3, 171.1; IR (KBr) 1398 (N=C−S), 1222 cm^{-1} (C−F); HPLC 6.08 min, 98.1%. Anal. ($C_{23}H_{18}FN_3S_2$) C, H, N.

5-(4-Fluoro-phenyl)-2-(4-methanesulfinyl-benzylsulfanyl)-4-pyridin-3-yl-pyrimidine (11b). Compound **11b** was prepared from 1-chloromethyl-4-methanesulfinyl-benzene and **8** according to the general procedure described above. Purification by column chromatography (basic alumina, ethyl acetate) yielded 0.14 g (40%) of **11b**: mp 128 °C; ^{1}H-NMR ($CDCl_3$, δ) 2.72 (s, 3H, CH_3), 4.52 (s, 2H, CH_2), 7.02−7.16 (m, 4H, 4-F−Ph), 7.22−7.32 (m, 1H, 3-Pyr C^5-H), 7.61−7.69 (m, 5H, 3-Pyr C^6-H and 4-MeS(O)−Ph), 8.56 (s, 1H, Pyr CH), 8.61 (m, 1H, 3-Pyr C^4-H), 8.59−8.70 (m, 1H, 3-Pyr C^2-H); ^{13}C-NMR ($CDCl_3$, δ) 34.0, 43.9, 116.2, 116.7, 123.4, 123.8, 128.3, 130.0, 130.1, 131.0, 131.1, 131.2, 138.0, 141.2, 144.6, 149.2, 149.4, 158.9, 160.3, 160.3, 165.3, 170.5; IR (KBr) 1397 (N=C−S), 1227 (C−F), 1042 cm^{-1} (S=O); HPLC 1.93 min, 99.6%. Anal. ($C_{23}H_{18}FN_3OS_2$) C, H, N.

5-(4-Fluoro-phenyl)-2-(4-methanesulfonyl-benzylsulfanyl)-4-pyridin-3-yl-pyrimidine (11c). Compound **11c** was prepared from 1-chloromethyl-4-methanesulfonyl-benzene and **8** according to the general procedure described above. The crude oil was extracted with diethyl ether. The organic extract was washed with H_2O and dried over Na_2SO_4, and the solvent was removed. Trituration of the oily residue with methanol gave 0.22 g (48%) of **11c**: mp 173 °C; ^{1}H-NMR ($CDCl_3$, δ) 3.05 (s, 3H, CH_3), 4.53 (s, 2H, CH_2), 7.02−7.18 (m, 4H, 4-F−Ph), 7.22−7.30 (m, 1H, 3-Pyr C^5-H), 7.68 (m, 3H, 3-Pyr C^6-H and 4-MeS(O)$_2$−Ph), 7.88 (d, 2H, 8.23 Hz, 4-MeS(O)$_2$−Ph), 8.55 (s, 1H, Pyr CH), 8.59−8.62 (m, 2H, 3-Pyr C^2-/C^4-H); ^{13}C-NMR ($CDCl_3$, δ) 34.8, 44.5, 116.2, 116.6, 123.1, 127.6, 128.4, 130.0, 130.9, 131.0, 131.1, 133.0, 137.3, 139.3, 144.5, 150.1, 158.9, 160.3, 160.8, 165.3, 170.1; IR (KBr) 1399 (N=C−S), 1301 (SO_2), 1223 (C−F), 1151 cm^{-1} (SO_2); HPLC 2.14 min, 99.7%. Anal. ($C_{23}H_{18}FN_3O_2S_2$) C, H, N.

4-(4-Fluoro-phenyl)-2-(4-methylsulfanyl-benzylsulfanyl)-5-pyridin-4-yl-pyrimidine (12a). Compound **12a** was prepared from 1-chloromethyl-4-methylsulfanyl-benzene and **9** according to the general procedure described above. Purification by column chromatography (basic alumina, DCM) yielded 0.23 g (61%) of **12a**: ^{1}H-NMR ($CDCl_3$, δ) 2.47 (s, 3H, CH_3), 4.44 (s, 2H, CH_2), 6.98−7.04 (m, 2H, 4-F−Ph), 7.13−7.22 (m, 4H, 4-Pyr and 4-F−Ph), 7.35−7.40 (m, 4H, 4-MeS−Ph), 8.50 (s, 1H, Pyr CH), 8.57−8.62 (m, 2H, 4-Pyr); ^{13}C-NMR ($CDCl_3$, δ) 15.9, 35.1, 115.4, 115.9, 124.1, 125.6, 126.7, 129.5, 131.8, 132.0, 132.3, 132.4, 134.2, 137.5, 145.3, 149.5, 158.2, 161.4, 162.8, 166.4, 172.1; IR (KBr) 1401 (N=C−S), 1227 cm^{-1} (C−F); HPLC 7.31 min, 98.4%. Anal. ($C_{23}H_{18}FN_3S_2$) C, H, N.

4-(4-Fluoro-phenyl)-2-(4-methanesulfinyl-benzylsulfanyl)-5-pyridin-4-yl-pyrimidine (12b). Compound **12b** was prepared from 1-chloromethyl-4-methanesulfinyl-benzene and **9** according to the general procedure described above. The crude oil was extracted with diethyl ether. The organic extract was washed with H_2O and dried over Na_2SO_4, and the solvent was removed. Trituration of the oily residue with *tert*-butylmethyl ether gave 0.12 g (28%) of **12b:** mp 142 °C; ^{1}H-NMR ($CDCl_3$, δ) 2.72 (s, 3H, CH_3), 4.52 (s, 2H, CH_2), 7.00−7.06 (m, 2H, 4-F−Ph), 7.15−7.18 (m, 2H, 4-Pyr), 7.33−7.40 (m, 2H, 4-F−Ph), 7.58−7.68 (m, 4H, 4-MeS(O)−Ph), 8.52 (s, 1H, Pyr CH), 8.59−8.62 (m, 2H, 4-Pyr); ^{13}C-NMR ($CDCl_3$, δ) 35.6, 44.6, 116.1, 116.5, 124.4, 124.5, 126.8, 130.7, 132.5, 132.6, 132.9, 133.0, 141.9, 144.9, 145.3, 150.9, 159.0, 162.0, 163.5, 167.0, 171.93; IR (KBr) 1399 (N=C−S), 1221 (C−F), 1039 cm^{-1} (S=O); HPLC 1.99 min, 97.6%. Anal. ($C_{23}H_{18}FN_3OS_2$) C, H, N.

4-(4-Fluoro-phenyl)-2-(4-methanesulfonyl-benzylsulfanyl)-5-pyridin-4-yl-pyrimidine (12c). Compound **12c** was prepared from 1-chloromethyl-4-methanesulfonyl-benzene and **9** according to the general procedure described above. Purification by column chromatography (basic alumina, 9:1 DCM/MeCN) yielded 0.25 g (56%) of **12c**: mp 151 °C; ^{1}H-NMR ($CDCl_3$, δ) 3.04 (s, 3H, CH_3), 4.53 (s, 2H, CH_2), 6.96−7.04 (m, 2H, 4-F−Ph), 7.11−7.17 (m, 2H, 4-Pyr), 7.32−7.39 (m, 2H, 4-F−Ph), 7.67 (d, 2H, 8.28 Hz, 4-MeS(O)$_2$−Ph), 7.88 (d, 2H, 8.32 Hz, 4-MeS(O)$_2$−Ph), 8.51 (s, 1H, Pyr CH), 8.59−8.70 (m, 2H, 4-Pyr); ^{13}C-NMR ($CDCl_3$, δ) 34.7, 44.4, 115.4, 115.8, 123.8, 126.2, 127.5, 129.9, 131.7, 131.9, 132.1, 132.2, 139.3, 144.0, 144.4, 150.2, 158.3, 161.3, 162.9, 166.3, 170.80; IR (KBr) 1402 (N=C−S), 1303 (SO_2), 1226 (C−F), 1149 cm^{-1} (SO_2); HPLC 2.21 min, 96.4%. Anal. ($C_{23}H_{18}FN_3O_2S_2$) C, H, N.

5-(4-Fluoro-phenyl)-2-(3-methylsulfanyl-benzylsulfanyl)-4-pyridin-4-yl-pyrimidine (13a). This compound was prepared from 1-chloromethyl-3-methylsulfanyl-benzene (0.18 g, 1.0 mmol) and **7** (0.28 g, 1.0 mmol) according to the general procedure described above. The crude oil of **13a** was used in the synthesis of **13b** without further purification.

5-(4-Fluoro-phenyl)-2-(3-methanesulfinyl-benzylsulfanyl)-4-pyridin-4-yl-pyrimidine (13b). A suspension of **13a** (0.35 g, 0.83 mmol) in glacial acetic acid (5 mL) was treated with 35% aqueous H_2O_2 (0.10 mL, 1.0 mmol). The mixture was stirred at room temperature for 5.5 h, diluted with H_2O (5 mL), adjusted to pH 9 with 25% aqueous ammonia, and extracted with DCM (3 times). The combined organic extracts were washed with saturated brine (3 times) and dried over Na_2SO_4, and the solvent was removed to yield an oily residue. The crude product was purified by column chromatography (silica gel 60, ethyl acetate) to give 0.15 g (42%) of **13b**: ^{1}H-NMR ($CDCl_3$, δ) 2.68 (s, 3H, CH_3), 4.53 (s, 2H, CH_2), 7.01−7.14 (m, 4H, 4-F−Ph), 7.27−7.30 (m, 2H, 4-Pyr), 7.46−7.62 (m, 3H, 3-MeS(O)−Ph C^4-/C^5-/C^6-H), 7.77−7.79 (m, 1H, 3-MeS(O)−Ph C^2-H), 8.58−8.60 (m, 3H, 4-Pyr and Pyr CH); ^{13}C-NMR ($CDCl_3$, δ) 35.3, 44.2, 116.4, 116.8, 122.8, 124.2, 124.4, 128.6, 129.8, 131.1, 131.2, 131.3, 131.4, 132.1, 140.0, 145.2, 146.3, 150.1, 159.4, 160.7, 161.4, 165.7, 170.8; IR (ATR) 1396 (N=C−S), 1222 (C−F), 1044 cm^{-1} (S=O). Anal. ($C_{23}H_{18}FN_3OS_2$) C, H, N.

5-(4-Fluoro-phenyl)-2-(2-methanesulfinyl-benzylsulfanyl)-4-pyridin-4-yl-pyrimidine (14). Compound **14** was prepared from 1-chloromethyl-2-methanesulfinyl-benzene and **9** according to the general procedure described above. Purification by column chromatography (silica gel 60, ethyl acetate) yielded 0.13 g (56%) of **14**: ^{1}H-NMR ($CDCl_3$, δ) 2.84 (s, 3H, CH_3), 4.57 (d, 1H, 13.8 Hz, CH_2), 4.68 (d, 1H, 13.8 Hz, CH_2), 7.08−7.27 (m, 2H, 4-F−Ph), 7.33−7.37 (m, 2H, 4-Pyr), 7.48−7.60 (m, 3H, 2-MeS(O)−Ph C^4-/C^5-/C^6-H), 8.07 (dd, 1H, 7.9 Hz, 2-MeS(O)−Ph C^3-H), 8.59−8.62 (m, 3H, 4-Pyr and Pyr CH); IR (ATR) 1397 (N=C−S), 1223 (C−F), 1035 cm^{-1} (S=O). Anal. ($C_{23}H_{18}FN_3OS_2$) C, H, N.

5-(4-Fluoro-phenyl)-6-pyridin-4-yl-2-thioxo-2,3-dihydro-1*H*-pyrimidin-4-one (15). Ethyl 2-(4-fluorophenyl)-3-oxo-3-(4-pyridyl)-propionate (3.1 g, 10.8 mmol) was reacted with thiourea (0.62 g, 8.2 mmol) at 180 °C for 20 min. The oily mixture was cooled to room temperature and taken up in acetone. Upon trituration, a precipitate formed which was filtered to yield 0.22 g (9%) of **15**: ^{1}H-NMR (DMSO-d_6, δ) 6.98−7.13 (m, 4H, 4-F−Ph), 7.24−7.29 (m, 2H, 4-Pyr), 8.49−8.52 (m, 2H, 4-Pyr), 12.68 (bs, 1H, exchangeable, NH/OH), 12.78 (bs, 1H, exchangeable, NH/OH); ^{13}C-NMR (DMSO-d_6, δ) 114.4, 114.8, 116.1, 123.8, 127.9, 128.0, 132.9, 133.0, 139.4, 148.0,

149.3, 158.9, 160.6, 163.8, 175.00; IR (KBr) 3408 (NH stretching), 1679 (HN−C═O), 1514 (HN−C═S), 1236 cm^{-1} (C−F).

5-(4-Fluoro-phenyl)-2-(4-methanesulfinyl-benzylsulfanyl)-6-pyridin-4-yl-pyrimidin-4-ol (16). A suspension of **15** (0.10 g, 0.33 mmol), 1-chloromethyl-4-methanesulfinyl-benzene (0.065 g, 0.34 mmol), and Na_2CO_3 (0.055 g, 0.52 mmol) in 90% ethanolic THF (10 mL) was heated to reflux for 4.5 h. After the mixture was cooled to room temperature, it was filtered, and the filtrate was concentrated in vacuo. The oily residue solidified upon trituration with diethyl ether. The crude product was washed with H_2O to yield 0.13 g (90%) of **16**: mp 101 °C; ^{1}H-NMR (CD_3OD, δ) 2.78 (s, 3H, CH_3), 4.48 (s, 2H, CH_2), 6.88−6.96 (m, 2H, 4-F−Ph), 7.08−7.12 (m, 2H, 4-F−Ph), 7.20−7.23 (m, 2H, 4-Pyr), 7.62 (d, 2H, 8.4 Hz, 4-MeS(O)−Ph), 7.71 (d, 2H, 8.4 Hz, 4-MeS(O)−Ph), 8.32−8.35 (m, 2H, 4-Pyr); ^{13}C-NMR (CD_3OD, δ) 35.1, 43.6, 115.4, 115.8, 119.6, 124.9, 126.0, 131.4, 133.9, 134.0, 134.2, 134.4, 144.0, 145.5, 149.5, 150.3, 159.6, 160.8, 165.6, 169.7; IR (KBr) 3399 (broad, OH), 1316 (N═C−S), 1220 (C−F), 1033 cm^{-1} (S═O); HPLC 1.25 min, 99.0%. Anal. ($C_{23}H_{18}FN_3O_2S_2$) C, H, N.

(3-Methylsulfanyl-phenyl)-methanol (17). To a cooled (0 °C) suspension of 95% $LiAlH_4$ (0.74 g, 18.5 mmol) in THF (20 mL) was slowly added a solution of 3-methylsulfanyl-benzoic acid (3.7 g, 22 mmol) in THF (35 mL). After the addition was complete (30 min), the reaction mixture was stirred for 3.5 h at room temperature. The reaction was quenched by dilution with cold water (50 mL), and the white precipitate was dissolved by addition of 10% sulfuric acid. The aqueous solution was extracted with diethyl ether (3 times). The combined organic extracts were washed with saturated brine and dried over Na_2SO_4, and the solvent was evaporated to yield 3.3 g (97%) of **17** as a yellowish oil: ^{1}H-NMR ($CDCl_3$, δ) 1.72 (s, 1H, exchangeable, OH), 2.49 (s, 3H, CH_3), 4.67 (s, 2H, CH_2), 7.10−7.32 (m, 4H, ar); IR (ATR) 3343 (OH stretching), 1202 (OH bending), 1013 cm^{-1} (C−O stretching).

1-Chloromethyl-3-methylsulfanyl-benzene (18). To a solution of **17** (3.3 g, 21 mmol) in DCM (20 mL) was added a solution of $SOCl_2$ (2.5 g, 21 mmol) in DCM (10 mL). The mixture was heated to reflux for 2 h. The reaction mixture was cooled to room temperature, consecutively washed with saturated aqueous $NaHCO_3$ and H_2O, and dried over Na_2SO_4, and the solvent was removed to yield an oily residue. Purification of the crude product by Kugelrohr distillation (130 °C, 0.07 mbar) gave 3.0 g (83%) of **18** as a colorless oil: ^{1}H-NMR ($CDCl_3$, δ) 2.49 (s, 3H, CH_3), 4.55 (s, 2H, CH_2), 7.16−7.28 (m, 4H, ar); IR (ATR) 705 cm^{-1} (C−Cl).

1-Chloromethyl-3-methanesulfinyl-benzene (19). A cooled (10 °C) solution of **18** (3.0 g, 17.4 mmol) in glacial acetic acid (25 mL) was treated with a solution of 35% aqueous H_2O_2 (1.8 g, 18.5 mmol) in glacial acetic acid (10 mL). After addition was complete (5 min), the mixture was stirred at room temperature for 2.25 h. The reaction was quenched by addition of ice (35 g), neutralized with 25% aqueous ammonia, and extracted with ethyl acetate (2 times). The combined organic extracts were washed with H_2O and dried over Na_2SO_4, and the solvent was removed to yield an oily residue. Purification of the crude product by Kugelrohr distillation (175 °C, 0.06 mbar) gave 2.4 g (73%) of **19** as a colorless oil: ^{1}H-NMR ($CDCl_3$, δ) 2.75 (s, 3H, CH_3), 4.64 (s, 2H, CH_2), 7.52−7.58 (m, 3H, C^4-/C^5-/C^6-H) 7.71 (m, 1H, C^2-H); ^{13}C-NMR ($CDCl_3$, δ) 44.0, 45.3, 123.4, 123.5, 129.8, 131.1, 139.2, 146.4; IR (ATR) 1043 (S═O), 707 cm^{-1} (C−Cl).

4-[5-(4-Fluoro-phenyl)-2-(4-methanesulfinyl-benzylsulfanyl)-3*H*-imidazol-4-yl]-pyridine (ML 3163). To a solution of 1-chloromethyl-4-methanesulfinyl-benzene (0.18 g, 1.0 mmol) in ethanol (15 mL) was added compound **20** (0.28 g, 1.0 mmol). The mixture was heated to reflux for 4 h. The solvent was removed, and the oily residue was extracted with ethyl acetate/methanol (9:1). When the organic extract was left at room temperature a precipitate formed which was filtered off to yield 0.18 g (41%) of ML 3163: mp 232 °C; ^{1}H-NMR (DMSO-d_6, δ) 2.71 (s, 3H, CH_3), 4.48 (s, 2H, CH_2), 7.22−7.53 (m, 6H, 4-Pyr and 4-F−Ph), 7.57−7.65 (m, 4H, 4-MeS(O)−Ph), 8.45−8.48 (m, 2H, 4-Pyr), 12.75 (bs, 1H, exchangeable, NH); IR (ATR) 1226 (C−F), 1039 cm^{-1} (S═O); HPLC 1.92 min, 97.9%. Anal. ($C_{22}H_{18}FN_3OS_2$) C, H, N.

4-[5-(4-Fluoro-phenyl)-2-(3-methylsulfanyl-benzylsulfanyl)-3*H*-imidazol-4-yl]-pyridine (21a). To a solution of **18** (0.69 g, 4.1 mmol) in ethanol (60 mL) was added compound **20** (1.1 g, 4.1 mmol). The mixture was heated to reflux for 11 h and stirred for another 60 h at room temperature. The yellow precipitate was filtered off and purified by trituration with ethanol to yield 1.2 g (73%) of **21a**: mp 218 °C; ^{1}H-NMR (DMSO-d_6, δ) 2.40 (s, 3H, CH_3), 4.46 (s, 2H, CH_2), 7.16−7.43 (m, 6H, 4-F−Ph and 3-MeS−Ph), 7.56−7.63 (m, 2H, 4-F−Ph), 7.90−7.93 (m, 2H, 4-Pyr), 8.66−8.69 (m, 2H, 4-Pyr), NH not detected; ^{13}C-NMR (DMSO-d_6, δ) 14.5, 36.0, 116.0, 116.5, 121.4, 124.7, 125.4, 126.2, 129.0, 130.6, 131.1, 131.3, 136.9, 138.2, 138.7, 141.3, 143.3, 148.9, 160.1, 165.1; IR (KBr) 1225 cm^{-1} (C−F).

4-[5-(4-Fluoro-phenyl)-2-(3-methanesulfinyl-benzylsulfanyl)-3*H*-imidazol-4-yl]-pyridine (21b). A suspension of **21a** (0.50 g, 1.2 mmol) in glacial acetic acid (7 mL) was treated with 35% aqueous H_2O_2 (0.13 mL, 1.3 mmol). The mixture was stirred at room temperature for 20.5 h, diluted with H_2O (5 mL), adjusted to pH 9 with 25% aqueous ammonia, and extracted with ethyl acetate (3 times). The combined organic extracts were washed with saturated brine (3 times) and dried over Na_2SO_4, and the solvent was removed to yield an oily residue which was crystallized from diethyl ether/ethyl acetate (1:1). The crude product was purified by column chromatography (RP-18, methanol) to give 0.16 mg (31%) of **13b**: mp 171 °C; ^{1}H-NMR (CD_3OD, δ) 2.67 (s, 3H, CH_3), 4.37 (s, 2H, CH_2), 7.13−7.21 (m, 2H, 4-F−Ph), 7.37−7.58 (m, 8H, 4-Pyr, 4-F−Ph and 3-MeS(O)-Ph), 8.40−8.43 (m, 2H, 4-Pyr); ^{13}C-NMR (CD_3OD, δ) 39.6, 43.7, 116.9, 117.3, 123.0, 123.9, 125.1, 130.9, 131.9, 132.0, 133.2, 141.4, 146.6, 149.8, 150.2, 162.0, 167.0; IR (KBr) 1228 (C−F), 1019 cm^{-1} (S═O); HPLC 2.28 min, 95.8%. Anal. ($C_{22}H_{18}FN_3OS_2$) C, H, N.

4-[5-(4-Fluoro-phenyl)-2-(2-methanesulfinyl-benzylsulfanyl)-3*H*-imidazol-4-yl]-pyridine (22). This compound was prepared from imidazole-2-thione **20** and 1-chloromethyl-2-methanesulfinyl-benzene as described in the synthesis of ML 3163 to yield 0.23 g (54%): mp 205 °C; ^{1}H-NMR (CD_3OD, δ) 2.87 (s, 3H, CH_3), 4.50 (d, 1H, 13.6 Hz, CH_2), 4.62 (d, 1H, 13.6 Hz, CH_2), 7.24−7.33 (m, 2H, 4-F−Ph), 7.47−7.62 (m, 5H, 4-F−Ph, 2-MeS(O)-Ph C^4-/C^5-/C^6-H), 7.95 (d, 1H, 7.2 Hz, 2-MeS(O)-Ph C^3-H), 7.99−8.03 (m, 2H, 4-Pyr), 8.55−8.58 (m, 2H, 4-Pyr); ^{13}C-NMR (CD_3OD, δ) 35.1, 43.5, 117.5, 117.9, 123.5, 125.0, 126.9, 130.7, 132.0, 132.4, 132.5, 132.8, 133.4, 136.7, 138.9, 142.3, 143.8, 145.3, 152.3, 162.7, 167.6; IR (KBr) 1213 (C−F), 1033 cm^{-1} (S═O); HPLC 2.11 min, 98.7%. Anal. ($C_{22}H_{18}FN_3OS_2$) C, H, N.

Biological Evaluation: PBMC and Whole Blood Assay.[24] Stock solutions of test compounds were prepared by serial dilution in DMSO (PBMC assay) or 70:30 Cremophor EL/ethanol (whole blood assay). Mononuclear cells were isolated from whole blood of healthy human donors by density gradient centrifugation, and the resulting suspension was adjusted to an approximate cell count of 10^6 mL^{-1} (PBMC assay). Cell samples (PBMC assay) or whole blood samples (whole blood assay) were preincubated for 15 min (37 °C, 5% CO_2) with test compounds (test samples) and 1% DMSO (control samples, PBMC assay) or 1% 70:30 Cremophor EL/ethanol (control samples, PBMC assay). DMSO (PBMC assay) or Cremophor EL and ethanol (70:30) (whole blood assay) were present at a concentration of 1% in all samples. Biosynthesis of cytokines was induced in all samples by stimulation with 1 μg/mL LPS (from *Escherichia coli*, serotype 026:B6, Sigma-Aldrich). All samples were stimulated for 4 h (37 °C, 5% CO_2). The cell reaction was terminated in an ice bath, and the samples were centrifuged. Concentrations of IL-1β and TNF-α in supernatants were determined by ELISA (Beckman Coulter Immunotech). The anti-cytokine activity of each compound was determined by blotting the percent reduction of cytokine concentration in test samples compared to the control samples on semilogarithmic paper over the concentration range of test

compounds (10^{-4} to 10^{-8} M). Results are given as IC_{50} values (μM). All assays were carried out at least in duplicate.

p38 MAP Kinase Assay.[25] Inhibitor dilutions were made in DMSO. Final DMSO concentrations did not exceed 1%. KB2 buffer was prepared by dissolution of p38 MAP kinase (16 μL) in KB1 buffer (7984 μL) containing 50 mM Tris (pH 7.5), 10 mM $MgCl_2$, 10 mM β-glycerolphosphate, 100 μg/mL of BSA, 1 mM DTT, 100 μM ATP, and 0.1 mM Na_3VO_4. Samples of KB2 buffer were preincubated for 5 min with test compounds in different concentrations (37 °C). The preincubated samples were transferred to a 96-well Immulon 4HBX plate coated with ATF-2 and incubated for 1 h (37 °C). Control samples were included containing only KB2 buffer. The 96-well plate was thoroughly washed with ultrapure H_2O and incubated with phospho-ATF-2-antibody (Cell Signaling Technology) for 1 h (37 °C). The 96-well plate was then thoroughly washed with ultrapure H_2O and incubated with alkaline phosphatase conjugated GAR antibody (Santa Cruz Biotechnology) for 1 h (37 °C). Next, the 96-well plate was thoroughly washed with ultrapure H_2O and blocking buffer containing Tween 20 (0.05%), BSA (0.25%), and NaN_3 (0.02%) in TBS; and, finally, the plate was washed again with ultrapure H_2O. 4-Nitrophenolphosphate was added to all wells, and the optical density was measured at 405 nm. Inhibition of p38 MAP kinase was determined by blotting the percent reduction of phospho-ATF-2 concentration in test samples compared to control samples on semilogarithmic paper over the concentration range of test compounds (10^{-4} to 10^{-8} M). Results are given as IC_{50} values (μM).

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